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Deletion of the *Telomerase Reverse Transcriptase* Gene and Haploinsufficiency of Telomere Maintenance in Cri du Chat Syndrome

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Cri du chat syndrome (CdCS) results from loss of the distal portion of chromosome 5p, where the *telomerase reverse transcriptase* (*hTERT*) gene is localized (5p15.33). hTERT is the rate-limiting component for telomerase activity that is essential for telomere-length maintenance and sustained cell proliferation. Here, we show that a concomitant deletion of the *hTERT* allele occurs in all 10 patients with CdCS whom we examined. Induction of hTERT mRNA in proliferating lymphocytes derived from five of seven patients was lower than that in unaffected control individuals ($P < .05$). The patient lymphocytes exhibited shorter telomeres than age-matched unaffected individuals ($P < .0001$). A reduction in replicative life span and a high rate of chromosome fusions were observed in cultured patient fibroblasts. Reconstitution of telomerase activity by ectopic expression of hTERT extended the telomere length, increased the population doublings, and prevented the end-to-end fusion of chromosomes. We conclude that hTERT is limiting and haploinsufficient for telomere maintenance in humans in vivo. Accordingly, the *hTERT* deletion may be one genetic element contributing to the phenotypic changes in CdCS.

Introduction

Human chromosomes terminate with telomeres consisting of TTAGGG repeat sequences and associated proteins. Telomeres are essential to genomic stability and integrity and are synthesized by the ribonucleoprotein enzyme telomerase (Blackburn 2001; Forsyth et al. 2002). Telomerase, an RNA-dependent DNA polymerase, contains two critical components: the RNA template (hTER) and the catalytic unit, or telomerase reverse transcriptase (hTERT). hTERT has been shown to be the rate-limiting component for telomerase activity, and its expression is required for telomerase activation in various human cells (Harrington et al. 1997; Kilian et al. 1997; Meyerson et al. 1997; Nakamura et al. 1997; Bodnar et al. 1998). Because hTERT/telomerase activation is critical to cellular immortalization and tumorigenesis, extensive efforts have been focused on the hTERT dysregulation in malignant cells (J. P. Liu 1999; Ducrest et al. 2002). Moreover, it is not possible to examine the regulation and role of hTERT in humans by using forward genetics; therefore, the in vivo physiological regulation and function of hTERT has not yet been studied.

Cri du chat syndrome (CdCS [MIM 123450]), one of the most frequent autosomal deletion syndromes, results mainly from loss of the distal portion of chromosome 5p, where the *hTERT* gene is localized (5p15.33) (Niebuhr 1978; Meyerson et al. 1997; Bryce et al. 2000; Zhang et al. 2000; Mainardi et al. 2001). We hypothesized that one allele of *hTERT* should be deleted in this syndrome, and, in that case, CdCS might provide a human genetic model to address the in vivo hTERT regulation/function. The present study demonstrates that a concomitant deletion of one *hTERT* copy indeed occurs in CdCS, consequently compromising hTERT expression and causing haploinsufficiency for telomere maintenance in the affected patients. Our findings suggest that hTERT is strictly required for normal cell growth and development in humans.

Subjects and Methods

Patients

The present study included 10 patients with median age 8.5 years (ranging from 1 mo to 35 years). The diagnosis of CdCS was based on clinical characteristics and cytogenetic analysis (Niebuhr 1978). The karyotyping data from all the patients are presented in table 1. The study was approved by the local ethics committee.

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Table 1

Karyotyping, Loss of the *hTERT* Allele, Telomere Length, and hTERT Expression in Patients with CdCS

Patient	Age (years)	Karyotyping	*hTERT* Status	Telomere Length[a]	TRFs[b] (kb)	hTERT mRNA Induction[c]
1	.1	46,XY,del(5)(p13)	+/−			
2	1	46, XX,del(5)(p15.1)	+/−	20,638 ± 6,141 (43,904 ± 546)		
3	4	46,XY,del(5)(p13)	+/−	15,819 ± 1,206 (46,549 ± 4,006)		.6 (3.1)
4	8	46,XY,rec(5)dup(5q)inv(5)(p14q35)	+/−	12,339 ± 3,901 (36,240 ± 2,290)	7.8 (9.5)	1.6 (1.7)
5	8.5	46,XX,del(5)(p13)	+/−	12,684 ± 1,886 (30,720 ± 1,080)		6.0 (5.4)
6	8.8	46,XX,del(5)(p15.1)	+/−	7,520 ± 1,823 (36,976 ± 5,230)		.5 (2.1)
7	10	46,XX,del(5)t(5;3)(p14;q31)	+/−	6,552 ± 635 (22,912 ± 3,030)		.7 (2.1)
8	12	46,XY,del(5)(p14)	+/−	13,461 ± 3,419 (26,146 ± 2,944)		3.2 (6.6)
9	14	46,XX,del(5)(p15.1)	+/−	4,337 ± 864 (30,223 ± 3,254)	6.8 (11)	.9 (3.0)
10	35	46,XX,del(5)(p14)	+/−			

[a] Telomere length in patient lymphocytes, analyzed by using Q-FISH (for details, see the "Subjects and Methods" section) and expressed as total fluorescence intensity. Telomere signal for each age-matched control is shown in parentheses.

[b] Assessed by using Southern blot. TRFs for age-matched control individuals are shown in parentheses.

[c] The levels of hTERT mRNA in activated T cells from patients, determined by using competitive RT-PCR (for details, see the "Subjects and Methods" section) and expressed as the ratio of hTERT signals:competitive signals normalized to β2-M expression. hTERT mRNA expression for each control individual is shown in parentheses.

FISH for the hTERT *Gene and Spectral Karyotyping (SKY) for Cytogenetic Analysis of Patient Fibroblasts*

The *hTERT* locus-specific probe and the reference probe, located at 5q31, were directly labeled with SpectrumOrange and SpectrumGreen fluorophore-conjugated dUTP with standard nick translation, respectively (Vysis). FISH was performed on the metaphase lymphocytes and fibroblasts, as described elsewhere (Zhang et al. 2000). In brief, the slides were fixed with paraformaldehyde, followed by dehydration in graded ethanol and subsequent hybridization. The samples were evaluated under a Zeiss epifluorescence microscope equipped with the corresponding wavelength filter, charge-coupled–device camera, and image-capturing and -analysis system. The metaphase fibroblasts at early and late population doublings (PDs) and their telomerase-expressing variant were analyzed for chromosome changes by using SKY, as described elsewhere (Zhang et al. 2000).

Telomere-Length Measurement

Telomere length was assessed by using quantitative FISH (Q-FISH), flow FISH, and Southern blot. The metaphase fibroblasts and lymphocytes derived from patients and age-matched unaffected control individuals were hybridized with the fluorescein isothiocyanate (FITC)–labeled peptide nucleic acid (PNA) telomere probe $(CCCTAA)_3$, as described by Lansdorp et al. (1996). Metaphase chromosomes were counterstained with 4,6-diamidino-2-phenylindole. Three-dimensional image volumes of the hybridized metaphases, measuring 768 × 768 × 5 pixels, were obtained using a Delta Vision system (Applied Precision). The maximum-intensity projections of the acquired images were analyzed using the Imp image-processing software (Center for Image Analysis at Uppsala University). Flow FISH was performed as described elsewhere but with minor modifications (Hultdin et al. 1998; Rufer et al. 1998). For Southern blot analysis, 5 μg of genomic DNA derived from the control and patient peripheral-blood cells was digested with the restriction enzymes *Hin*fI and *Rsa*I and was resolved in 0.7% agarose gels. DNA was transferred onto nylon membranes, was hybridized with the digoxin (DIG)–labeled $(CCCTAA)_3$ probe, and was detected with the DIG detection kit, following the manufacturer's protocol (Roche Diagnostics Scandinavia AB).

Cell Culture, Competitive RT-PCR for hTERT mRNA, and Telomerase Activity Assay

Lymphocytes isolated from peripheral blood from patients and unaffected control individuals were cultured in the presence of anti-CD3 (1 μg/ml) and -CD28 (0.5 μg/ml) antibodies (R & D Systems, Norakemi AB) for 48 h. Dermal fibroblasts derived from patients and unaffected control individuals were maintained in 15% fetal calf serum–containing RPMI 1640 medium, and the number of divisions was counted. Competitive RT-PCR for hTERT mRNA was performed as described elsewhere (Xu et al. 1999). The primer sequences specific for hTERT mRNA (GenBank accession number AF015950) are as follows: forward (5′-CGG AAG AGT GTC TGG AGC AA) (1784–1803) and reverse (5′-GGA TGA AGC GGA GTC TGG A) (1928–1910). In the present study, 2,500 hTERT-competitive molecules were used per reaction with 36 PCR cycles. As an internal control, β2-microglobulin (β2-M) RT-PCR was run in parallel, to monitor RNA integrity and efficiency of cDNA synthesis. To determine telomer-

ase activity, we used a commercial Telomerase PCR ELISA kit (Roche Diagnostics Scandinavia AB), following the manufacturer's protocol, and 28 PCR cycles.

Infection of Fibroblasts with hTERT Retroviral Vectors

The hTERT retroviral plasmid pBABE-puro-hTERT has been described elsewhere (Counter et al. 1998), and the hTERT retroviral-producing cells were made by transfection of the plasmid DNA into PA317 packaging cells. Fibroblasts at PD10 from two patients with CdCS were infected with retroviral supernatants and were then selected in the presence of puromycin (0.75 μg/ml) for 10 d. The infection of the cells with the parental pBABE-puro was performed in parallel, as a control.

β-Galactosidase (β-Gal) Staining of Fibroblasts

β-Gal staining of patient fibroblasts was performed as described elsewhere (Dimri et al. 1995). In brief, patient fibroblasts and their hTERT-expressing variants were grown on slides at 37°C overnight. The cells were rinsed with PBS once, were fixed in 3% formaldehyde for 5 min, and were incubated with freshly prepared β-Gal staining solution at 37°C for 24 h.

Results

The hTERT *Gene Deletion and Accelerated Shortening of Telomeres in Lymphocytes from Patients with CdCS*

We first examined the *hTERT* gene status in patients with CdCS by FISH with a locus-specific probe (Zhang et al. 2000). As expected, lymphocytes or fibroblasts derived from all 10 patients showed only one copy of *hTERT* (fig. 1*A*). Since haploinsufficiency for telomere maintenance has been seen in mouse embryonic stem cells (MESCs) carrying a single *TERT* copy, we asked whether this is the case in patients with CdCS. Lymphocytes from eight patients and age-matched unaffected control individuals were analyzed for telomere length by using Q-FISH and flow FISH (Lansdorp et al. 1996; Hultdin et al. 1998; Rufer et al. 1998). Telomeric sequences were visualized by the hybridization of a PNA FITC-labeled telomere probe to metaphase cells, and the total telomere fluorescence intensity was then measured (figs. 1*B* and 1*C*). In patients and control individuals, telomere-signal intensity tended to decrease with increasing age (figs. 1*B*–1*D* and table 1), consistent with early observations (Harley et al. 1990; Hastie et al. 1990; Allsopp et al. 1992; Lansdorp et al. 1996). In all the patients, the telomere-specific signal was significantly weaker than in their age-matched controls (figs. 1*B*–1*D* and table 1). To validate the result achieved by Q-FISH, we further applied Southern blot methods to determine telomere restriction fragments (TRFs) in lymphocytes derived from two patients. A reduction in TRFs in the patient samples was similarly observed, as shown in figure 1*E* and table 1. These results demonstrate an accelerated telomere shortening due to the *hTERT* gene deletion in patients with CdCS, supporting haploinsufficiency for telomere stabilization in humans.

Premature Senescence and the End-to-End Fusion of Chromosomes in Cultured CdCS Fibroblasts Can Be Prevented by Ectopic Expression of hTERT

Progressive loss of telomeres with cellular replications limits the in vitro and maybe the in vivo life span of human somatic cells. When telomeres become critically short, normal somatic cells are signaled to enter into a senescent stage characterized by a permanent cell-growth arrest (Blackburn 2001). To investigate whether shorter telomeres result in defects in cell-proliferation potential in CdCS, we determined the number of in vitro PDs of patient fibroblasts. Dermal fibroblasts were obtained from two patients (1 mo and 35 years old, respectively) and grown under a standard culture condition. Fibroblasts divided only ~25 (in the 1-mo-old patient) and ~20 times (in the 35-year-old patient) before they became senescent, the life span being much shorter than that of the fibroblasts from an unaffected adult control individual (~50 PDs). This finding supports an impaired replicative capacity in cells from patients with CdCS. To test whether the reduced PDs are telomere-length dependent, we reconstituted telomerase activity in the patient fibroblasts at PD10 by infecting them with a viral hTERT expression vector (Counter et al. 1998), and we then monitored changes in their replicative capacity, senescence-associated features, and telomere sizes. Concurrent with the increase in the number of PDs, the fibroblasts infected with the parental viral vector started to grow slowly and eventually stopped dividing. Consistently, these cells exhibited β-Gal staining, an established characteristic for senescent cells (Dimri et al. 1995) (fig. 2*A*), and showed minimal DNA synthesis, as determined by incorporation of [^{3}H]-thymidine (data not shown). In contrast, the telomerase-expressing fibroblasts displayed no senescent signs, even at PD30. A new telomere-length equilibrium was established in telomerase-expressing cells, and substantial (fourfold) increases in telomere sizes were demonstrated by using Q-FISH and flow-FISH assays (fig. 2*B* and data not shown). So far, the hTERT-expressing patient fibroblasts have doubled nearly 200 times and continue to proliferate. The observed result thus suggests that a telomere-mediated mechanism contributes to the premature senescence of CdCS-derived cells.

Given that telomeres play an essential role in the protection of linear chromosome ends from degradation and illegitimate recombination, loss of telomeres is believed to be associated with genomic instability (Counter et al.

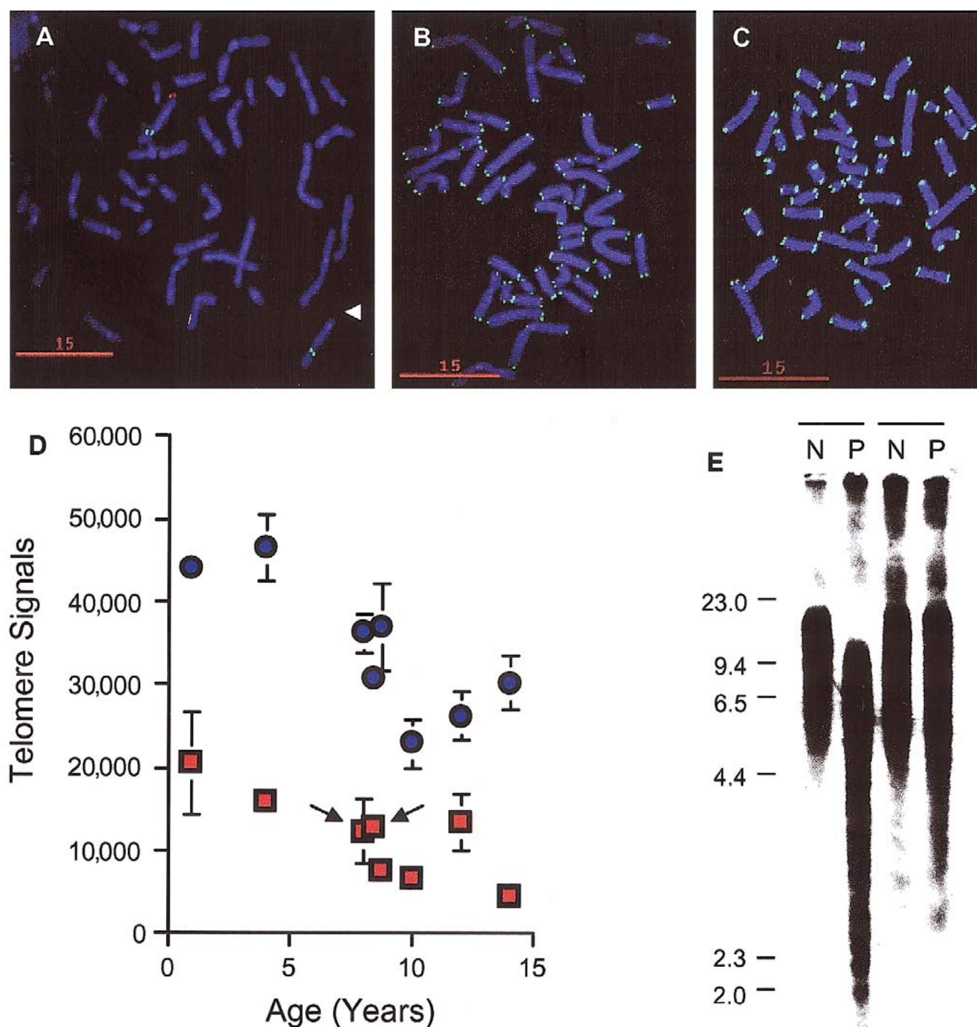

Figure 1 Deletion of the *hTERT* gene and accelerated telomere shortening in patients with CdCS. *A,* Deletion of one *hTERT* allele in CdCS. A representative lymphocyte metaphase from a patient with CdCS is shown. The *hTERT* and 5q31 marker signals are labeled in red and green, respectively. The arrowhead indicates the chromosome 5 lacking the *hTERT* signal. Length (in μM) is represented by the scale bar. *B* and *C,* Visualization of telomeres on lymphocyte chromosomes from a patient with CdCS and an age-matched unaffected control individual, respectively, by using Q-FISH. Length (in μM) is represented by scale bars. *D,* Telomere length expressed as the total fluorescence intensity, assessed by using the Imp image-processing software, in metaphase lymphocytes from eight patients with CdCS (*red square*) and age-matched unaffected control individuals (*blue circle*) (CdCS: 11,668 ± 1,887; control individuals: 34,208 ± 2,923 [$P < .0001$, by Student's *t* test]). Bars indicate SEM (in some cases, the bar is invisible owing to SEM values that are too small). Arrowheads indicate two patients whose T cells expressed comparable levels of hTERT/telomerase activity when compared with matched control cells. *E,* Telomere length as determined using Southern blot. Typical smear signals of human telomeres are shown. The positions (in kb) of DNA molecular markers are indicated at left. "N" and "P" denote the age-matched control individuals and patients, respectively.

1992; Artandi et al. 2000). Indeed, a high rate of chromosomal abnormalities, such as end-to-end fusion, is found in telomerase-deficient mice or MESCs in which mouse telomerase RNA (mTER) or mouse telomerase reverse transcriptase (mTERT) is knocked out (Blasco et al. 1997; Y. Liu et al. 2002). We further sought to determine additional cytogenetic alterations in CdCS cells that exhibit abnormally short telomeres. As described above (see the "Subjects and Methods" section), the fibroblasts were analyzed by using SKY (Zhang et al. 2000). Cells from the 1-mo-old patient showed a karyotype that, at both early and late PDs, was normal except for a partial 5p deletion; however, in late-passage fibroblasts (PD18) from the 35-year-old patient, it was determined, by using SKY, that 45% of the metaphases exhibited chromosome fusions (one to three fusion events/metaphase) (fig. 2C). In accordance with this, we observed that a fraction of chromosomes lacked clear telomere signals at their termini (data not shown). Thus, potential genomic instability becomes evident with increasing ages in CdCS. When telo-

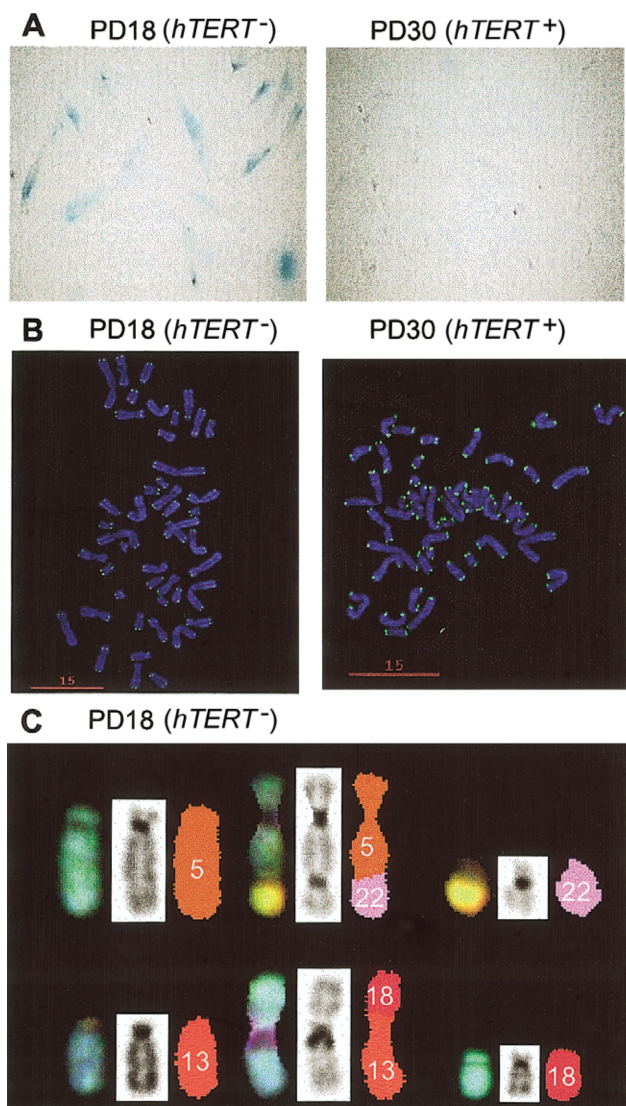

Figure 2 Extension of life span, stabilization of telomeres, and prevention of chromosome fusions by expression of telomerase activity in fibroblasts from patients with CdCS. Telomerase-reconstituted fibroblasts at PD30 (*right*) from a 35-year-old patient, together with their parental cells at PD18 (*left*), were analyzed for β-Gal staining (Senescence biomarker) (*A*), telomere length (*B*), and chromosome fusions (*C*). *C*, Representative SKY results from one metaphase. *Top*, Fusion between chromosomes 5 and 22 (*middle*). The other chromosome 5 with a partial 5p deletion and the normal chromosome 22 are shown at right and left, respectively. *Bottom*, Fusion event between chromosomes 13 and 18 (*middle*). Normal chromosomes 13 and 18 from the same cell are shown at right and left, respectively.

merase activity was reconstituted in patient fibroblasts at PD10, with telomere lengthening as visualized by Q-FISH (fig. 2*B*), aberrant fusions were rarely seen (data not shown), indicating that telomerase expression prevents chromosome fusions from occurring in CdCS.

Diminished hTERT Induction in Activated Lymphocytes Derived from Patients with CdCS

We then sought to determine whether the *hTERT* deletion occurring in CdCS affects the gene expression and telomerase activation. It is known that hTERT expression and telomerase activity are induced in activated lymphocytes (Forsyth et al. 2002). We thus stimulated patient T cells with anti-CD3 and -CD28 antibodies, followed by measurement of hTERT mRNA expression. Efficient activation of lymphocytes was demonstrated by the presence of CD25 (a T-cell activation marker) on >90% of the treated CD3-positive T cells. The stimulated T cells from five of seven patients exhibited only minimal amounts of hTERT mRNA induction—amounts that were one-fifth to one-half those in T cells from unaffected individuals (fig. 3*A*). However, in two patients, levels of hTERT mRNA did not differ from those in matched control individuals, as demonstrated by a number of repeated assays. It was also evident that induction of hTERT expression varied considerably within patients and unaffected control individuals. In five patients, telomerase activity was further analyzed using a telomeric-repeat–amplification protocol (TRAP). There was an overall correlation between telomerase activity and hTERT expression—lower in the patients with diminished hTERT mRNA induction but indistinguishable in the patients without reduction of hTERT mRNA when compared with the matched control individuals (fig. 3*B*). However, there was no significant difference in telomerase activity between patient and control cells ($P > .05$, by Student's t test). Variation in the abundance of telomerase activity from person to person and inability to identify subtle differences by the PCR-based TRAP assay may explain these findings.

Discussion

In the present study, we have demonstrated that a single *hTERT* copy is incapable of maintaining telomere length in patients with CdCS, providing evidence that hTERT/telomerase is limiting in vivo and is haploinsufficient for telomere maintenance in humans. Consistent with this, it has recently been shown that MESCs heterozygous for the mTERT disruption, unlike their wild-type counterparts, display progressive telomere attrition with cellular proliferation, indicative of the haploinsufficiency of TERT in mice, too (Y. Liu et al. 2000, 2002). These observations, together with similar findings on yeasts (Lendvay et al. 1996; Lingner et al. 1997), suggest that such a regulatory pathway of telomere length is evolutionarily conserved.

A close correlation between the dosages of *hTERT* copies and the expression levels of hTERT/telomerase has previously been observed in various human malignant cells. The tumor cells carrying *hTERT* ampli-

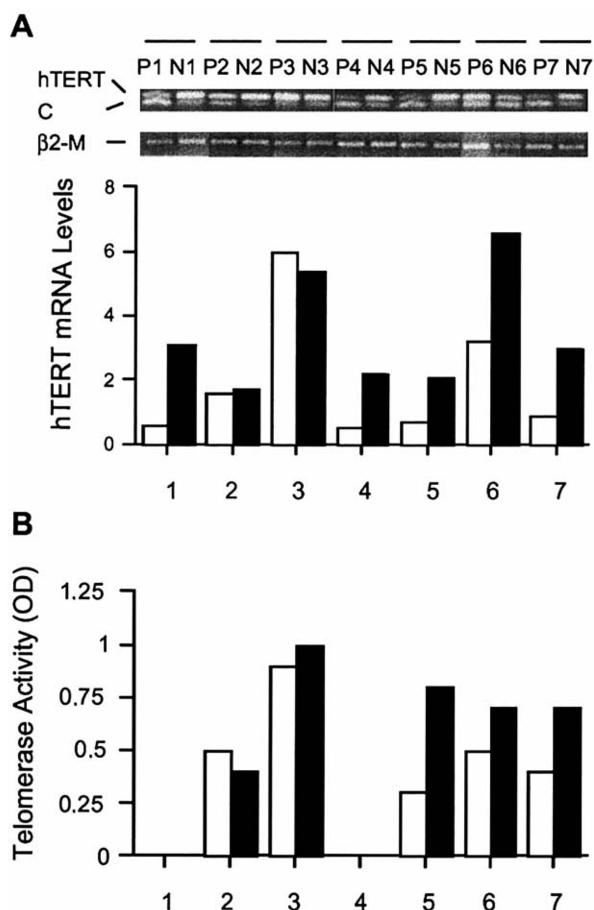

Figure 3 hTERT mRNA expression and telomerase activity in activated T lymphocytes derived from patients with CdCS. Unblackened and blackened columns show patients with CdCS and unaffected control individuals, respectively. *A,* hTERT mRNA levels in activated T cells from patients with CdCS. *Top,* hTERT mRNA signals as revealed by a competitive RT-PCR. β2-M RT-PCR was performed as internal controls. P1–P7 = patients in order of age; N1–N7 = matched unaffected control individuals; C = hTERT competitor. *Bottom,* Relative levels of hTERT mRNA which were calculated from signal ratio of hTERT mRNA and its competitor normalized to β2-M expression (as shown at top) (patients with CdCS: 1.9 ± 0.8; control individuals: 3.4 ± 0.7 [$P < .05$, by Student's *t* test]). *B,* Telomerase activity in activated T cells from patients and unaffected control individuals as measured by using a telomerase PCR-ELISA kit (patients with CdCS: 0.5 ± 0.3; control individuals: 0.7 ± 0.2 [$P > .05$, by Student's *t* test]). Relative levels of telomerase activity were arbitrarily expressed as absorbance (OD) at 450 nm.

fication frequently express higher levels of hTERT mRNA/protein, whereas those with the loss of one *hTERT* allele exhibit a significant reduction in hTERT/telomerase expression (Zhang et al. 2000, 2002; Saretzki et al. 2002). In accordance with these findings, hTERT induction was compromised by the loss of the *hTERT* allele in activated lymphocytes derived from five of seven patients with CdCS; consequently, insufficient telomerase activity led to accelerated telomere shortening. However, two of the patients studied had indistinguishable levels of hTERT mRNA and telomerase activity in their proliferating lymphocytes when compared with matched control individuals, as demonstrated by repeated quantitative measurements, and, unexpectedly, their lymphocytes still exhibited abnormally shorter telomeres, despite high hTERT expression and telomerase activity. At face value, the apparent dissociation between the *hTERT* gene copy number and mRNA expression and the discordance between telomerase activity and telomere maintenance that occur in these two patients are rather confusing. However, the current assays measure only a relative difference in amounts of hTERT mRNA and telomerase activity between the patients and unaffected control individuals, whereas it is most likely that maintenance of telomere length requires an absolute (rather than relative) abundance of hTERT/telomerase activity in each individual. This may be one potential explanation underlying the observed paradox. In addition, inability to identify subtle differences in telomerase activity by the PCR-based TRAP assay may also be misleading. An unexpected observation was similarly made in EMSCs in which one *mTERT* allele is disrupted (i.e., $mTERT^{+/-}$): There was no detectable decrease in expression of mTERT and telomerase activity, but the capacity for telomere elongation in those $mTERT^{+/-}$ EMSCs was impaired when compared with that of their wild-type counterparts (Y. Liu et al. 2000).

Conceivably, it is very difficult to assess whether TERT haploinsufficiency leads to telomere dysfunction by using a mouse model: First, mouse cells possess very big telomere sizes, and an extremely long time might be required to exhaust telomeres in crosses between $mTERT^{+/-}$ mice. Second, in $mTERT^{+/-}$ MESCs in which limited levels of telomerase activity are constitutively expressed, telomerase is specifically recruited to and elongates the shortest telomeres, to maintain critical telomere-length equilibrium (Y. Liu et al. 2002). The phenotype of telomere dysfunction is therefore prevented, despite a reduction of average telomere sizes in $mTERT^{+/-}$ MESCs. However, these may not be the case in human cells with relative shorter telomeres (~10 kb). During late fetal development or after birth, hTERT is stringently repressed, and telomerase activity is completely switched off in most human somatic cells. Thus, critically short telomeres in somatic CdCS cells cannot be preferentially compensated, and eventual onset of telomere dysfunction seems possible. The present study indeed demonstrates a reduced replicative life span and a potentially high frequency of chromosome fusions in CdCS, as evidence for phenotypic consequences of hTERT haploinsufficiency. The observation that chromosome fusions occur in adult but not pediatric patients is likely to reflect the phenotypic lag that has been seen in $mTER^{-/-}$ mice, in which genomic

instability is triggered in late (rather than early) generations (Blasco et al. 1997; Artandi et al. 2000). It should be pointed out, however, that this finding is informative (rather than conclusive) because of our limited sample size. More extended investigations are required in order to ascertain the hTERT haploinsufficiency-mediated replicative defect and genomic instability in CdCS.

Recent evidence suggests that TER may be limiting in vivo, too. Although a defective telomere elongation in *mTER*$^{+/-}$ mice is controversial (Niida et al. 1998; Hathcock et al. 2002), deletion or mutation of the *hTER* allele, as well as reduction in hTER expression/telomerase activation, has been found in a human disease, dyskeratosis congenita (DKC [MIM 305000]) (Mitchell et al. 1999; Vulliamy et al. 2001). Patients with DKC have premature shortening of telomeres in their somatic cells, and the disease onset triggered by telomere dysfunction is more frequently seen in second or later generations, likely owing to a phenotypic lag. These observations, together with the present finding, suggest that both *hTER* and *hTERT* are haploinsufficient for telomere maintenance. Given that both the deficiency and the overabundance of telomerase activity contribute to formation of mammalian malignancies (Blasco et al. 1997; Hahn et al. 1999; Artandi et al. 2000, 2002; González-Suárez et al. 2001) and that normal mammalian growth and development are rigidly dependent on optimal levels of telomerase activity (Vulliamy et al. 2001), such a finely tuned control may be very important to human health.

It is currently unclear to what extent the loss of the *hTERT* allele contributes to the clinical features of CdCS. During early stages of embryonic development, hTERT and telomerase are widely activated to prevent telomere attrition, so that high cellular-proliferation capacities necessary for organ growth are maintained (Forsyth et al. 2002). Insufficient or suboptimal hTERT expression resulting from the *hTERT* deletion in CdCS may affect normal fetal development. For instance, growth retardation and mental deficiencies observed in these patients could be attributable to accelerated telomere shortening during early development. It is also possible that haploinsufficiency for telomere stabilization leads to premature gray hair and small testes with spermatogonia, as well as other related conditions, in adult patients with CdCS (Breg et al. 1970; Niebuhr 1978; Van Buggenhout et al. 2000; Collins and Eaton-Evans 2001; Mainardi et al. 2001). In addition, it has been noticed that accelerated telomere shortening results in an increased incidence of cancers in DKC. According to the available literature, however, patients with CdCS do not seem to carry an increased risk of developing cancer. Because almost no patients with CdCS have children (Niebuhr 1978; Martinez et al. 1993), telomere dysfunction, as seen in them, owing to a phenotypic lag, may be much less pronounced than that observed in patients with DKC, thus not leading to a measurable increase in malignancy development and bone marrow failure.

It should be emphasized that deletion of the *hTERT* allele could not explain all characteristics of CdCS on the basis of the phenotype of mTERT-knockout mice (Yuan et al. 1999; Y. Liu et al. 2000). Concomitant loss of other genes at the end of the 5p region also plays crucial roles in the development of CdCS. Nevertheless, the deletion of the *hTERT* may be one genetic element contributing to phenotype changes in CdCS, based on the present finding. Thus, profound insights into the effect of hTERT haploinsufficiency in patient cells will lead to a better understanding of both in vivo hTERT regulation/telomerase biology and potential mechanisms underlying the phenotypic features of CdCS.

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Electronic-Database Information

The accession number and URLs for data presented herein are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for the hTERT mRNA sequence [accession number AF015950])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for CdCS and DKC)

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