Rothmund-Thomson Syndrome Type 1 vs. Type 2

Webinar Series, #3

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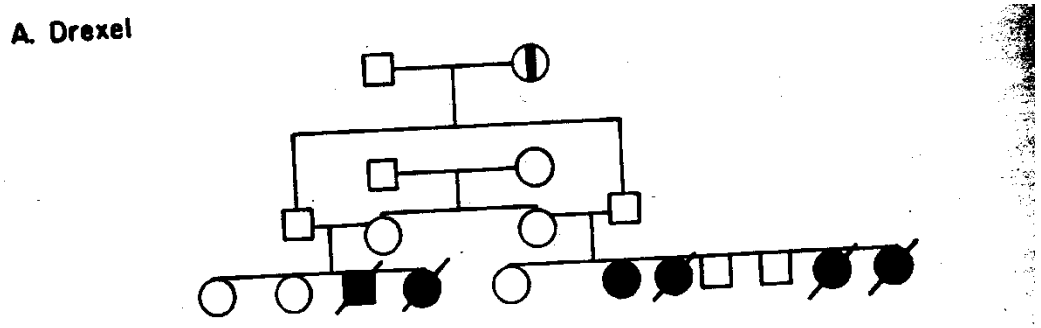
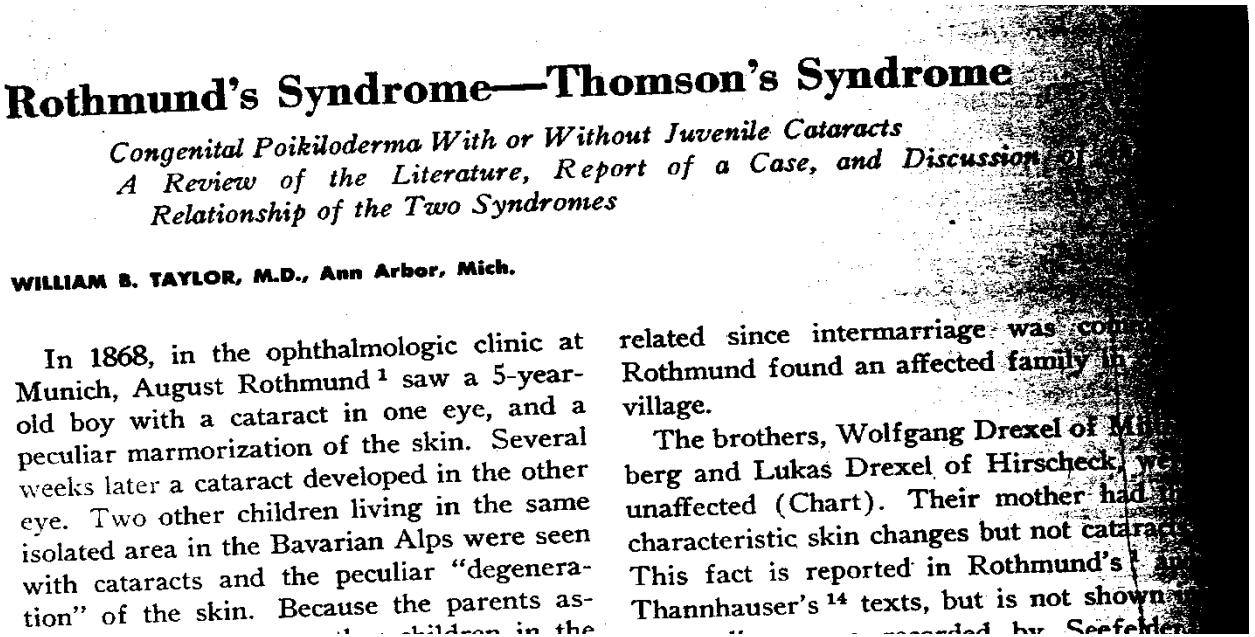
History of RTS

- 1868: Auguste Rothmund first described the characteristic rash and *cataracts* in brothers from an inbred Austrian families



- 1923: M. Sydney Thomson described the rash and *skeletal anomalies* in sisters in England; coined term “poikiloderma congenitale”

- 1957: William Taylor united the two disorders, proposed eponym "Rothmund-Thomson Syndrome"



Rothmund's Syndrome—Thomson's Syndrome

Congenital Poikiloderma With or Without Juvenile Cataracts
A Review of the Literature, Report of a Case, and Discussion of the
Relationship of the Two Syndromes

WILLIAM B. TAYLOR, M.D., Ann Arbor, Mich.

In 1868, in the ophthalmologic clinic at Munich, August Rothmund [1] saw a 5-year-old boy with a cataract in one eye, and a peculiar marmorization of the skin. Several weeks later a cataract developed in the other eye. Two other children living in the same isolated area in the Bavarian Alps were seen with cataracts and the peculiar "degeneration" of the skin. Because the parents as-

related since intermarriage was co
Rothmund found an affected family in
village.

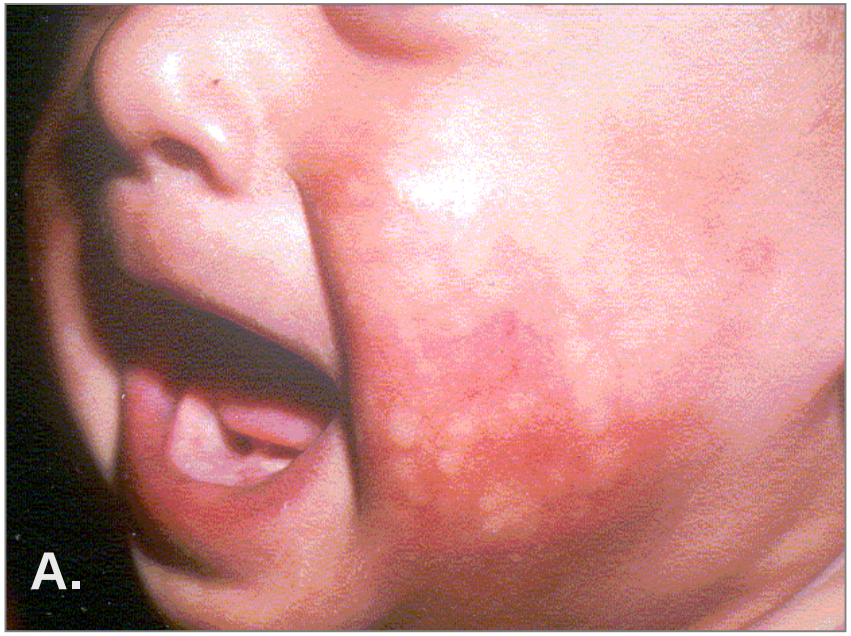
The brothers, Wolfgang Drexel of M
berg and Lukas Drexel of Hirscheck, we
unaffected (Chart). Their mother had
characteristic skin changes but not cataract
This fact is reported in Rothmund's
Thannhauser's [14] texts, but is not shown

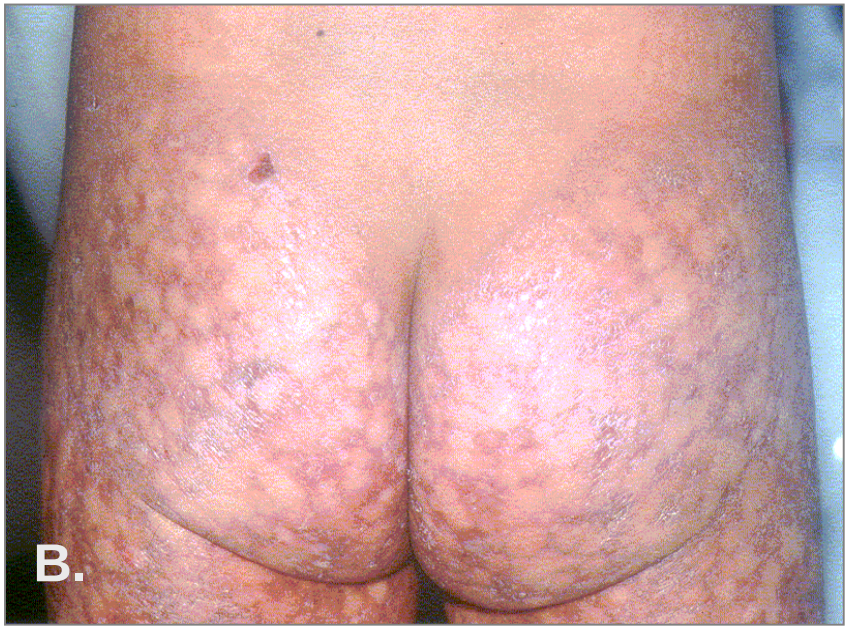
Taylor WB, AMA Arch Dermatol;75:236-244 (1957)

Summary of Clinical Findings in 41 RTS Subjects Baylor College of Medicine Study

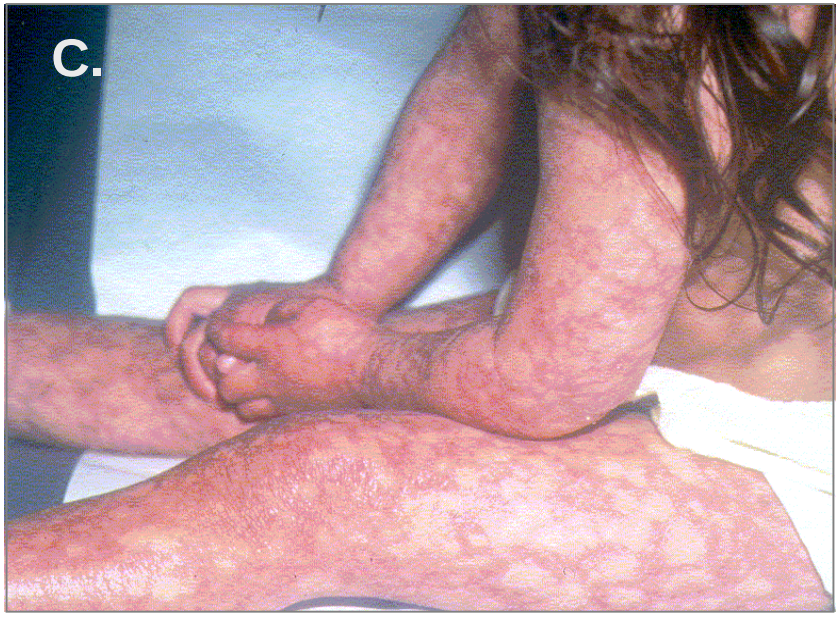
Rash	**41/41**	**100%**
Small stature	**25/38**	**66%**
Skeletal dysplasia	**15/20**	**75%**
Radial ray defect	**8/40**	**20%**
Sparse scalp hair	**15/30**	**50%**
Sparse brows/lashes	**19/26**	**73%**
GI disturbance	**7/41**	**17%**
Cataracts	**2/32**	**6%**
Skin cancer	**1/41**	**2%**
Osteosarcoma	**13/41**	**32%**

Wang *et al* (2001) Am J Med Genet; 102:11-17

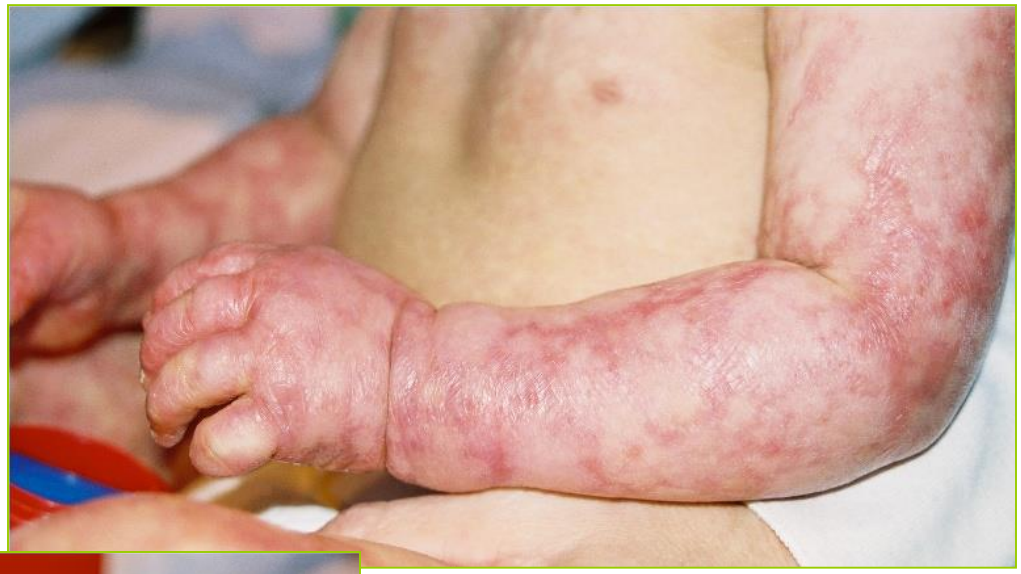


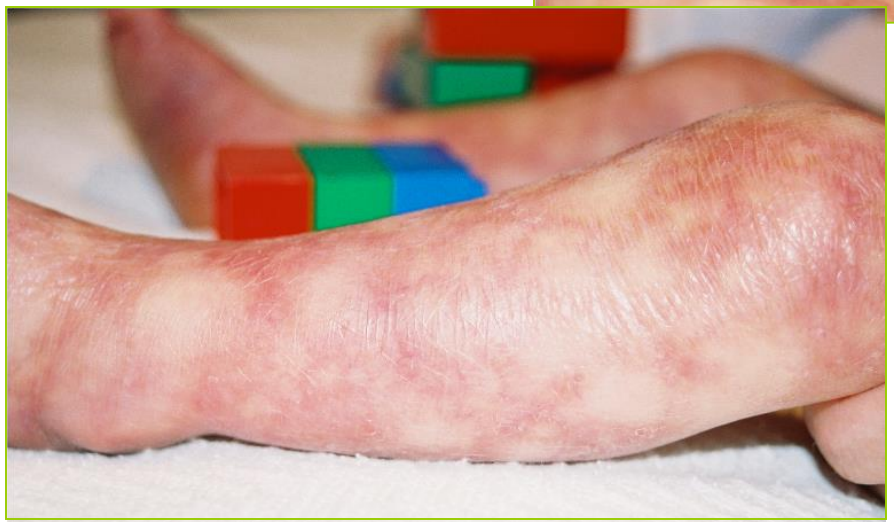


Characteristic rash of RTS. The *acute phase* of the rash starts on the cheeks during infancy (A) and spreads to buttocks (B) and extremities (C). The *chronic phase* persists as *poikiloderma*.

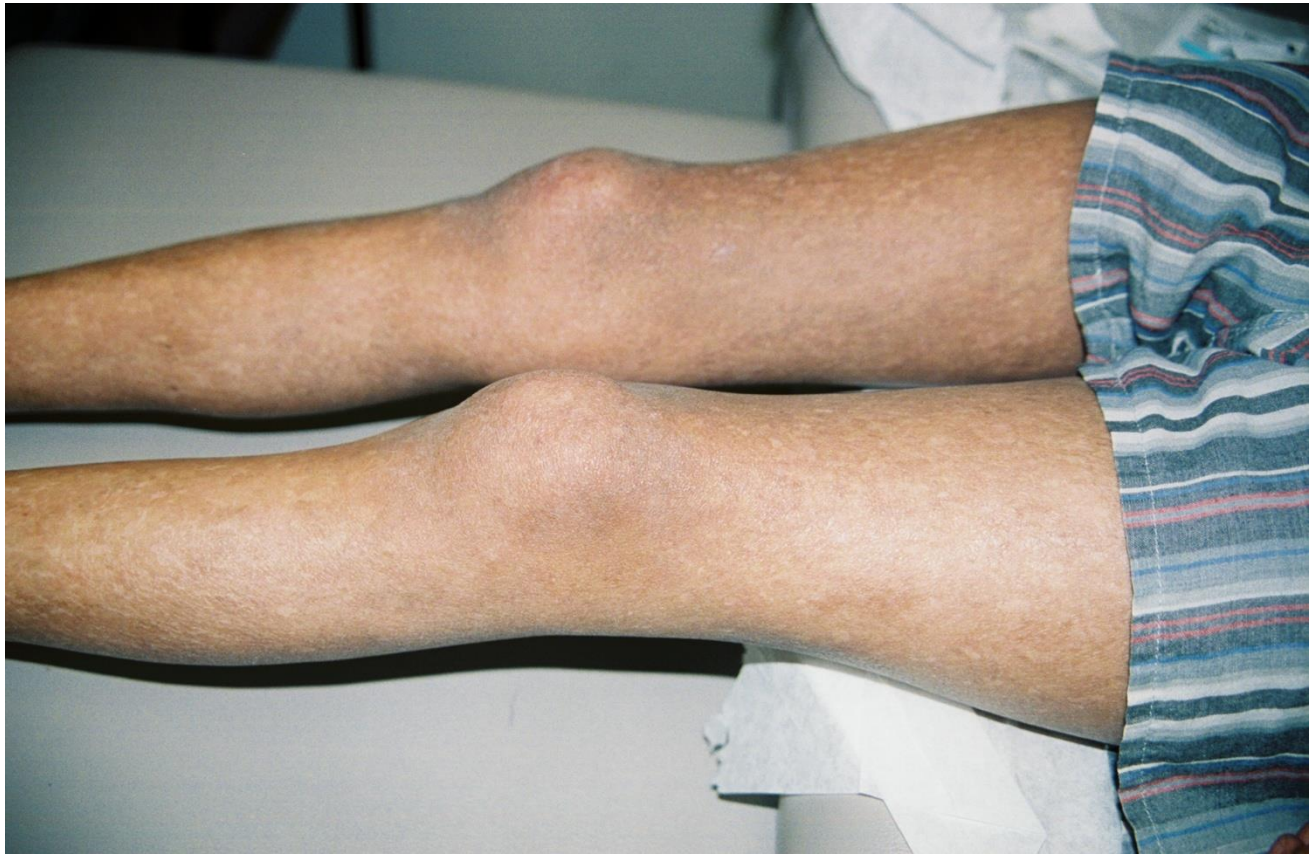


RTS rash-extremities

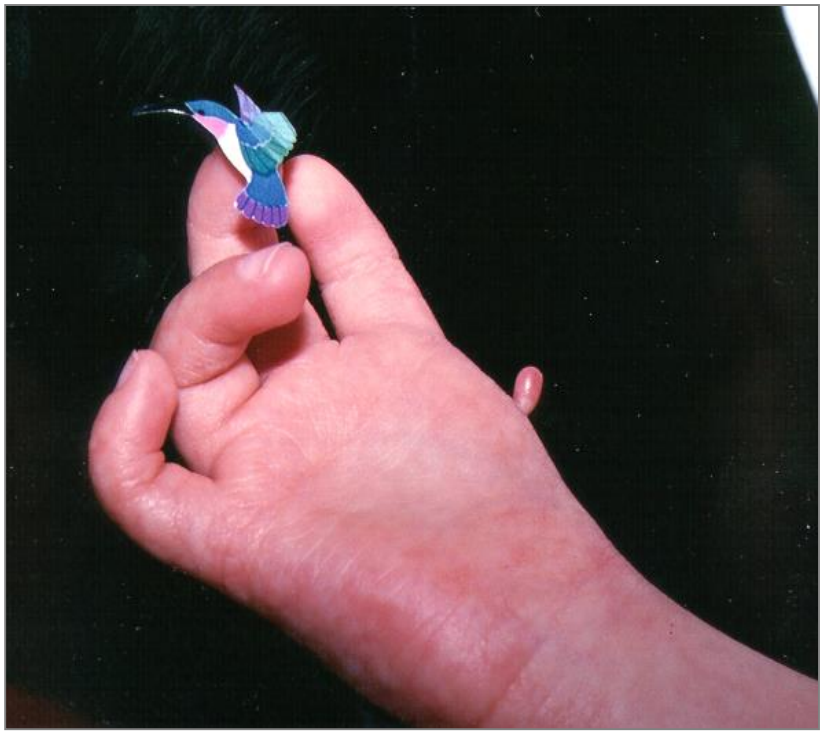


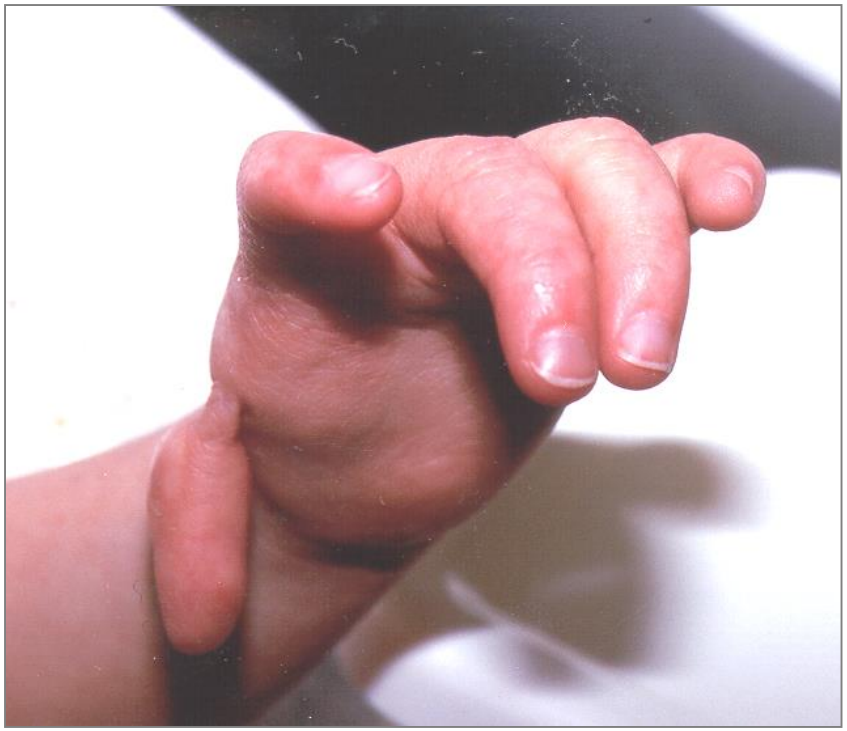


RTS rash-extremities

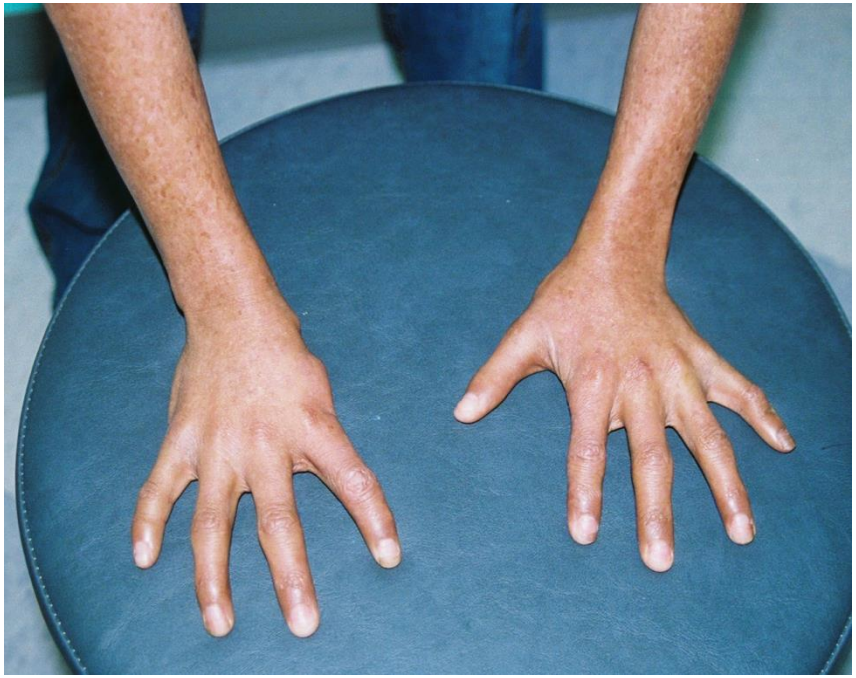


Radial Ray Defects in RTS

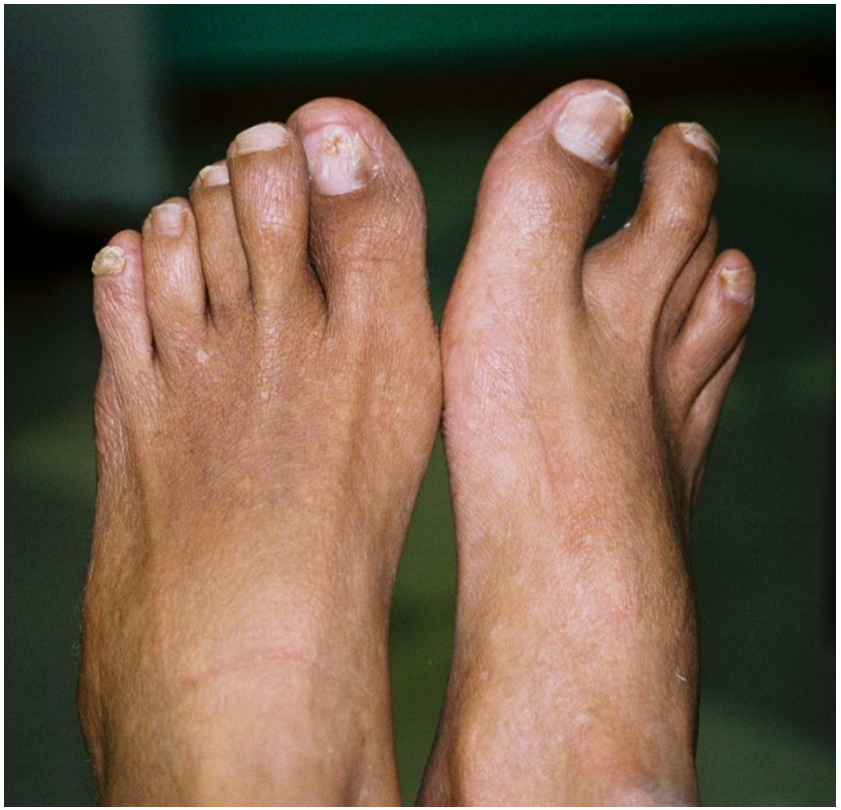


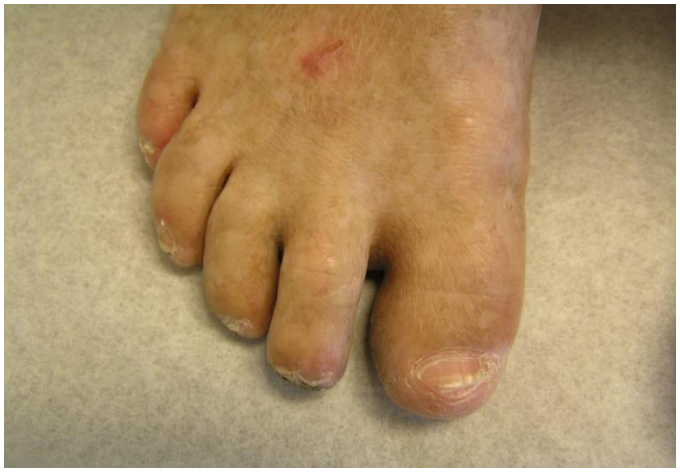


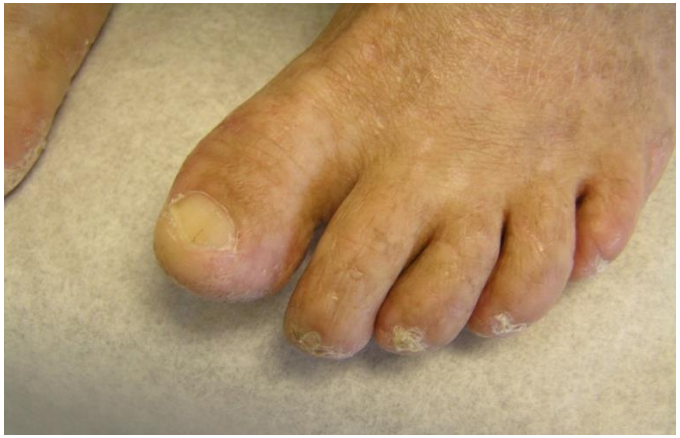
Radial Ray Defects in RTS



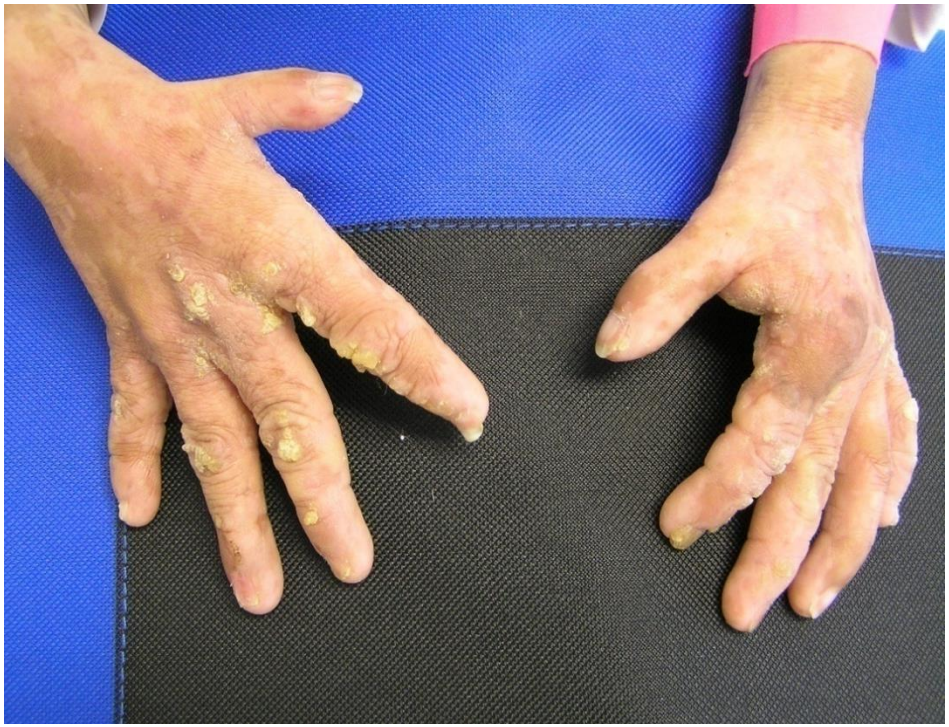
Nail abnormalities in RTS

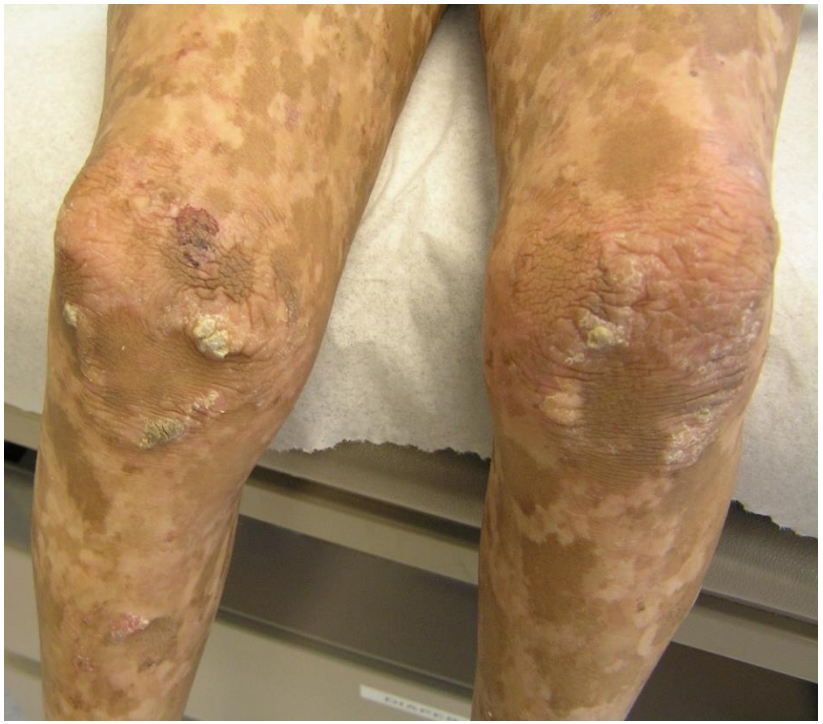




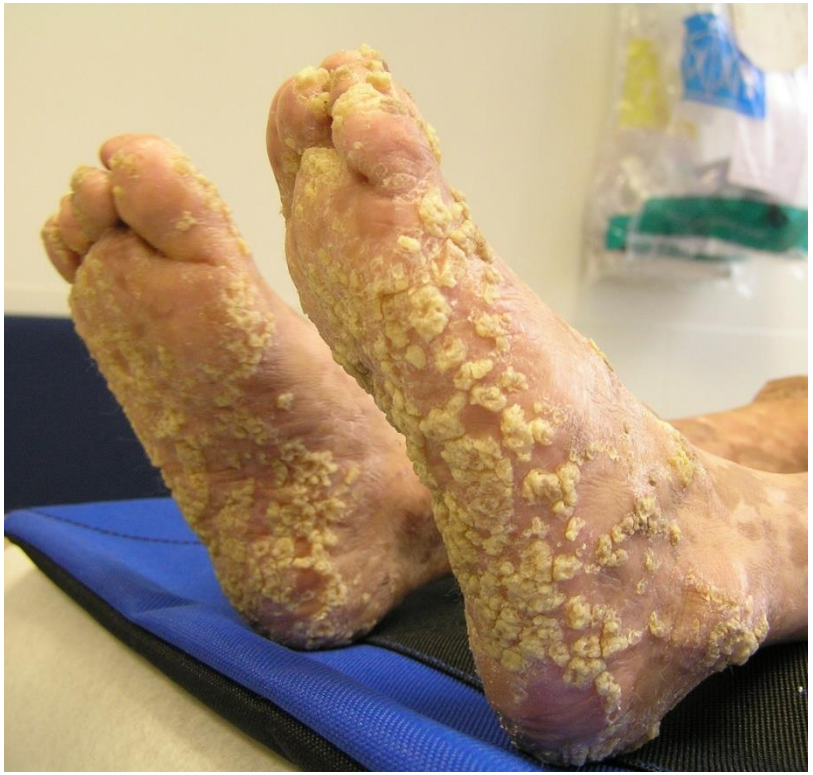


RTS Hyperkeratosis





RTS Hyperkeratosis



Clinical Diagnostic Criteria for RTS

DEFINITE RTS

- **Characteristic rash with or without other features:**
  - ***Acute phase:*** **Begins in infancy (usually 3-6 months) as erythema/blisters on cheeks, often sun-sensitive, spreads to extensor surfaces of extremities, buttocks**
  - ***Chronic phase:*** **Poikiloderma consisting of reticulated hypo- and hyper-pigmentation, atrophy, telangiectases**

Wang *et al* (2001) Am J Med Genet; 102:11-17

Clinical Diagnostic Criteria for RTS

PROBABLE RTS

- ***Atypical rash*** **plus two or more features of RTS**

- **Positive family history of RTS**
- **Osteosarcoma**
- **Skin malignancy**
- **Radial ray defect**
- **Other skeletal dysplasias**
- **Juvenile cataract**
- **Small stature**
- **Sparse scalp hair**
- **Sparse brows and/or lashes**
- **Dental abnormalities**
- **Nail abnormalities**

- ***Hearing loss, gastrointestinal problems, immune deficiency…***

Wang *et al* (2001) Am J Med Genet; 102:11-17

Enrollment of RTS Families

	Type 1 Probands	Type 2 Probands	Type 1 Family Members	Type 2 Family Members	TOTAL
Female	21	33	50	83	187
Male	29	45	34	76	184
TOTAL	**50**	**78**	**84**	**159**	**371**

Baylor College of Medicine Study, November 2020

Texas Children's Cancer and Hematology Centers

Baylor College of Medicine

Kitao *et al*. (1999) Nature Genetics; 22: 82-84

letter

Mutations in *RECQL4* cause a subset of cases of Rothmund-Thomson syndrome

Saori Kitao[1], Akira Shimamoto[1], Makoto Goto[2], Robert W. Miller[3], William A. Smithson[4], Noralane M. Lindor[4] & Yasuhiro Furuichi[1]

Rothmund-Thomson syndrome (RTS; also known as poikiloderma congenitale) is a rare, autosomal recessive genetic disorder characterized by abnormalities in skin and skeleton, juvenile cataracts, premature ageing and a predisposition to neoplasia[1–4]. Cytogenetic studies indicate that cells from

The coding sequence of *RECQL4*, consisting of 3,627 bases and encoding a protein with 1,208 amino acids, has been published[18]; exon and intron junctions have also recently been identified (unpublished data). We amplified all exon regions of *RECQL4* from patients by PCR and compared their sequences

- 3 out of 7 RTS cases had mutations in ***RECQL4***

RECQL4

- Genetic locus: *RECQL4* at 8q24.3
- Encodes member of RECQ DNA helicase family
- RECQ helicases: DNA unwinding enzymes involved in basic cellular functions (DNA replication, recombination, transcription, repair)
- Important for maintaining *genomic stability*

Cellular Roles of RECQL4

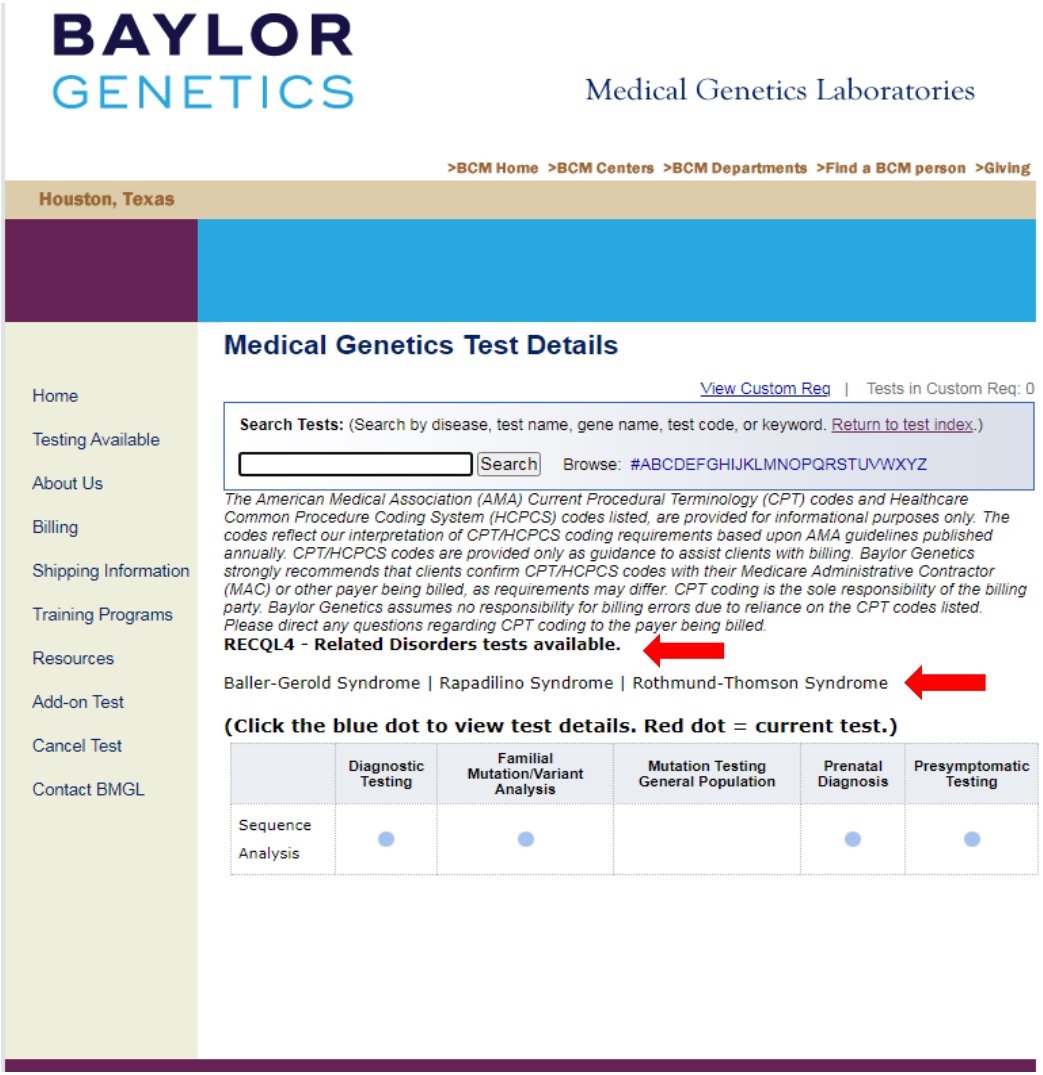
- Initiation of DNA replication
- DNA repair: double strand break and base excision repair
- Telomere maintenance
- Maintenance of mitochondrial integrity
- Response to oxidative stress

1st Question:

Does the presence of *RECQL4* mutations correspond to increased risk of osteosarcoma in RTS patients?

Sequence RECQL4 gene in RTS patients to determine mutation frequency

- Mutation testing done initially in the lab as part of research
- Now widely available as a clinical test



Association Between Osteosarcoma and Deleterious Mutations in the RECQL4 Gene in Rothmund–Thomson Syndrome

Lisa L. Wang, Anu Gannavarapu, Claudia A. Kozinetz, Moise L. Levy, Richard A. Lewis, Murali M. Chintagumpala, Ramon Ruiz-Maldanado, Jose Contreras-Ruiz, Christopher Cunniff, Robert P. Erickson, Dorit Lev, Maureen Rogers, Elaine H. Zackai, Sharon E. Plon

Background: **Rothmund–Thomson syndrome (RTS) is an autosomal recessive disorder associated with an increased predisposition to osteosarcoma. Children with RTS typically present with a characteristic skin rash (poikiloderma), small stature, and skeletal dysplasias. Mutations in the RECQL4 gene, which encodes a RecQ DNA helicase, have been reported in a few RTS patients. We examined whether a predisposition to developing osteosarcoma among an international cohort of RTS patients was associated with a distinctive pattern of mutations in the RECQL4 gene.** ***Methods:*** **We obtained clinical information about and biologic samples from 33 RTS patients (age range = 1–30 years). Eleven patients were diagnosed with osteosarcoma. All 21 exons and 13 short introns of the RECQL4 gene were sequenced from the genomic DNA of all subjects. Kaplan–Meier survival analysis was used to estimate the incidence of osteosarcoma among patients with and without mutations predicted to produce a truncated RECQL4 protein.** ***Results:*** **Twenty-three RTS patients, including all 11 osteosarcoma patients, carried at least one of 19 truncating mutations in their RECQL4 genes. The incidence of osteosarcoma was 0.00 per year in truncating mutation-negative patients (100 person-years of observation) and 0.05 per year in truncating mutation-positive patients (230 person-years of observation) (P = .037; two-sided log-rank test).** ***Conclusions:*** **Mutations predicted to result in the loss of RECQL4 protein function occurred in approximately two-thirds of RTS patients and are associated with risk of osteosarcoma. Molecular diagnosis has the potential to identify those children with RTS who are at high risk of this cancer. [J Natl Cancer Inst 2003;95: 669–74]**

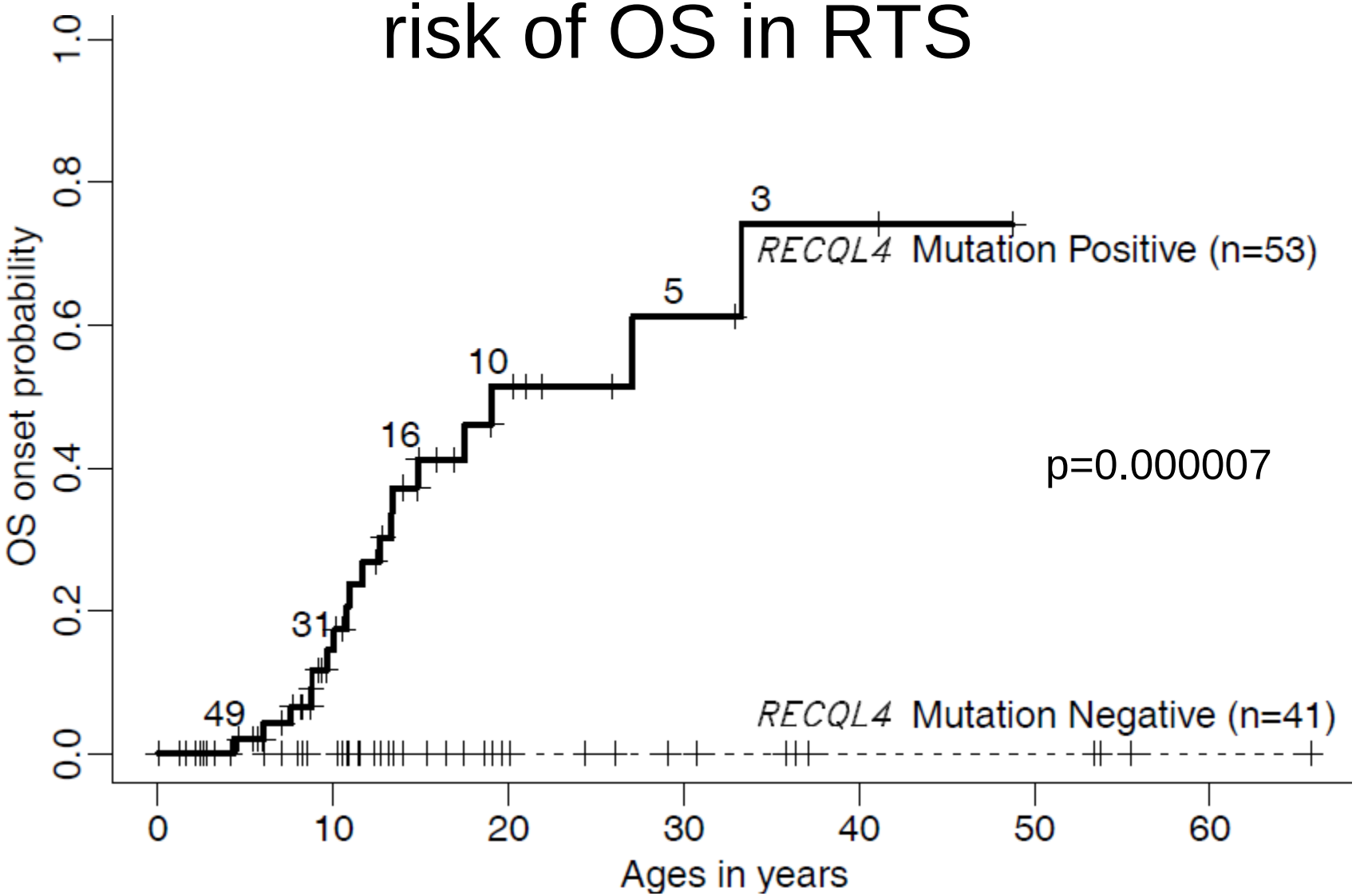
eral juvenile cataracts. However, evaluation of an international cohort of 41 RTS probands revealed a different clinical profile, which included a prevalence of osteosarcoma at approximately 0.30 *(2)*. Currently no clinical or molecular marker predicts which RTS patients will develop osteosarcoma, a malignancy that carries a substantial mortality rate despite available surgery and chemotherapy *(5)*.

In 1999, Kitao et al. *(6)* used a pure candidate gene approach to show that mutations in the RECQL4 gene, which is located on human chromosome 8q24.3, occurred in two of the six RTS kindreds they examined. The RECQL4 protein belongs to the RecQ family of DNA helicases, which includes proteins encoded by genes that are disrupted in Bloom syndrome and Werner syndrome, two clinically related cancer predisposition syndromes *(7)*. DNA helicases are enzymes that unwind DNA and are involved in many basic cellular processes; interruption of their functions may reduce genomic stability and thus contribute to tumorigenesis *(8,9)*. No complementation or linkage studies have been reported that might indicate whether mutations in more than one gene (termed genetic heterogeneity) are responsible for RTS, and no studies of RECQL4 gene mutations in sporadic osteosarcoma have been reported. We performed comprehensive DNA sequence analysis of the RECQL4 gene from 33 RTS patients to examine the spectrum of RECQL4 mutations in RTS and to assess whether RTS patients with osteosarcoma have a distinctive pattern of mutation.

Affiliations of authors: L. L. Wang, A. Gannavarapu, M. M. Chintagumpala (Texas Children's Cancer Center and Department of Pediatrics), C. A. Kozinetz (Department of Pediatrics), M. L. Levy (Departments of Pediatrics and Dermatology), R. A. Lewis (Departments of Pediatrics, Ophthalmology, and Molecular and Human Genetics), Baylor College of Medicine, Houston, TX; R. R. Maldanado, Department of Dermatology, National University of Mexico, Mexico

2003

Presence of *RECQL4* mutations increases risk of OS in RTS



Lu et al, Adv Exp Med Biol. 2014;804:129-45

2nd Question:

Does the presence of *RECQL4* mutations correspond to increased risk of skeletal defects in RTS patients?

Type 2 RTS and skeletal defects

Radiographic Abnormalities in Rothmund-Thomson Syndrome and Genotype–Phenotype Correlation with *RECQL4* Mutation Status

Amy R. Mehollin-Ray[1]
Claudia A. Kozinetz[2]
Alan E. Schlesinger[1]
R. Paul Guillerman[1]
Lisa L. Wang[3]

OBJECTIVE. The purpose of this study was to summarize the radiographic skeletal findings in patients with Rothmund-Thomson syndrome (RTS) and to determine whether there is an association between the presence of skeletal abnormalities and the mutational status of the *RECQL4* gene.

SUBJECTS AND METHODS. Twenty-eight subjects with RTS underwent skeletal surveys and *RECQL4* DNA mutation testing. Radiographs were reviewed by two radiologists. *RECQL4* mutation testing by DNA sequencing of the gene was performed by a diagnostic laboratory. Genotype–phenotype analysis by Fisher's exact test was performed to investigate whether there was a correlation between mutation status and skeletal abnormalities.

RESULTS. Twenty-one (75%) of the subjects had at least one significant skeletal abnormality, the more common being abnormal metaphyseal trabeculation, brachymesophalangy, thumb aplasia or hypoplasia, osteopenia, dislocation of the radial head, radial aplasia or hypoplasia, and patellar ossification defects. Three subjects had a history of destructive bone lesion (osteosarcoma). Genotype–phenotype analysis showed a significant correlation between *RECQL4* mutational status and the presence of skeletal abnormalities ($p < 0.0001$).

CONCLUSION. Skeletal abnormalities are frequent in persons with RTS. Many of these abnormalities are not clinically apparent but are detectable on radiographs. The presence of skeletal abnormalities correlates with *RECQL4* mutation status, which has been found to correlate with risk of osteosarcoma. Skeletal surveys aid in both diagnosis and management of RTS.

Keywords: bone abnormality, *RECQL4* mutation, Rothmund-Thomson syndrome, skeletal dysplasia

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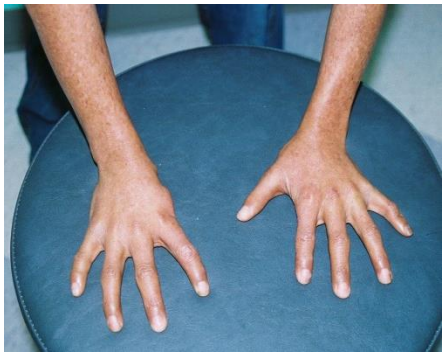
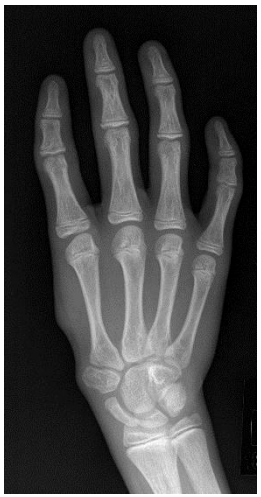
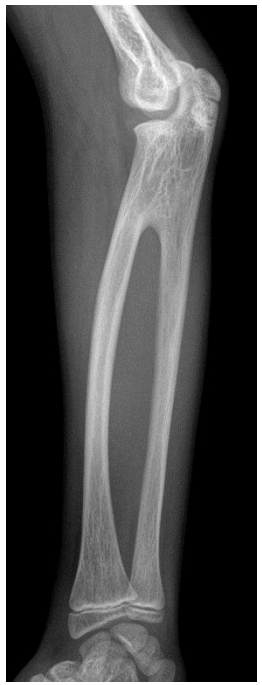
Rothmund-Thomson syndrome (RTS) is an autosomal recessive disorder with heterogeneous clinical features, including a characteristic rash (poikiloderma), small stature, clinically overt skeletal abnormalities but did not thoroughly discuss the entire skeletal system. In a review [1] of the cases of 41 patients with RTS, 75% of the 20 patients who under-







2008

Skeletal Findings in RTS

- 75% (21/28) had major skeletal abnormalities
- The most common findings were:
  - Abnormal metaphyseal trabeculation (64%)
  - Brachymesophalangy (64%)
  - 1st metacarpal or thumb agenesis/hypoplasia (43%)
  - Osteopenia (25%)
  - Radial agenesis/hypoplasia (21%)
  - Radioulnar synostosis (18%)
  - Ulnar hypoplasia (18%).
  - History of OS (11%)

Mehollin-Ray et al, Am J Roentgenol. 2008 Aug;191(2):W62-6

RTS Classification

- Type II RTS
  - Poikiloderma
  - Mutations in *RECQL4*
  - Increased risk for OS
  - Association with skeletal defects
- Type I RTS
  - Poikiloderma
  - No mutations in *RECQL4*
  - Less risk of cancer

Wang *et al* (2003) J Natl Cancer Inst; 95(9):669-74

Type 1 RTS

- Cause unknown at the time
- Likely due to gene(s) other than *RECQL4*
- Classic poikiloderma and skin findings
- Less cancer risk
- More juvenile cataracts
- Exome sequencing project with Dr. Philippe Campeau (BCM-Montreal)

A cause for Type 1 RTS

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REPORT

Mutations in *ANAPC1*, Encoding a Scaffold Subunit of the Anaphase-Promoting Complex, Cause Rothmund-Thomson Syndrome Type 1

Norbert F. Ajeawung,[1,6] Thi Tuyet Mai Nguyen,[1,6] Linchao Lu,[2,6] Thomas J. Kucharski,[3] Justine Rousseau,[1] Sirinart Molidperee,[1] Joshua Atienza,[1] Isabel Gamache,[1] Weidong Jin,[2] Sharon E. Plon,[2,4] Brendan H. Lee,[4] Jose G. Teodoro,[3] Lisa L. Wang,[2,*] and Philippe M. Campeau[1,5,*]

Rothmund-Thomson syndrome (RTS) is an autosomal-recessive disorder characterized by poikiloderma, sparse hair, short stature, and skeletal anomalies. Type 2 RTS, which is defined by the presence of bi-allelic mutations in *RECQL4*, is characterized by increased cancer susceptibility and skeletal anomalies, whereas the genetic basis of RTS type 1, which is associated with juvenile cataracts, is unknown. We studied ten individuals, from seven families, who had RTS type 1 and identified a deep intronic splicing mutation of the *ANAPC1* gene, a component of the anaphase-promoting complex/cyclosome (APC/C), in all affected individuals, either in the homozygous state or in *trans* with another mutation. Fibroblast studies showed that the intronic mutation causes the activation of a 95 bp pseudoexon, leading to mRNAs with premature termination codons and nonsense-mediated decay, decreased ANAPC1 protein levels, and prolongation of interphase. Interestingly, mice that were heterozygous for a knockout mutation have an increased incidence of cataracts. Our results demonstrate that deficiency in the APC/C is a cause of RTS type 1 and suggest a possible link between the APC/C and RECQL4 helicase because both proteins are involved in DNA repair and replication.

Analysis of the clinical and molecular features of individuals with Rothmund-Thomson syndrome (RTS [MIM: 268400]), including assessing the prevalence of osteosarcoma and the mutational status of the *RECQL4* gene (MIM: 603780), resulted in the definition of two distinct ancestry. All individuals presented with classical RTS type 1 features, including poikiloderma, abnormal hair and nails, bilateral juvenile cataracts, and an absence of *RECQL4* mutations (see Table 1 and Figure 1A for photos and Figure 1B for pedigrees). Additional features in our

- Mutations in *ANAPC1* identified in 10/18 subjects (7/14 families) with Type 1 RTS

2019

Causative genes in RTS

RTS	Type 1		Type 2
Gene defect	*ANAPC1*	*Unknown*	*RECQL4*
# of individuals	10	40	78

Type 1 and Type 2 RTS Clinical Features

	Type 1	Type 2
Poikiloderma	+	+
Sparse hair/brows/lashes	+	+
Bone defects	+	++
Osteopenia/osteoporosis	+	+
Gastrointestinal problems	+	++
Cataracts	++(juvenile)	+
Squamous or basal cell skin cancer	+	+
Osteosarcoma	+	+
Hearing problems	+	++

Thank you