Dyskeratosis Congenita: the Prototypic Telomere Biology Disorder

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NCI's Inherited Bone Marrow Failure Syndromes Study

- **Family Study**
  - Fanconi Anemia
  - **Dyskeratosis Congenita (DC)**
  - Diamond-Blackfan Anemia
  - Shwachman-Diamond Syndrome
- **Questionnaires**
- **Medical Record Review**

- **Evaluation at the NIH Clinical Center**
  - IBMFS Team
  - Genetic Counseling
  - Subspecialists
  - Biospecimens

Blanche Alter, MD, MPH
Neelam Giri, MD

http://marrowfailure.cancer.gov

NCI DC Cohort Study

	# Families	% Families Seen at NIH
DC	75	60%
HH & RS	11	70%
DC-like	21	43%
Total	107	57%

DC = Diagnostic Triad or 1 of the triad, + BMF + 2 other findings (Vulliamy et al, Blood, 2006, 107(7):2680-5)
HH = Hoyeraal Hreidarsson syndrome
RS = Revesz syndrome
DC-Like = Clinical features suggestive of DC and short telomeres but do not quite meet DC criteria (Savage and Bertuch, Genet Med, 2010, 12(12):753-754)

June 2015

Telomeres Preserve Chromosomal Integrity

- Long nucleotide $(TTAGGG)_n$ repeats and protein structure at chromosome ends
- Shorten with each cell division eventually leading to genetic instability, cell crisis and cell death
- Cancer cells survive despite critically short telomeres and chromosomal instability
- Genes important in telomere biology are evolutionarily conserved and have low nucleotide diversity in human populations

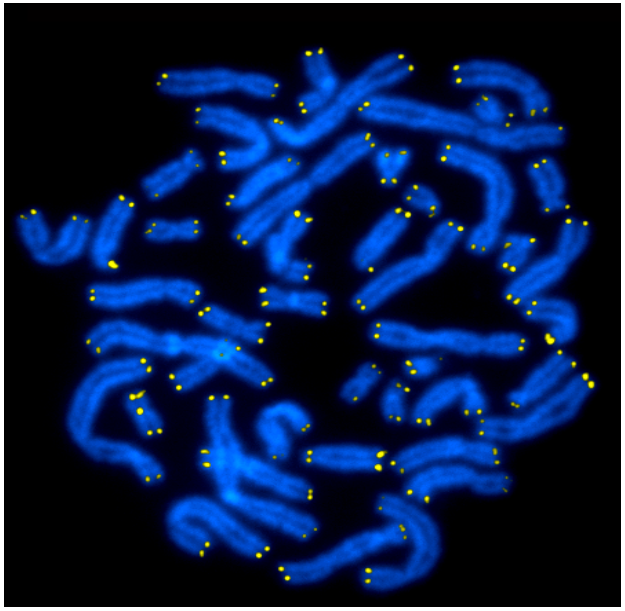
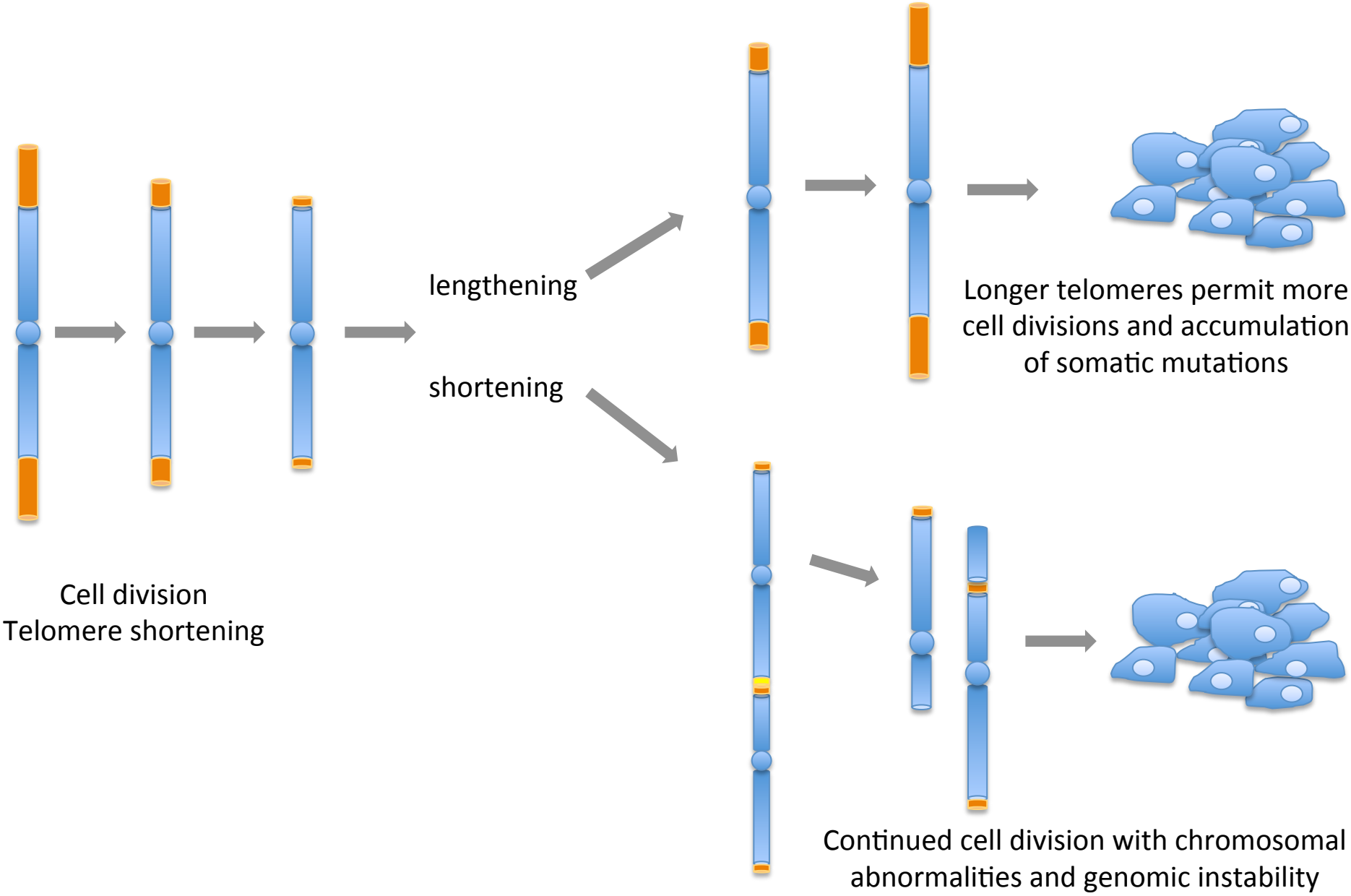
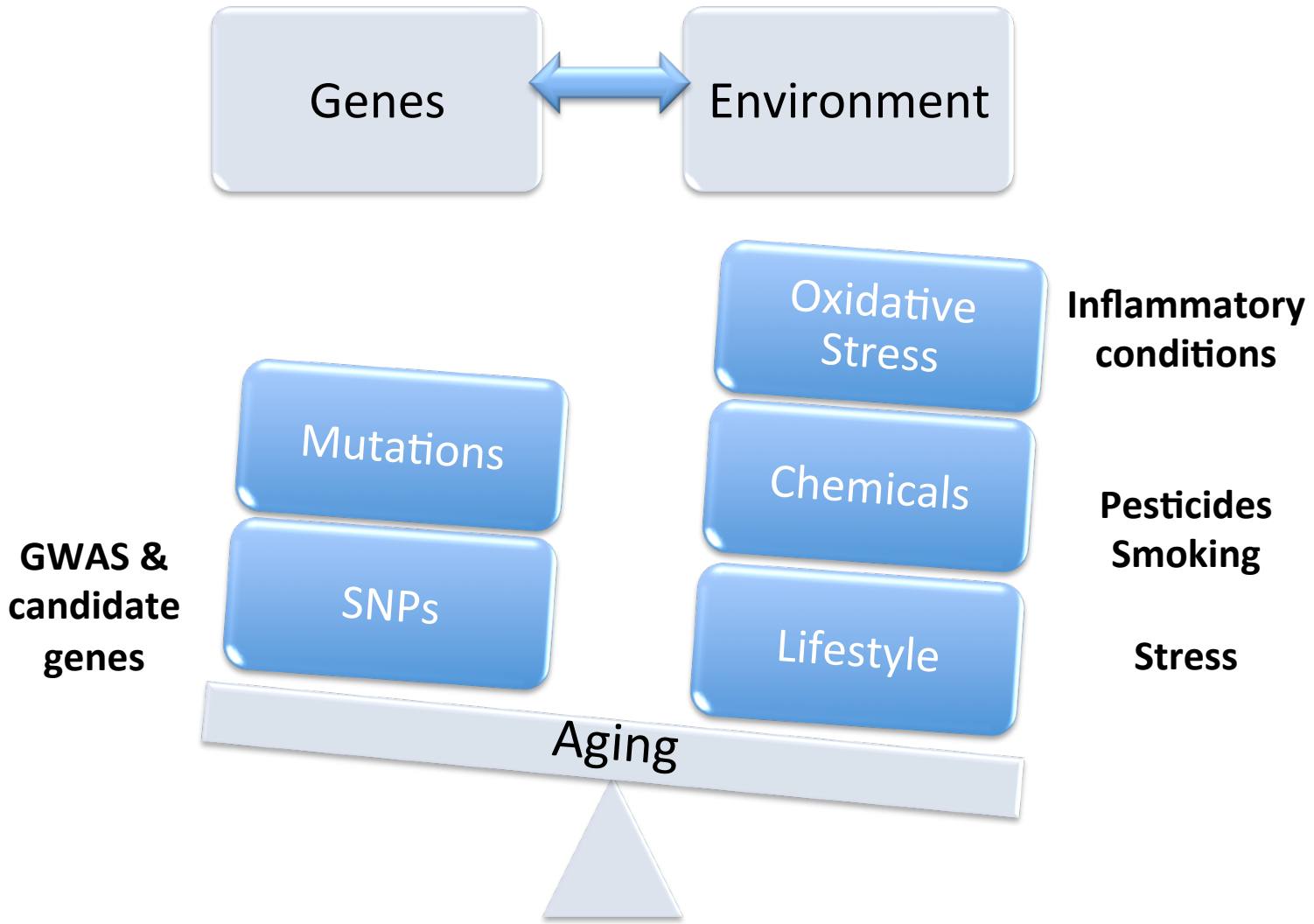


Photo: Dr. Peter Lansdorp

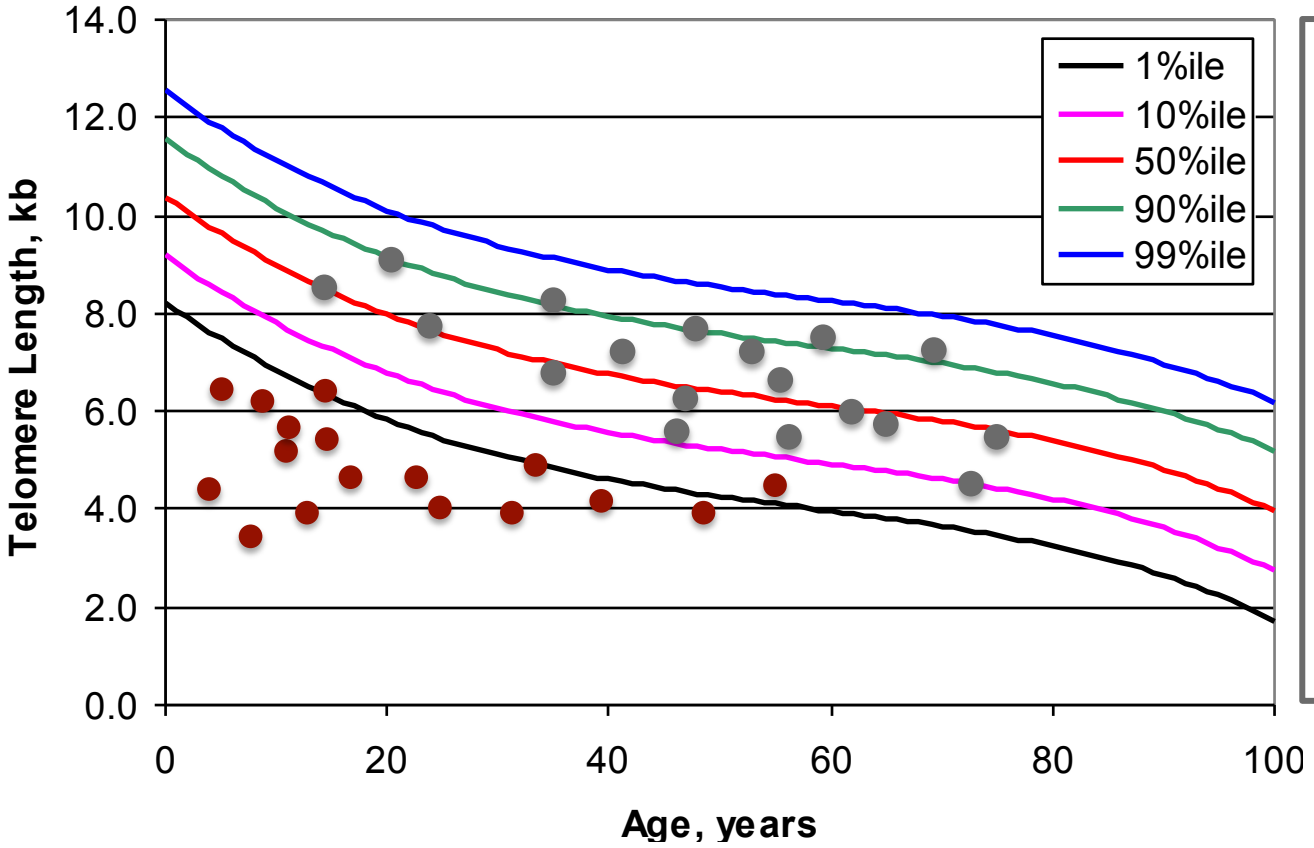
Telomeres: Dual Roles in Carcinogenesis



Telomere Length Regulation



The Telomere Length Continuum



Normal Range

- Associated with common disease
- Statistical Differences in Populations
- Methodologic limitations

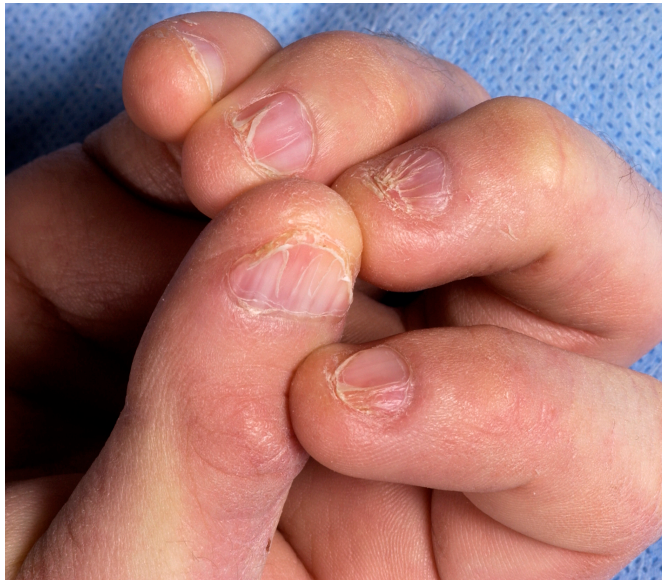
Very Short

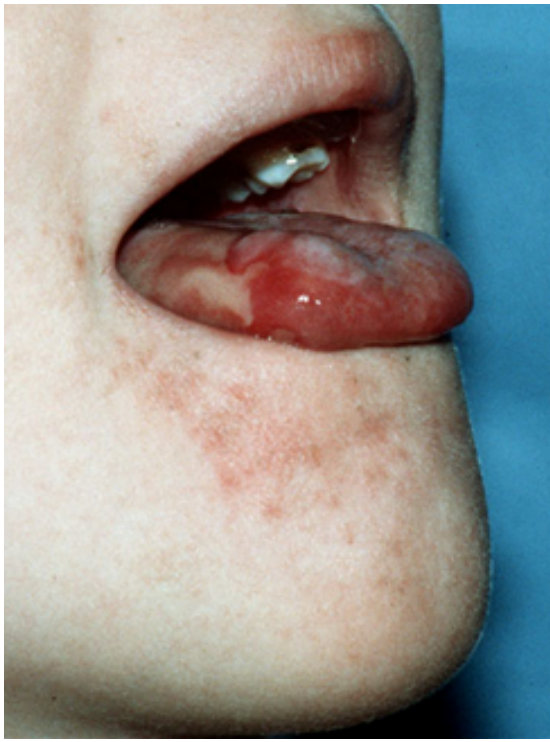
- Dyskeratosis Congenita & Related Telomere Biology Disorders
- <1st % diagnostic

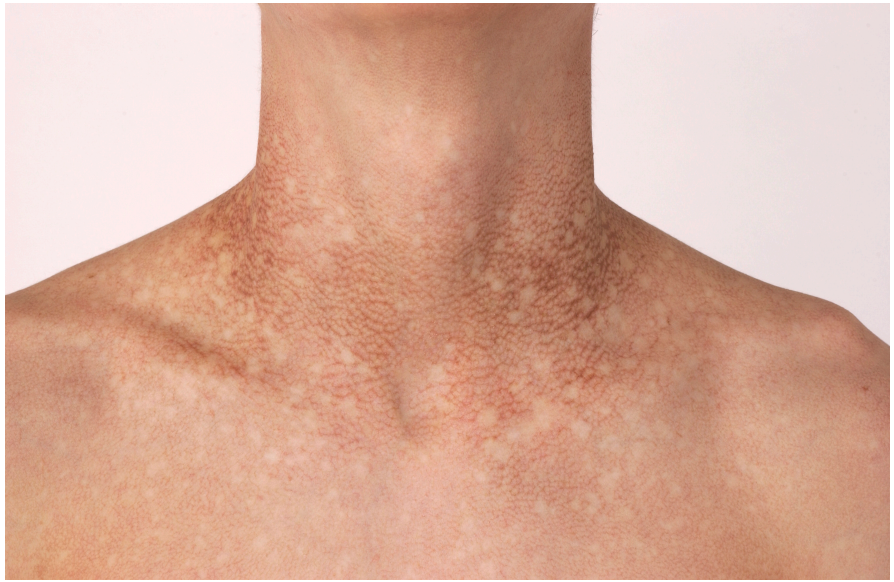
Dyskeratosis Congenita

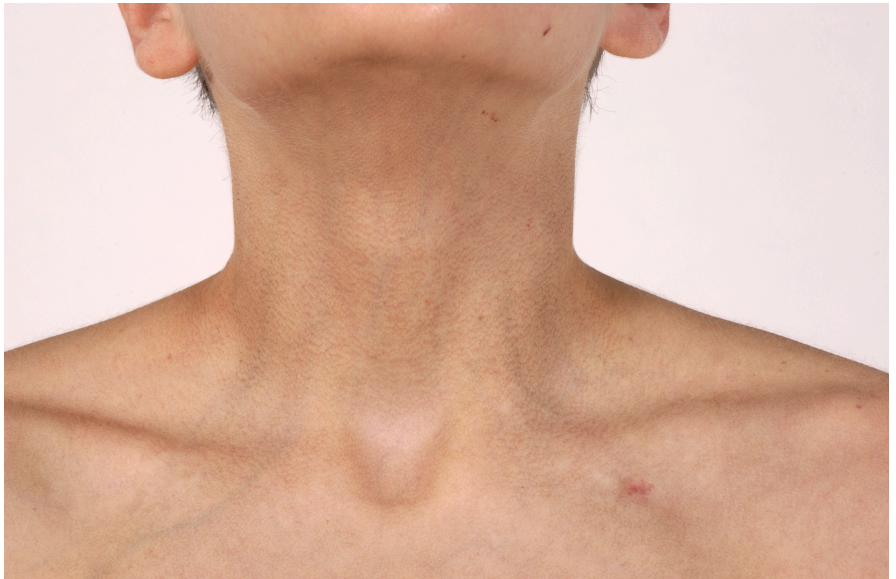
- **An inherited bone marrow failure syndrome complicated by multiple medical problems and elevated risk of cancer**
- **Early Descriptions**
  - 1906, Poikilodermia vascularis atrophicans, at a conference in Bern
  - 1908, Abnormal skin, sparse hair, latticework described by Prof. E. Jacobi
  - 1910, first publication, Prof. Ferdinand Zinsser
    - 2 brothers with skin, nail and tongue abnormalities
  - 1926, Report by Engman
  - 1930, Report by Cole "Dyskeratosis congenita with pigmentation"
  - 1956, Costello & Buncke, detailed description of clinical features
  - **1963, first report of a female case by Sorrow &Hitch**
- **Initially thought to be X-linked recessive but also has autosomal dominant and autosomal recessive inheritance patterns**

The Dyskeratosis Congenita Diagnostic Triad





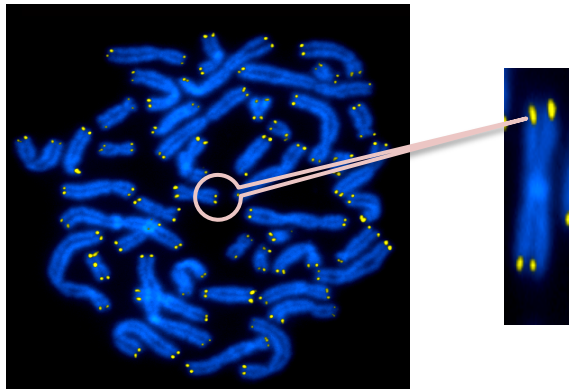




Other Medical Problems: Variable Age & Severity

- **Bone Marrow Failure**
- **Pulmonary Fibrosis**
- **Liver Fibrosis**
  - Non-infectious, non-alcoholic
- **Gastrointestinal**
  - Non-specific enteropathy
  - Esophageal stenosis
- **Urogenital**
  - Urethral stenosis
- **Ophthalmologic**
  - Lacrimal duct stenosis
  - Exudative retinopathy
- **Cancer**
  - Head & Neck
  - Leukemia
  - Anogenital
- **Neurologic**
  - Microcephaly
  - Cerebellar hypoplasia
  - Development delay
  - Psychiatric
- **Orthopedic**
  - Osteoporosis
  - Avascular necrosis
- **Hair**
  - Early graying
  - Early alopecia

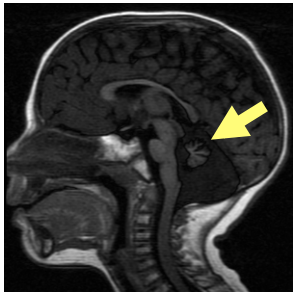
Extremely <u>short</u> telomeres unify the phenotype



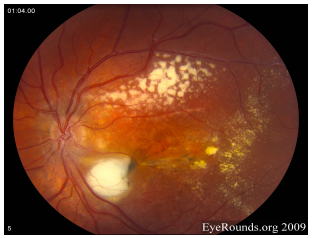
Traditional diagnosis:
Diagnostic Triad <u>or</u> 1 of the triad, + BMF + 2 other findings, Vulliamy et al, *Blood,* 2006, 107(7):2680-5

DC-related Telomere Biology Disorders:

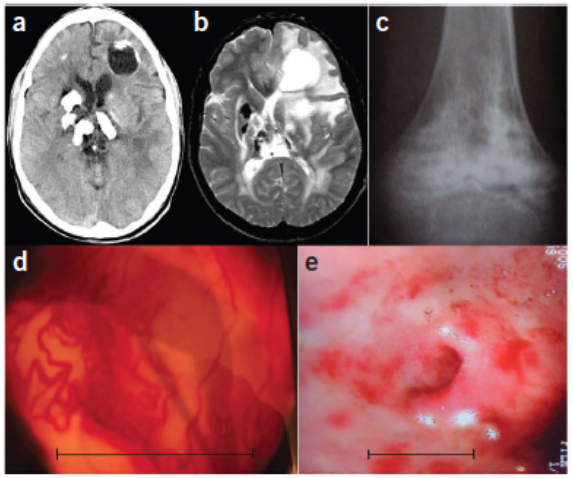
Often earlier onset, distinct complications



- **Hoyeraal Hreidarsson (HH) Syndrome**
  - Cerebellar hypoplasia
  - IUGR
  - Immune Deficiency



- **Revesz Syndrome**
  - Bilateral exudative retinopathy
  - Intracranial calcifications
  - IUGR



- **Coats Plus/CRMCC**
  - Retinal telangiectasias
  - Exudative retinopathy
  - Intracranial calcifications and/or cysts
  - Leukodystrophy
  - GI vascular ectasias
  - Osteopenia, fractures, poor bone healing

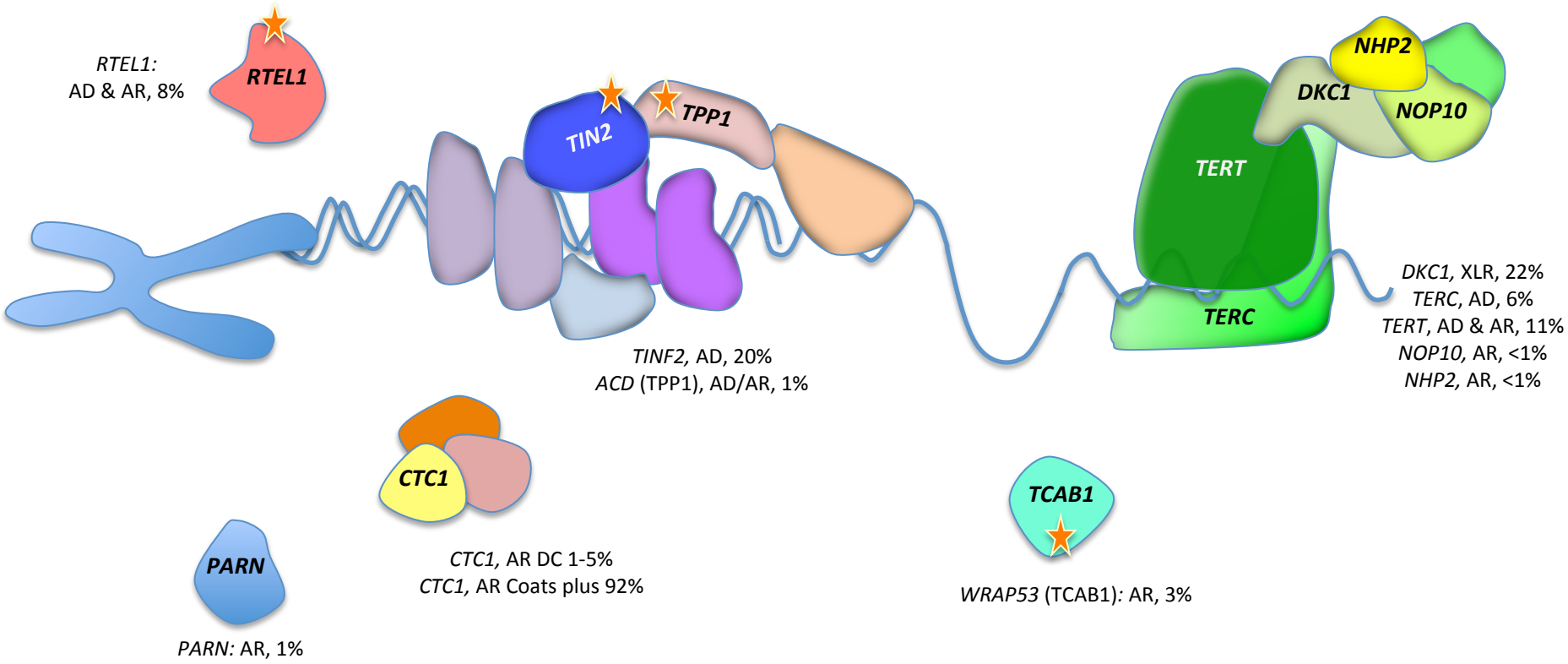
Anderson et al, Nature Genetics 2012

DC-related Telomere Biology Disorders:
Often later onset, fewer complications

- **Apparently isolated disease**
  - Pulmonary Fibrosis
  - Aplastic Anemia
  - Liver Disease
  - Head and Neck Squamous Cell Carcinoma
- **Classic Features of DC may not be present**
  - May be subtle or develop with time
- **Family History may or may not be present**
  - Take the time to ask detailed questions
  - Genotype-phenotype correlations exist

DC-associated Genes, 11 to date

Mutation in ~70% of classic DC cases



RTEL1: AD & AR, 8%

TINF2, AD, 20%
ACD (TPP1), AD/AR, 1%

DKC1, XLR, 22%
TERC, AD, 6%
TERT, AD & AR, 11%
NOP10, AR, <1%
NHP2, AR, <1%

CTC1, AR DC 1-5%
CTC1, AR Coats plus 92%

PARN: AR, 1%

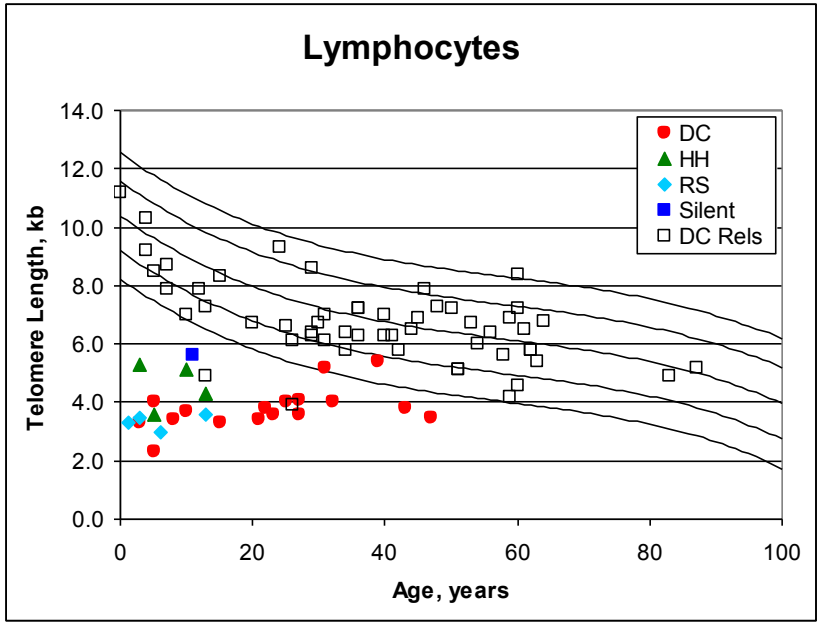
WRAP53 (TCAB1)*:* AR, 3%

% based on NCI cohort and literature review
AD, autosomal dominant; AR, autosomal recessive; XLR, X-linked recessive

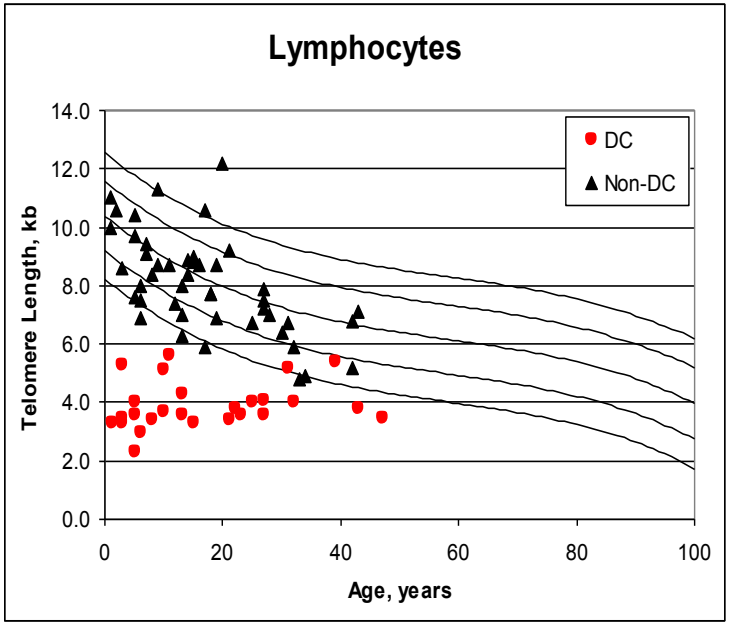
★ Discovered through the NCI cohort

Development of a Diagnostic Test

DC vs. healthy relatives



DC vs. other IBMFS

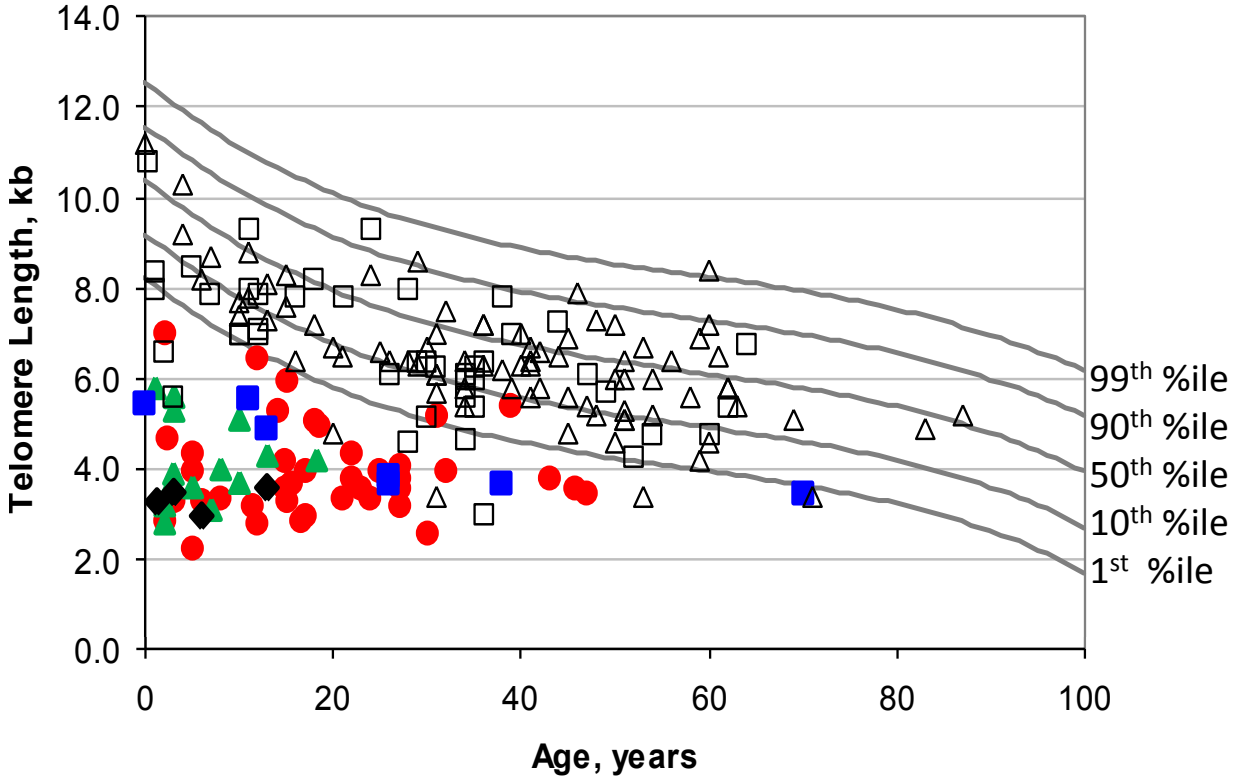


DC vs. other IBMFS	Sensitivity	Specificity
If 4/6 leukocyte subsets <1st percentile	92%	98%

Alter et al. Blood 2007;110(5):1439-1447

Development of the Diagnostic Test:

Leukocyte flow-FISH Telomere Length
CLIA-Certified, Gold Standard



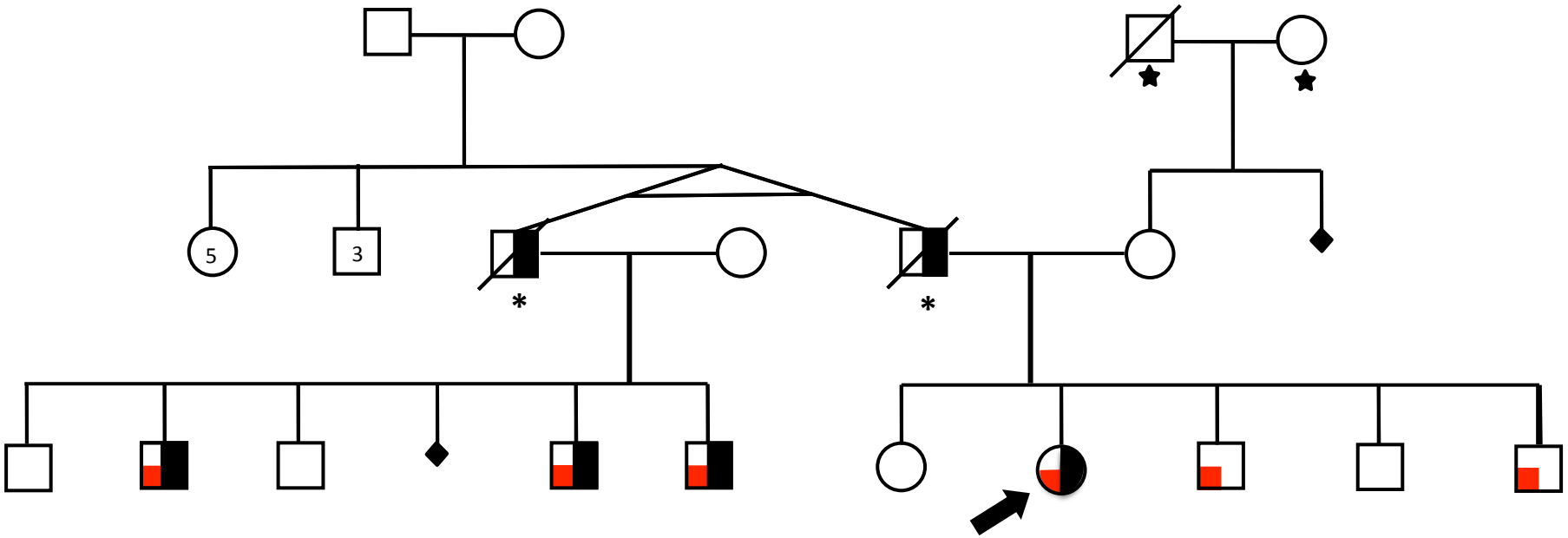
DC
HH
RS
Silent
DC Rels

Lymphocyte telomeres <1st %ile for age are >95% sensitive and specific

Alter et al, Haematologica 2012;97(3):353-9
Alter et al. Blood 2007;110(5):1439-1447

Genome-Wide Linkage Scan in DC

Autosomal dominant inheritance. *DKC1, TERC,* and *TERT* normal.



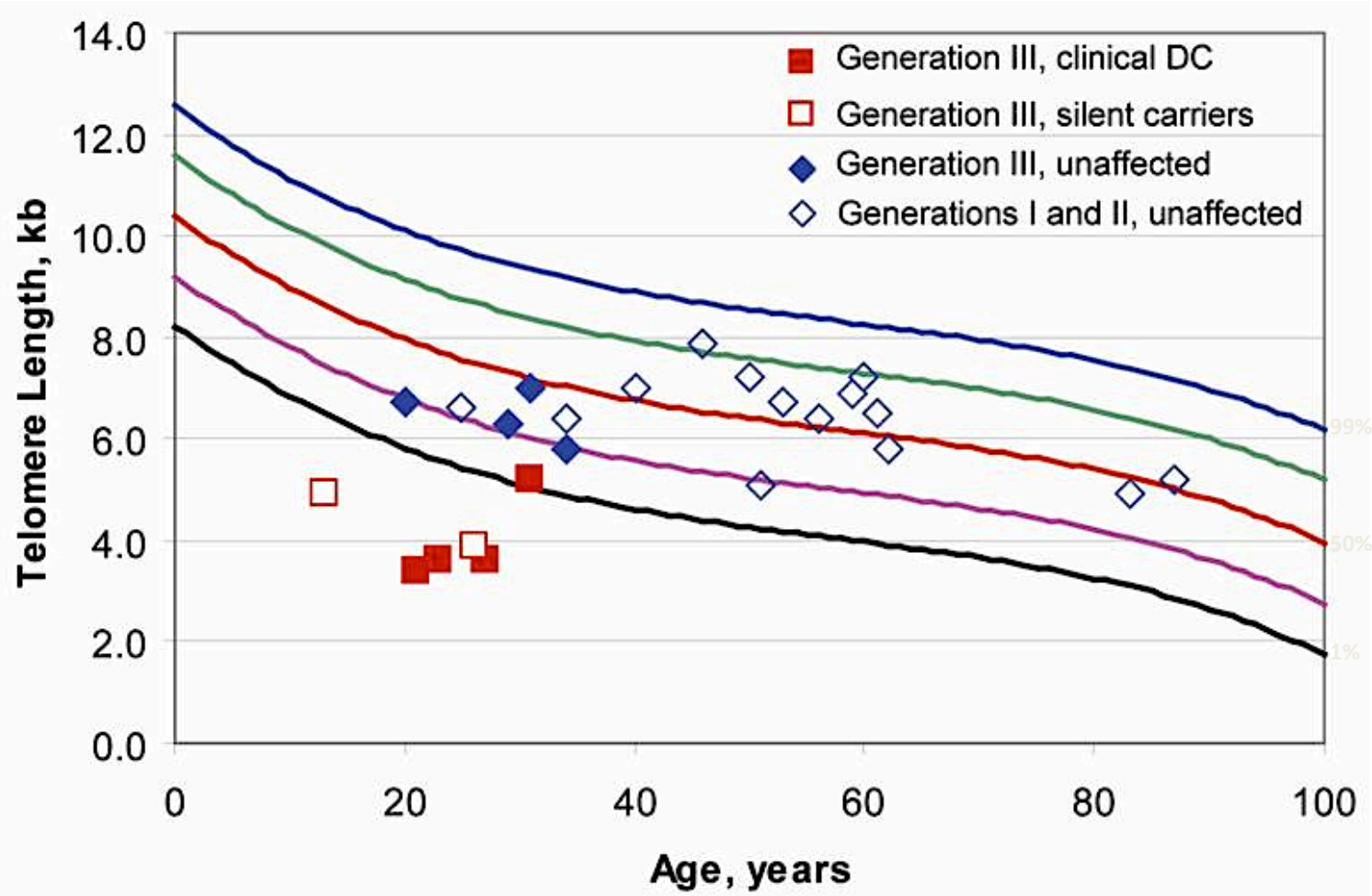
Clinical DC

Telomere length in WBC subsets <1st %ile

◆ miscarriage

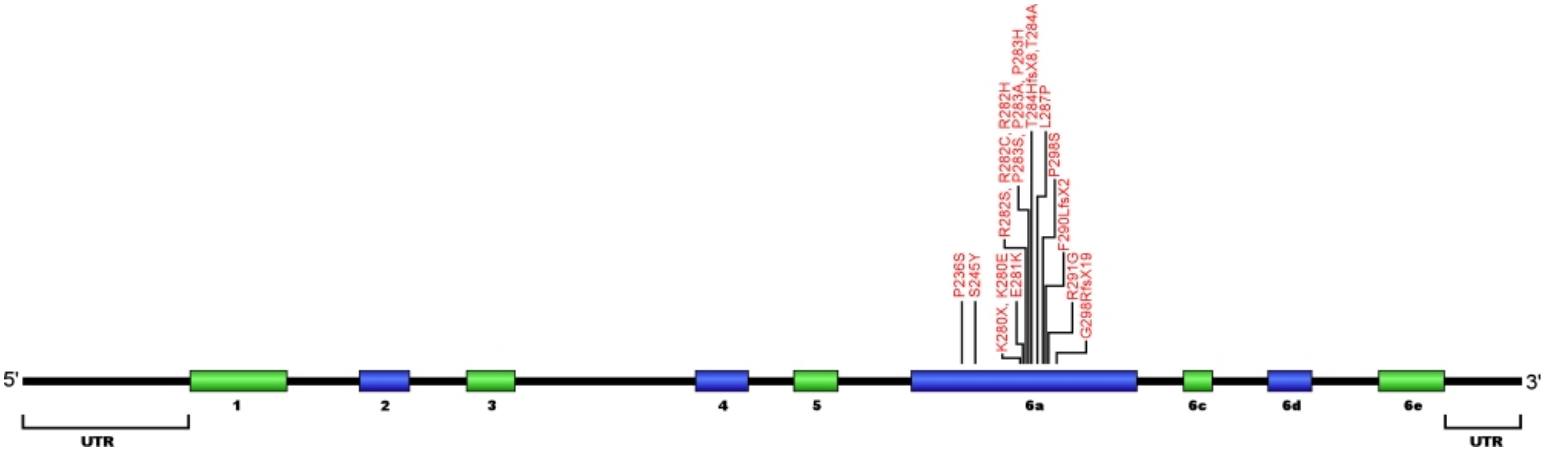
* Telomere length not measured

Lymphocyte Telomere Lengths: Family A



Autosomal dominant *TINF2* mutations cause DC

- Evidence for linkage at 14q11.2
- Autosomal dominant mutations in *TINF2* tracked with phenotype (telomeres <1st %ile for age)
- Affected unrelated individuals with DC have mutations

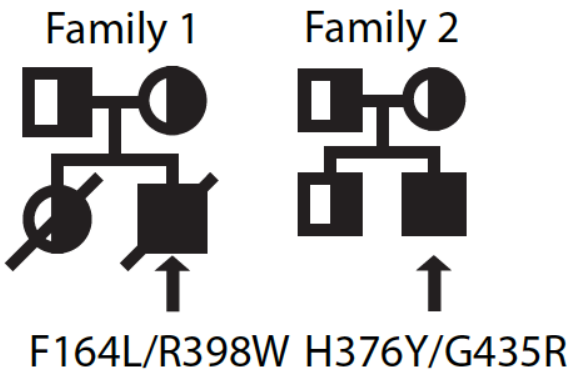


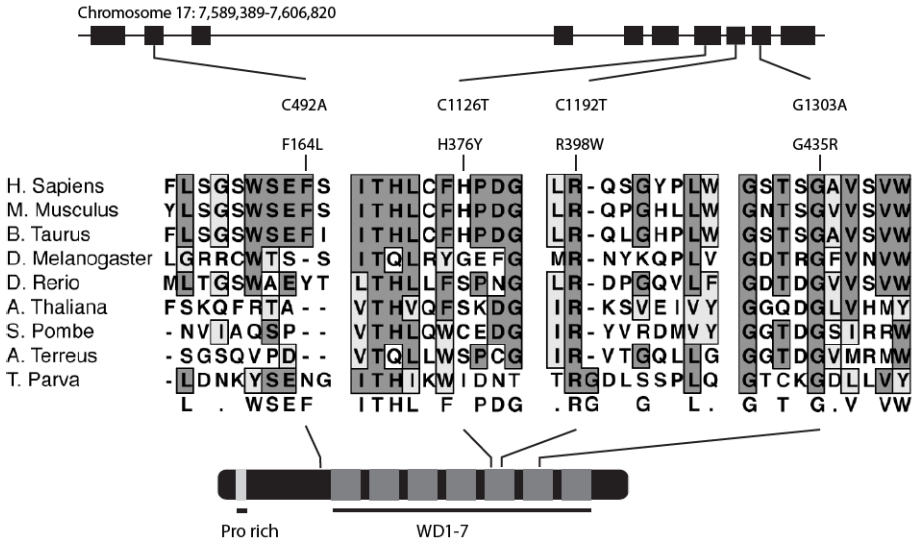
- *TINF2* mutations cause 12-20% of DC

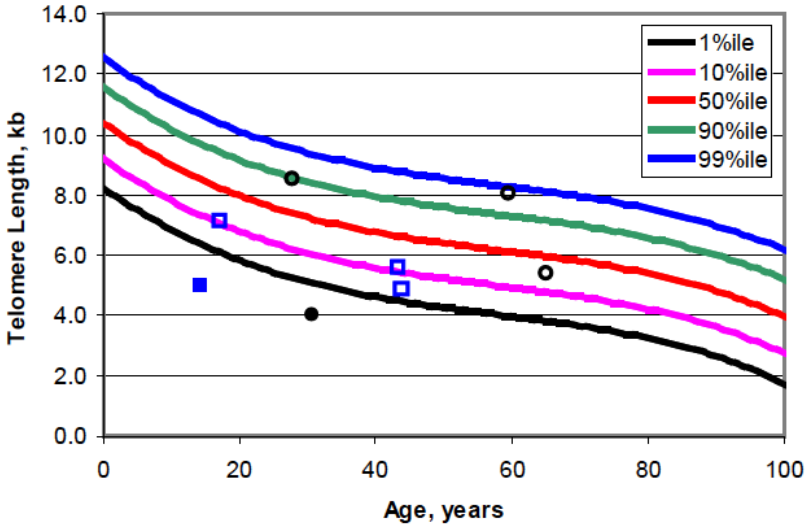
Savage et al, Am J Hum Genet. 2008 Feb;82(2):501-9

http://telomerase.asu.edu/

Uncovering Genetic Causes of DC: ***WRAP53*** **(TCAB1)**
Telomerase Cajal Body Protein 1



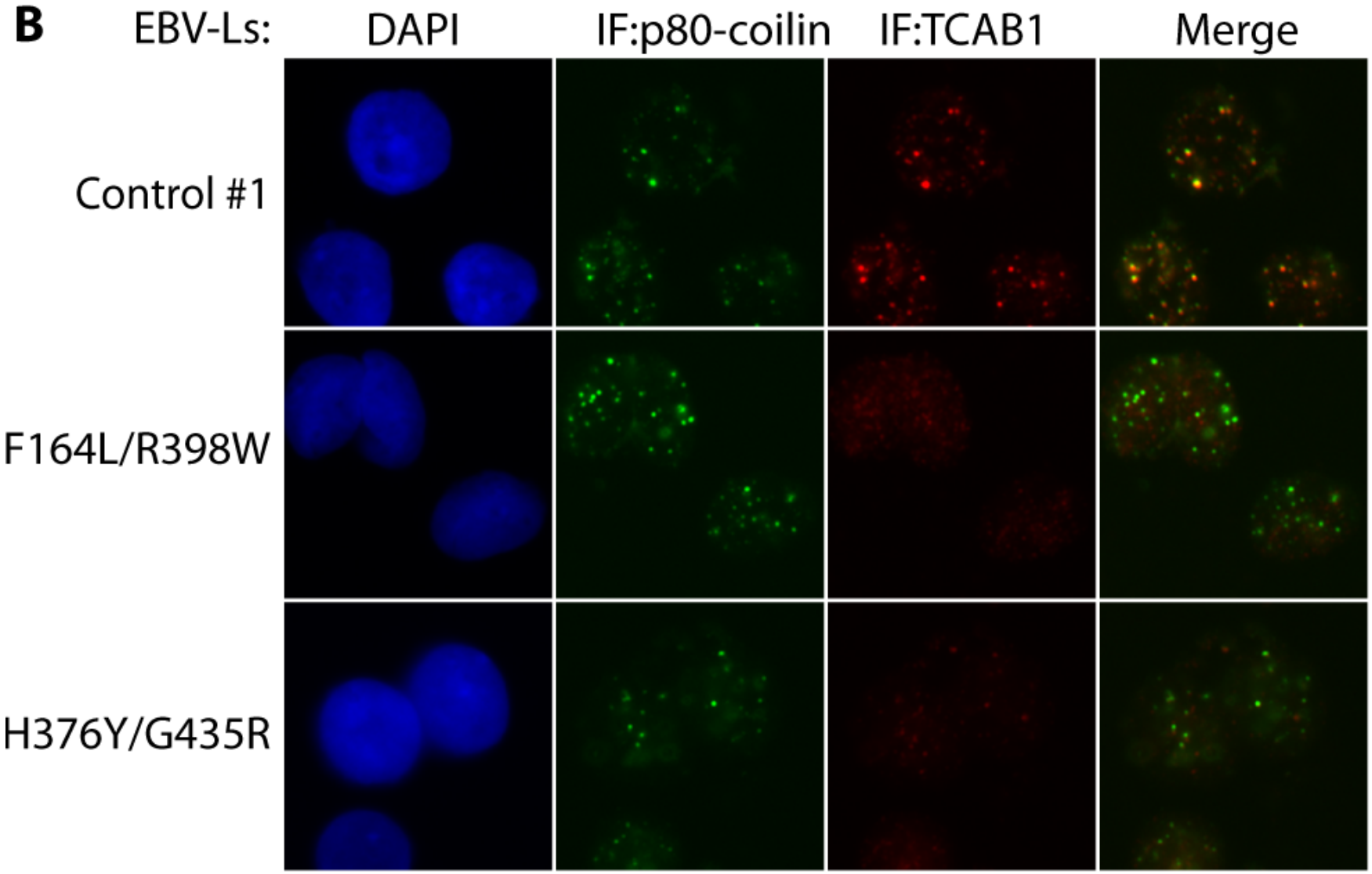




- TCAB1: essential for telomerase assembly and trafficking
- Mutations result in telomerase mislocalization

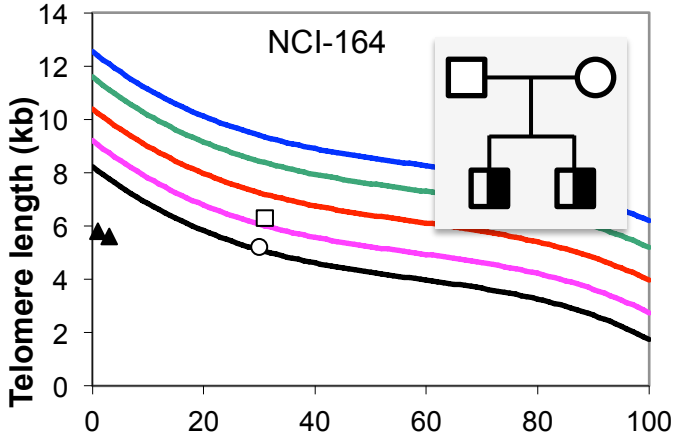
Zhong F, Savage SA, et al, Genes Dev. 2011 Jan 1;25(1):11-6

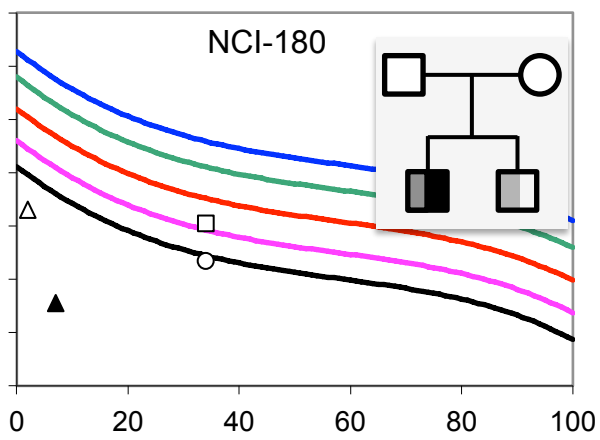
Uncovering Genetic Causes of DC: ***WRAP53*** **(TCAB1)**
Telomerase Cajal Body Protein 1



Zhong F, Savage SA, et al, Genes Dev. 2011 Jan 1;25(1):11-6

Uncovering Genetic Causes of DC: ***RTEL1***





Variant Filtering Strategy

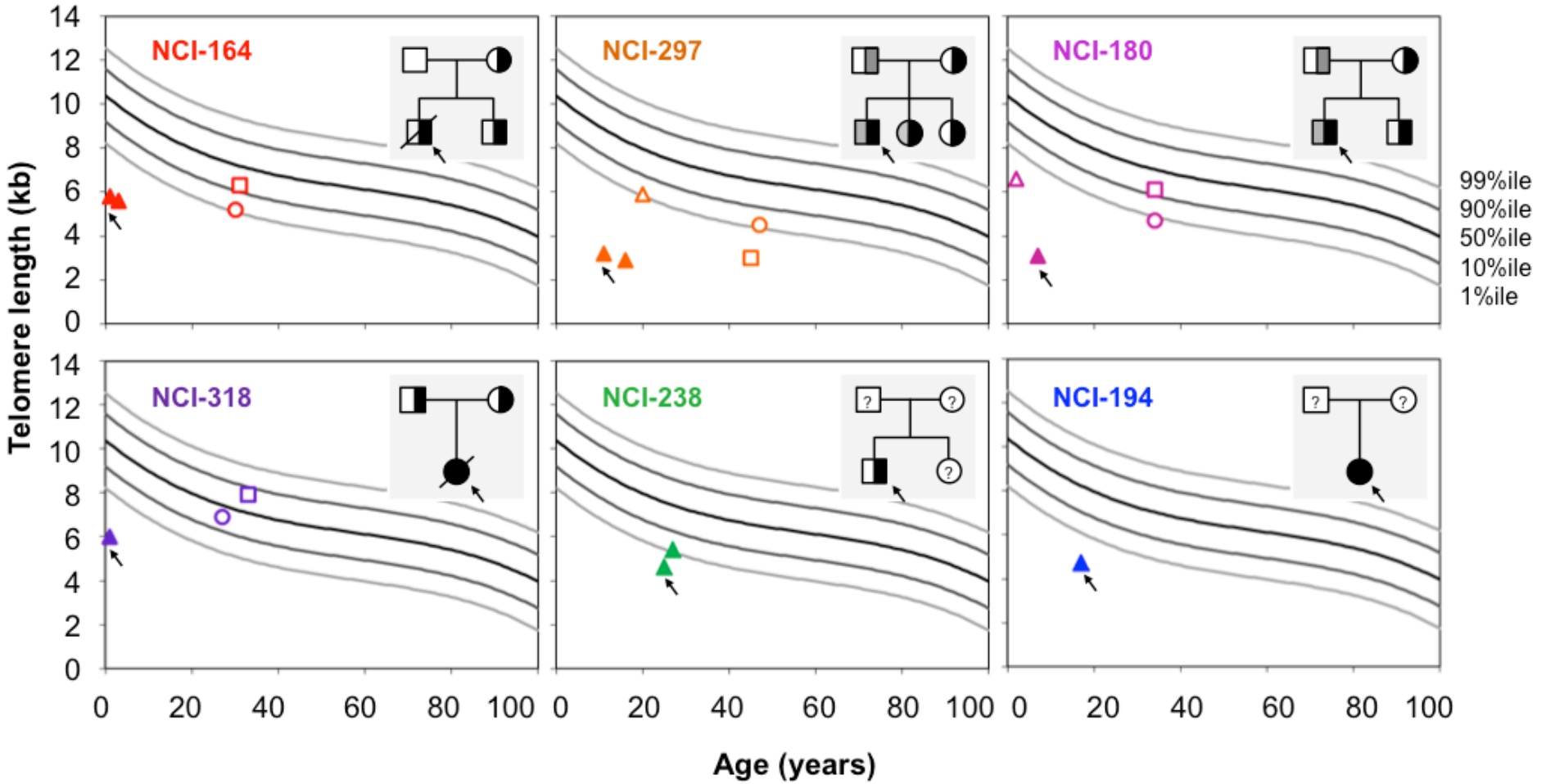
Total variants → Remove synony-mous SNPs → In ESP <4x → In internal control group <3x → Segmental duplications → In proband → In family members with short telomeres

Technical Validation and Functional Characterization

Exome Sequencing, Cancer Genomics Research Lab, DCEG, NCI. Nimbelgen v2 Capture Arrays, Illumina HiSeq

Bari Ballew, PhD

Uncovering Genetic Causes of DC: ***RTEL1***



Graph legend:

- ▲ Affected child
- △ Healthy child
- ○ Mother
- □ Father

Pedigree legend:

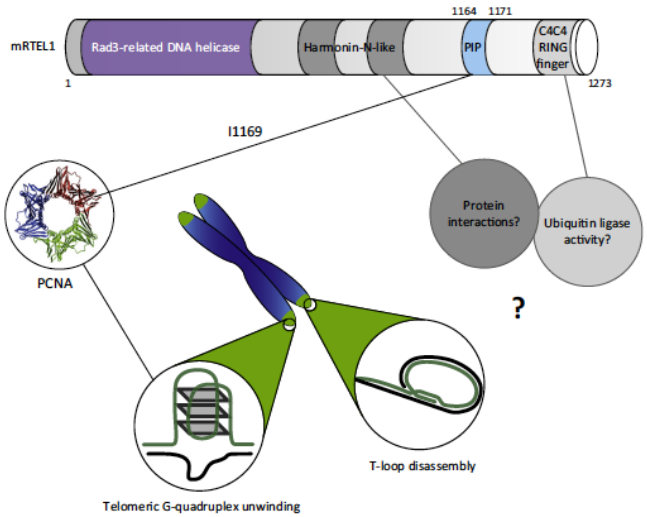
- □ No mutation
- ◨ Heterozygote
- ■ Homozygote
- ◧ Compound heterozygote
- ↖ Proband

Ballew et al, Hum Genet 2013Apr;132(4):473-80. Epub Feb 2013

Other groups subsequently reported *RTEL1* as a DC gene
- Walne et al. Am J Hum Genet. 2013 Mar 7;92(3):448-53
- Le Guen et al. Hum Molec Genet 2013 Aug 15;22(16)3239-49
- Deng et al. Proc Natl Acad Sci USA 2013 Sep 3;110(36)

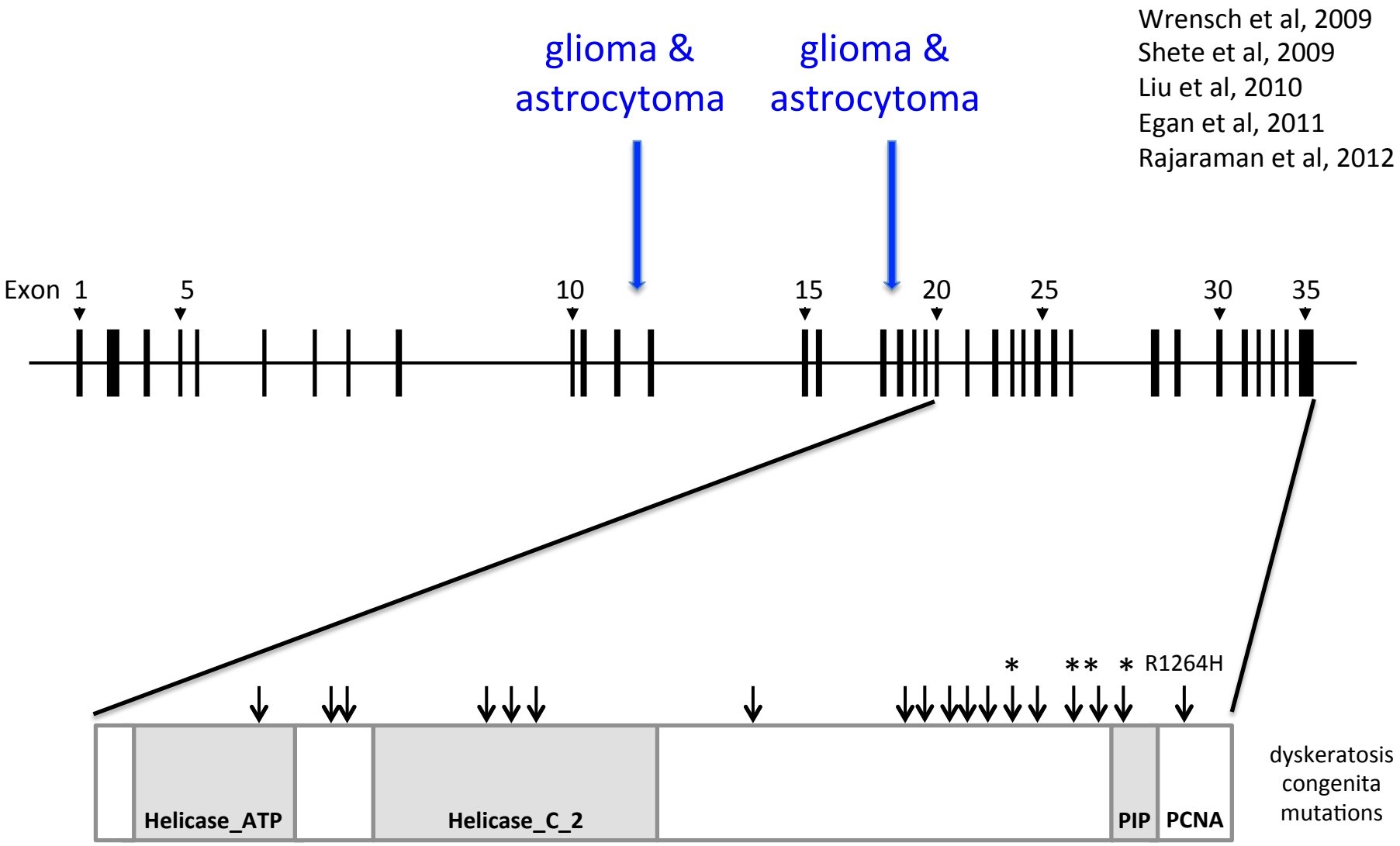
RTEL1:
Regulator of Telomere Elongation Helicase 1

- Essential DNA helicase
- Metabolism of DNA secondary structures
- Essential for DNA replication
- Controls recombination in mitotic and meiotic cells
- Maintains telomere homeostasis

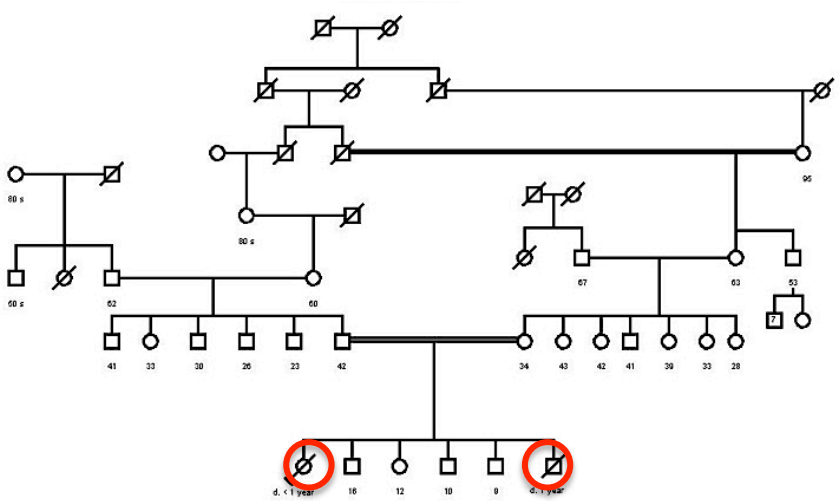


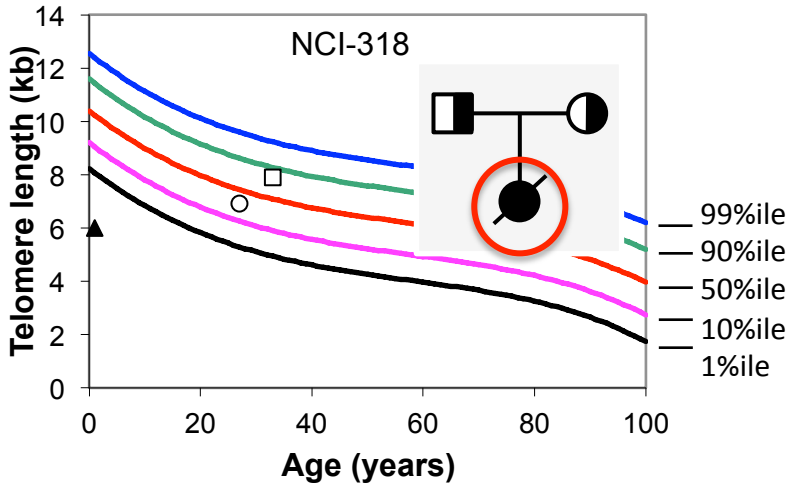
Vannier et al. Trends Cell Biol 2014 Feb 25

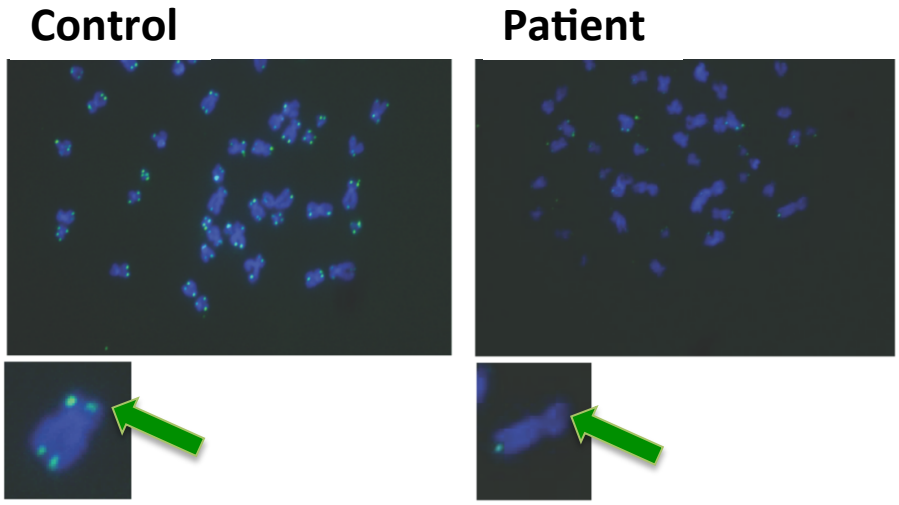
RTEL1 and Cancer Risk



HH Due to Recessive Founder *RTEL1* p.R1264H Mutations

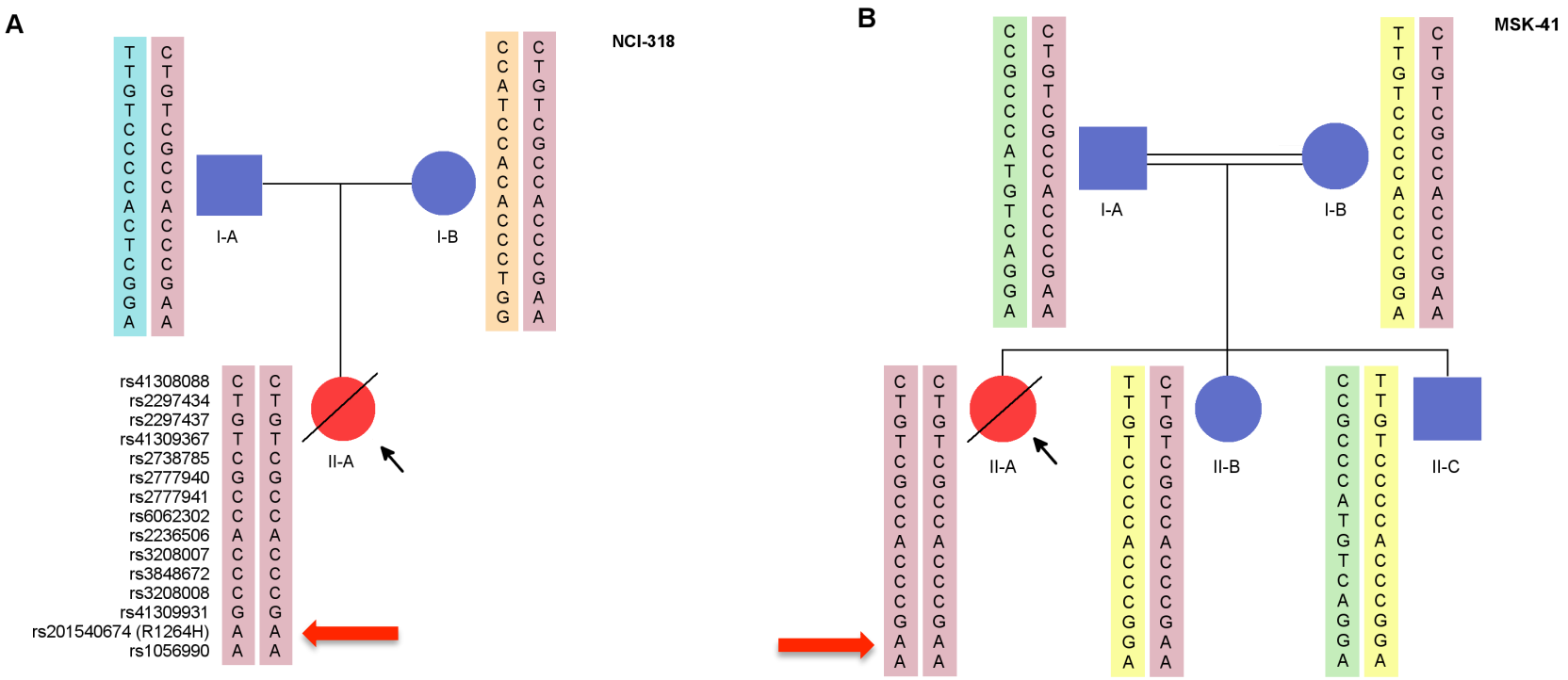






Ballew, Joseph, De et al, PLoSGenetics 2013;9(8):e1003695

p.R1264H *RTEL1* Mutations Are Carried on a Common Haplotype



p.R1264H minor allele frequency 1 in 9,600 individuals of European ancestry in ESP, 1000Genomes, and dbSNP

Ballew, Joseph, De et al, PLoSGenetics 2013;9(8):e1003695

Carrier Frequency of *RTEL1* p.R1264H

- Genotyped 1,048 individuals of Orthodox Ashkenazi Jewish (AJ) heritage
  - Dor Yeshorim program, Center for Jewish Genetics
  - Verified to be unrelated
- Genotyped 2,240 individuals from AJ general population
  - Mount Sinai Genetic Testing Laboratory
  - Verified to be unrelated

Fedick et al, Clin Genet 2014 Jul 22 [Epub ahead of print]

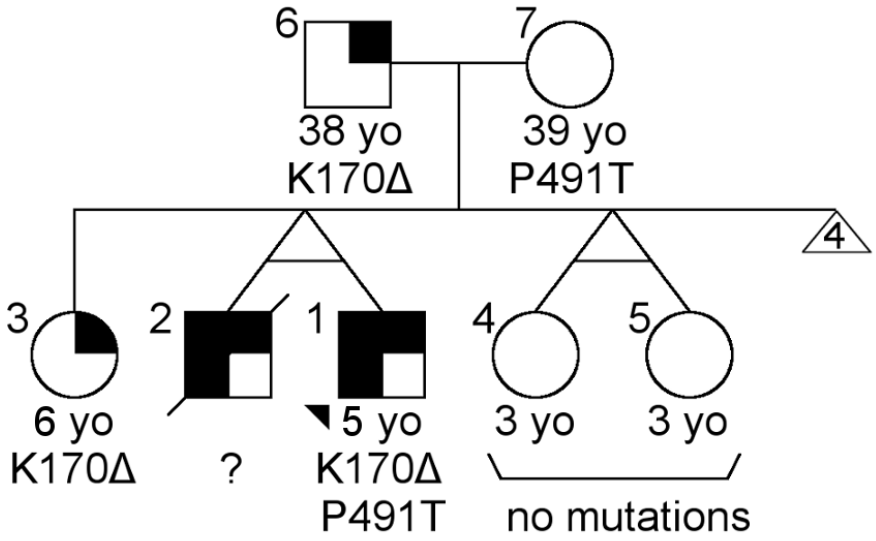
Carrier Frequency of *RTEL1* p.R1264H

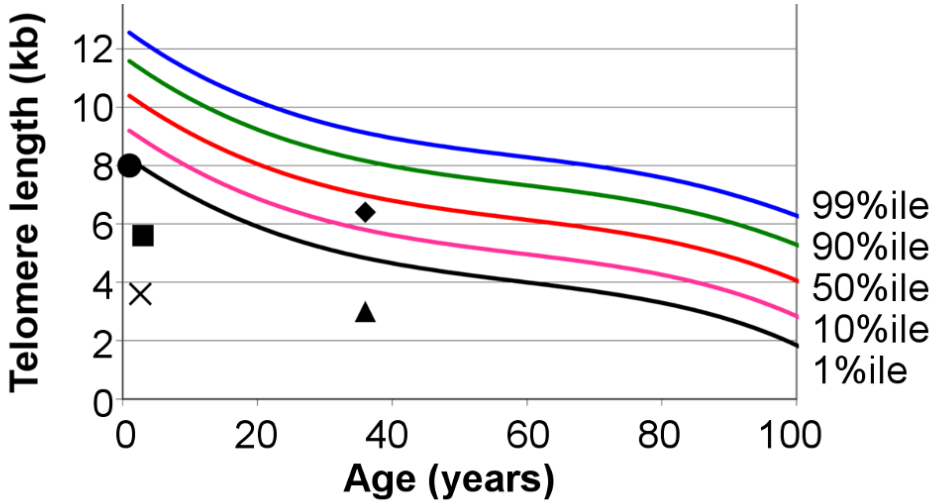
- **1% of Orthodox AJ individuals**
  - 10/1032 or ~1 in 100
- **0.45% in general AJ population**
  - 10/2,227 or ~1 in 223
- 1 in 100 is similar to that of other genetic disorders considered for screening in this population
  - Bloom's Syndrome, 1 in 110
  - Joubert Syndrome 2, 1 in 92
  - Mucolipidosis IV, 1 in 100
  - Fanconi Anemia, *FANCC,* 1 in 89

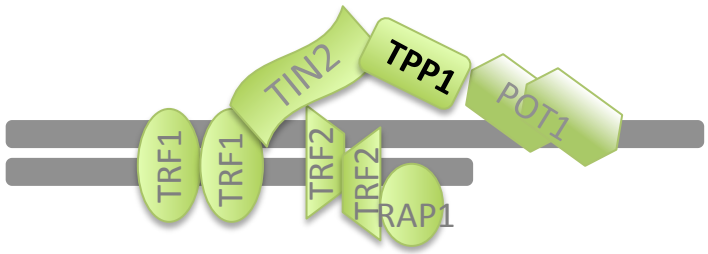
Fedick et al, Clin Genet 2014 Jul 22 [Epub ahead of print]

Uncovering Genetic Causes of DC: **TPP1**

Telomere Protection Protein 1 (encoded by *ACD*)



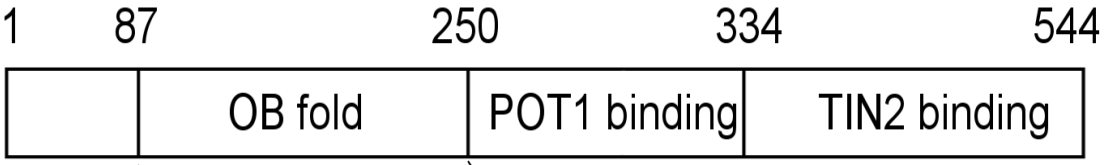


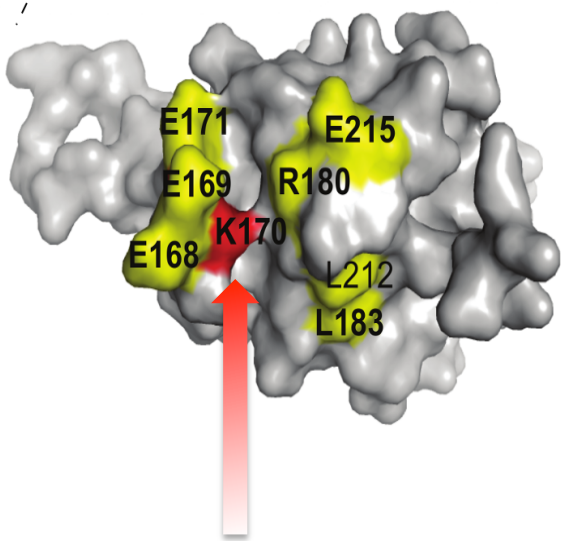


Kocak et al. Genes Development 2014;28:2090-2102

Uncovering Genetic Causes of DC: **TPP1**

Telomere Protection Protein 1 (encoded by *ACD*)

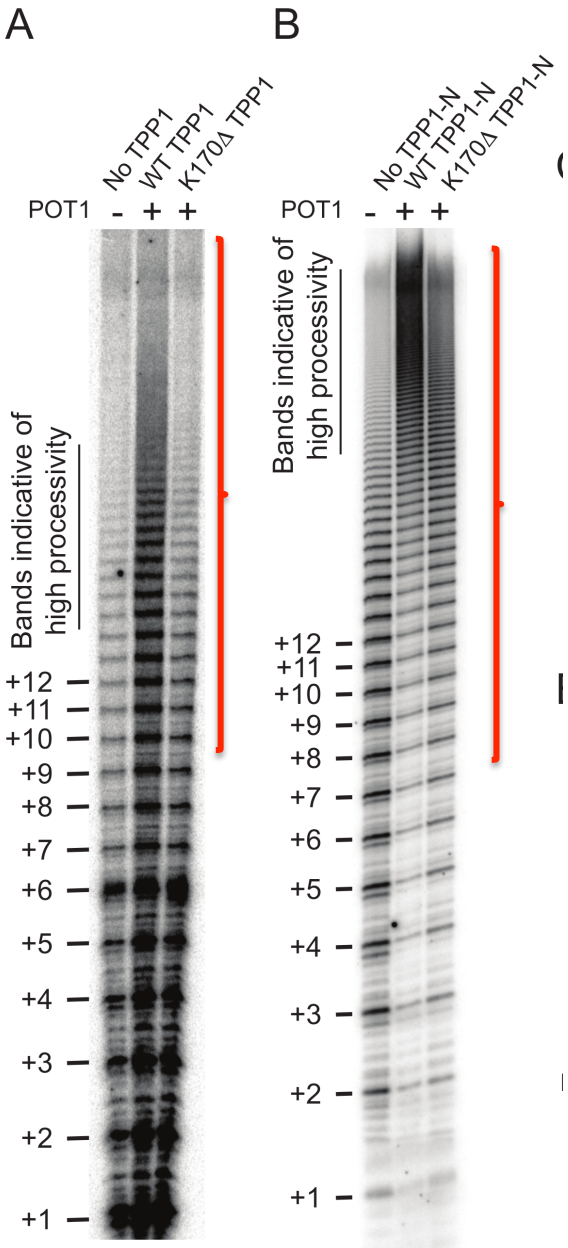




K170del in TEL-patch

Markedly reduced telomerase processivity

Modifier mutation at p.P491T



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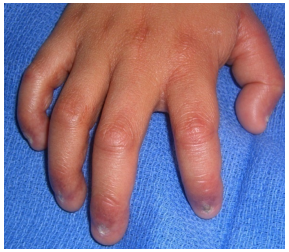
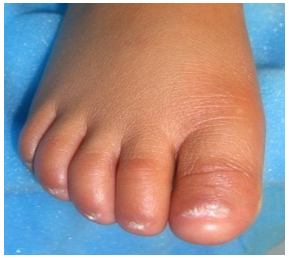
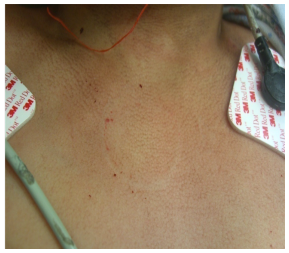
Complicated Presentation and Phenotype Progression

6 years



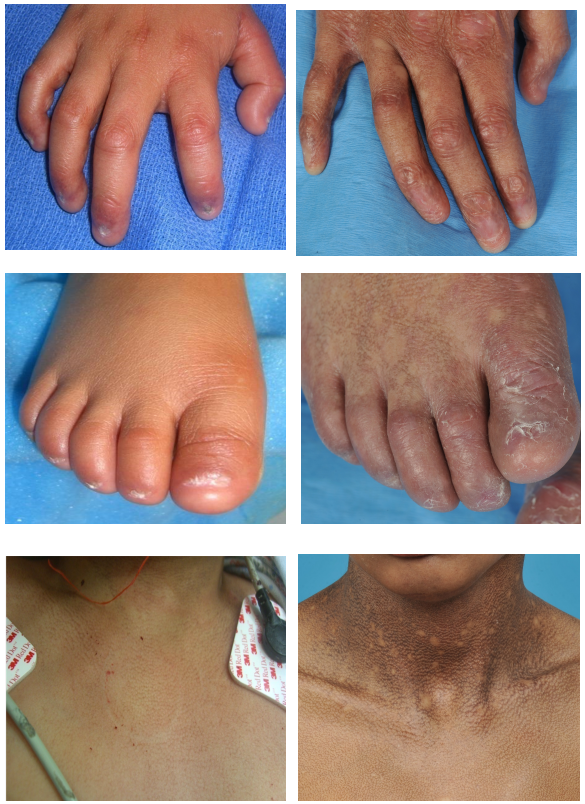




Burris AM, et al. submitted

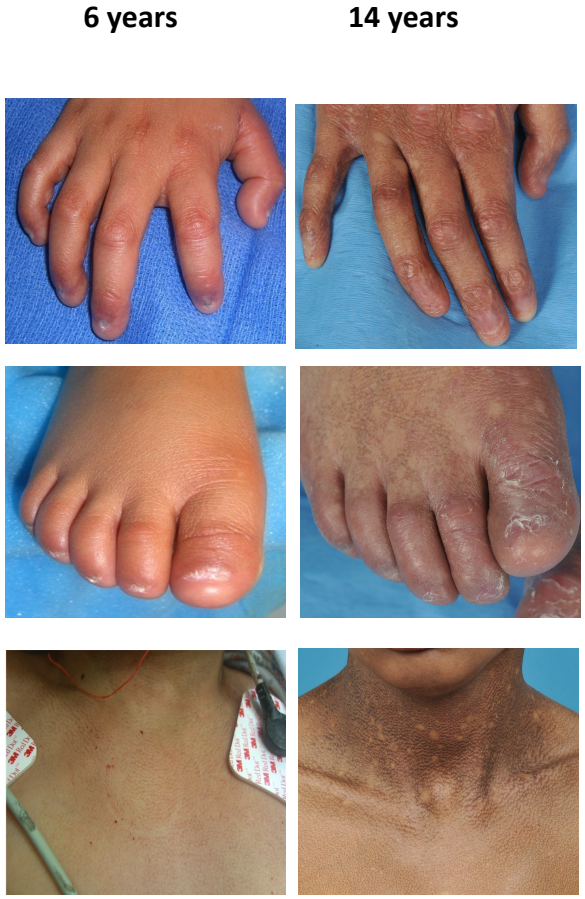
Complicated Presentation and Phenotype Progression

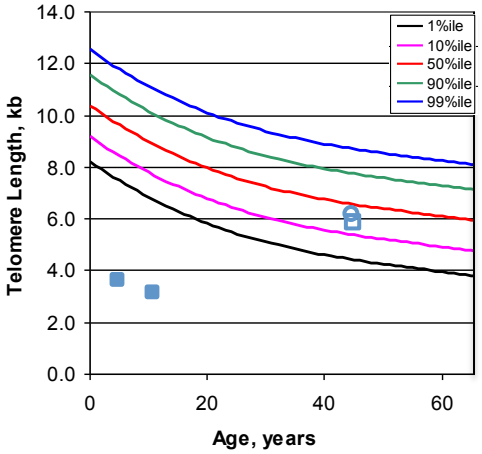
6 years **14 years**



Burris AM, et al. submitted

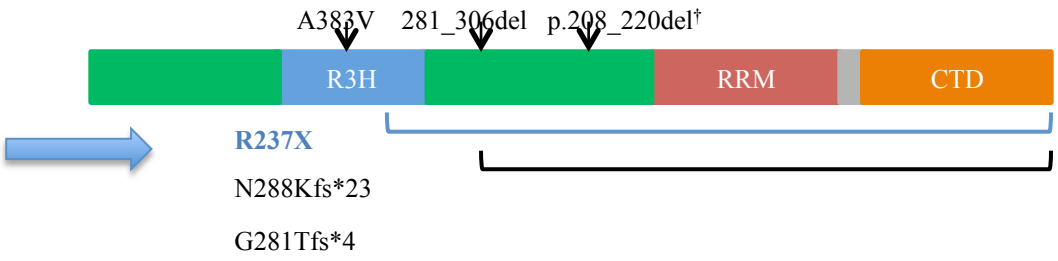
Complicated Presentation and Phenotype Progression





PARN
poly(A)-specific ribonuclease

Novel promoter mutation and partial gene deletion

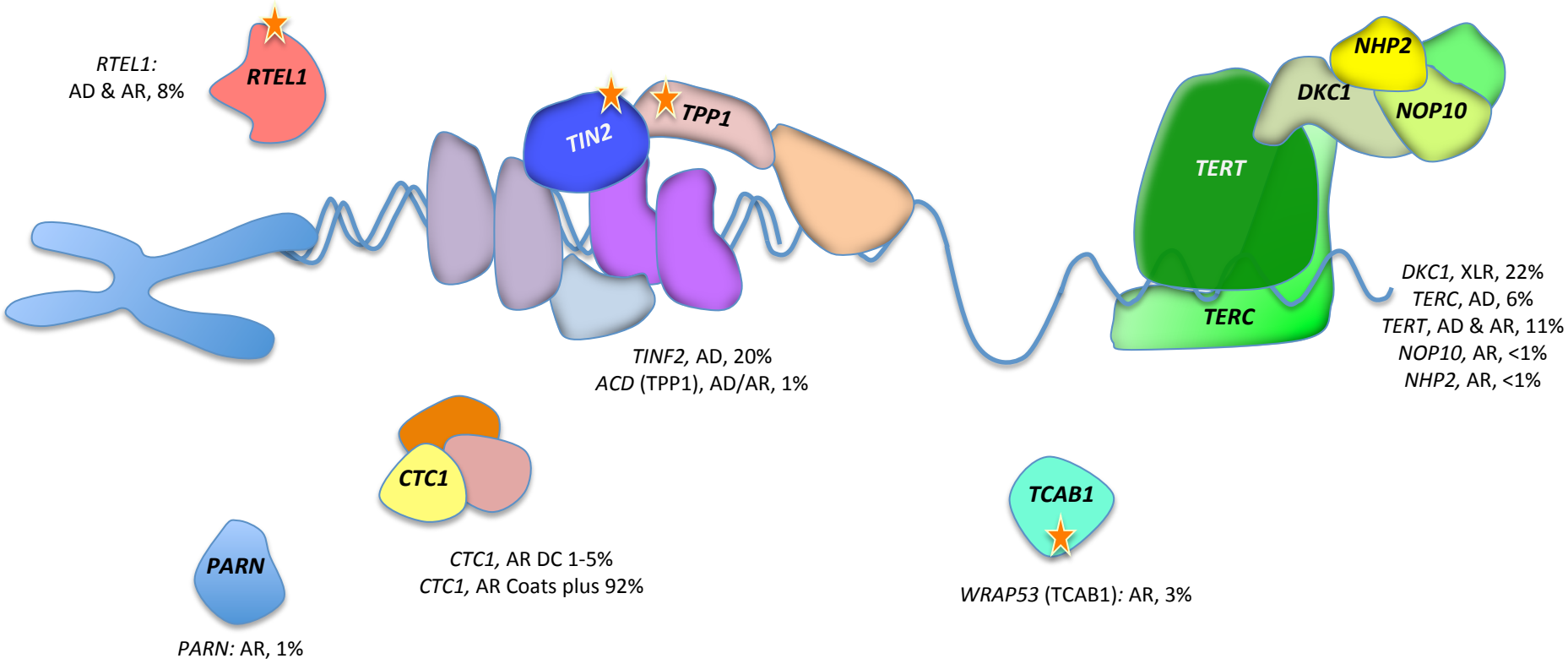


RNA-binding domains: R3H domain [red], RRM domain [RNA recognition motif, blue], two catalytic nuclease domains [green]; C-terminal domain [CTD, orange]; predicted bipartite NLS motif in grey

Burris AM, et al. submitted

DC-associated Genes, 11 to date

Mutation in ~70% of classic DC cases



RTEL1: AD & AR, 8%

TINF2, AD, 20%
ACD (TPP1), AD/AR, 1%

DKC1, XLR, 22%
TERC, AD, 6%
TERT, AD & AR, 11%
NOP10, AR, <1%
NHP2, AR, <1%

CTC1, AR DC 1-5%
CTC1, AR Coats plus 92%

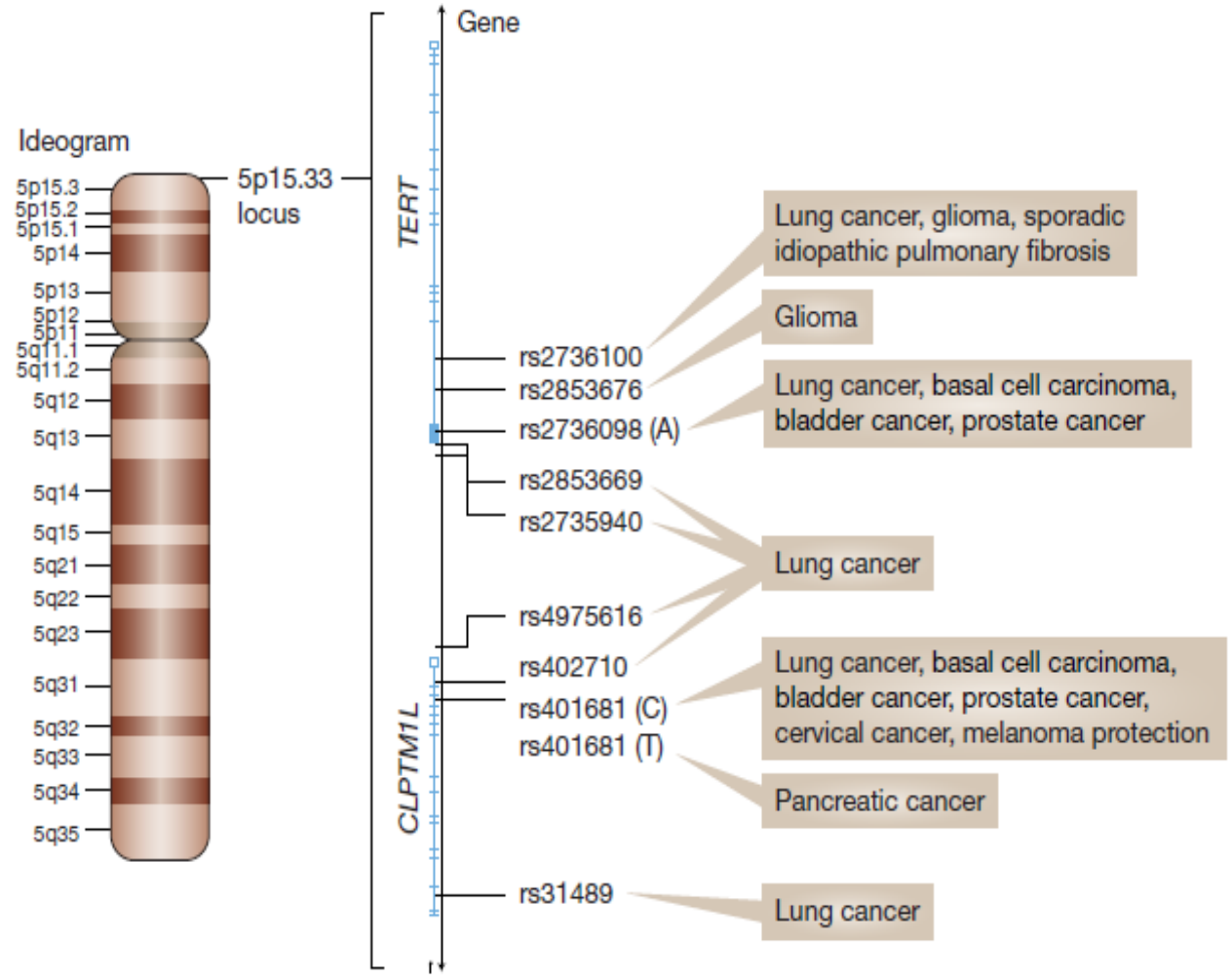
PARN: AR, 1%

WRAP53 (TCAB1)*:* AR, 3%

% based on NCI cohort and literature review
AD, autosomal dominant; AR, autosomal recessive; XLR, X-linked recessive

★ Discovered through the NCI cohort

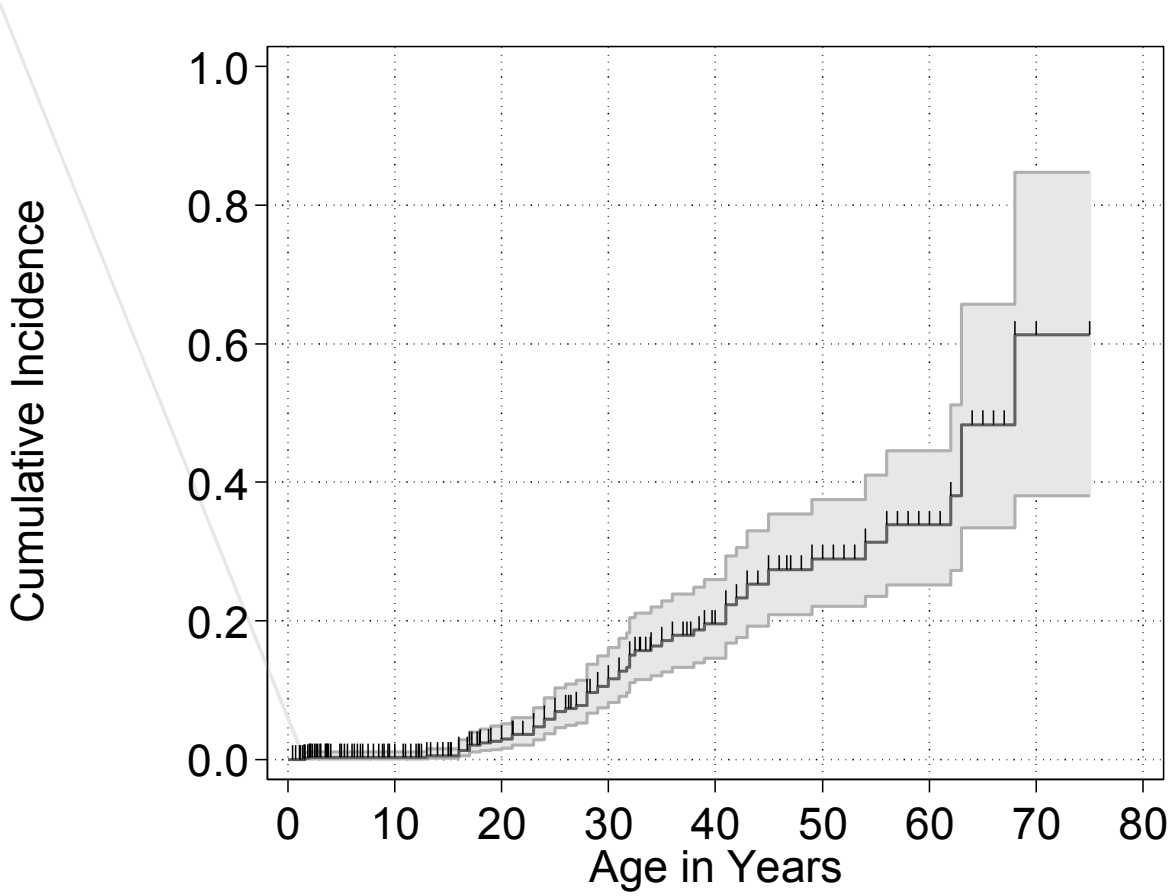
TERT-CLPTM1L SNPs and Cancer



Cancer risk variants at the 5p15.33 locus

Baird DM Expert Rev Mol Med 2010;12:e16

Cumulative incidence of cancer among 775 patients with DC reported in the literature

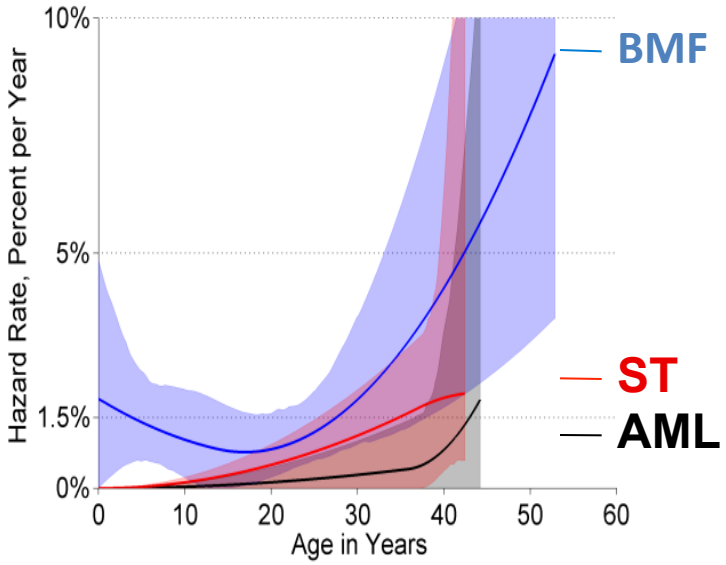


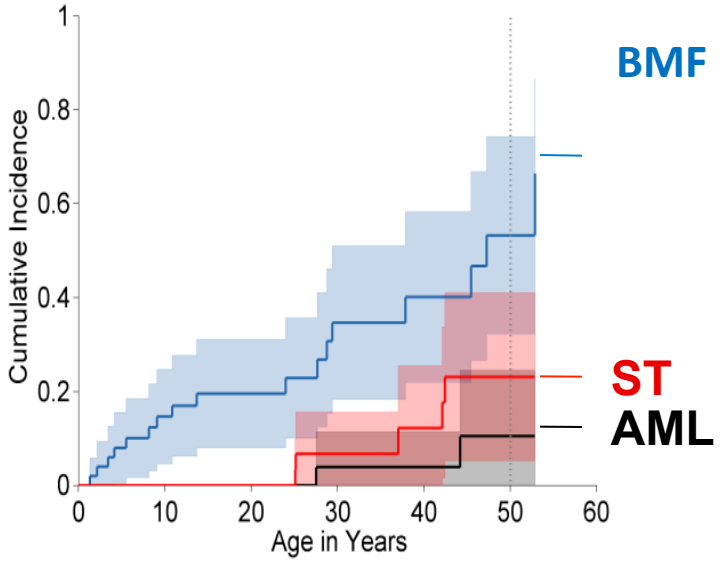
Relative Risk of Cancer in DC

Cancer Type	# observed (O)	# expected (E)	O/E ratio
All sites	7	0.6	11*
All solid tumors	5	0.5	8*
HNSCC	3	0	1154*
Cervix	1	0.02	43
Non-Hodgkin lymphoma	1	0.03	34
Skin basal cell	1	NA	NA
Leukemia	2	0.01	196*
MDS	5	0	2663*

775 patients with DC , 60 of whom had 69 cancers. All cancers were in patients who had not received a stem cell transplant. The observed number of cancers was compared with the expected number in the general population (O/E ratio) based on SEER, after adjustment for age, sex, race, and birth cohort (www.seer.cancer.gov). *$p<0.05$, i.e. statistically significant. From Alter et al, Blood 2009; 113(26): 6549-6557

Annual hazard and cumulative incidence of competing adverse events in patients with DC





BMF, bone marrow failure

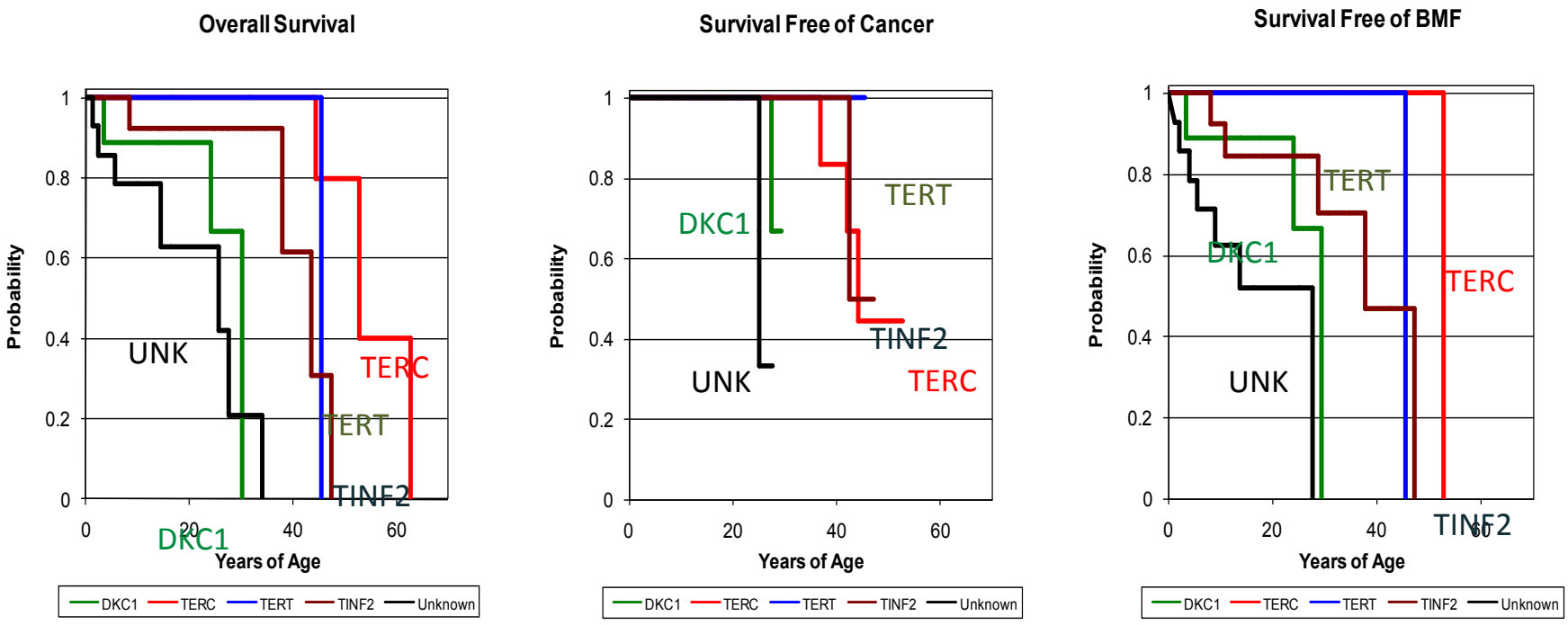
ST, solid tumors

AML, acute myeloid leukemia

Alter et al, Blood 2009; 113(26):6549-6557

Alter et al, Br J Hemat 2010; 150(2):179-88

Outcome by DC Genotype

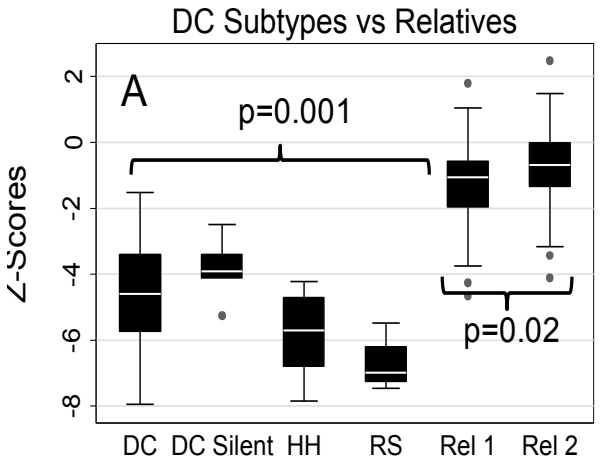


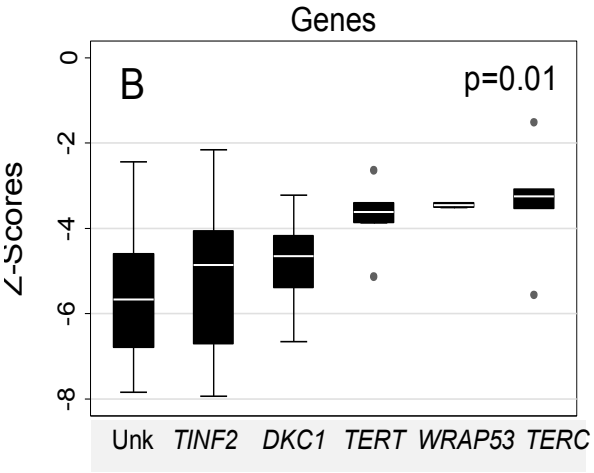
Unknown and *DKC1* are most severe, followed by *TERT* and *TINF2*; *TERC* is less severe; $p<0.05$. But, all have high probabilities.

Alter et al, Haematologica 2012;97(3):353-9

Phenotypes with Age-adjustment:
Telomere Length Z-Scores

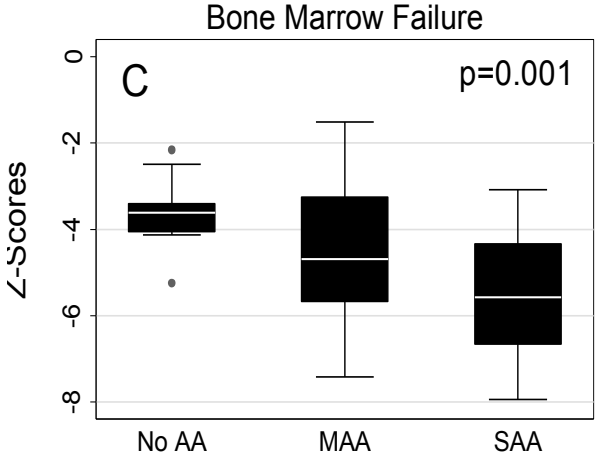
Low Z-score: severe DC

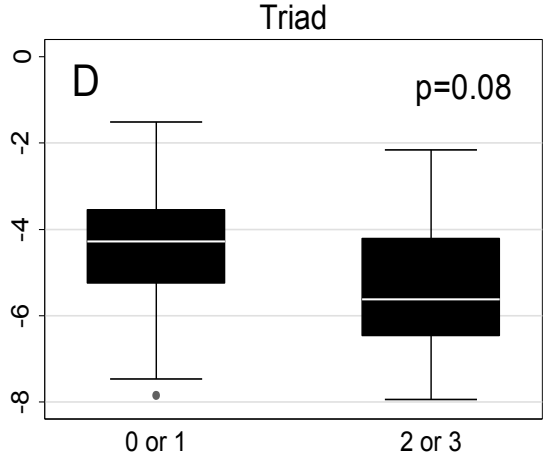




Low Z-score: unknown, *TINF2, DKC1*

Low Z-score: severe BMF





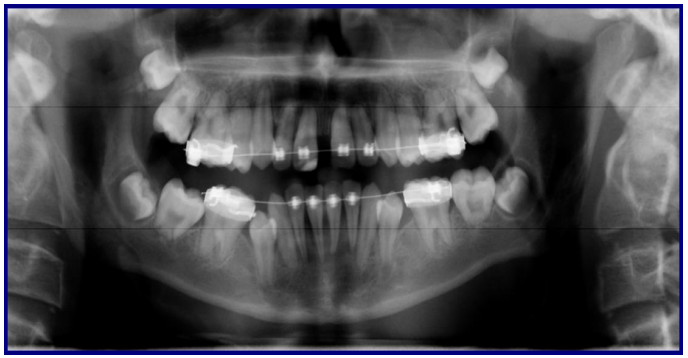
Low Z-score: triad features

Z-score: mean = 0 SD; 1st %ile = -2.33 SD

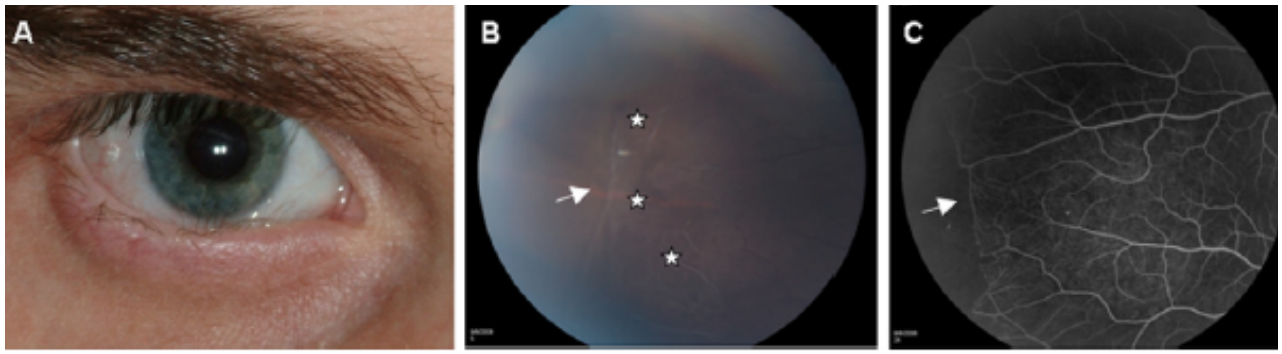
Alter et al, Haematologica 2012;97(3):353-9

Refining the Phenotype of DC

- **Neuropsychiatric Conditions**
  - Rackley et al, Psychosomatics 2012;53:230-5
- **Antibody response to HPV vaccine**
  - Alter et al, Vaccine 2014;32(10):1169-73
- **HPV in SCC**
  - Alter et al, Int J Cancer 2013;133(6):1513-5
- **Cytokine production**
  - Matsui et al, Br J Haematol 2013;163(1):81-92
- **Anti-Mullerian hormone levels**
  - Sklavos et al, JCEM 100(2):E197-203



Atkinson et al, Oral Dis 2008;14(5): 419-27



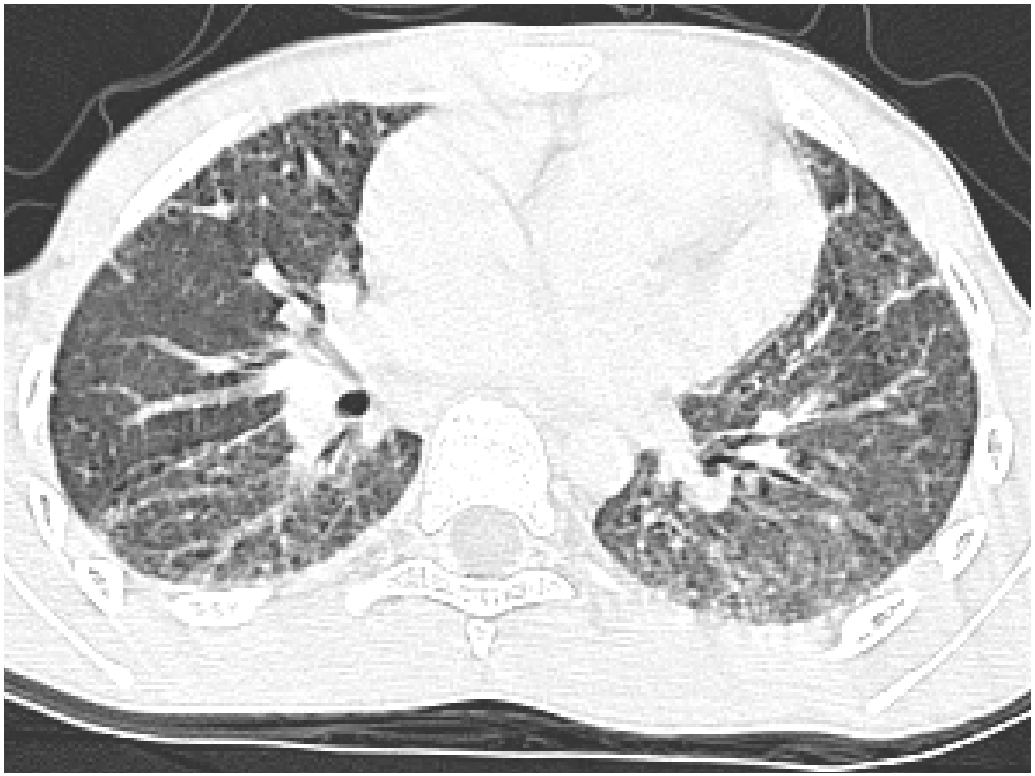
Tsilou et al, Ophthalmology. 2010;117(3):615-22

Pulmonary Complications in DC

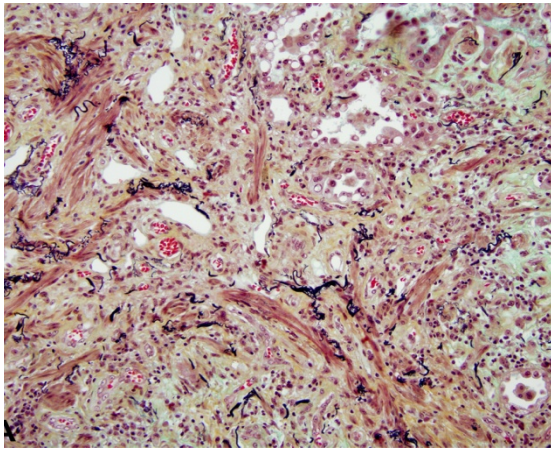
- 4 y.o. male with severe aplastic anemia
  - Matched-related HSCT
- At 6 y.o. DC triad noted
- Pulmonary symptoms at age 11
  - Pulmonary fibrosis diagnosed
- Germline mutation in *TINF2* identified at age 11
- Bilateral lung transplant at 13 y.o.

Giri et al, BMC Blood Disorders 2011;11:3

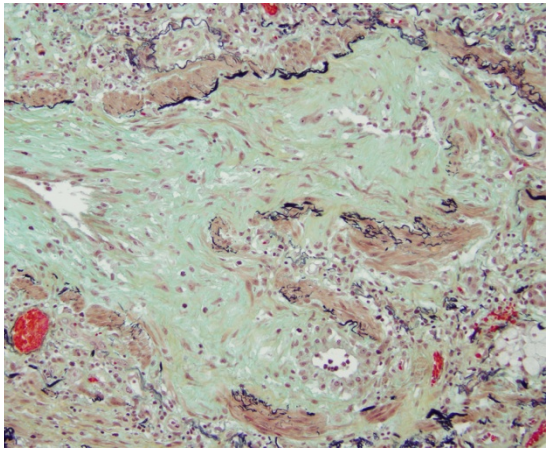
Pulmonary Complications in DC



Ground glass opacities & fibrosis

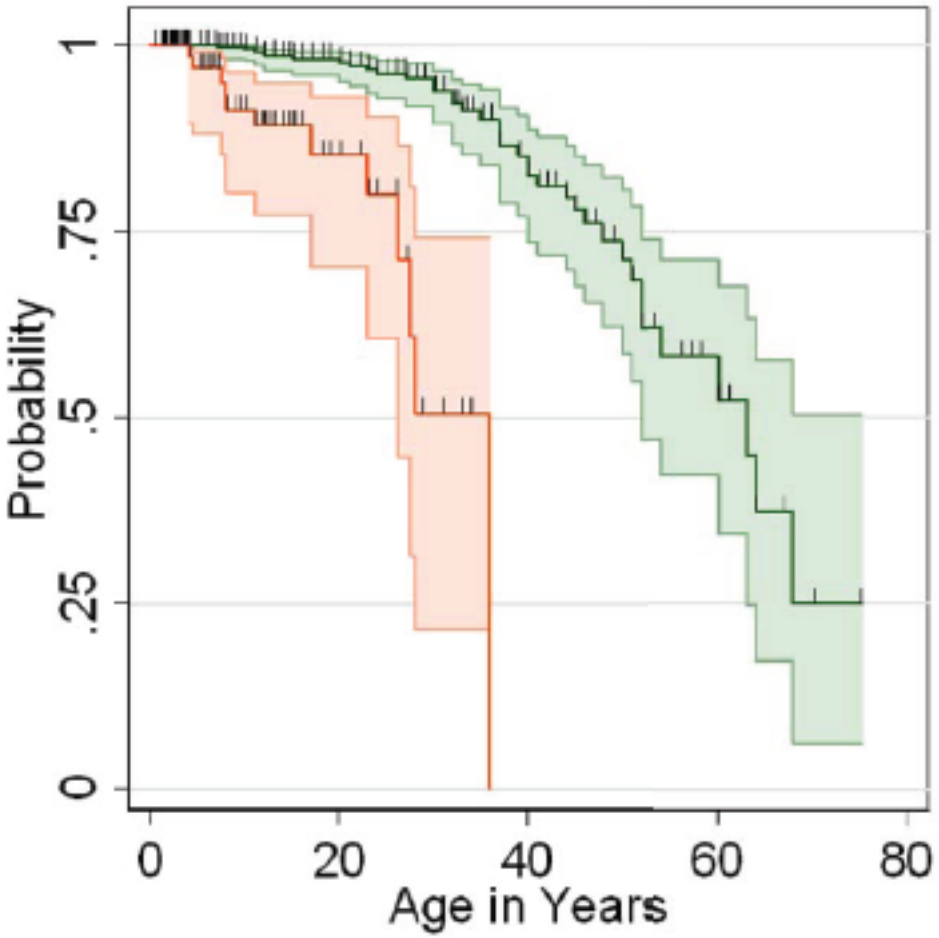


Diffuse fibrosis



Bronchiolitis obliterans

Pulmonary Complications in DC



Patients with DC who received a bone marrow transplant (red) had pulmonary symptoms younger than patients who did not have a HSCT (green).

Giri et al, BMC Blood Disorders 2011;11:3

Management of DC

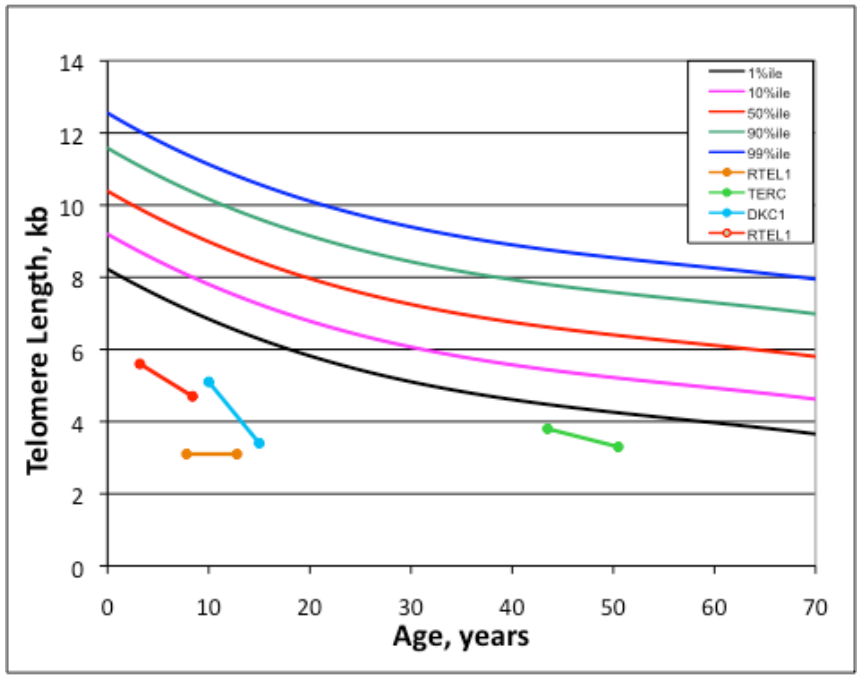
- Bone Marrow Failure
  - Supportive
    - Based of FA recommendations
    - Monitor CBCs, consider annual bone marrow
  - Androgens
    - Oxymethalone
    - Danazol

Androgens for Bone Marrow Failure in DC

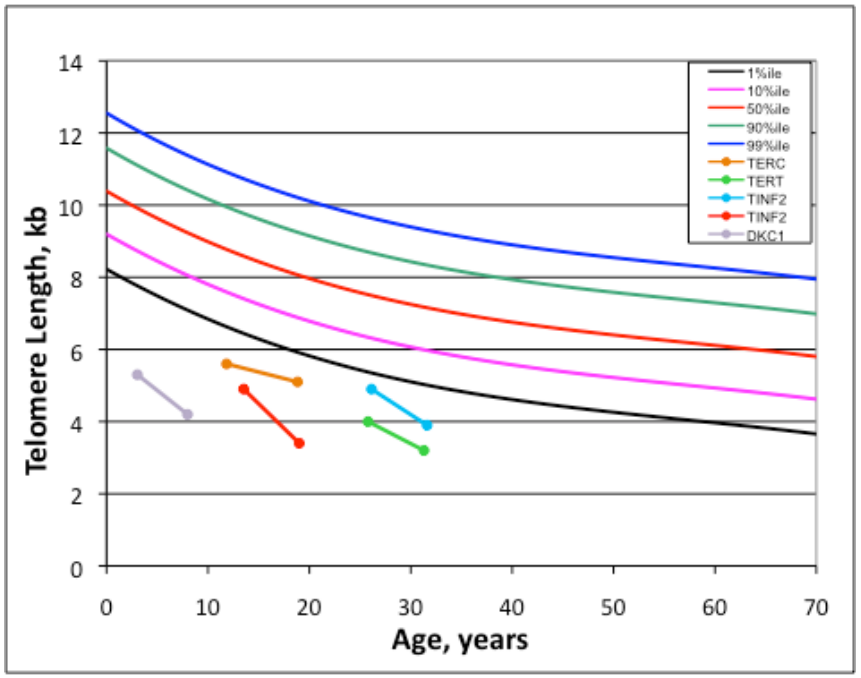
- 11 of 16 (69%) had a hematologic response
- No statistically significant differences in liver-related toxicities
- Two patients on androgens and simultaneous G-CSF developed splenic peliosis
- Significant lipid profile abnormalities on androgen therapy
  - Increased total cholesterol in 54%
  - All patients had extremely low HDL, and high LDL
  - 38% had high triglycerides after treatment

Khincha et al, Br J Haematol. 2014;165(3):349-57

Telomere length declined in both androgen treated and untreated patients



Treated



Not Treated

Khincha et al, Br J Haematol. 2014;165(3):349-57

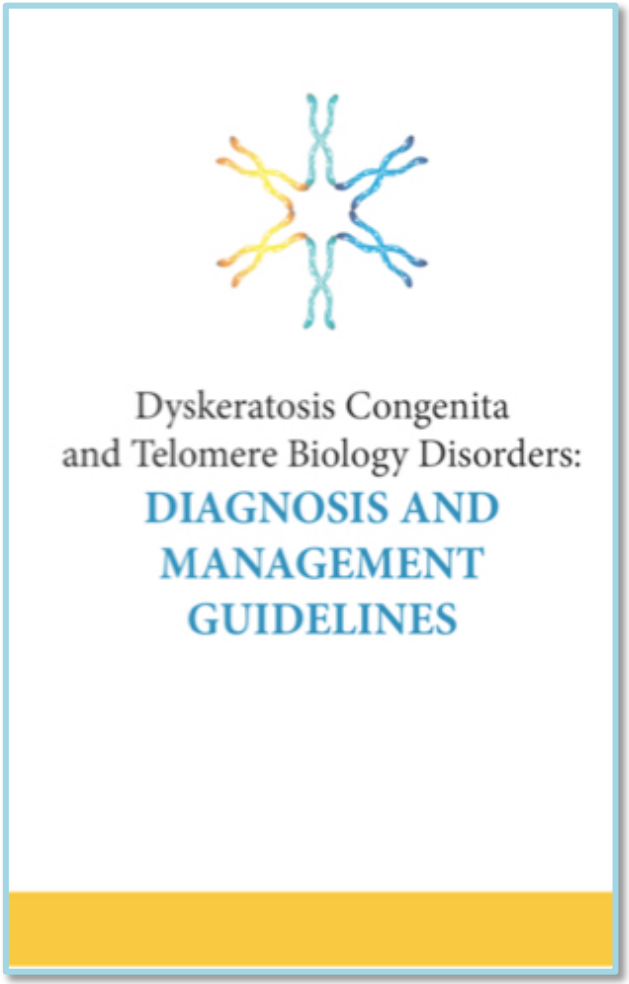
Management of DC

Dyskeratosis Congenita Outreach, Inc.

A Community for Telomere Biology Disorders

Our mission is to provide information and support services to families worldwide affected by Dyskeratosis Congenita and Telomere Biology Disorders, to encourage the medical community's research in finding causes and effective treatments, and to facilitate improved diagnosis by educating medical providers.

https://www.dcoutreach.org



Management of DC

- Bone Marrow Failure: HSCT
  - Telomere length testing of potential related donors
    - Silent carriers have been identified
    - Graft failure is reported
  - High rates of morbidity and mortality in the past
  - Modified, non-myeloablative protocols
    - Cyclophosphamide, Fludarabine, Alemtuzumab, 200cGY TBI, modified lung shielding
      - Dietz et al, Bone Marrow Transplant 2011; 46(1):98-104
    - Fludarabine and Alemtuzumab
      - S. Agarwal, unpublished data
    - Busulfan, Fludarabine, Cyclophosphamide
      - F. Boulad, unpublished data

Management of DC

- Cancer Risk: Screening and Early Detection
  - Annual ENT exam
  - Dental exam at least every 6 months
  - Based on Fanconi anemia guidelines
- Other
  - Pulmonary Function Tests
  - Bone density
  - Ophthalmology
  - Dermatology
  - Developmental delay

Acknowledgements

Patients & Families

NCI's IBMFS study team

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Shahinaz Gadalla MD, PhD
Bari Ballew, PhD
Payal Khincha, MD

NCI Cancer Genomics Research Laboratory & Exome Sequencing Group

Telomere Length Flow-FISH

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G. Baerlocher, University Bern, Switzerland

Outcomes after HSCT

J. Tolar, University of Minnesota
National Marrow Donor Program
CIBMTR

Functional Studies

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T. de Lange: Rockefeller University
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P. Lansdorp: University of British Columbia
J.K. Nadakumar: University of Michigan
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S. Smith: New York University
J. Wong: University of British Columbia

Epidemiology

I. De Vivo: Harvard
R. Hayes: NYU
M. Alavanja: NCI
L. Hou: Northwestern
A. Aviv: UMDNJ

And many more…