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Understanding Telomere Biology Through Studies of Dyskeratosis Congenita

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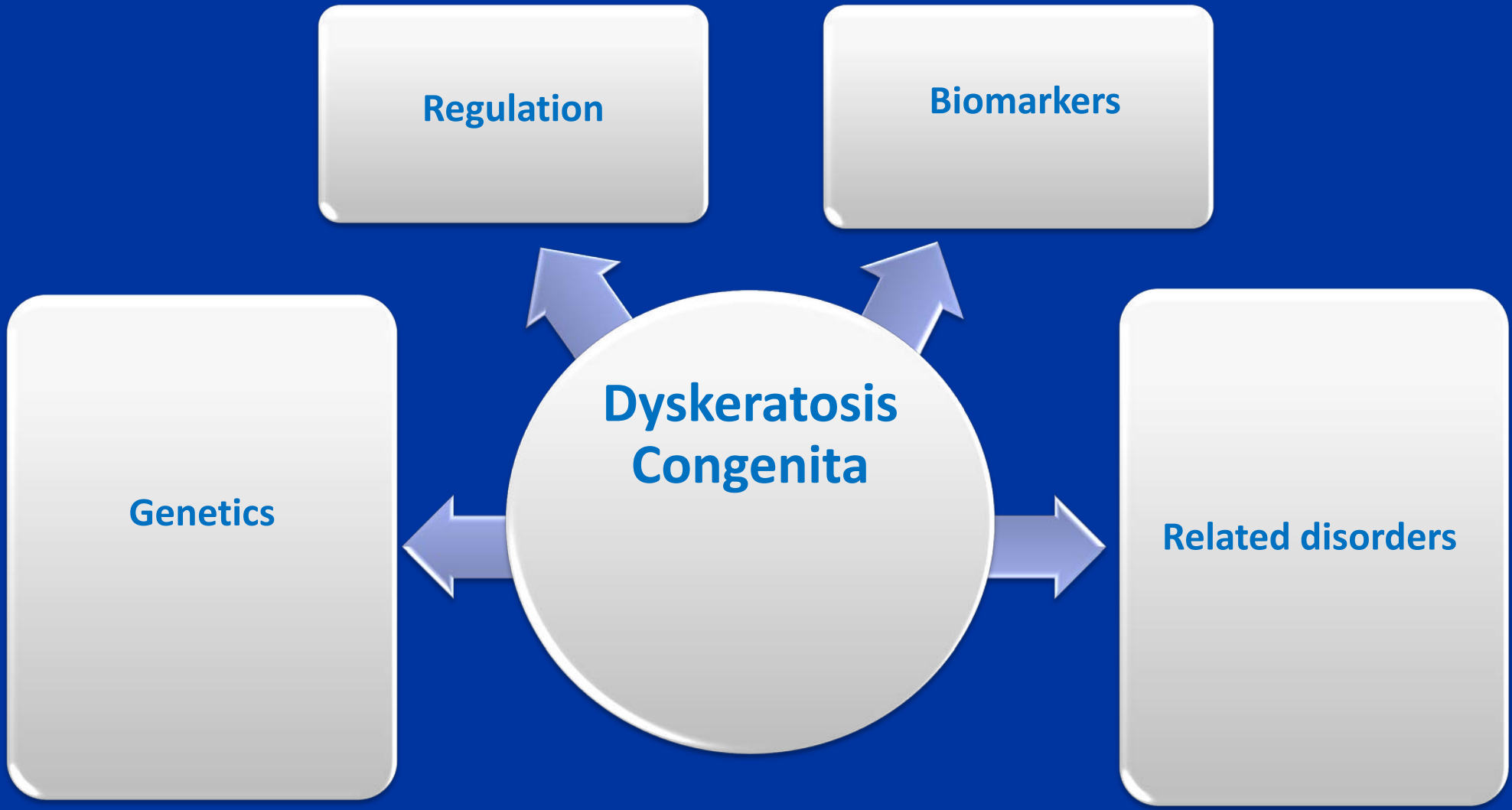
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

Dyskeratosis congenita:

a highly informative model of telomere biology and cancer predisposition



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

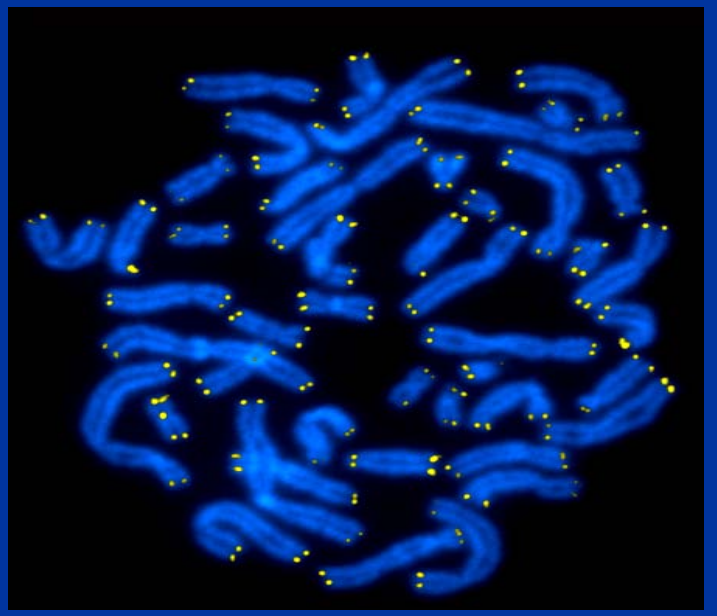


Photo: Dr. Peter Lansdorp

Evolutionary Conservation of Telomere Biology Genes

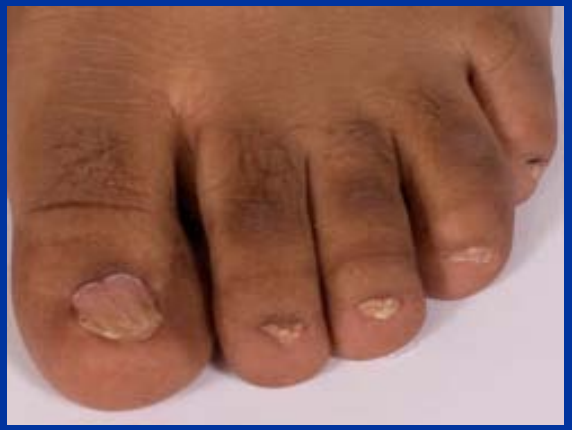
- *Hypothesis:* Genetic variation in telomere biology genes is limited because of their critical roles in genomic stability

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

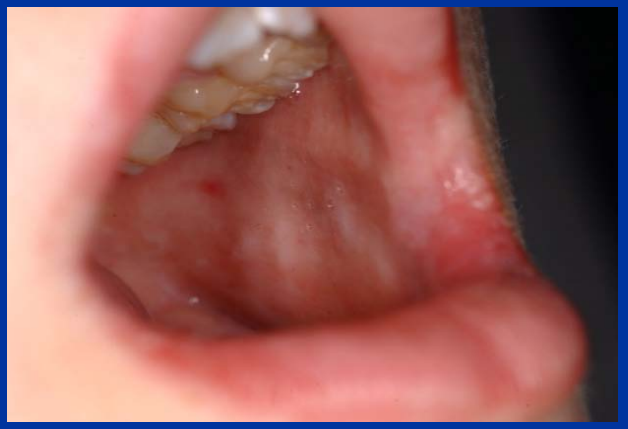
Savage SA et al, Human Mutation 2005;26(4):343-350

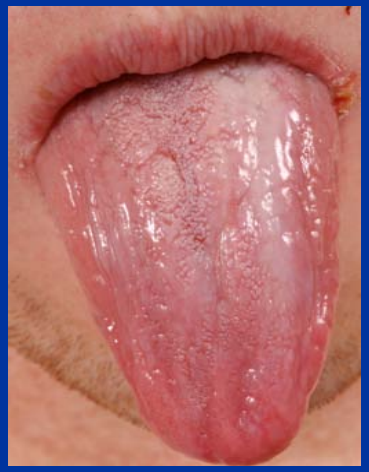
Dyskeratosis Congenita:
a disorder of telomere biology

Nail Dystrophy

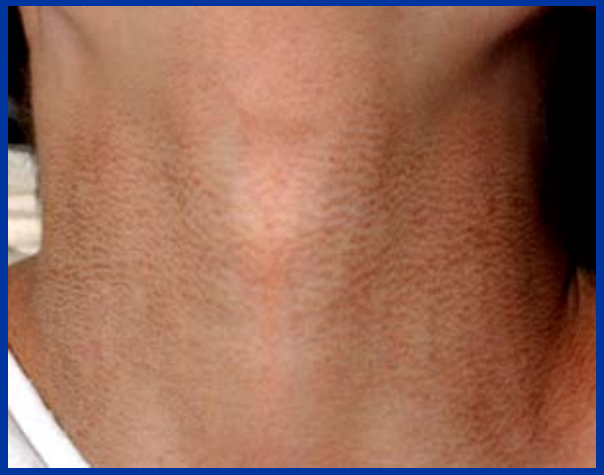


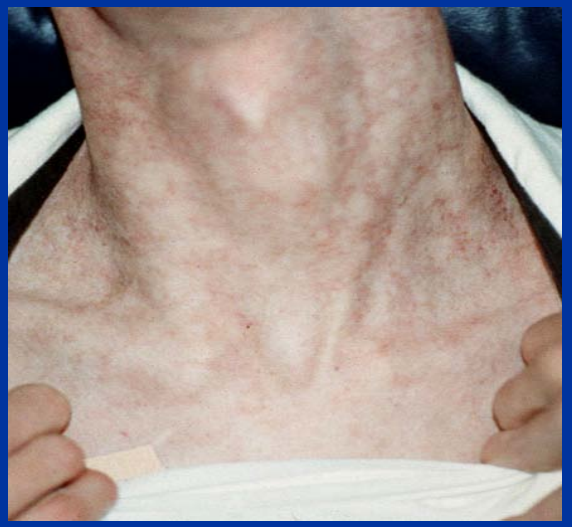
Oral Leukoplakia





Skin Pigmentation





In addition to the triad…

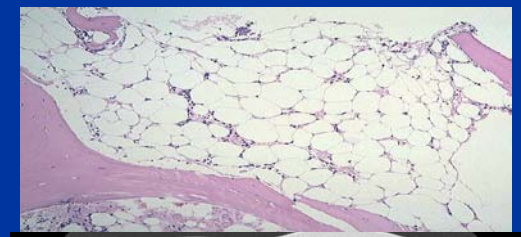
Bone Marrow Failure

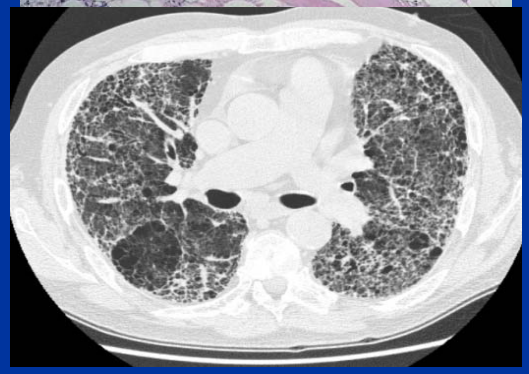
Pulmonary Fibrosis

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 <u>short</u> telomeres are the unifying feature

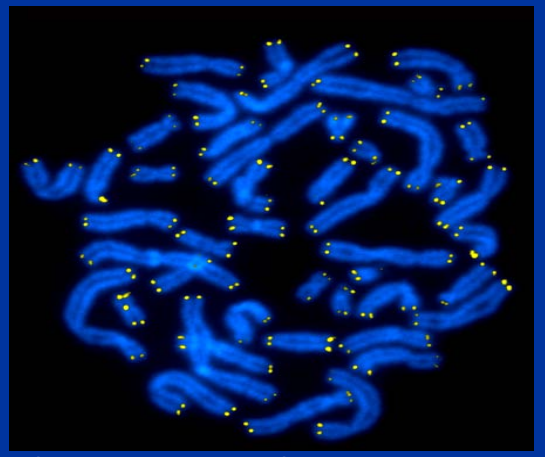
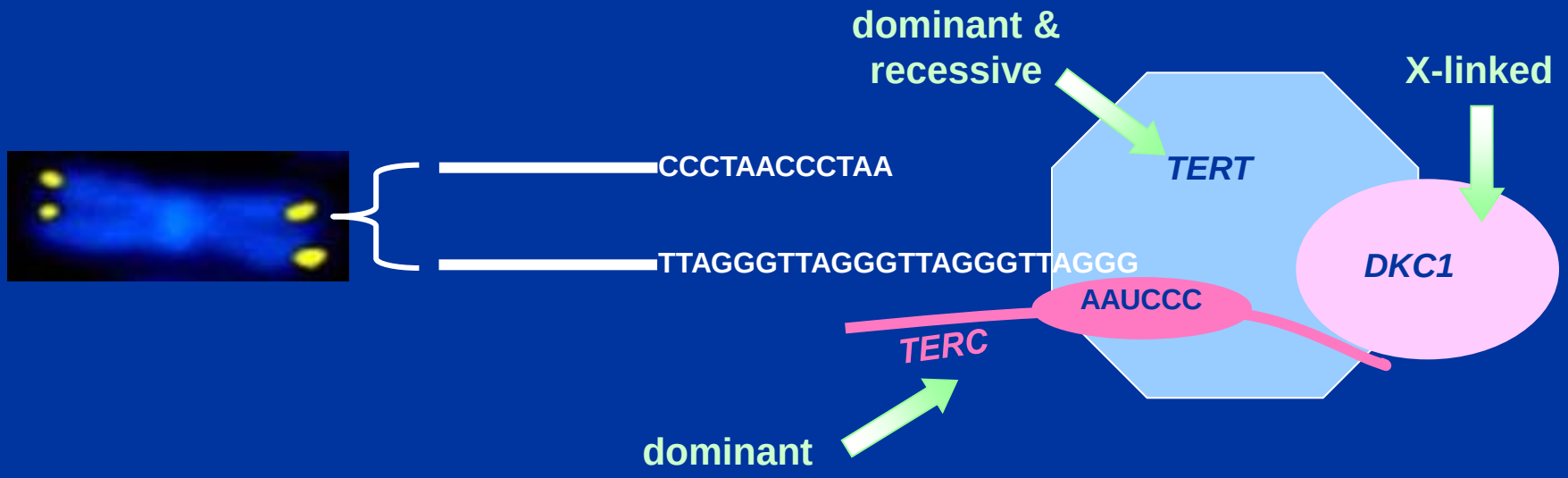


Photo: Dr. Peter Lansdorp

Traditional diagnosis: Diagnostic Triad <u>or</u> 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.

Etiologic Investigation of Cancer Susceptibility in Inherited Bone Marrow Failure Syndromes (IBMFS)

- Systematic evaluation of affected individuals and their families in North America
  - Fanconi Anemia
  - **Dyskeratosis Congenita (DC)**
  - Diamond-Blackfan Anemia
  - Shwachman-Diamond Syndrome
  - And others

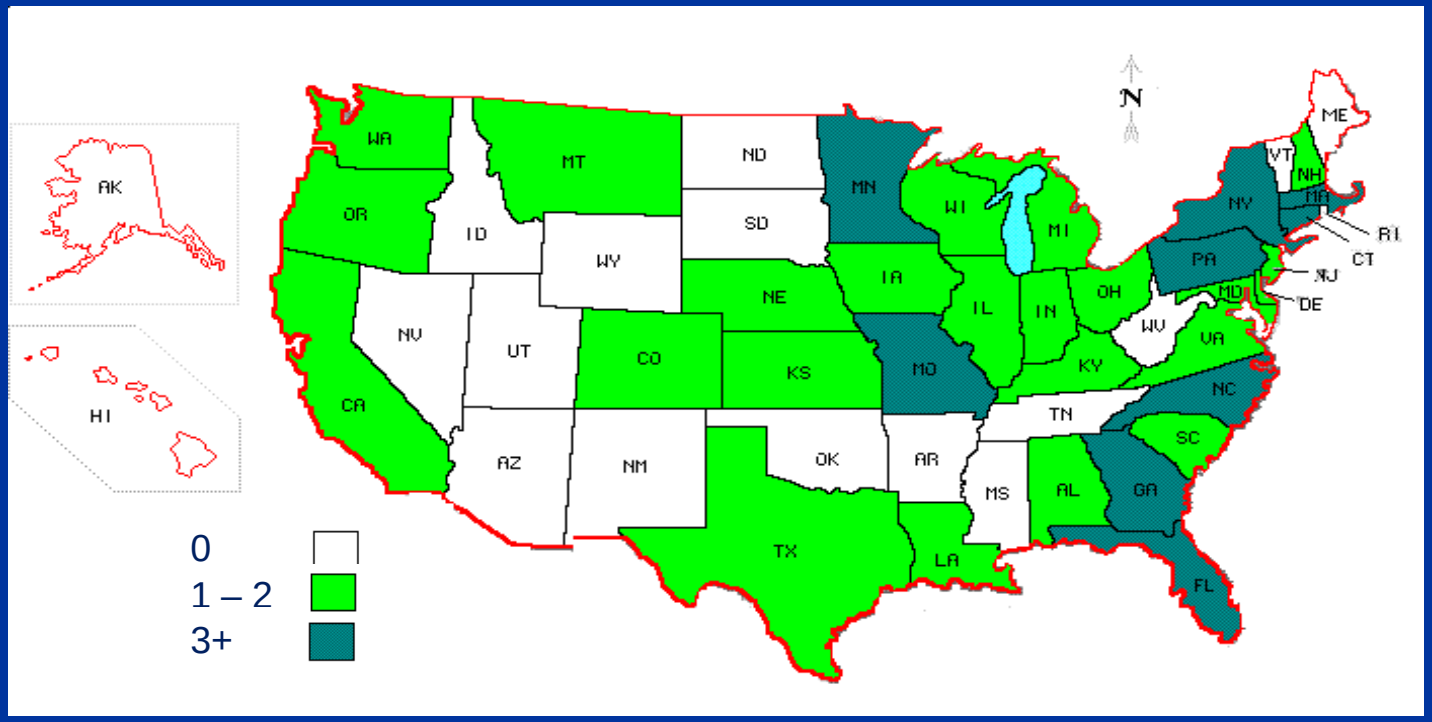
- Opened in January 2002
- http://marrowfailure.cancer.gov
- Principal Investigator: Blanche P. Alter, MD, MPH
- Staff Clinician: Neelam Giri, MD
- DC: Sharon Savage, MD

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

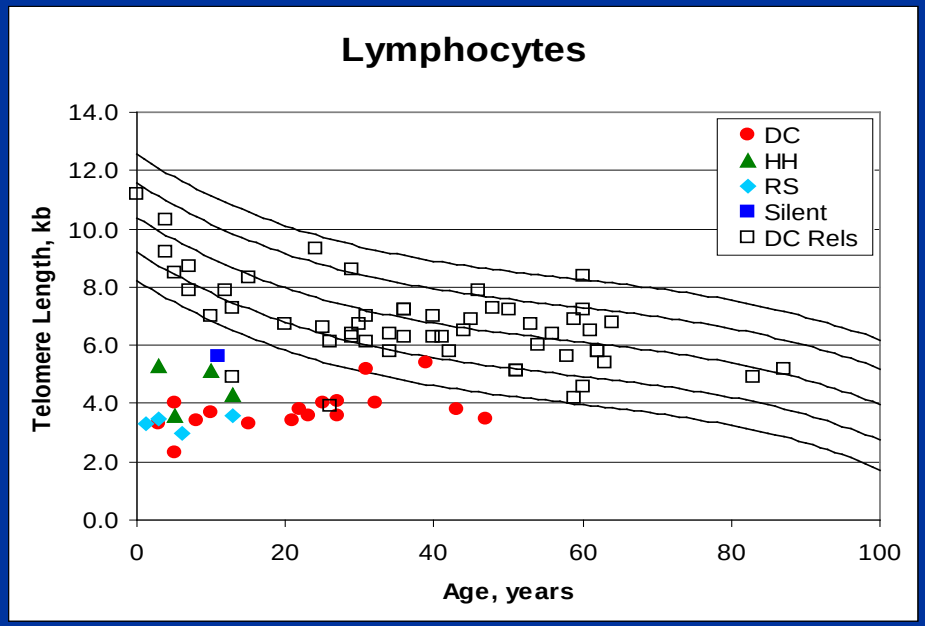
NCI Cohort	# Families	# Affected Individuals	# Healthy Relatives
Clinic	35	46	86
Field	11	24	101



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

DC vs. healthy relatives

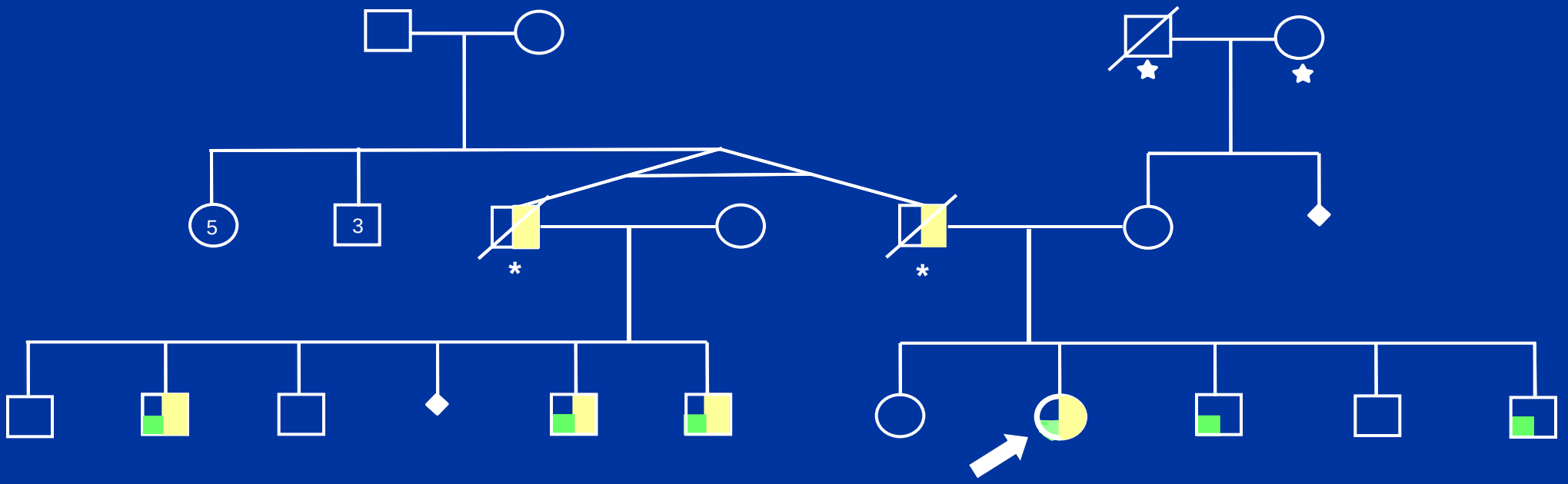


DC vs. other IBMFS	Sensitivity	Specificity
If 4/6 leukocyte subsets <1st percentile	92%	98%

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

Genome-Wide Linkage Screen in DC

Heterogeneous, autosomal dominant, *DKC1, TERC,* and *TERT* normal



Clinical DC

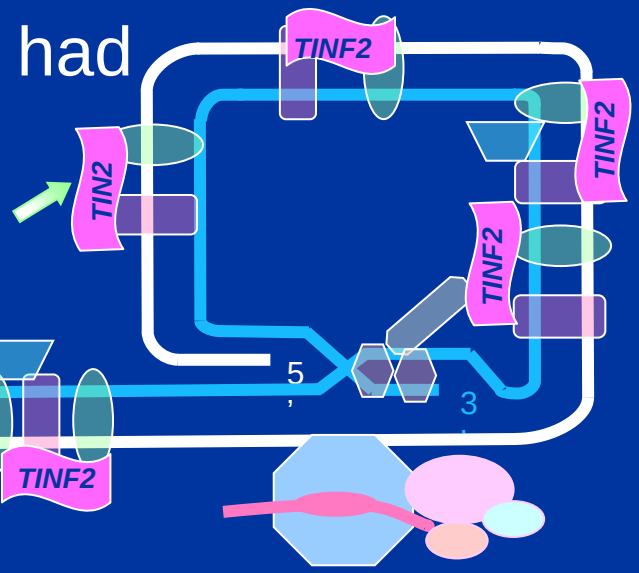
Telomere length in WBC subsets <1st %ile

◆ 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



Telomerase complex

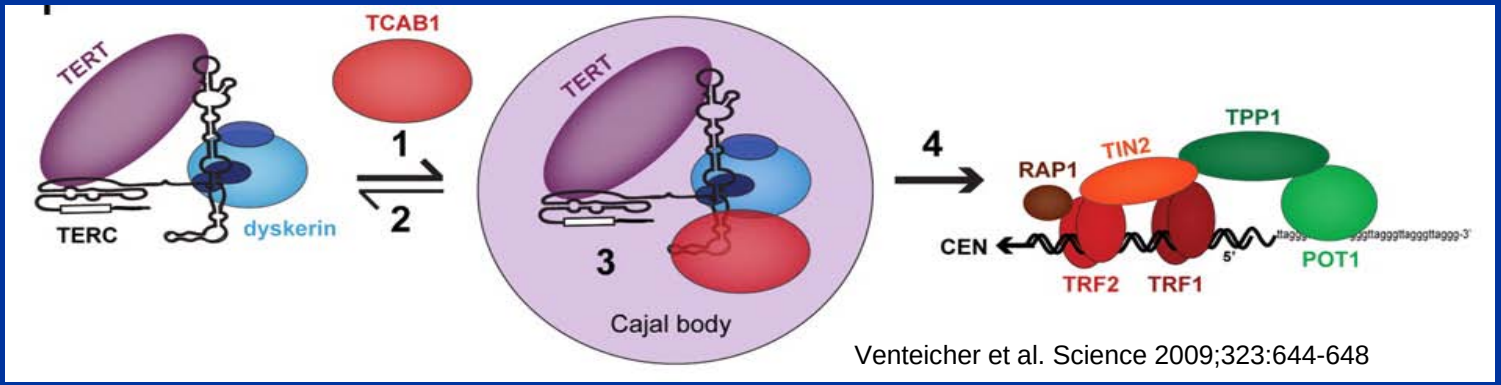
Savage et al., Am J Hum Genet. 2008;82(2):501-9

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.

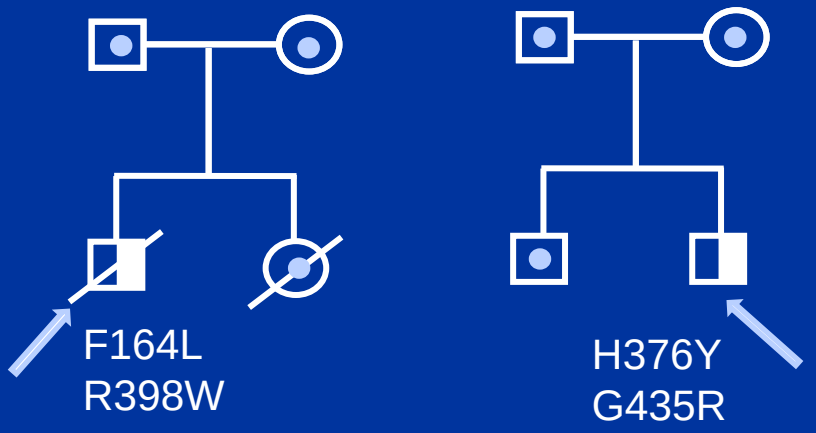
Savage et al, J Med Genet 2010: in press

TCAB1 in Dyskeratosis Congenita



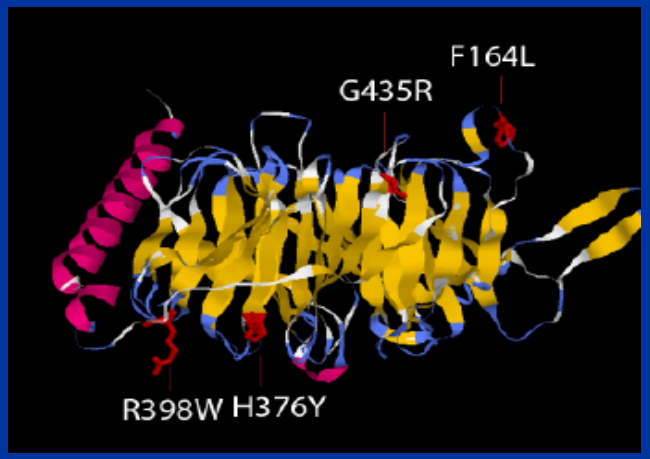
Venteicher et al. Science 2009;323:644-648

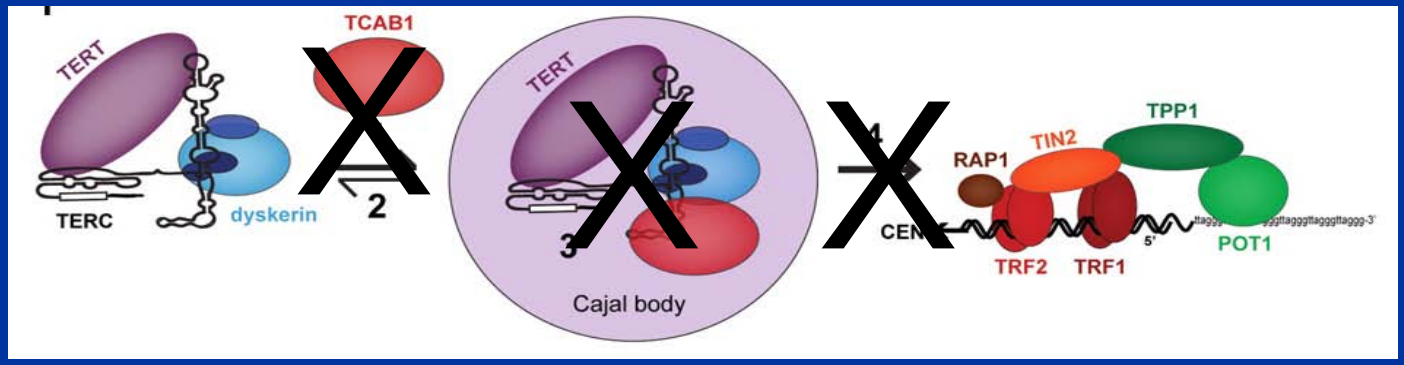
- Cajal body: sites of mRNA & rRNA processing in the nucleus
- *TCAB1* is located in Cajal bodies and is required for telomerase trafficking
- Mutations in *TCAB1* cause DC
  - 2 of 9 families studied have autosomal recessive mutations



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

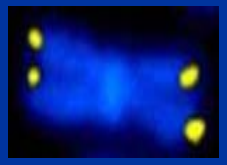


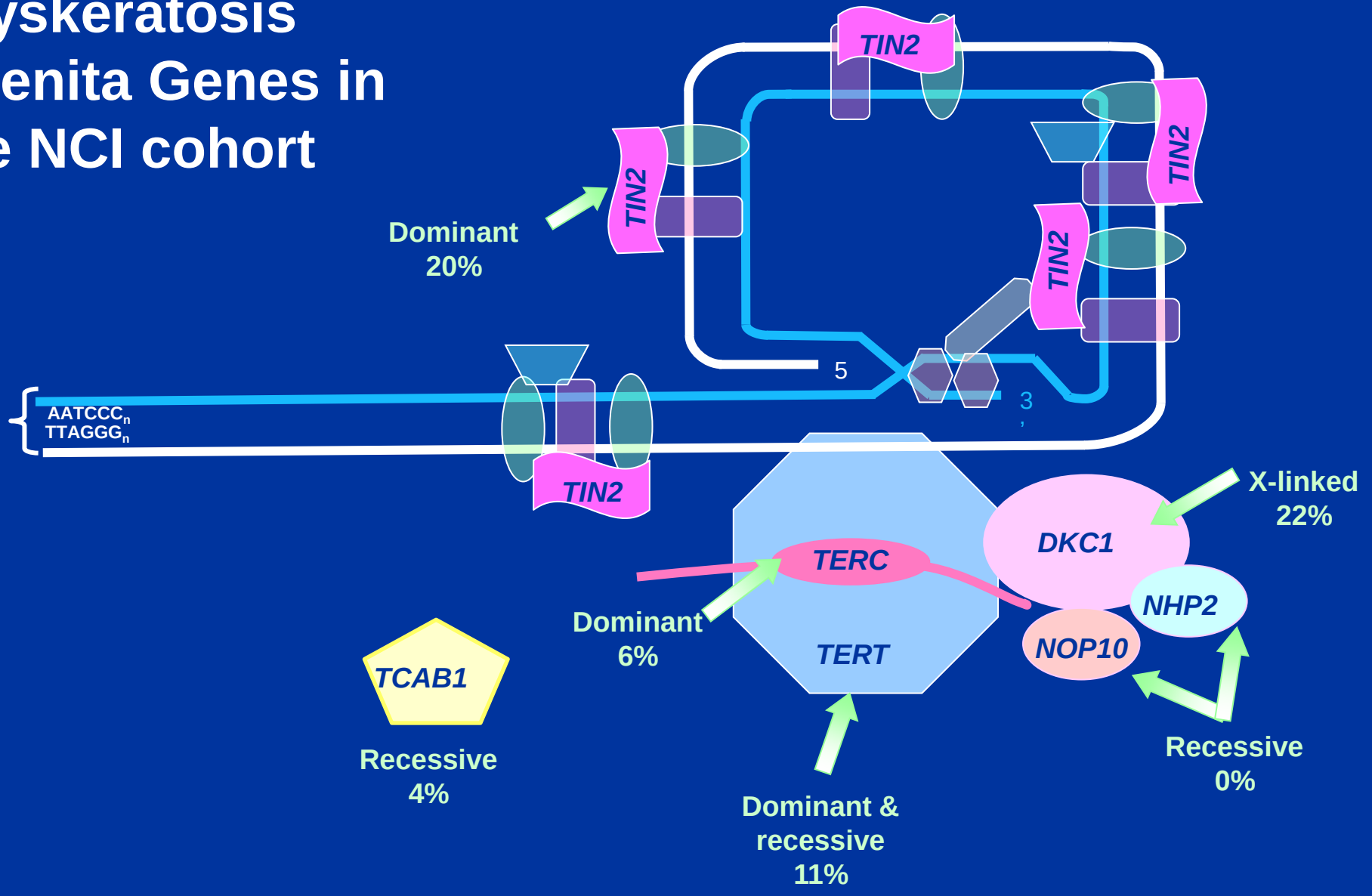


Zhong F, Savage SA, et al, Genes Devel 2010; in press

Study highlighted in the “Best of” session at the American Society of Hematology annual meeting

Dyskeratosis Congenita Genes in the NCI cohort

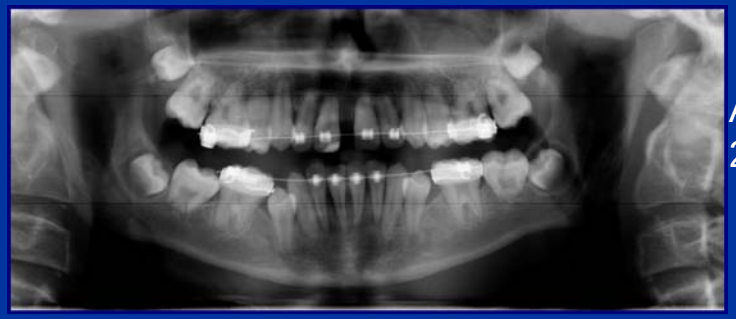




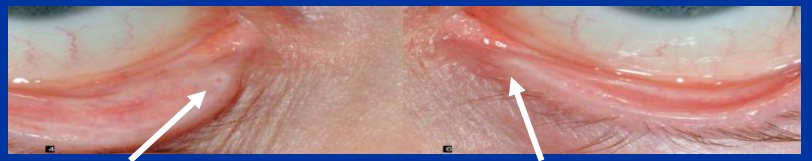
~ 1/3 of our families do not have a mutation in one of these genes

Refining the Phenotype of DC

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



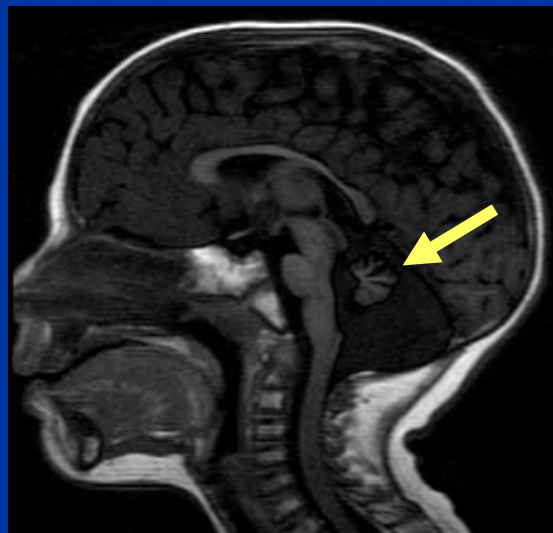
Atkinson et al, Oral Dis 2008;14(5):419-27.



Tear duct present Tear duct absent

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

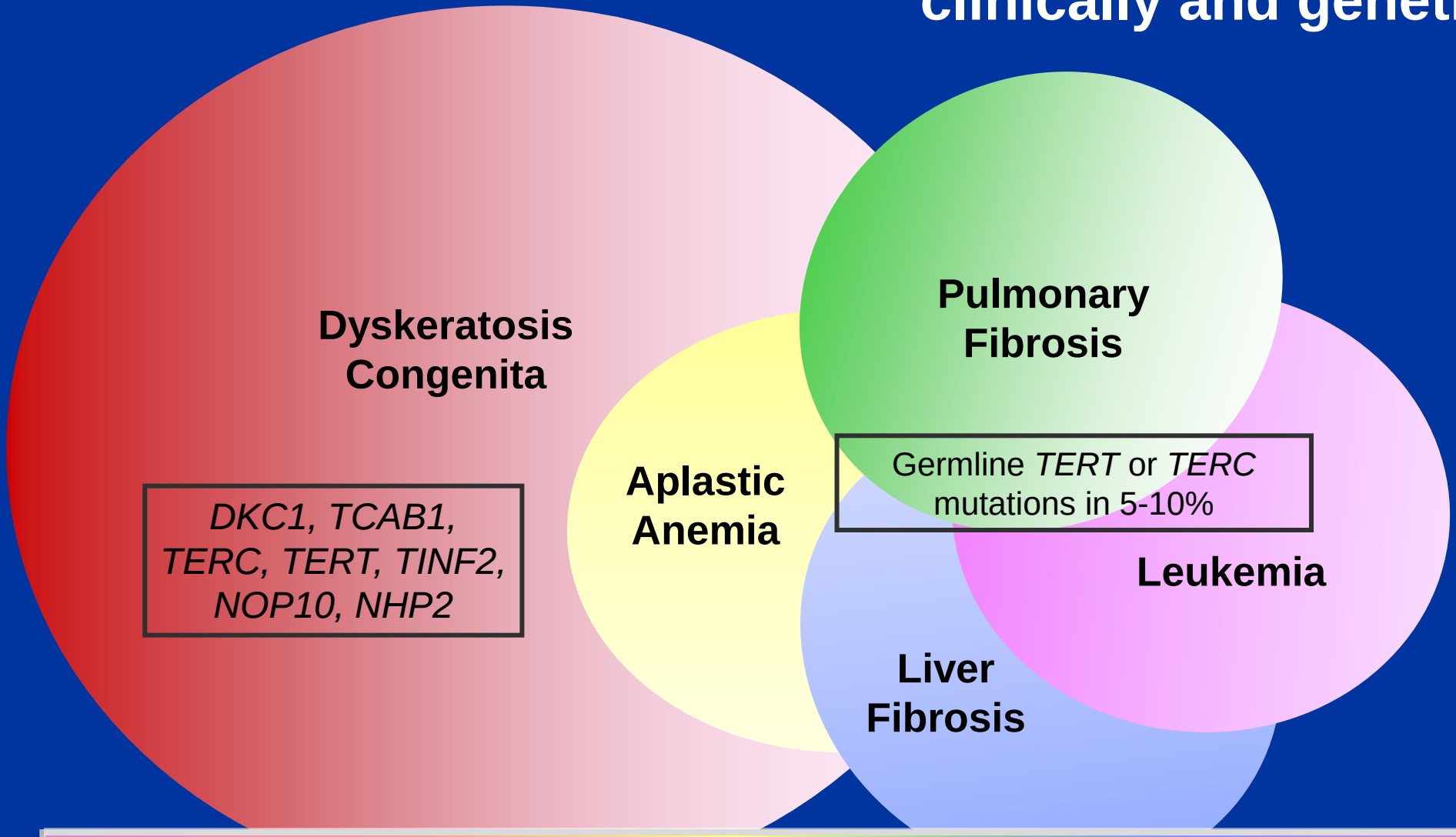
Unpublished data on MRI findings in the brain: John Butman, MD, Clinical Center, NIH, Neelam Giri, MD, Clinical Genetics Branch, DCEG, NCI



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



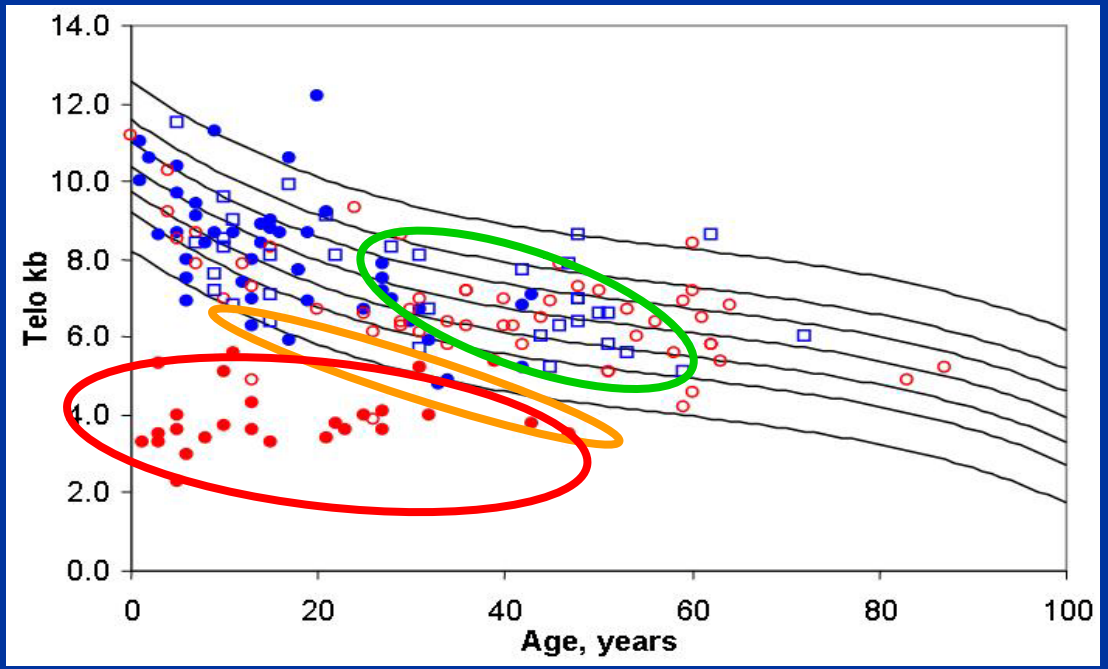
The clinical spectrum is growing.
Signs & symptoms of DC develop at variable rates.

Savage and Bertuch, Genetics in Medicine 2010: in press

The Telomere Length Continuum

Normal range

- Marker of common disease risk
- Statistically significant differences



***VERY* short, <1st%**

Dyskeratosis Congenita

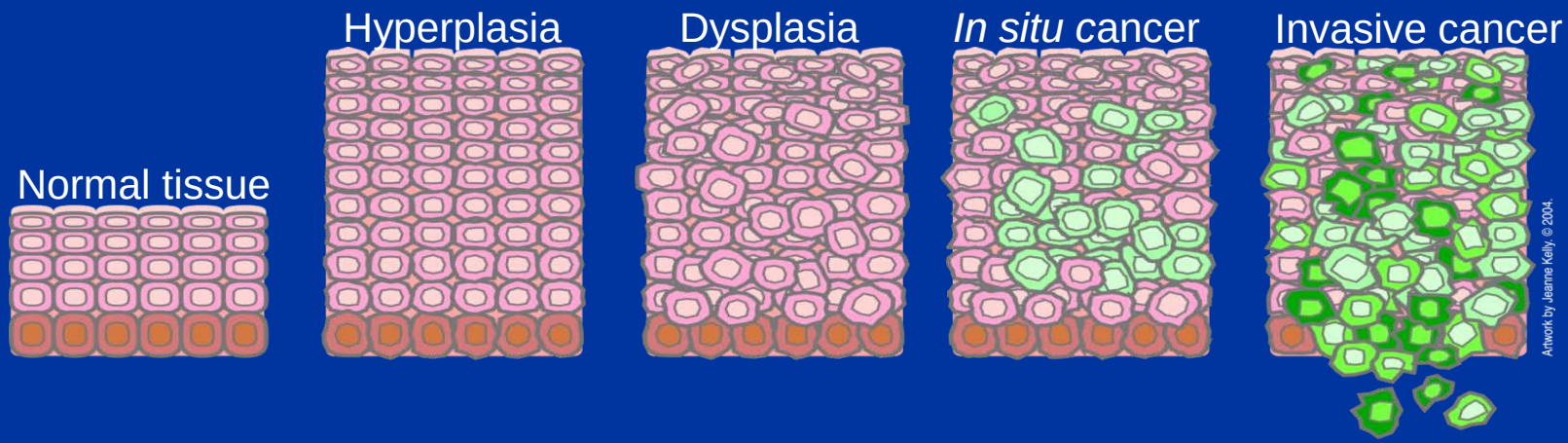
- Rare, highly penetrant disease causing mutations

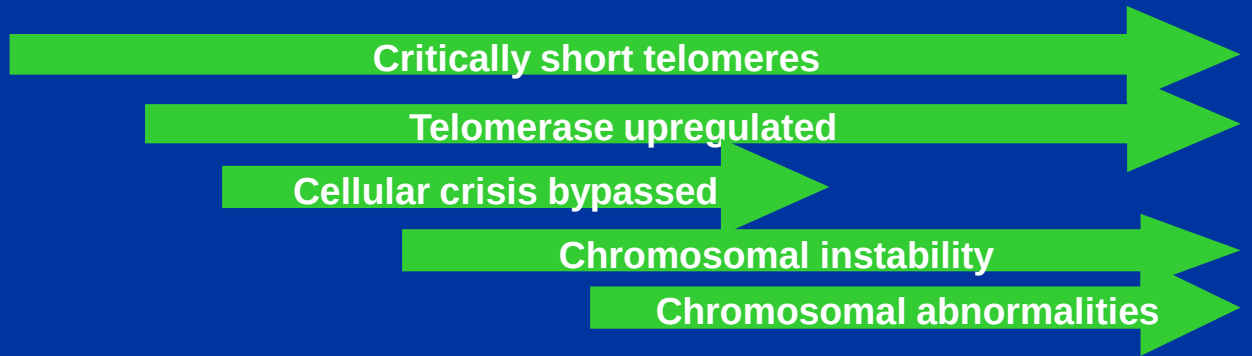
Short, <10th%

- Aplastic anemia, leukemia, pulmonary fibrosis
- Mutations in *TERT, TERC*

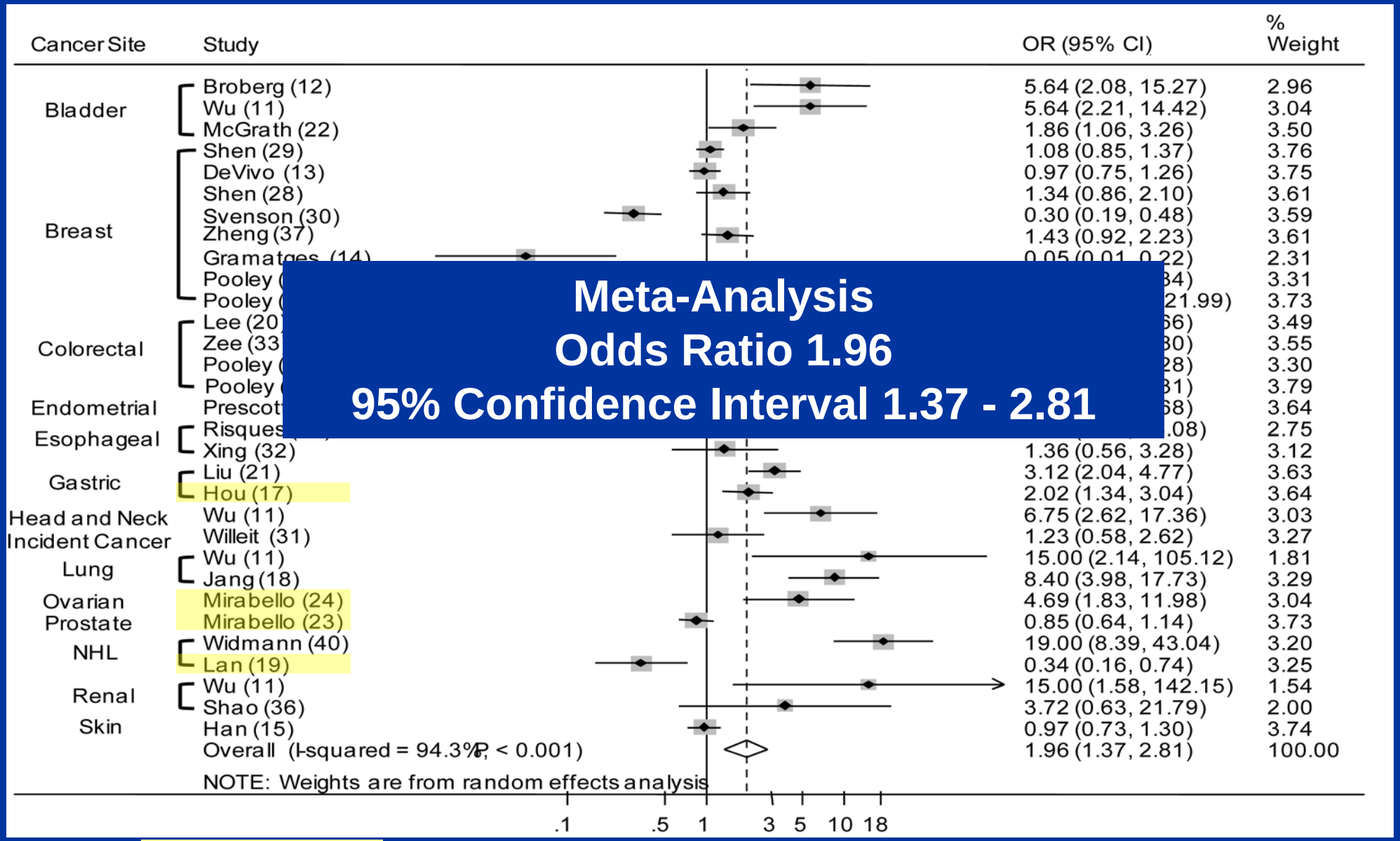
Telomere Length and Cancer Risk in the General Population

- 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



DCEG, NCI studies

Wentzensen I, Mirabello L, Pfeiffer R, Savage SA: unpublished data

Dyskeratosis congenita:

a highly informative model of telomere biology and cancer predisposition

Regulation

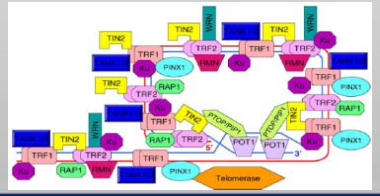
- Chromosomal stability
- Epigenetics

Biomarkers

- Short telomeres and increased cancer risk in populations

Genetics

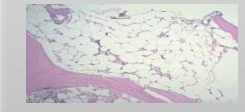
- Rare mutations cause DC
  - *DKC1, TERC, TERT, NHP2, NOP10, TINF2, TCAB1*
- Common variants contribute to cancer in the general population (GWAS)

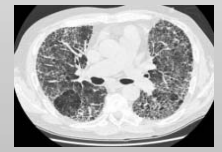


Dyskeratosis Congenita

Related disorders

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





Photos with permission

Acknowledgements & Collaborators

- **Patients & Families**
  - www.dcoutreach.com

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  - G. Daley: Harvard Medical School
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- **function**
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- **function**
  - S. Artandi: Stanford University