Welcome to the 2020 Webinar #1

Dr. Lisa L. Wang – The History of RTS Research and Clinical Studies
Mission Statement

• Promote awareness of Rothmund-Thomson Syndrome and related syndromes to the general public and to healthcare professionals

• Provide education and support to affected families worldwide

• Provide education to healthcare professionals who may encounter patients affected with these disorders

• Promote research aimed at understanding the molecular and cellular basis of RTS and the clinical manifestations

• Raise funds through events and contributions that will support the overall efforts of the group
Ideas for Future RTS Webinars

• Coping with a rare disease during the COVID pandemic
• Dermatology challenges with Dr. Moise Levy
• The differences between Type 1 and Type 2 RTS and genetic testing
• Orthopedic issues and challenges
• Cancer concerns, monitoring and treatment
RTSF Medical Advisory and Board Members

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  - Caroline Brain, MD (Pediatric Endocrinology)
The Future of RTS Research

Lisa L. Wang, MD
Texas Children’s Hospital
Baylor College of Medicine
Houston, TX

&

John Kimmel,
Founder and Chair, Rothmund-Thomson Syndrome Foundation
Chantilly, VA
1st RTS “Sharing and Caring” Conference
October 17-19, 2007
Houston, TX

• **Day 1:** Educational Lectures
  - Genetics of RTS (Sharon E. Plon, MD, PhD)
  - Dermatologic Manifestations of RTS (Mo Levy, MD)
  - RTS and Bone Disease (Lisa L. Wang, MD)
  - Radiologic Findings in RTS (Amy Mehollin-Ray, MD)
  - Sharing and Caring Session—Families

• **Day 2:**
  - RTS Support Group Planning
Outline

• History of RTS and RTS Research
  • Milestones in RTS Research
  • Current and Future Goals
History of RTS

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

- **1923:** M. Sydney Thomson described the rash and *skeletal anomalies* in sisters; coined term “poikiloderma congenitale”
• **1957**: William Taylor united the two disorders, proposed eponym “Rothmund-Thomson Syndrome”
How we got started

- Patient diagnosed with osteosarcoma (OS) in 1999
- Also carried diagnosis of Rothmund-Thomson Syndrome (RTS)
- Sister also had RTS and died of metastatic OS
- QUESTION: Is there any link between these two rare diseases?
History of Baylor College of Medicine (BCM) RTS Study

• **First IRB Protocol**
  - Includes subjects with RTS and other disorders
  - Includes family members
  - First subject enrolled 01/19/1999
  - Yearly recontact letters

• **Second IRB Protocol** approved 08/22/2000
  - H-9106: “The Molecular Basis and Clinical Spectrum of Rothmund-Thomson Syndrome”
History of BCM RTS Study

- General Clinical Research Center (GCRC) Study approved 09/20/2000
  - Includes subjects with a diagnosis of RTS
  - First subject enrolled 07/25/2001
  - 36 subjects enrolled - came to Texas Children’s Hospital
  - Comprehensive evaluation including Genetics, Dermatology, Ophthalmology, Oncology, labs, skeletal surveys

- With these protocols approved and in place, we could begin to collect medical information and biologic samples to start generating data.
Clinical Manifestations in a Cohort of 41 Rothmund-Thomson Syndrome Patients

Lisa L. Wang, Moise L. Levy, Richard A. Lewis, Murali M. Chintagumpala, Dorit Lev,
Maureen Rogers, and Sharon E. Plan

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5Institute of Medical Genetics, Wolfson Medical Center, Holon, Israel
6Children’s Hospital Medical Centre, Westmead NSW, Australia

Rothmund-Thomson syndrome (RTS) is a rare autosomal recessive genodermatosis characterized by a poikilodermatous rash starting in infancy, small stature, skeletal abnormalities, juvenile cataracts, and predisposition to specific cancers. We have identified a contemporary cohort of 41 patients to better define the clinical profile, diagnostic criteria, and management of patients with RTS. Patients with the diagnosis of RTS were ascertained by referrals from dermatology, ophthalmology, genetics, and oncology or from direct contact with the patient’s family. Medical information was obtained from interviews with physicians, patients, and their parents and a review of medical records. The age range at ascertainment was 9 months to 42 years (28 males and 13 females; M:F 2:1). All subjects displayed a characteristic rash. Thirteen subjects had osteosarcoma (OS) (38%), eight had radial defects (20%), seven had gastrointestinal findings (17%), two had cataracts (8%), and one had skin cancer (2%). Twenty-two of 28 patients without OS were less than 15 years old and thus remain at significant risk for this tumor. This case-series study reveals a clinical profile of RTS that includes a higher prevalence of OS and fewer cataracts, compared to older reports. The frequency of clinical anomalies in a contemporary cohort of RTS patients and revises guidelines for diagnosis and management of RTS.

KEY WORDS: cancer; cataract; chromosomal instability; genetics; osteosarcoma; poikiloderma; radial ray defect; rash

INTRODUCTION

Rothmund-Thomson syndrome (RTS) (OMIM 268400) is a rare autosomal recessive disorder first described in 1868 by German ophthalmologist Auguste Rothmund in his family members who had a peculiar rash and bilateral juvenile cataracts [Rothmund, 1868]. Sydney Thomson, a British dermatologist, coined the term “poikilodermia congenita” in 1923 for patients with a similar rash and skeletal abnormalities, but no cataracts [Thomson, 1923]. In 1957, William Taylor suggested that the two disorders were the same and proposed the combined eponym Rothmund-Thomson syndrome [Taylor, 1957].

RTS is characterized primarily by a sun-sensitive rash that usually begins between 3 and 6 months, but may appear soon after birth or as late as 2 years. The
Summary of Clinical Findings in 41 RTS Subjects
Baylor College of Medicine Study

<table>
<thead>
<tr>
<th>Condition</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rash</td>
<td>41/41</td>
<td>100%</td>
</tr>
<tr>
<td>Small stature</td>
<td>25/38</td>
<td>66%</td>
</tr>
<tr>
<td>Skeletal dysplasia</td>
<td>15/20</td>
<td>75%</td>
</tr>
<tr>
<td>Radial ray defect</td>
<td>8/40</td>
<td>20%</td>
</tr>
<tr>
<td>Sparse scalp hair</td>
<td>15/30</td>
<td>50%</td>
</tr>
<tr>
<td>Sparse brows/lashes</td>
<td>19/26</td>
<td>73%</td>
</tr>
<tr>
<td>Cataracts</td>
<td>2/32</td>
<td>6%</td>
</tr>
<tr>
<td>Skin cancer</td>
<td>1/41</td>
<td>2%</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>13/41</td>
<td>32%</td>
</tr>
</tbody>
</table>

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

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

Genetic Basis of RTS

• When we started our research, the cause of RTS was unknown.

• It was known to be an inherited disorder and transmitted in an **autosomal recessive** pattern.

• In 1999, a gene for RTS was discovered.
2: Finding the cause of RTS

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

Mutations in \textit{RECQL4} cause a subset of cases of Rothmund-Thomson syndrome

Saori Kitao\textsuperscript{1}, Akira Shimamoto\textsuperscript{1}, Makoto Goto\textsuperscript{2}, Robert W. Miller\textsuperscript{3}, William A. Smithson\textsuperscript{4}, Noralane M. Lindor\textsuperscript{4} \& Yasuhiro Furuichi\textsuperscript{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\textsuperscript{1-4}. Cytogenetic studies indicate that cells from the coding sequence of \textit{RECQL4}, consisting of 3,627 bases and encoding a protein with 1,208 amino acids, has been published\textsuperscript{18}; exon and intron junctions have also recently been identified (unpublished data). We amplified all exon regions of \textit{RECQL4} from patients by PCR and compared their sequences.

- 3 out of 7 RTS cases had mutations in \textit{RECQL4}
3: Sequence RTS patients to see how many have mutations in \textit{RECQL4}

- Mutation testing done initially in the lab as part of research
- Helped to develop a clinical test for RTS
- Now widely available in the U.S.
Characterizing different \textit{RECQL4} mutation types in RTS

\textbf{Report}

\textbf{Intron-Size Constraint as a Mutational Mechanism in Rothmund-Thomson Syndrome}

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Departments of \textsuperscript{1}Pediatrics, \textsuperscript{2}Molecular and Human Genetics, and \textsuperscript{3}Dermatology and the \textsuperscript{4}Human Genome Sequencing Center, Baylor College of Medicine, Houston

Rothmund-Thomson syndrome (RTS) is an autosomal recessive disorder caused by deleterious mutations in the \textit{RECQL4} gene on chromosome 8. The \textit{RECQL4} gene structure is unusual because it contains many small introns <100 bp. We describe a proband with RTS who has a novel 11-bp intronic deletion, and we show that this mutation results in a 66-bp intron too small for proper splicing. Constraint on intron size may represent a general mutational mechanism, since human-genome analysis reveals that \textasciitilde 15\% of genes have introns <100 bp and are therefore susceptible to size constraint. Thus, monitoring of intron size may allow detection of mutations missed by exon-by-exon approaches.

- Mutation testing can be tricky
- Need to examine intronic regions
- Make sure proper test is performed
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 Canniff, 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 dysplasia. 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 biological 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 (106 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.
**RECQL4 mutation status and OS in RTS**

Type 2 RTS:
- **RECQL4 mutations**
- Increased risk of OS compared to Type 1

Type 1 RTS:
- No **RECQL4 mutations**

*p = 0.000007*

4: Determining if *RECQL4* mutations correlate with other features of RTS: Skeletal defects

- Skeletal defects correlate with *RECQL4* mutations
- Skeletal surveys are useful
5: Managing osteosarcoma in RTS patients

- Overall, OS in RTS is similar to OS in general population.
- In general, patients tolerate treatment fairly well.
- Difficult to predict a priori response to therapy.
- Do not decrease doses up front.
6: Are RTS patients more sensitive to DNA-damaging agents?

Implications for:
• Sun protection (UV)
• Radiology tests, screening for cancer
• Prediction of side effects from cancer treatment

Sensitivity of RECQL4-deficient fibroblasts from Rothmund–Thomson syndrome patients to genotoxic agents

Weidong Jin · Hao Liu · Yiqun Zhang · Subhendu K. Otta · Sharon E. Plon · Lisa L. Wang

Abstract RECQ helicase protein-like 4 (RECQL4) is a member of the human RECQ family of DNA helicases. Two-thirds of patients with Rothmund–Thomson syndrome (RTS) carry biallelic inactivating mutations in the RECQL4 gene. RTS is an autosomal recessive disorder characterized by arikloodema, sparse hair, small stature, skeletal abnormalities, cataracts, and an increased risk of cancer. Mutations in two other RECQ helicases, BLM and WRN, are responsible for the cancer predisposition conditions Bloom and Werner syndromes, respectively. Previous studies have shown that BLM and WRN-deficient cells demonstrate increased sensitivity to hydroxyurea (HU), camptothecin (CPT), and 4-nitroquinoline 1-oxide (4NQO). Little is known about the function of the RECQL4 protein. Our results show that primary fibroblasts from RTS patients carrying two deleterious RECQL4 mutations, compared to wild type (WT) fibroblasts, have increased sensitivity to HU, CPT, and doxorubicin (DOX), modest sensitivity to other DNA damaging agents including ultraviolet (UV) irradiation, ionizing radiation (IR), and cisplatin (CDDP), and relative resistance to 4NQO. The RECQ family of DNA helicases has been implicated in the regulation of DNA replication, recombination, and repair. Because HU, CPT, and DOX exert their effects primarily during S phase, these results support a greater role for the RECQL4 protein in DNA replication as opposed to repair of exogenous damage.

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7: RECQL4 Spectrum of Disorders

1. RTS
2. Baller-Gerold Syndrome (BGS)
3. RAPADILINO

2006, 2008
Diagram showing overlapping and unique clinical features of the RECQL4–associated disorders. Osteosarcomas and lymphomas have been described in both RTS and RAPADILINO, but osteosarcomas predominate in RTS, while lymphomas are more common in RAPADILINO. There has been only one case of lymphoma reported in a patient with BGS.
History of BCM
RTS Study

• **Third IRB Protocol** approved 10/11/2007

• **Clinical Research Center (CRC) Study** approved 11/2010
  – First subject enrolled in 2010
  – 29 subjects enrolled
These mice with Recql4 mutations had skeletal features similar to RTS patients; however, they did not develop osteosarcoma unless crossed with p53 deficient mice.
9: Do RTS patients have altered bone metabolism?

Some RTS patients have decreased bone mineral density (osteoporosis) and may need monitoring (DXA scans).

2017
10: Finding a cause for Type 1 RTS

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

Lisa L Wang, MD1 and Sharon E Plon, MD, PhD, FACMG2

Created: October 6, 1999; Revised: June 4, 2020.

Summary

Clinical characteristics

Rothmund-Thomson syndrome (RTS) is characterized by a rash that progresses to poikiloderma; sparse hair, eyelashes, and/or eyebrows; small size; skeletal and dental abnormalities; juvenile cataracts; and an increased risk for cancer, especially osteosarcoma. A variety of benign and malignant hematologic abnormalities have been reported in affected individuals. The rash of RTS typically develops between ages three and six months (occasionally as late as age two years) as erythema, swelling, and blistering on the face, subsequently spreading to the buttocks and extremities. The rash evolves over months to years into the chronic pattern of reticulated hypopigmentation, telangiectasias, and punctate atrophy (collectively known as poikiloderma) that persist throughout life. Hyperkeratotic lesions occur in approximately one third of individuals. Skeletal abnormalities can include radial ray defects, ulnar defects, absent or hypoplastic patella, and osteopenia.
What is on the horizon for RTS research?
What is Needed to Continue Research on RTS?

• Researchers

• Patients and Families

• Funding

- Basic science research
- Clinical & translational research
- Infrastructure

- Registry - clinical database; yearly questionnaires
- Biologic material for laboratory studies

- Major funding – e.g., National Institutes of Health (NIH)
- Foundation grants
Academic medicine - Physician scientist

- 80% research effort – grants
- 20% clinical effort - see patients

Grants must pay for:

- Personnel – salaries including own
- Supplies
- Animal costs
- Maintenance of lab (liquid nitrogen, CO2, glasswashing, etc.)
RTS Registry

• Information entered into database
• Yearly recontact
• This will allow us to
  – describe the natural history of RTS
  – understand the full clinical manifestations in RTS patients and their relatives
  – Identify areas of research study
Roadblocks to Federal Funding

- Limited resources – diminishing payline; Covid-19 impact
- Fierce competition – more common diseases, larger labs, PhD scientists
- The vicious cycle:

- Funding
- Preliminary data
- Personnel salaries, supplies
- Publications
- Submit grant – 6-9 month process
How Can We Sustain Research on RTS?

- **Researchers**
  - Continue to submit grants and conduct research

- **Patients and Families**
  - Play an active role in research!
  - Fill out questionnaires and provide clinical information
  - Provide biologic samples when possible

- **Funding**
  - Local fundraisers to support RTSF and RTS research
  - Facebook fundraisers
  - Workplace, company support
Challenges

• Raising money to support clinical research
  - Research is expensive
  - Research dollars are limited and competitive, especially for rare diseases
  - The Foundation needs to increase fundraising

• Building the patient registry and increasing the number of families involved in clinical trials
  - There have been tremendous advances, but there is much more to be done

• Fresh ideas, energetic members, renewed commitment