Understanding Telomere Biology Through Studies of Dyskeratosis Congenita

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Dyskeratosis congenita: a highly informative model of telomere biology and cancer predisposition

- Regulation
- Biomarkers
- Genetics
- Related disorders
Telomeres Preserve Chromosomal Integrity

- Long nucleotide (TTAGGG)_n repeats at chromosome ends
- Many proteins regulate telomere length and stabilize structure
- Shorten with each cell division
- Erosion causes genetic instability, cell crisis and cell death
- Cancer cells survive despite critically short telomeres and chromosomal instability

Photo: Dr. Peter Lansdorp
Evolutionary Conservation of Telomere Biology Genes

- **Hypothesis:** Genetic variation in telomere biology genes is limited because of their critical roles in genomic stability

- Population genetic study showed that these genes are highly conserved between ethnic groups and species

Dyskeratosis Congenita: a disorder of telomere biology

Nail Dystrophy

Oral Leukoplakia

Skin Pigmentation
In addition to the triad...

- Bone Marrow Failure
- Pulmonary Fibrosis
- Head and Neck Cancer
- Anogenital Cancer
- Leukemia
- Liver Fibrosis
- Dental Disease
- Hyperhidrosis
- Esophageal Stenosis
- Urethral Stenosis
- Cerebellar Hypoplasia
- Tear duct stenosis
- Microcephaly
- Osteoporosis
- Avascular Necrosis
- Developmental Delay
- Early Gray Hair
- Hair Loss

Abnormally short telomeres are the unifying feature.

Traditional diagnosis: Diagnostic Triad or 1 of the triad, + BMF + 2 other findings. Vulliamy et al, Blood, 2006, 107(7):2680-5
Dyskeratosis Congenita Genetics: The Telomere Connection (c. 2006)

These mutations result in short telomeres, but only ~40% of patients have a mutation in one of these genes.
• Systematic evaluation of affected individuals and their families in North America
  – Fanconi Anemia
  – **Dyskeratosis Congenita (DC)**
  – Diamond-Blackfan Anemia
  – Shwachman-Diamond Syndrome
  – And others

• Opened in January 2002
• http://marrowfailure.cancer.gov
• Principal Investigator: Blanche P. Alter, MD, MPH
• Staff Clinician: Neelam Giri, MD
• DC: Sharon Savage, MD
IBMFS Study: Levels of Participation

- Epidemiology Questionnaires
  - Family history
  - Individual history

- Medical Record Review

- Evaluation at the NIH Clinical Center
  - IBMFS Team
  - Genetic Counseling
  - Subspecialists
  - Biospecimens
### Dyskeratosis Congenita Families

<table>
<thead>
<tr>
<th>NCI Cohort</th>
<th># Families</th>
<th># Affected Individuals</th>
<th># Healthy Relatives</th>
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</thead>
<tbody>
<tr>
<td>Clinic</td>
<td>35</td>
<td>46</td>
<td>86</td>
</tr>
<tr>
<td>Field</td>
<td>11</td>
<td>24</td>
<td>101</td>
</tr>
</tbody>
</table>

#### Map of Dyskeratosis Congenita Families

- The map uses different shades of green to represent the number of affected individuals across various states. The legend indicates the range of affected individuals per family: 0 (white), 1-2 (light green), 3+ (dark green).
Development of the First (and only) Diagnostic Test for DC

- Varied clinical presentations
- Signs and symptoms often progress with age
- <50% have an identifiable mutation
- Telomeres are very short for their age

<table>
<thead>
<tr>
<th>DC vs. other IBMFS</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>If 4/6 leukocyte subsets &lt;1st percentile</td>
<td>92%</td>
<td>98%</td>
</tr>
</tbody>
</table>

Genome-Wide Linkage Screen in DC
Heterogeneous, autosomal dominant, *DKC1*, *TERC*, and *TERT* normal

Clinical DC
Telomere length in WBC subsets <1\textsuperscript{st} %ile

◆ Spontaneous abortion

* Telomere length unknown
**TINF2 mutations cause DC**

- Evidence for linkage at 14q11.2 led to the identification of mutations in *TINF2*, a component of the shelterin telomere protection complex
  - Mutations present only in those with telomeres <1st percentile
  - No mutations in 298 healthy controls
  - Additional unrelated individuals with DC had mutations
- Mutations located in a small, highly conserved region of exon 6

Sequence Analysis of the Shelterin Telomere Protection Complex

- **TERF1, TERF2, POT1, TERF2IP, and ACD** sequenced in 9 patients with DC, and 7 DC-like
- Variants in patients were compared to 380 control subjects
- Mutations in these genes do not appear to be a common cause of dyskeratosis congenita.

**TCAB1 in Dyskeratosis Congenita**

- Cajal body: sites of mRNA & rRNA processing in the nucleus
- *TCAB1* is located in Cajal bodies and is required for telomerase trafficking
- **Mutations in TCAB1 cause DC**
  - 2 of 9 families studied have autosomal recessive mutations

Venteicher et al. Science 2009;323:644-648

- Heterozygous carrier
- Affected with DC

- F164L R398W
- H376Y G435R
DC-associated *TCAB1* Mutations Result in Defective Telomerase Trafficking

- Occur in conserved loop residues
- Reduce TCAB1 levels in Cajal bodies
- Prevents localization of the telomerase enzyme complex (*TERT, TERC, DKC1*) to Cajal bodies


Study highlighted in the “Best of” session at the American Society of Hematology annual meeting
Dyskeratosis Congenita Genes in the NCI cohort

~ 1/3 of our families do not have a mutation in one of these genes
Refining the Phenotype of DC

- Detailed clinical characterization at the NIH Clinical Center
  - Dental
  - ENT
  - Ophthalmology
  - Radiology
  - Psychiatry
  - Pulmonology
  - And others


Unpublished data on MRI findings in the brain: John Butman, MD, Clinical Center, NIH, Neelam Giri, MD, Clinical Genetics Branch, DCEG, NCI
Major Contributions from NCI’s Dyskeratosis Congenita Study

• Developed the diagnostic test
  – Telomere length

• Discovered 2 of the 7 causative genes
  – TINF2: The first component of the shelterin telomere protection complex associated with any disease
  – TCAB1: The first study showing that telomerase mislocalization can cause disease

• Quantified cancer risk

• Refined the extent of medical complications

• Helped families create a support group: DC Outreach

• Created basis for population-based studies of the contribution of aberrations in telomere biology to cancer risk
Telomere biology disorders overlap clinically and genetically. Dyskeratosis Congenita includes DKC1, TCAB1, TERC, TERT, TINF2, NOP10, NHP2. The clinical spectrum is growing. Signs & symptoms of DC develop at variable rates. Germline TERT or TERC mutations in 5-10%. Savage and Bertuch, Genetics in Medicine 2010: in press.
The Telomere Length Continuum

**Normal range**
- Marker of common disease risk
- Statistically significant differences

**Short, <10th%**
- Aplastic anemia, leukemia, pulmonary fibrosis
- Mutations in *TERT, TERC*

**VERY short, <1st%**
- Dyskeratosis Congenita
- Rare, highly penetrant disease causing mutations
Telomere Length and Cancer Risk in the General Population

- Individuals in the general population with shorter germline telomeres may have increased cancer risk because of telomere dysregulation.
# Surrogate Tissue Telomere Length and Cancer Risk

Meta-Analysis
Odds Ratio 1.96
95% Confidence Interval 1.37 - 2.81

<table>
<thead>
<tr>
<th>Cancer Site</th>
<th>Study</th>
<th>OR (95% CI)</th>
<th>% Weight</th>
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<tbody>
<tr>
<td>Bladder</td>
<td>Broberg (12)</td>
<td>5.64 (2.08, 15.27)</td>
<td>2.96</td>
</tr>
<tr>
<td></td>
<td>Wu (11)</td>
<td>5.64 (2.21, 14.42)</td>
<td>3.04</td>
</tr>
<tr>
<td></td>
<td>McGrath (22)</td>
<td>1.86 (1.06, 3.26)</td>
<td>3.50</td>
</tr>
<tr>
<td></td>
<td>Shen (29)</td>
<td>1.08 (0.85, 1.37)</td>
<td>3.76</td>
</tr>
<tr>
<td></td>
<td>De Vivo (13)</td>
<td>0.97 (0.75, 1.26)</td>
<td>3.75</td>
</tr>
<tr>
<td></td>
<td>Shen (28)</td>
<td>1.34 (0.86, 2.10)</td>
<td>3.61</td>
</tr>
<tr>
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<td>Svenson (30)</td>
<td>0.30 (0.19, 0.48)</td>
<td>3.59</td>
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<tr>
<td></td>
<td>Zheng (37)</td>
<td>1.43 (0.92, 2.23)</td>
<td>3.61</td>
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<tr>
<td></td>
<td>Gramatges (14)</td>
<td>0.05 (0.01, 0.22)</td>
<td>2.31</td>
</tr>
<tr>
<td></td>
<td>Pooley (66)</td>
<td>1.96 (1.37, 2.81)</td>
<td>3.31</td>
</tr>
<tr>
<td></td>
<td>Pooley (69)</td>
<td>1.96 (1.37, 2.81)</td>
<td>3.31</td>
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<td>Lee (20)</td>
<td>1.96 (1.37, 2.81)</td>
<td>3.31</td>
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<td>Zee (33)</td>
<td>1.96 (1.37, 2.81)</td>
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<td>Pooley (28)</td>
<td>1.96 (1.37, 2.81)</td>
<td>3.30</td>
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<td>Pooley (31)</td>
<td>1.96 (1.37, 2.81)</td>
<td>3.79</td>
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<td></td>
<td>Prescot (68)</td>
<td>1.96 (1.37, 2.81)</td>
<td>3.64</td>
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<td>Xing (32)</td>
<td>1.36 (0.56, 3.28)</td>
<td>3.12</td>
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<td>Risques (20)</td>
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<tr>
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<td>Liu (21)</td>
<td>3.12 (2.04, 4.77)</td>
<td>3.63</td>
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<tr>
<td></td>
<td>Hou (17)</td>
<td>2.02 (1.34, 3.04)</td>
<td>3.64</td>
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<tr>
<td></td>
<td>Wu (11)</td>
<td>6.75 (2.62, 17.36)</td>
<td>3.03</td>
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<tr>
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<td>Willeit (31)</td>
<td>1.23 (0.58, 2.62)</td>
<td>3.27</td>
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<tr>
<td></td>
<td>Wu (11)</td>
<td>15.00 (2.14, 105.12)</td>
<td>1.81</td>
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<tr>
<td></td>
<td>Jang (18)</td>
<td>8.40 (3.98, 17.73)</td>
<td>3.29</td>
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<td>Mirabelle (24)</td>
<td>4.69 (1.83, 11.98)</td>
<td>3.04</td>
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<td>Mirabelle (23)</td>
<td>0.85 (0.64, 1.14)</td>
<td>3.73</td>
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<td>Widmann (40)</td>
<td>19.00 (8.39, 43.04)</td>
<td>3.20</td>
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<td></td>
<td>Lan (19)</td>
<td>0.34 (0.16, 0.74)</td>
<td>3.25</td>
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<tr>
<td></td>
<td>Wu (11)</td>
<td>15.00 (1.58, 142.15)</td>
<td>1.54</td>
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<tr>
<td></td>
<td>Shao (36)</td>
<td>3.72 (0.63, 21.79)</td>
<td>2.00</td>
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<tr>
<td></td>
<td>Han (15)</td>
<td>0.97 (0.73, 1.30)</td>
<td>3.74</td>
</tr>
<tr>
<td></td>
<td>Overall (I-squared = 94.3%, p &lt; 0.001)</td>
<td>1.96 (1.37, 2.81)</td>
<td>100.00</td>
</tr>
</tbody>
</table>

NOTE: Weights are from random effects analysis

DCEG, NCI studies

Wentzensen I, Mirabelle L, Pfeiffer R, Savage SA: unpublished data
Dyskeratosis congenita: a highly informative model of telomere biology and cancer predisposition

Related disorders
- Wide phenotypic spectrum, short telomeres, mutations in TERT or TERC
- Aplastic anemia, leukemia, pulmonary fibrosis, liver disease

Genetics
- Rare mutations cause DC
  - *DKC1, TERC, TERT, NHP2, NOP10, TINF2, TCAB1*
- Common variants contribute to cancer in the general population (GWAS)

Regulation
- Chromosomal stability
- Epigenetics

Biomarkers
- Short telomeres and increased cancer risk in populations

Photos with permission
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  – www.dcoutreach.com

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  – Blanche Alter, MD, MPH
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  – S. Artandi: Stanford University
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• function
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• function
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• function
  – S. Artandi: Stanford University