Mutations of the Human Telomerase RNA Gene (TERC) in Aplastic Anemia and Myelodysplastic Syndrome

Hiroki Yamaguchi, M.D. 1, Gabriela M. Baerlocher, M.D. 2, Peter M. Lansdorp, M.D., PhD 2,  3, Stephen J. Chanock, M.D. 4, Olga Nunez, RN 1, Elaine Sloand, M.D. 1, and Neal S. Young, M.D. 1

1 Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA

2 Terry Fox Laboratory, BC Cancer Research Center, Vancouver, BC, Canada

3 Department of Medicine, University of British Columbia, Vancouver, BC, Canada

4 Section on Genomic Variation, Pediatric Oncology Branch, National Cancer Institutes, Advanced Technology Center, Gaithersburg, MD, USA

Correspondence: Dr. Neal S. Young

Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health

10 Center Drive, MSC 1652, Bethesda, MD 20892-1652, USA

Tel: 301-496-5093, Fax:301-496-8396

E-mail: youngn@nhlbi.nih.gov

Running head; Mutations of TERC in AA and MDS

Hematopoiesis (Brief Report)

Word count; Abstract; 155, Text; 1286
ABSTRACT

Mutations in the human telomerase RNA (TERC) occur in the autosomal dominant form of dyskeratosis congenita (DKC). Because of the possibility that TERC mutations might underlie seemingly acquired forms of bone marrow failure, we examined blood samples from a large number of patients with aplastic anemia (AA), paroxysmal nocturnal hemoglobinuria (PNH), and myelodysplasia (MDS). Only three of a total of 210 cases showed heterozygous TERC mutations: both n305 (G-A) and n322 (G-A) were within the CR4-CR5 domain; n450 (G-A) was localized to the boxH/ACA domain. However, the clinical characteristics of only one patient (with a mutation at n305 (G-A)) suggested DKC; her blood cells contained short telomeres and her sister also suffered from bone marrow failure. Another 21 patients with short telomeres did not show TERC mutations. Our results suggest that cryptic DKC, at least secondary to mutations in the TERC gene, is an improbable diagnosis in patients with otherwise typical AA, PNH, and MDS.

Corresponding author’s E-mail: youngn@nhlbi.nih.gov
INTRODUCTION

Aplastic anemia (AA), pancytopenia and a hypocellular bone marrow, may be acquired or constitutional\(^1\). Constitutional types of marrow failure, Fanconi anemia and dyskeratosis congenita (DKC), typically present in the first or second decade of life, and frequently with associated physical anomalies\(^2\)-\(^3\). Detailed analysis of large families, collected in a DKC registry, allowed identification of several putatively etiologic genes in DKC. Mutations in the \textit{DKC1} gene occur in the X-linked form of the disease\(^4\)-\(^5\); \textit{DKC1} encodes dyskerin, which binds to the human telomerase RNA (\textit{TERC})\(^6\). Subsequently, mutations in \textit{TERC} were identified in autosomal dominant DKC\(^7\). The involvement of these genes has implicated the telomerase complex in the pathophysiology of DKC\(^8\)-\(^10\).

By analogy with Fanconi anemia, a diagnosis of DKC has been sought in patients with seemingly acquired AA. In a small study by Vulliamy et al., abnormalities in the \textit{TERC} were identified in more than 10\% of patients with AA, apparently “acquired” late in life\(^11\). We identified two families in which the probands presented with “acquired” AA but in whom stem cells could not be mobilized from matched sibling donors\(^12\). In each kindred a new mutation in the \textit{TERC} and short telomere were observed in multiple family members, associated with very mild blood count abnormalities but no physical anomalies\(^12\). Because of the possibility that \textit{TERC} mutations might underlie other cases of bone marrow failure, we undertook to examine a large number of blood sample from patients with AA, paroxysmal nocturnal hemoglobinuria (PNH), and myelodysplasia (MDS).
MATERIALS AND METHODS

Patients

Blood samples, previously collected and frozen, had been obtained from a total of 210 patients with bone marrow failure syndromes (150 AA, 55 MDS, and 13 PNH). Living patients provided informed consent for genetic testing according to protocols approved by the Institutional Review Board of the National Heart, Lung, and Blood Institute. For controls, we analyzed a panel of genomic DNA from 194 normal donors, comprising 123 Caucasian, 23 Hispanic, 24 black, and 24 Asian individuals (http://snp500cancer.nci.nih.gov).

Telomere fluorescence in situ hybridization and flow cytometry

The average length of telomere repeats at chromosome ends in individual peripheral blood leukocytes was measured by flow-FISH as previously reported\textsuperscript{13, 14}.

Mutation analysis of the \textit{TERC} gene

The PCR primers for amplification of around chromosome \textit{3q26.3} including promoter and transcription region of \textit{TERC} were designed. We defined the first nucleotide of \textit{TERC} transcription region was n1. The TERCNF1-1; (n-1660) GAGCTCATATAGAGCAGAACAAG, and TERC4; GGTGACGGATGCGCACGAT (n502), and TERC4; (n-150) TCATGGCCGGAAATTGGAACT, and TERC7-1; TGCACTTGTGTAGTTCAGGAG (n1939), were amplified to detect deletions of the \textit{TERC} gene. PCR amplification of the genomic DNA was performed by Advantage-GC2PCR kit (BD Biosciences Clontech, CA). The PCR conditions were as follows;
preheating at 94 C for 3 min, followed by 35 cycles of 94 C for 30 sec, 58 C for 50 sec, and 68 C for 3 min, a final extension at 68 C for 5 min.

We designed two pair primers for sequencing. Primer sequences are as follow, TERCF4 and TERCseqR1: GTTTGCTCTAGAATGAACGGTGG, TERCseqF2; GTCTAACCCTAACTGAGAAGGGC, and TERC4. Reactions for direct sequencing of the PCR products were performed with BigDye Terminator ver3.1 (Perkin-Elmer Cetus, CA).

RESULTS and DISCUSSION

TERC abnormalities in unselected bone marrow failure patients

We sought large deletions in TERC by PCR, but none were found (data not shown) (but our screening method would not detect novel deletions of TERC whose borders fell outside the limits of our primers). Direct sequencing showed only three heterozygous TERC mutations among 210 patients samples examined, for an approximate frequency in the range of 1.5% (Table 1). Both the n305 (G-A) and n322 (G-A) were within the CR4-CR5 domain; n450 (G-A) was located in the box H/ACA domain (Table 1, Figure 1). Of 22 patients in whom telomere length was previously determined to be short compared to age-matched controls15, only one showed a TERC mutation (n305 (G-A)) (Table 2).

All vertebrate telomerase RNA share four highly conserved structural regions: a pseudoknot domain, a CR4-CR5 domain, a boxH/ACA domain, and a CR7 domain6,16. The pseudoknot and CR4-CR5 domains are known to be necessary for telomerase
activity. For the patient with the n305 (G-A) mutation, a sister also suffered from bone marrow failure, and we had previously measured an extremely short telomere length for the patient, and by history she had failed to respond to immunosuppressive therapy (Table 2). Mouse mutations in the homologous P6 region of CR4-CR5 domain of murine telomere RNA, corresponding to the J6/5 unpaired region of human telomere RNA, abolish ability to reconstitute an active telomerase complex. Therefore, telomere shortening and pancytopenia in this patient were likely secondary to this single nucleotide substitution.

Because telomeres are believed to play an important role in the maintenance of chromosome integrity, genetic instability has been blamed on age-related loss of telomeric DNA in hematopoietic stem cells, in the pathogenesis of de novo MDS. However, we found only a single MDS patient with TERC mutation (n322 (G-A)), at the J6/5 unpaired region of the CR4/CR5 domain (Table 1, Figure 1). No telomere length data were available for this archived case. While it remains possible that telomeric shortening was related to the hematologic disease, TERC mutations in general were certainly not prevalent in the MDS patients that we studied. For the n450 (G-A) substitution, a pathogenic role is uncertain in the absence of either telomere data or a suggestive family history (Table 2); furthermore, this patient had a more typical course for acquired AA, with a good and sustained response to immunosuppressive therapy (Table 2). Additionally, we found the n467 (T-C) substitution at 3’ downstream region in a single AA patient (Table 1); the biologic significance of this substitution is unclear.
The absence of these particular mutations, even in the large number of normal controls that we examined (n=388 chromosomes), strongly suggests that these are rare mutations and not common single nucleotide polymorphisms (SNPs)\(^{20}\). However testing of the ability of these mutated TERC to diminish telomerase activity in transfected cells in vitro is required to establish its pathogenic role.

**Polymorphisms in TERC**

We analyzed 194 normal controls of different self-described ethnicity to determine if the variants represented polymorphisms\(^{20}\). Most important was the n58 (G-A) substitution, which was observed in 5 African Americans, and the n228 (G-A), found in a single African-American (Table 1). We also found the n-21 (C-T) substitution at 5’ upstream region. Among patients, two black MDS and a single black AA patient showed the n58 (G-A) polymorphism, and a single AA the n228 (G-A) polymorphism\(^{21}\) (Table 1). For the n228 (G-A) polymorphism, this base pair is disrupted within a hypervariable paired region (Figure 1) and would not be predicted to be important in the required secondary structure of TERC.

Our results and data on a small number of patients reported in correspondences\(^{22,23}\) stand particularly in contrast to those reported by Vulliamy et al\(^{11}\), for the sequence substitution that they identified in their cases of idiopathic and presumed constitutional AA--n58 (G-A)--is unlikely to represent a true mutation, being present in a large proportion of African-American normals. Subtracting these cases, the English series contains only two patients with a clinical diagnosis of constitutional AA with TERC mutations, at n72 (C-G) and n110-113 (deletion GACT)\(^{11}\).
Finally, we and others have reported short telomeres in a substantial proportion of patients with AA\textsuperscript{15,24,25}. While many such examples were studied in the current study, \textit{TERC} mutations were not found; of course, other components of the telomerase complex might be affected and explain the telomere shortening. Mutations in appropriate crucial regions of these genes could result in a similar phenotype to those associated with \textit{TERC} abnormalities. Therefore, we cannot rigorously exclude cryptic DKC due to mutations in telomerase complex components other than \textit{TERC} in marrow failure syndromes.

**REFERENCES**


Table 1  *TERC* mutations and polymorphisms

<table>
<thead>
<tr>
<th></th>
<th>n-21C/T*</th>
<th>n58G/A</th>
<th>n228G/A</th>
<th>n305G/A</th>
<th>n322G/A</th>
<th>n450G/A</th>
<th>n467T/C**</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA or AA/PNH (n=150) and PNH (n=5)</td>
<td>0/155</td>
<td>1/155</td>
<td>1/155</td>
<td>1/155</td>
<td>0/155</td>
<td>1/155</td>
<td>1/155</td>
</tr>
<tr>
<td>MDS (n=55)</td>
<td>0/55</td>
<td>2/55</td>
<td>0/55</td>
<td>0/55</td>
<td>1/55</td>
<td>0/55</td>
<td>0/55</td>
</tr>
<tr>
<td>total</td>
<td>0/210</td>
<td>3/210</td>
<td>1/210</td>
<td>1/210</td>
<td>1/210</td>
<td>1/210</td>
<td>1/210</td>
</tr>
<tr>
<td>Normal control***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>1/123</td>
<td>0/123</td>
<td>0/123</td>
<td>0/123</td>
<td>0/123</td>
<td>0/123</td>
<td>0/123</td>
</tr>
<tr>
<td>Hispanic</td>
<td>0/23</td>
<td>0/23</td>
<td>0/23</td>
<td>0/23</td>
<td>0/23</td>
<td>0/23</td>
<td>0/23</td>
</tr>
<tr>
<td>Black</td>
<td>0/24</td>
<td>5/24</td>
<td>1/24</td>
<td>0/24</td>
<td>0/24</td>
<td>0/24</td>
<td>0/24</td>
</tr>
<tr>
<td>Asian</td>
<td>0/24</td>
<td>0/24</td>
<td>0/24</td>
<td>0/24</td>
<td>0/24</td>
<td>0/24</td>
<td>0/24</td>
</tr>
<tr>
<td>total</td>
<td>1/194</td>
<td>5/194</td>
<td>1/194</td>
<td>0/194</td>
<td>0/194</td>
<td>0/194</td>
<td>0/194</td>
</tr>
</tbody>
</table>

* 21bp upstream from 5' transcription region of *TERC*. ** 16bp downstream from 3' transcription region of *TERC*. *** Normal control is SNP500 (http://snp500cancer.nci.nih.gov)
Table 2 Summary of patients background with *TERC* mutation

<table>
<thead>
<tr>
<th>Case No./Mutation</th>
<th>Sex/Age/Race</th>
<th>Diagnosis</th>
<th>Family history</th>
<th>Chromosome abnormality</th>
<th>Telomere length in leukocytes (in kb)</th>
<th>Treatment response</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1 n305G/A</td>
<td>F/15/H</td>
<td>mAA</td>
<td>Affected twin sister</td>
<td>Normal</td>
<td>4.6</td>
<td>No response to ATG+CSA</td>
</tr>
<tr>
<td>C35 n450G/T</td>
<td>F/31/W</td>
<td>sAA</td>
<td>Negative</td>
<td>Normal</td>
<td>11</td>
<td>Response to ATG+CSA</td>
</tr>
<tr>
<td>C131 n322 G/A</td>
<td>M/77/W</td>
<td>MDS RA</td>
<td>Negative</td>
<td>del(5)(q15;q13)</td>
<td>Not done</td>
<td>No response to Epo or CSA</td>
</tr>
</tbody>
</table>

Figure 1  Mutations and polymorphisms of Human telomerase RNA (TERC) gene.

The schematic of the TERC gene showing the positions of mutations and polymorphisms identified in patients and normal controls.
Mutations of the human telomerase RNA gene (TERC) in aplastic anemia and myelodysplastic syndrome

Hiroki Yamaguchi, Gabriela M Baerlocher, Peter M Lansdorp, Stephen J Chanock, Olga Nunez, Elaine Sloand and Neal S Young