TELOMERE LENGTH AND MALIGNANT TRANSFORMATION IN APLASTIC ANEMIA

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TELOMERE LENGTH MEASUREMENT

Calado & Young, Blood 2008
TELOMERE ELONGATES TELOMERES BY ADDING TTAGGGG REPEATS
TELOMERES ARE SHORT IN ACQUIRED APLASTIC ANEMIA

RAPID COMMUNICATION

Progressive Telomere Shortening in Aplastic Anemia

By Sarah E. Ball, Frances M. Gibson, Sian Rizzo, Jennifer A. Tooze, Judith C.W. Marsh, and Edward C. Gordon-Smith

Telomere length in leukocyte subpopulations of patients with aplastic anemia

Tim H. Brümmendorf, Jaroslaw P. Maciejewski, Jennifer Mak, Neal S. Young, and Peter M. Lansdorp
DYSKERATOSIS CONGENITA
Bone Marrow Failure Associated with Mucocutaneous Triad

Nail dystrophy  Reticular skin hyper- or hypopigmentation  Leukoplakia

X-linked dyskeratosis congenita is caused by mutations in a highly conserved gene with putative nucleolar functions

A telomerase component is defective in the human disease dyskeratosis congenita

Nina S. Heiss1, Stuart W. Knight2, Tom J. Velliamo2, Sabine M. Klauck1, Stefan Wiemann3, Philip J. Mason3, Annemarie Poustka1 & Inderjeet Doka2

Letters to Nature

nature genetics volume 19 may 1998

James R. Mitchell, Emily Wood & Kathleen Collins
## ACQUIRED APLASTIC ANEMIA

### Telomerase Mutations in Patients without Clinical Features of DKC

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**The NEW ENGLAND JOURNAL of MEDICINE**

Cohort of 205 patients with acquired aplastic anemia.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Patient</th>
<th>Diagnosis</th>
<th>Family History</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala 202</td>
<td>41 yr Male Hispanic</td>
<td>Severe AA</td>
<td>--</td>
</tr>
<tr>
<td>Ala 202</td>
<td>75 yr Female Hispanic</td>
<td>Moderate AA</td>
<td>brother MDS</td>
</tr>
<tr>
<td>His 412</td>
<td>56 yr Male White</td>
<td>Moderate AA</td>
<td>mother MDS</td>
</tr>
<tr>
<td>Val 694</td>
<td>53 yr Male White</td>
<td>Moderate AA</td>
<td>sister MDS/AML</td>
</tr>
<tr>
<td>Tyr 772</td>
<td>34 yr Male Hispanic</td>
<td>Severe AA</td>
<td>sister “anemia”</td>
</tr>
<tr>
<td>Val 1090</td>
<td>64 yr Female Hispanic</td>
<td>Severe AA</td>
<td>--</td>
</tr>
</tbody>
</table>

Mutations in TERT, the Gene for Telomerase Reverse Transcriptase, in Aplastic Anemia

Hiroki Yamaguchi, M.D., Rodrigo T. Calado, M.D., Ph.D., Hinh Ly, Ph.D., Sachiko Kajigaya, Ph.D., Gabriela M. Baerlocher, M.D., Stephen J. Chanock, M.D., Peter M. Lansdorp, M.D., Ph.D., and Neal S. Young, M.D.

Yamaguchi et al., NEJM 2005
ACQUIRED APLASTIC ANEMIA

Telomeres Are Short in Patients in with Telomerase Mutations

Yamaguchi et al., NEJM 2005
FUNCTIONAL ASSAYS OF TELOMERASE ACTIVITY

Primary cells

Transfection of *TERT* Mutants

Co-transfection

**Codon**

202 1090 control - +

WT 202 694 772 1090 - +

WT:Codon

202 441 694 772

Yamaguchi et al., NEJM 2005
HEMATOPOIESIS IN “NORMAL” RELATIVES WITH TERC MUTATIONS

Hematology:

- normal peripheral blood counts
- mild anemia with macrocytosis
- mild thrombocytopenia

Hematopoiesis:

- severely hypoplastic
- ↓ CD34 number
- ↓ colony formation
- ↑ erythropoietin, thrombopoietin

proband
affected sister
affected niece
unaffected brother

Fogarty et al., Lancet 2003
TELOMERE LENGTH IN PATIENTS WITH ACQUIRED APLASTIC ANEMIA

183 Consecutive Patients Treated with IST

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**Diagram Description:**

- The graph compares telomere length (T/S ratio) with age in healthy controls and patients with aplastic anemia.
- Healthy controls are represented by yellow dots.
- Patients with aplastic anemia are represented by blue squares.

- **Axes:**
  - Y-axis: Telomere length (T/S ratio) ranging from 0.0 to 2.5.
  - X-axis: Age (years) ranging from 0 to 100.

- **Trends:**
  - Longer telomeres (yellow dots) tend to have higher T/S ratios and do not show a significant decrease with age.
  - Shorter telomeres (blue squares) show a decrease in T/S ratio with age, indicating a shorter telomere length.

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**Conclusion:**

The study reveals a significant difference in telomere length between healthy controls and patients with aplastic anemia, with shorter telomeres observed in patients treated with IST.
Telomere Length and Relapse

Scheinberg et al. JAMA 2010

Telomere length
1st (shortest) quartile
2nd quartile
3rd quartile
4th (longest) quartile

Probability of Relapse

Follow-up, years

No. at risk

TL 1st quartile: 26, 21, 15, 13, 10, 7, 4
TL 2nd quartile: 25, 23, 19, 14, 11, 8, 5
TL 3rd quartile: 27, 27, 26, 19, 14, 13, 10
TL 4th quartile: 26, 25, 22, 18, 16, 14, 10
## Telomere Length and Clonal Evolution Multivariate Analysis

<table>
<thead>
<tr>
<th></th>
<th>Coefficient</th>
<th>SD</th>
<th>Relative Risk</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Telomere length</td>
<td>-1.475</td>
<td>0.539</td>
<td>0.229</td>
<td>0.006</td>
</tr>
<tr>
<td>Age</td>
<td>0.020</td>
<td>0.011</td>
<td>1.020</td>
<td>0.078</td>
</tr>
<tr>
<td>ARC</td>
<td>0.192</td>
<td>0.374</td>
<td>1.211</td>
<td>0.610</td>
</tr>
<tr>
<td>ALC</td>
<td>0.083</td>
<td>0.428</td>
<td>1.087</td>
<td>0.850</td>
</tr>
<tr>
<td>ANC</td>
<td>-0.446</td>
<td>0.326</td>
<td>0.640</td>
<td>0.170</td>
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<tr>
<td>Platelet</td>
<td>-0.262</td>
<td>0.283</td>
<td>0.769</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Scheinberg et al. *JAMA* 2010
**EVOLUTION RATE BY TELOMERE LENGTH**

![Graph A](image1)

- Telomere length
- 1st (shortest) quartile
- > 1st quartile

Log rank P = 0.009

<table>
<thead>
<tr>
<th>Follow-up, years</th>
<th>No. at risk</th>
<th>TL &lt; 1st quartile</th>
<th>TL &gt; 1st quartile</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>46</td>
<td>137</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>42</td>
<td>124</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>30</td>
<td>120</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>26</td>
<td>94</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>17</td>
<td>73</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>14</td>
<td>62</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>9</td>
<td>42</td>
</tr>
</tbody>
</table>

**MONOSOMY 7 RATE BY TELOMERE LENGTH**

![Graph B](image2)

- Telomere length
- 1st (shortest) quartile
- > 1st quartile

Log rank P = 0.002

<table>
<thead>
<tr>
<th>Follow-up, years</th>
<th>No. at risk</th>
<th>TL &lt; 1st quartile</th>
<th>TL &gt; 1st quartile</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>46</td>
<td>137</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>43</td>
<td>128</td>
</tr>
<tr>
<td>2</td>
<td></td>
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<td>3</td>
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<tr>
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<td>66</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>10</td>
<td>45</td>
</tr>
</tbody>
</table>

Scheinberg et al. *JAMA* 2010
SURVIVAL PROBABILITY BY TELOMERE LENGTH

A

Probability of Survival

0.0 0.2 0.4 0.6 0.8 1.0

Follow-up, years

No. at risk

TL < 1st quartile 46 43 34 29 20 16 10
TL > 1st quartile 137 128 125 102 80 66 45

Log rank P=0.008

Survival Probability by Telomere Length & ARC

B

Probability of Survival

0.0 0.2 0.4 0.6 0.8 1.0

Follow-up, years

No. at risk

TL < 1st quartile, ARC low 24 22 15 11 8 7 4
TL > 1st quartile, ARC low 95 86 84 69 55 47 33
TL < 1st quartile, ARC high 22 21 19 18 12 9 6
TL > 1st quartile, ARC high 42 42 41 33 25 19 12

Log rank P<0.001

Scheinberg et al. JAMA 2010
INCREASED TELOMERE-FREE ENDS IN MARROW CELLS OF AA PATIENTS WITH SHORTER TELOMEREs

INCREASED TELOMERE-FREE ENDS IN MARROW CELLS OF AA PATIENTS WITH SHORTER TELOMEREs

Calado et al. Leukemia 2012 in press
APLASTIC ANEMIA PATIENTS WITH SHORTER TELOMERES AT DIAGNOSIS ALSO DISPLAY MORE ANEUPLOIDY

Calado et al. Leukemia 2012 in press
CHROMOSOMAL INSTABILITY IN APLASTIC ANEMIA PATIENTS WITH SHORTER TELOMERES

Calado et al. Leukemia 2012 in press
TERT MUTATIONS IN ACUTE MYELOID LEUKEMIA

Constitutional hypomorphic telomerase mutations in patients with acute myeloid leukemia

Rodrigo T. Calado,1 Joshua A. Regal,1 Mark Hills,2 William T. Yewdell,3 Leandro F. Dalmazzo,4 Marco A. Zago,5 Peter M. Lansdorp,6 Donna Hogge,7 Stephen J. Chanock,8 Elihu H. Estey,9 Roberto P. Falcão,5 and Neal S. Young1

1Hematology Branch, National Heart, Lung, and Blood Institute, and 2Laboratory of Translational Genomics, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; 3Center of Cell-Based Therapy, Department of Internal Medicine, University of São Paulo at Ribeirão Preto Medical School, SP 1408-900, Ribeirão Preto, Brazil; 4Leukemia Department, University of Texas MD Anderson Cancer Center, Houston, TX 77030; 5Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada V5Z 4E6; and 6Department of Medicine, University of British Columbia Cancer Center, Vancouver, BC, Canada V6T 1Z4

TERT-AML: 2 inv(16), 1 complex, t(5;11)(q35;q13) + del(10)(p15), 3 t(15;17)

MD Anderson Cohort

N= 89 selected AML by cytogenetics (528 healthy controls; P=0.028)

4 TERT mutations: 2 heterozygous for A1062T
   1 homozygous for 411E deletion
   1 heterozygous for V299M

Mutations associated with trisomy 8 and inv(16)

TERT mutations are constitutional and dominant loss-of-function.

Calado et al., PNAS 2009
DYSKERATOSIS CONGENITA

*Predisposes to Cancer*

Cumulative incidence of cancer

Cumulative incidence of MDS

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<table>
<thead>
<tr>
<th>Types of cancers and observed/expected (O/E) ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer</td>
</tr>
<tr>
<td>--------------</td>
</tr>
<tr>
<td>All sites</td>
</tr>
<tr>
<td>Tongue</td>
</tr>
<tr>
<td>AML</td>
</tr>
<tr>
<td>Cervical SCC</td>
</tr>
</tbody>
</table>

*P<0.05

Alter et al., Blood 2009
A Genome-wide Association Study of Lung Cancer Identifies a Region of Chromosome 5p15 Associated with Risk for Adenocarcinoma

Maria Teresa Landi,1,* Nilanjan Chatterjee,1 Kai Yu,1 Lynn R. Goldin,1 Alisa M. Goldstein,1 Melissa Rotunno,1 Lisa Mirabello,1 Kevin Jacobs,1 William Wheeler,2 Meredith Yeager,1 Andrew W. Bergen,3 Qizhai Li,1,4 Dario Consonni,5 Angela C. Pesatori,5 Sholom Wacholder,1 Michael Thun,6 Ryan Diver,6 Martin Oken,7 Jarmo Virtamo,8 Demetrius Albanes,1 Zhaoming Wang,1 Laurie Burdette,1 Kimberly F. Doheny,9 Elizabeth W. Pugh,9 Cathy Laurie,10 Paul Brennan,11 Rayjean Hung,12 Valerie Gaborieau,11 James D. McKay,11 Mark Lathrop,13 John McLaughlin,12 Ying Wang,12 Ming-Sound Tsao,14 Margaret R. Spitz,15 Yufei Wang,16 Hans Krokan,17 Lars Vatten,17 Frank Skorpen,17 Egil Arnesen,18 Simone Benhamou,19 Christine Bouchard,20 Andres Metsapalu,21 Tonu Vooder,21 Mari Nelis,21 Kristian Välk,21 John K. Field,22 Chu Chen,23 Gary Goodman,23 Patrick Sulem,24 Gudmar Thorleifsson,24 Thorunn Rafnar,24 Timothy Eisen,25 Wiebke Sauter,26 Albert Rosenberger,29 Heike Bickeböller,29 Angela Risch,30 Jenny Chang-Claude,30 H. Erich Wichmann,26,27,28 Kari Stefansson,24 Richard Houlston,16 Christopher I. Amos,15 Joseph F. Fraumeni, Jr.,1 Sharon A. Savage,1 Pier Alberto Bertazzi,5 Margaret A. Tucker,1 Stephen Chanock,1 and Neil E. Caporaso1
Telomere Erosion

Senescence

Apoptosis

Chromosomal Instability

Marrow Failure

Malignant Transformation

↑p53

Chromosomal Instability

→ Malignant Transformation
Telomere erosion and cancer risk

- Dyskeratosis congenita
  - Tongue SCC, MDS, AML

- Inflammatory bowel disease
  - Colorectal cancer

- Aplastic anemia
  - MDS, AML

- Barrett’s esophagitis
  - Adenocarcinoma

- TERT locus and general cancer risk
  - Lung cancer, basal cell carcinoma, urinary bladder cancer, cervical cancer, glioma
ANDROGEN THERAPY FOR APLASTIC ANEMIA

Blood, Vol. 34, No. 3 (Sept.) 1969

Anabolic Androgenic Steroids in the Treatment of Acquired Aplastic Anemia

By L. Sanchez-Medal, A. Gomez-Leal, Lorenzo Duarte and Maria Guadalupe Rico

16% to 48%
**SEX HORMONES INCREASE TELOMERASE ACTIVITY IN CULTURED HUMAN LYMPHOCYTES**

![Graph showing the increase in telomerase activity with various sex hormones.](https://example.com/graph.png)

- Telomerase Activity (TPG units)
- Androgens:
  - Methyltrienolone (synthetic)
  - Nandrolone
  - 6β-Hydroxy-Testosterone
  - β-Estradiol

Calado *et al.*, *Blood*, 2009
CONCLUSIONS IV

Clinical Implications: Treatment

• Selection of sibling donors for HSCT: mutation status
  • Telomere length measurement (silent carrier)
  • Mutation screening

• Androgens as potential therapy for aplastic anemia patients with telomerase mutations
  • Response in 60% of dyskeratosis congenita patients
  • Response in some AA patients
  • Androgens in pulmonary fibrosis, cirrhosis?
  • Androgens for iatrogenic telomere shortening (Post-HSCT)?
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**Emory University:**
Hinh Ly

**University of Arizona:**
Thomas Boyer

_National Heart, Lung and Blood Institute_