Complex inheritance pattern of dyskeratosis congenita in two families with two different mutations in the telomerase reverse transcriptase gene

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Running title: Complex inheritance of DC in TERT
Abstract

Heterozygous mutations in the telomerase components \textit{TERT} the reverse transcriptase and \textit{TERC} the RNA template cause autosomal dominant dyskeratosis congenita due to telomere shortening. Anticipation, whereby the disease severity increases in succeeding generations due to inheritance of shorter telomeres is a feature of this condition. Here we describe two families in which two \textit{TERT} mutations are segregating. Both families contain compound heterozygotes. In one case the proband is homozygous for a novel mutation causing a P704S substitution while his father’s second allele encodes a H412Y mutation. The proband in the second family has mutant alleles Y846C and H876Q. Transfection studies show co-dominant expression of the mutated alleles with no evidence of a dominant negative effect or of intragenic complementation. Thus in these families the expression of both \textit{TERT} alleles and the inherited telomere length contribute to the clinical phenotype.
Introduction

Mutations in genes encoding components of the telomerase ribonucleoprotein complex resulting in very short telomeres have been identified in patients with dyskeratosis congenita (DC), a rare inherited bone marrow failure syndrome.\(^1\)\(^-\)\(^6\) X-linked DC is caused by mutations in the *DKC1* gene, encoding a protein necessary for the stabilization of the *TERC* RNA. Individuals with autosomal dominant DC (AD DC) are heterozygous for mutations in the telomerase RNA *TERC* or the gene encoding the catalytic subunit TERT.\(^2\)\(^-\)\(^5\) In contrast to patients with X-linked DC, who usually develop severe disease with a high penetrance, disease penetrance and expressivity in AD DC are highly variable and, in addition to the gene mutation, the inheritance of short telomeres is required for the manifestation of the disease.\(^7\)\(^,\)\(^8\) Here we demonstrate that the inheritance of AD DC may be complex. We report a DC patient homozygous for a *TERT* mutation and compound heterozygotes in two separate families with apparent co-dominance of the two mutations.

Materials and Methods

Clinical and genetic information was obtained through our ongoing study on the molecular mechanisms of bone marrow failure (http://bmf.im.wustl.edu). The study is approved by the Washington University School of Medicine Institutional Review Board. Informed consent was provided according to the Declaration of Helsinki. DNA for mutation analysis was extracted from peripheral blood cells (Qiagen, CA). Telomere length measurements in peripheral blood mononuclear (PBMC) by flow-FISH and direct DNA sequencing were previously described.\(^8\) Primers used are shown in Table 1 included in supplementary data.
The mutations identified were introduced in the p3.1+ TERT plasmid\(^9\) by using the QuickChange XL site-directed mutagenesis kit (Stratagene, CA). Four \(\mu\)g of wild type (WT) or mutant TERT plasmid were transfected into WI-38 VA-13 cells at 80% confluence in the presence of equal amount of pUC TERC using lipofectamine 2000 (Invitrogen, CA).\(^10\) In co-transfection experiments 2\(\mu\)g of each mutant TERT plasmid were used. Thirty-six hours after transfection telomerase activities were determined in cell lysates at protein concentrations of 40, 10, 2.5, and 0.625ng using a Q-PCR based TRAP assay as previously described.\(^11\)

**Results and discussion**

Figure 1A shows the pedigrees of families 199 and 284. Patient 199.1 is a 31-year-old man of Scottish descent. His clinical manifestations include short stature, elfin appearance, esophageal stricture, leukoplakia of the buccal mucosa, anus and penis, abnormal pigmentation of his neck, trunk and back, hyperkeratosis of his palms, ridged finger nails, avascular necrosis of both hips, tooth loss, chronic diarrhea, learning difficulties, pulmonary infiltrates, and progressive bone marrow failure (Fig. 1B). His 61-year-old father was diagnosed with osteoporosis at the age of 60. His 60-year-old mother is healthy. Both parents have normal peripheral blood cell counts. The paternal grandmother (age, 84 years) has a history of anemia, osteoporosis, and pulmonary fibrosis. The maternal grandmother was reported to have died at the age of 60 years old because of pulmonary fibrosis.

Mutation analysis revealed that patient 199.1 is homozygous for a C to T transition in exon 5 of the TERT gene (cDNA nt C2110T) causing a proline to serine substitution at
amino acid 704 (P704S). Functional analysis in WI-38 VA-13 cells demonstrated that the
TERT P704S mutation severely reduces telomerase activity to 13% of normal \( (P<0.0001; \)
Fig. 2A). Both parents are heterozygous for the TERT P704S mutation (Fig. 1A).
Interestingly, however, the father carries a second \( TERT \) mutation in exon 2. This
C1234T mutation (H412Y), has been previously described in an unrelated family.\(^3\) This
mutation reduced telomerase activity to 36% of normal in our transfection experiments
\( (P=0.0004; \) Fig. 2A). Co-expression of WT TERT with either the P704S or H412Y
variants did not show evidence of a dominant negative effect. Whereas the co-expression
of the two \( TERT \) mutations resulted in an intermediate telomerase activity of 22%
\( (P=0.0007; \) Fig. 2B), suggesting a synergic effect on telomerase activity and no
intragenic complementation. Careful analysis of the family tree revealed that the parents
are fourth cousins, explaining the presence of the TERT P704S mutation in both parents.

Telomere length measurement in family 199 revealed that patient 199.1 has very
short telomeres (below the 1st percentile of the normal telomere length distribution, Fig.
2C). Interestingly, the father (199.2), who is compound heterozygous for the TERT
P704S and H412Y mutations, has also very short telomeres, whereas the mother (199.3),
who is heterozygous for TERT P704S mutation, has a normal telomere length.

Patient 284.1 is an 8-year-old girl of European descent, originally diagnosed with
moderate but progressive aplastic anemia. Both of her parents are healthy with no
abnormalities in the peripheral blood. Family history was negative for blood diseases,
pulmonary fibrosis, or cancer.

Mutation analysis revealed two different \( TERT \) gene sequence alterations. The
A2537G in exon 9 (Y846C) and C2628G mutation in exon 10 (H876Q). Further analysis
showed that the TERT Y846C mutation was inherited from the mother, whereas the TERT H876Q mutation was inherited from her father, indicating that patient 284.1 is a compound heterozygote for the two TERT gene mutations (Fig 1A). Both TERT gene mutations result in a significantly reduce telomerase activity after transfection into WI-38 VA-13 cells to 10% (P<0.0001) and 50% (P=0.00036) of normal (Fig. 2A), whereas the co-transfection of the two mutants results in a telomerase activity of 38% (P=0.004; Fig. 2B).

Telomere length in peripheral blood cells from patient 284.1 was very short, below the 1st percentile of normal and so were those measured in her mothers (284.2) and in one of her uncles (284.4) both of whom carry the TERT Y846C mutation. Telomere length in her father (284.3) heterozygous for the TERT H876Q mutation was between the 1st and 5th percentile of normal (Fig. 2C).

In conclusion, we have identified three novel and one recurrent TERT gene mutation in two families who were thought to have sporadic DC and idiopathic aplastic anemia. All four mutations are hypomorphic mutations, impairing, but not eliminating telomerase activity. Homozygous hypomorphic TERT mutations have recently been found to cause disease in two consanguineous families.12 Here we demonstrate that in a non-consanguineous family compound heterozygosity for TERT can cause disease and that the involvement of TERT in the pathogenesis of DC is probably more complex than initially anticipated. Our data indicate that in compound heterozygosity or homozygosity for hypomorphic TERT mutation the mutant alleles are co-dominant and suggest that severity of telomerase dysfunction and the inheritance of short telomeres determine the clinical phenotype and onset of disease. Co-dominant inheritance has also been found in
one family with two hypomorphic *TERC* gene mutations, whereas compound heterozygosity or homozygosity for *TERC* or *TERT* null mutations have never been reported, suggesting that in humans, in contrast to mice, biallelic *TERC* or *TERT* null mutations are probably not compatible with life. The consideration that both sides of the family may be affected even in non-consanguineous families might have important implications for the patient in the selection of a potential sibling donor as well as for the prognosis and management of other family members carrying one or two of the identified gene mutations.

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**Authorship**

Contribution: H.Y.D. designed and performed the experiments, analyzed the data, and drafted the manuscript; M.B. and P.J. M. design the experiments and drafted the manuscript. E.P. carried out some of the experiments; D.B.W., P.M., J.J.F., and S.J.B. aided in the collection of the family’s clinical data.

The authors declare no competing financial interests.
**References**


Figure legends

Figure 1 A) Pedigrees and identified TERT gene mutations in families 199 and 284. Circles, females; squares, males; white, wild type; color, mutant as indicated in the chart. Half filled symbols indicate heterozygosity. The TERT gene haplotype is shown. 1 B) Clinical manifestations in patient 199.1. are characteristic of DC including hyperpigmentation of the back and reticular hyperpigmentation of neck, ridged and brittle fingernails, and leukoplakia of the tongue (Camera; Canon EF-S 18-55mm, Japan).

Figure 2A) In vitro telomerase activity of the mutant TERT proteins in WI-38 VA-13 cells. WI-38 VA-13 cells were transfected with a plasmid expressing the mutant TERT cDNA sequences and a plasmid expressing the WT TERC RNA. Telomerase activity was determined using a Q-PCR based TRAP assay. Activity is shown in comparison to the activity obtained after transfection with WT TERT cDNA (=1.0). Telomerase activity in 293T cells served as a positive control. 2B) In vitro telomerase activity of two mutants or one mutant and WT TERT proteins in WI-38 VA-13 cells. WI-38 VA-13 cells were co-transfected with two plasmids expressing two different mutants, or one mutant and the WT TERT cDNA sequences. Equal amounts of plasmid expressing the TERC RNA were co-transfected in all experiments. Activity is shown in comparison to the activity obtained after the transfection of equal amounts of the WT TERT (=1.0). Experiments were performed four times and co-transfection experiments twice. The comparison of telomerase activity between the variants was performed by ANOVA analysis followed by post hoc test. Statistically significant reduction of telomerase activity (P< 0.05) is indicated with a star.
2C) Telomere lengths measured in PBMN cells. Lines represent percentiles (1%-99%) of telomere length measured in 234 healthy individuals between the ages of 0.3 to 94 years old. The telomere lengths of family 199 are represented as circles and those of 284 as triangles. Filled symbols indicate those with mutations (see text and Fig. 1), empty symbols indicate family members carrying two wild type alleles.
Figure 1

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B

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Figure 2

A

B

C
Complex inheritance pattern of dyskeratosis congenita in two families with two different mutations in the telomerase reverse transcriptase gene

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