Constitutional Telomerase Mutations Are Genetic Risk Factors for Cirrhosis

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Some patients with liver disease progress to cirrhosis, but the risk factors for cirrhosis development are unknown. Dyskeratosis congenita, an inherited bone marrow failure syndrome associated with mucocutaneous anomalies, pulmonary fibrosis, and cirrhosis, is caused by germline mutations of genes in the telomerase complex. We examined whether telomerase mutations also occurred in sporadic cirrhosis. In all, 134 patients with cirrhosis of common etiologies treated at the Liver Research Institute, University of Arizona, between May 2008 and July 2009, and 528 healthy subjects were screened for variation in the *TERT* and *TERC* genes by direct sequencing; an additional 1,472 controls were examined for the most common genetic variation observed in patients. Telomere length of leukocytes was measured by quantitative polymerase chain reaction. Functional effects of genetic changes were assessed by transfection of mutation-containing vectors into telomerase-deficient cell lines, and telomerase activity was measured in cell lysates. Nine of the 134 patients with cirrhosis (7%) carried a missense variant in *TERT*, resulting in a cumulative carrier frequency significantly higher than in controls ($P = 0.0009$). One patient was homozygous and eight were heterozygous. The allele frequency for the most common missense *TERT* variant was significantly higher in patients with cirrhosis (2.6%) than in 2,000 controls (0.7%; $P = 0.0011$). One additional patient carried a *TERC* mutation. The mean telomere length of leukocytes in patients with cirrhosis, including six mutant cases, was shorter than in age-matched controls ($P = 0.0004$). *Conclusion:* Most *TERT* gene variants reduced telomerase enzymatic activity *in vitro*. Loss-of-function telomerase gene variants associated with short telomeres are risk factors for sporadic cirrhosis. (HEPATOLOGY 2011;53:1600-1607)

Abbreviations: NASH, nonalcoholic steatohepatitis; qPCR, quantitative polymerase chain reaction; SNP, single nucleotide polymorphism.

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Telomeres, the natural ends of linear chromosomes, cap and protect chromosomes against damage and from being mistaken for double-stranded DNA breaks.[1] When a cell divides, telomeres shorten due to DNA polymerase's inability to fully replicate the 3′ ends of chromosomes, the "end-under-replication problem."[2] If telomeres become critically short, cellular signaling cascades involving p53 and p21 are activated, resulting in cell senescence or apoptosis.[3,4] To counter telomere attrition, cells with high proliferative capacity express telomerase, a reverse transcriptase enzyme that adds DNA repeats to telomeres.[5] Components of the telomerase complex include the reverse transcriptase enzyme, TERT, the RNA component that serves as template for telomere elongation, encoded by *TERC,* and associated proteins, including dyskerin (encoded by *DKC1*).[6,7] Loss-of-function heterozygous or homozygous mutations in genes of

the telomerase complex have been implicated in human diseases caused by a deficient tissue regeneration capacity,[3] including dyskeratosis congenita,[8,9] aplastic anemia,[10,11] and familial idiopathic pulmonary fibrosis,[12,13] and also with acute myeloid leukemia.[14,15] Common genetic variants in the region of the *TERT* gene on chromosome 5p15.33 have been associated with susceptibility to brain tumors, lung cancer, pancreatic cancer, and additional cancers.[16–20]

Dyskeratosis congenita is a rare genetic disease in which patients develop bone marrow failure and exhibit a mucocutaneous triad of abnormal reticular skin pigmentation, leukoplakia, and nail dystrophy.[21] Most cases are X-linked and caused by mutations in the *DKC1* gene. Dyskeratosis congenita also may be autosomal dominant, in which heterozygous mutations in the telomere biology genes *TERT, TERC*, or *TINF2* are etiologic, or autosomal recessive, due to mutations in *NOLA2* or *NOLA3*, coding telomerase-associated proteins.[22,23] The observation that lung disease, mainly pulmonary fibrosis, is present in up to 20% of patients with dyskeratosis congenita led to the association of telomerase mutations with familial idiopathic pulmonary fibrosis.[12] Approximately 7% of dyskeratosis patients have a concurrent diagnosis of hepatic disease, including cirrhosis.[21] Fatal liver complications are a relatively common cause of death after hematopoietic stem-cell transplantation for bone marrow failure in dyskeratosis congenita, whereas fatal liver complications are infrequent following transplant for other disorders, suggestive of a related underlying mechanism.[24] In families of patients with telomerase mutation and aplastic anemia, severe hepatic disease in relatives tracks to mutation status.[25]

The relationship between telomere shortening and risk of cirrhosis has been examined in an experimental model of mice null for the telomerase reverse transcriptase gene. *Tert*-deficient mice had reduced regenerative activity following partial hepatectomy as well as more hepatic fibrosis and inflammation after exposure to carbon tetrachloride (CCl_4) compared to normal mice.[26] In human cirrhosis, hepatocytes display excessive telomere shortening and senescence.[27,28] These findings in experimental animals and observations in humans suggest that reduced telomerase activity may contribute to the development of cirrhosis. In the present study we sought to determine whether germline missense sequence variants in the telomerase complex genes are more frequent in patients with nonfamilial cirrhosis due to a variety of causes.

Patients and Methods

Patients and Controls. Adults with cirrhosis who were patients at the liver clinic at the University of Arizona were recruited for the study. The diagnosis of cirrhosis was established by liver biopsy or clinical evidence of cirrhosis and portal hypertension (i.e., ascites, varices, or computed tomography [CT] findings of cirrhosis). None of the patients had a family history of liver disease or the classical manifestation of dyskeratosis congenita (bone marrow failure, ungual dystrophy, abnormal skin pigmentation). Patients or their legal guardians provided informed consent for genetic testing, as specified by protocols approved by the Institutional Review Board of the University of Arizona (IRB No. 08-0347-04). DNA was extracted from buccal mucosa swab using the Gentra Puregene buccal cell core kit (Qiagen, Gaithersburg, MD) or peripheral blood leukocytes using the automated Maxwell 16 System (Promega, Madison, WI). Samples from 528 healthy subjects were analyzed as controls for genetic screening, as described (control group 1).[11] An additional 1,472 healthy subjects were screened for variations in *TERT* exon 15 (control group 2; 751 individuals from the Human Genome Diversity Panel Project, 477 drawn from the NCI Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial [PLCO] Cohort, and 244 blood donors at the NIH Clinical Center; Supporting Tables 1, 2). DNA from peripheral blood leukocytes for telomere length measurement also was obtained from 175 healthy volunteers ranging in age from 0 to 99 years (control group 3; median 35.8 years old; Table 1).

Mutational Analysis. Bidirectional sequencing of *TERC* and *TERT* was performed as described.[11,14]

Telomere Length Measurement. Mean telomere length was measured in peripheral blood leukocytes by quantitative polymerase chain reaction (qPCR), as described.[29,30] PCR was conducted in triplicate in a 7500 Real Time PCR System (Applied Biosystems, Foster City, CA), and analysis was completed using SDSv1.3. The telomere length for each sample was determined using the telomere to single copy gene ratio (T/S ratio) with the calculation of the ΔC_t $[C_t^{(\text{telomere})}/C_t^{(\text{single gene})}]$. The T/S ratio for each sample (x) was normalized to the mean T/S ratio of reference sample $[2^{-(\Delta Ctx - \Delta Ctr)} = 2^{-\Delta\Delta Ct}]$, which was used for the standard curve, both as a reference sample and as a validation sample.

Functional Analysis. Functional analysis was performed for the *TERC* mutation 37A→G and *TERT* mutations P529L and T882I, as described.[14,25] In vitro mutagenesis was performed on the wildtype vector by Mutagenex (Somerset, NJ). Telomerase activity was measured using the fluorescent telomerase repeat

Table 1. Demographic Characteristics of 134 Patients with Hepatic Cirrhosis

	Cirrhotic Patients (n = 134)	Healthy Controls (n = 175)
	Number of Patients (%)	Number of Subjects (%)
Gender		
Male	86 (64)	89 (51)
Female	48 (36)	86 (49)
Ethnicity		
Caucasian	84 (63)	101 (58)
Hispanic	37 (28)	20 (11)
Native American	4 (3)	0
Asian	4 (3)	6 (4)
Black	0	21 (12)
Mixed ethnicity	5 (4)	27 (12)
Etiology of liver disease		
Hepatitis C virus	50 (37)	–
Alcohol	33 (25)	–
NASH	12 (9)	–
Hepatitis C and alcohol	11 (8)	–
Autoimmune hepatitis	6 (4)	–
Primary biliary cirrhosis	7 (5)	–
Primary sclerosing cholangitis	4 (3)	–
Hepatitis B virus	4 (3)	–
Budd-Chiari	1 (1)	–
Congenital hepatic fibrosis	1 (1)	–
Hepatitis B + fatty liver	1 (1)	–
Hepatitis C + B cell lymphoma	1 (1)	–
Sarcoidosis	1 (1)	–
Wilson's disease	1 (1)	–
Cause unknown	1 (1)	–
Age, median	56 years (range, 21 to 74 years)	36 years (range, 0 to 99 years)

NASH, nonalcoholic steatohepatitis.

amplification protocol (TRAPeze XL, Chemicon), as described.[14,25]

Statistical Analysis. Fisher's exact test was used to evaluate differences in the cumulative frequency of missense variations between patients and controls. For the comparison of *TERT* codon A1062T variant allele frequency (most common variant in patients) between patients and an extended number of controls (control group 2; n = 1472), the χ^2 test was employed, as it is not possible to calculate Fisher's test with such large sample groups. For the analysis of differences between patients and controls, telomere length was corrected for age. A subject's "predicted telomere length" was computed using $(a + b \times Age)$, where a and b were the least squares estimates of the slope and intercept for the linear model of telomere length versus age. Age-adjusted telomere length for each subject was computed by subtracting the subject's "predicted telomere length" from his/her observed telomere length. The differences in telomere length between the two groups were evaluated using Student's t test. A P-value <0.05 was considered statistically significant.

Results

Patients. From May 2008 to April 2009, 149 patients with hepatic cirrhosis were enrolled in the study. Thirteen patients did not donate buccal mucosa and/or peripheral blood for DNA extraction; one patient withdrew from the study; and the DNA sample from one patient was not adequate for amplification. Thus, samples from 134 patients were available for analysis; their characteristics are described in Table 1. In 67 patients (50%) the diagnosis of cirrhosis was established by liver biopsy; in the remaining patients cirrhosis was diagnosed based on clinical parameters for cirrhosis and portal hypertension.

Gene Variants. Among the 134 patients with cirrhosis, one heterozygous mutation in *TERC* was found in one patient and four missense gene variants in *TERT* were identified in nine patients (cumulative carrier frequency for *TERT* missense variants, 7%). Eight patients were heterozygous and one was homozygous

Table 2. Functional Gene Variants in TERC and TERT in Patients with Hepatic Cirrhosis

		Patients with Hepatic Cirrhosis (n = 134)	Controls (n = 528)
Gene	Location of Variation	No. of Heterozygotes; No. of Homozygotes (Allele Frequency)	
TERC	N. 37A→G	1;0 (0.004)	0;0
	Total	1;0 (0.004)	0;0
TERT	Exon 2, codon 441 (Glu) deletion†	1;0 (0.004)	1;0 (0.001)
	Exon 3, codon 530 CCG/CTG (Pro/Leu)	1;0 (0.004)	0;0
	Exon 10, codon 882 ACC/ATC (Thr/Ile)	1;0 (0.004)	0;0
	Exon 15, codon 1062 GCC/ACC (Ala/Thr)	5;1 (0.026)‡	7;0 (0.007)
	Total	8;1 (0.037)*	8;0 (0.008)

*The cumulative frequency of *TERT* gene variants was significantly greater in patients than in controls ($P = 0.0009$ by Fisher's exact test).

†Nucleotides GGA 1378 to 1380 (GenBank accession number NM_003219) were deleted, abolishing codon 441.

‡The A1062T polymorphism allele frequency was significantly greater in patients than in 528 controls (control group 1; *$P = 0.012$, by Fisher's exact test). As this TERT* gene variant was the most common among patients and controls, we screened an additional 1472 subjects as controls (control group 2); A1062T variant allele frequency was again significantly higher in patients than in control group 2 ($P = 0.0011$, by χ^2 test). As the A1062T allele frequency was similar in both control groups (0.007), we combined both groups for comparison: the allele frequency was 3.7 times higher in patients than in 2000 controls (0.026 vs. 0.007, respectively; $P = 0.001$ by χ^2 test).

Table 3. Clinical Profile of Patients with Hepatic Cirrhosis Carrying TERC and TERT Gene Variants

Patient	Gene Variant	Age (yr)	Sex	Ethnicity	Cause of Cirrhosis	Clinical Follow-up
A	TERC N. 37A→G	54	F	Caucasian	Hepatitis C	Died of head and neck cancer
B	TERT codon 441E deletion	56	M	Caucasian	Alcohol	Died of progressive liver disease
C	TERT codon P530L	62	F	Caucasian	NASH	–
D	TERT codon T882I	48	F	Native American	Alcohol	–
E	TERT codon A1062T	51	F	Caucasian	Alcohol	–
F	TERT codon A1062T (homozygous)	64	F	Caucasian	Primary biliary cirrhosis	–
G	TERT codon A1062T	56	M	Caucasian	Hepatitis C	–
H	TERT codon A1062T	62	M	Caucasian	Hepatitis C	–
I	TERT codon A1062T	47	M	Caucasian	Wilson's disease	Liver transplantation
J	TERT codon A1062T	59	F	Caucasian	Hepatits C	–

NASH, nonalcoholic steatohepatitis.

for the *TERT* codon Ala1062Thr gene variant (Tables 2, 3).

A 54-year-old female patient with hepatitis C virus-associated cirrhosis was heterozygous for the *TERC* n.37A→G mutation, which has been previously described in one patient with dyskeratosis congenita[31] and in one patient with idiopathic pulmonary fibrosis,[13] but not in healthy individuals in our study or in other series.[32–34] A 56-year-old male patient with alcoholic cirrhosis was heterozygous for a codon 441Glu deletion in the N-terminal region of *TERT* (Fig. 1A), previously described in aplastic anemia,[11] acute myeloid leukemia (in homozygosity),[14] and in one healthy subject.[11] Two individuals carried novel *TERT* mutations: a 62-year-old woman with nonalcoholic steatohepatitis (NASH) who was heterozygous for a *TERT* codon Pro530Leu located in the N-terminal region, and a 48-year-old woman with alcoholic cirrhosis, heterozygous for a *TERT* codon Thr882Ile in the Reverse Transcriptase Motif D (Fig. 1A). These novel mutations were not found in 528 control subjects.

Five patients were heterozygous and one was homozygous for the *TERT* codon Ala1062Thr, located in the C-terminal region and adjacent to Motif E-III (Fig. 1A). The primary etiology for cirrhosis for these patients was chronic hepatitis C virus infection in three; alcoholic cirrhosis in one; primary biliary cirrhosis in one (homozygous); and Wilson's disease in another. The *TERT* codon Ala1062Thr gene variant has been previously described in aplastic anemia,[11] acute myeloid leukemia,[14] idiopathic pulmonary fibrosis,[12] and healthy individuals.[11] As the *TERT* codon Ala1062Thr gene variant was observed in low frequency in the 528 control subjects (allele frequency, 0.007), we screened an additional 1,472 controls for this gene variant (total number of controls, 2,000); the allele frequency for the *TERT* codon Ala1062Thr variant was 3.7 times higher in cirrhosis patients than in healthy controls (0.026 versus 0.007, respectively; $P = 0.001$, χ^2 test; Table 2). Silent single nucleotide polymorphisms (SNPs) and intronic SNPs showed similar allele frequencies in patients and healthy controls[35] (Supporting Table 3). The overall cumulative frequency of *TERT* gene missense variants in patients with hepatic cirrhosis was significantly greater than in 528 healthy controls ($P = 0.0009$, Fisher's exact test; Table 2). Of note, none of the patients with mutations had hepatocellular carcinoma. One had undergone liver transplantation and two died during the study period (of head and neck cancer and one of progressive liver disease; Table 3).

Germline origin of gene variants was demonstrated by analysis of DNA obtained from peripheral blood leukocytes and buccal mucosa in all patients tested, except for two patients, one with the *TERC* n. 37A→G mutation and another with the *TERT* 441E deletion, who died before the study was complete.

Telomere Length. Leukocyte telomere length in the six patients with cirrhosis and mutations who were tested was below the median based on a reference group of 175 healthy individuals varying in age from 0 to 99 years, as measured by qPCR (Fig. 1B). The leukocyte telomere lengths of the two patients carrying novel *TERT* mutations (Patients C and D) were in the shortest quartile for healthy controls.

Leukocyte telomere length was also measured for 44 patients with cirrhosis without identifiable telomerase missense mutations from whom peripheral blood leukocytes were collected; they had significantly shorter telomeres in comparison to controls (Fig. 1C; $P = 0.0004$). The mean age-adjusted telomere length in patients with cirrhosis was −0.114 (95% confidence interval, −0.162, −0.06), compared to 0.001 (95%, −0.04, 0.04) in controls. Eighty-two percent of patients with cirrhosis had telomere lengths below the median for their age.

Telomerase Activity. To evaluate whether mutations in *TERC* and *TERT* decreased telomerase enzymatic

activity (its ability to synthesize telomeric repeats), telomerase-deficient VA13 cells were transfected with plasmids containing wildtype or mutant *TERT* and *TERC* constructs (or transfected with an empty vector). Novel *TERT* codon Pro530Leu and codon Thr882Ile mutations produced significant reduction in telomerase activity as compared to wildtype *TERT* (Fig. 1D). In our transfection experiments, *TERC* 37A→G mutation resulted in increased telomerase enzymatic activity in comparison to wildtype *TERC*. However, previous studies indicated that this mutation modulates telomerase activity from 75%[13] to 100%[31] of wildtype function. *TERT* 441Glu deletion has been previously found to generate ≈40% of wildtype telomerase activity, whereas the telomerase activity produced by the *TERT* codon Ala1062Thr variant is ∼60% of wildtype *TERT*.[14]

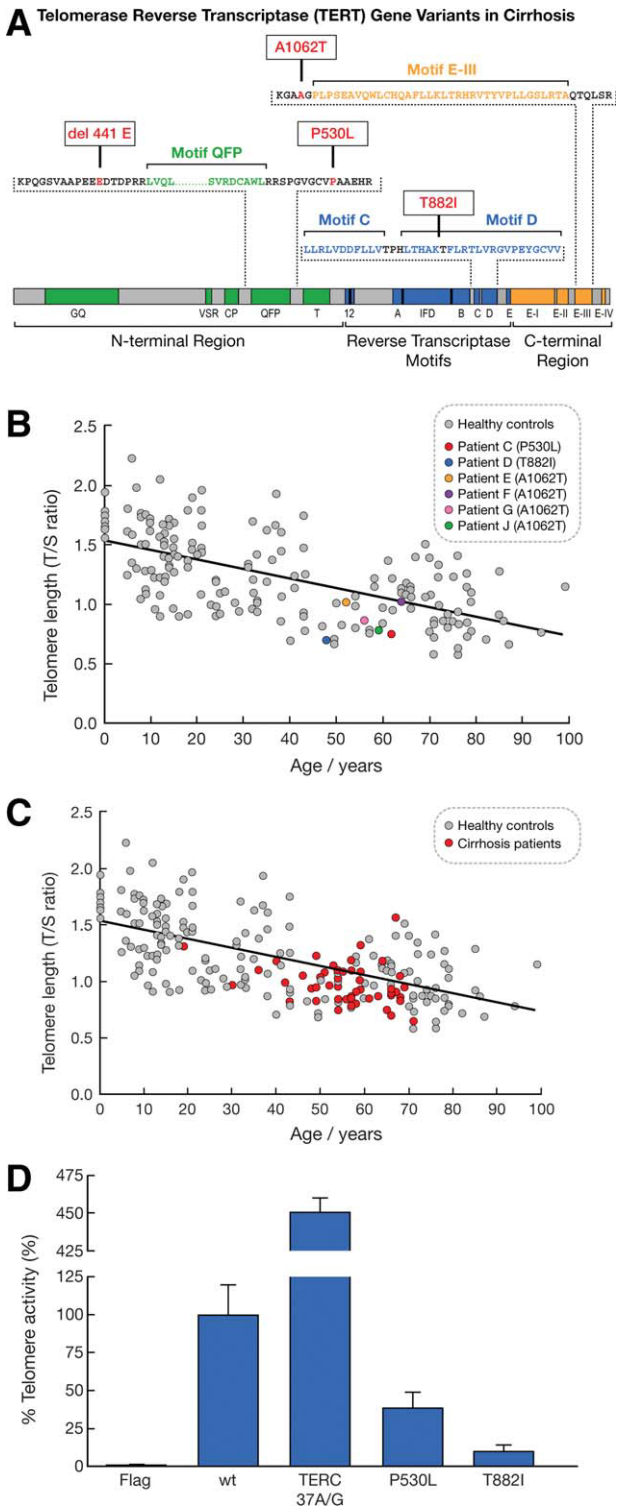

Fig. 1. (A) Schematic domain structure of TERT, indicating three major regions: N-terminal, reverse transcriptase motifs, and C-terminal. Mutation codon location and amino acid substitutions caused by mutations are shown. Abbreviations for amino acid residues: A, alanine; E, glutamic acid; H, I, isoleucine; L, leucine; P, phenylalanine; T, threonine. (B) Leukocyte telomere length was measured by qPCR. Telomere length of 175 healthy controls is shown in gray and patients with telomerase mutation and hepatic cirrhosis are depicted in different colors. (C) Telomere length of patients with cirrhosis without identifiable mutations (red) was significantly shorter than in healthy controls (gray; $P = 0.0004$). Eighty-two percent of patients with cirrhosis had their telomere lengths below the median. (D) Telomerase activity of the empty (Flag), wildtype, or mutated *TERT* or TERC expression vectors in the telomerase-negative VA13 cell line was measured by the fluorescent telomeric repeat-amplification protocol (TRAP) assay. Telomerase activity was considered 100% for the wildtype. Telomerase activity in each experiment was corrected for *TERT* or *TERC* messenger RNA (mRNA) levels as measured by reverse-transcription polymerase chain reaction (RT-PCR).

Discussion

In this study we found that missense variants in genes encoding components of the telomerase complex occurred at increased frequency in sporadic cirrhosis, suggesting that telomerase deficiency causing accelerated telomere shortening may predispose to cirrhosis and that the clinical spectrum of "telomere diseases"[3] may be broader and more common than previously suspected. We also confirmed that patients with cirrhosis have shorter telomeres of peripheral blood leukocytes than age-matched controls, further implicating telomere dysfunction as a molecular event in the pathophysiology of cirrhosis.

Mutations in telomerase complex genes have been associated with the inherited bone marrow failure syndrome dyskeratosis congenita, apparently acquired aplastic anemia, and familial idiopathic pulmonary fibrosis.[3] Less than 10% of patients with dyskeratosis congenita eventually develop severe liver disease with several histopathologic findings, especially after hematopoietic stem-cell transplant. In pedigrees of patients with bone marrow failure and telomerase deficiency, loss-of-function mutations correlate with an unusually

high prevalence of severe hepatic disease, mainly represented by cirrhosis and nodular regenerative hyperplasia.[25] In the present work we determined that telomerase mutations also are associated with nonfamilial cirrhosis with an identifiable etiologic factor, and that telomerase mutations might contribute to cirrhosis development in these patients. That mutations may contribute to fibrosis progression is further indicated by the recent observation by others of an absence of telomerase mutations in 200 individuals with chronic hepatitis C virus infection who did not progress to cirrhosis (K.L. Rudolph, pers. commun.).

Hepatic fibrosis in combination with the formation of regenerative nodules is the pathologic hallmark of cirrhosis.[36] The most common causes of cirrhosis in the developed world are hepatitis C virus infection and chronic alcohol abuse. However, only a portion of patients with chronic hepatitis C or who abuse alcohol eventually develops cirrhosis, suggesting host factors play a critical role in disease progression.[37] Numerous attempts to identify genetic risk factors for the development of cirrhosis have had limited success. Most reports have focused on candidate variants that might alter the primary pathologic process, such as oxidant stress and immunologic response, with inconsistent results.[38–42] One of the better-studied risk factors are mutations in keratins as susceptibility markers for cirrhosis. Mutations in keratins 8 and 18 have been found in patients with cirrhosis due to a variety of causes. A 3.35-fold increase in frequency of mutations in the keratin genes was found relative to controls,[43] which is somewhat less than the 4.63-fold increase found in *TERT* in the current study. It is important to note that, in contrast to the studies of keratins and other genes, where many of the mutations were of uncertain functional significance, in our study all of the mutations in *TERT* were shown to reduce telomerase activity leading to shorten telomeres.

Telomerase-deficient murine models have provided some insights into possible mechanisms that might explain the current observations. Short telomeres rather than telomerase insufficiency causes impairment of regeneration and pathological phenotypes in the mouse.[44] Also, in the telomerase "knockout" model, excessively short and dysfunctional telomeres predispose the mouse to chemically induced cirrhosis, and exogenous telomerase expression in hepatocytes ameliorates hepatic function and fibrosis in response to liver chemical injury, indicating a role of telomeres in pathogenesis of cirrhosis.[26] In addition, human cirrhosis due to chronic liver injury may improve once liver injury is eliminated[45–49] and hepatocyte regenerative capacity and reduced synthesis of collagen are critical in this process. Excessive telomere shortening may impair this repair process.

Telomere length was measured in peripheral blood samples from 50 patients with cirrhosis (37% of patients) and was significantly shorter than in healthy controls (Fig. 1C). It has been reported previously that telomeres are shorter in cirrhotic than in noncirrhotic hepatocytes regardless of disease etiology.[27,28] Short telomeres in both hepatocytes and peripheral blood leukocytes indicate the constitutive essence of telomere attrition in cirrhosis and implicate short and dysfunctional telomeres as a molecular mechanism for cirrhosis. Excessive telomere shortening (caused by telomerase gene mutations or other factors) may impair the hepatocyte regenerative ability in response to chronic injury, thus facilitating fibrosis progression. For example, as telomeres are eroded with aging (Fig. 1B), shorter telomeres in older humans may contribute to the more rapid rate of progression to cirrhosis with hepatitis C virus infection in the more elderly.[50] In agreement with our findings, in families with idiopathic pulmonary fibrosis and telomerase mutations, short telomeres have been hypothesized to limit pneumocyte proliferation, causing loss of alveolar cells and, secondarily, fibrosis.[12,13] Alternatively, in cirrhosis, short telomeres may affect stellate cell differentiation into myofibroblasts upon injury, thereby affecting the severity of fibrosis. Additionally, telomere attrition in inflammatory cells may induce a profibrotic response or contribute to the myofibroblast differentiation of cells of bone marrow origin.

Environmental factors may influence disease expression. Patients with X-linked dyskeratosis congenita, caused by *DKC1* mutations, have extremely short telomeres due to *DKC1* gene hemizygosity and present a severe and multiorgan phenotype, including mucocutaneous anomalies, bone marrow failure, and pulmonary and hepatic fibrosis.[3] In patients with telomerase deficiency due to telomerase mutations, enzyme function is reduced by haploinsufficiency and telomere shortening may be less intense and clinical phenotype may be less pronounced.[51] In patients with *TERT* or *TERC* mutations, aplastic anemia or pulmonary fibrosis may be the only clinical presentation.[11,12] Most patients with telomerase mutations and aplastic anemia do not have respiratory failure, and most patients with pulmonary fibrosis do not have cytopenias, suggesting that environmental factors contribute to disease development in a susceptible patient; for example, most patients with telomerase mutations and pulmonary fibrosis are smokers.[12,13] In pedigrees of telomerase

mutations, liver disease and aplastic anemia presented alone in different affected individuals, further suggesting a role for environmental factors. In one study, ≈3% of patients with idiopathic pulmonary fibrosis also had cryptogenic cirrhosis, indicating some overlap between clinical features.[52]

In conclusion, telomerase mutations resulting in telomere erosion appear to be a genetic risk factor for human cirrhosis and may predispose affected subjects to disease progression in combination with environmental injury, further supporting telomere attrition as a causal event in cirrhosis pathophysiology. Establishing how shortened telomeres increase the risk of cirrhosis may allow for the design of future therapies to reduce the risk of hepatic fibrosis in susceptible populations. Patients with mutations also may be appropriate targets for more aggressive forms of therapy to treat their primary disease given their increased risk of cirrhosis.

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