Rothmund-Thomson syndrome: insights from new patients on the genetic variability underpinning clinical presentation and cancer outcome

Elisa Adele Colombo1,*, Andrea Locatelli2, Laura Cubells Sánchez3, Sara Romeo4,5, Nursel H Elcioglu6,7, Isabelle Maystadt8, Altea Esteve Martínez3, Alessandra Sironi9,10, Laura Fontana1, Palma Finelli9,10, Cristina Gervasini1, Vanna Pecile11 and Lidia Larizza1.

1 Dipartimento di Scienze della Salute, Università degli Studi di Milano, 20142 Milan, Italy; 2 UO Dermatologia e Venereologia, Asst Papa Giovanni XXIII, 24127 Bergamo, Italy; 3 Department of Dermatology, Consorcio Hospital General Universitario de Valencia, 46014 Valencia, Spain; 4 Institute of Clinical Sciences, Imperial College London, London W12 0NN, UK; 5 MRC London Institute of Medical Sciences, Imperial College London, London W12 0NN, UK; 6 Department of Pediatric Genetics, Marmara University Medical School, 34890 Istanbul, Turkey; 7 Eastern Mediterranean University, Cyprus, Turkey; 8 Centre de Génétique Humaine, Institut de Pathologie et de Génétique, 6041 Charleroi (Gosselies), Belgium; 9 Laboratory of Medical Cytogenetics and Molecular Genetics, IRCCS Istituto Auxologico Italiano, 20149 Milan, Italy; 10 Department of Medical Biotechnology and Translational Medicine, University of Milan, 20133 Milan, Italy; 11 Institute for Maternal and Child Health, Foundation IRCCS Burlo Garofolo Institute, 34137 Trieste, Italy.

* Correspondence: elisaadele.colombo@unimi.it; Tel.: +39-02-50323200

Table S1. RECQL4 pathogenic variants in RECQL4-mutated patients who developed cancer

| Tumors (Age at onset) | Patient code | Pathogenic variant 1 | Pathogenic variant 2 | Ref. |
|-----------------------|--------------|----------------------|----------------------|------|
| Lymphoma (24y)        | RAPA r704    | c.806G>A             | c.1390+2del          | IVS7 | [8] |
| HL (35y)              | RTS 1        | c.1048_1049del       | c.1391-1G>A          | IVS7 | [27] |
| OS (23y)              | III-1        | c.1048_1049del       | c.1878+32_1878+55del | IVS11|     |
| OS (19y)              | III-2        | c.1048_1049del       |                      |      |     |
| SCC and BSC (≤39y)    | RTS Pt 15    | c.1078C>T            | c.1222C>T           | ex. 6| [26]|
| OS (15y)              | RAPA r504    | c.1390+2del          | c.1390+2del         | IVS7 | [8] |
| OS (10y)              | RAPA Pt 7    | c.1390+2del          | c.1390+2del         | IVS7 | [8] |
| Lymphoma (21y)        | RAPA r903    | c.1390+2del          | c.1390+2del         | IVS7 | [8] |
| Lymphoma (25y)        | RAPA r904    | c.1390+2del          | c.1390+2del         | IVS7 | [8] |
| Lymphoma (33y)        | RAPA Pt 6    | c.1390+2del          | c.3599_3600del      | ex. 21| [8] |
| OS (21y)              | RTS II-1     | c.1391-1G>A          | c.[1568G>C;1573delT]a | ex. 9| [8] |
| OS (20y)              | RTS II-2     | c.1391-1G>A          | c.[1568G>C;1573delT]a | ex. 9| [8] |
| OS (9y)               | RTS FCP-153  | c.1391-1G>A          | c.[1568G>C;1573delT]a | ex. 9| [8] |
| OS (c2y)              | RTS Pt 12    | c.1391-1G>A          | c.2085del           | ex. 13| [26]|
| OS (11y)              | RTS FCP-102  | c.1483+25del         | c.1483+25del        | IVS8 | [8] |
| OS (12y)              | and sibling  |                      |                      |      |     |
| OS (14y)              | RTS IV-4     | c.1483+27del         | c.1483+27del        | IVS8 | [8] |
| MFH (15y)             | RTS IV-5     |                      |                      |      |     |
| OS (10y)              | c.[1568G>C;1573delT] | ex. 9 | c.2269C>T           | ex. 14| [30]|
| OS (4y)               | RTS FCP-129  | c.[1568G>C;1573delT]a | ex. 9 | c.2269C>T           | ex. 14| [8] |
| OS (12y)              | RTS AS517    | c.[1568G>C;1573delT]a | ex. 9 | c.2269C>T           | ex. 14| [8, 28]|
| OS (14y; 17y)         | Family C II-6| c.[1568G>C;1573delT] | ex. 9 | c.3021_3022del      | ex. 17| This work|
| OS (31y)              | RTS II-3     | c.1650del            | c.2269C>T           | ex. 14| [8] |
| OS (15y)              | RTS II-6     | c.1650del            | c.2269C>T           | ex. 14| [8] |
| Lymphoma (9y)         | RTS          | c.1704+1G>A          | IVS10               | ex. 12| [32]|

Patients with malignancy carrying at least one alteration affecting the helicase domain (exons 8-15)
| Age    | Diagnosis       | RTQT/FRQ | Mutation Type | Exon     | Reference |
|--------|-----------------|----------|---------------|----------|-----------|
| OS (14y) | Leukemia (21y)  |          | c.1718delA    | ex. 11   | c.1878+32del | VS11 [8] |
| OS (8y)  |                  | RTS FCP-210 |                |          |            |
| OS (7y)  |                  | RTS FCP-136 | c.1878+5>G>A  | IVS11    | c.2476C>T  | ex. 15 [8] |
| Lymphoma (2y) |          | RTS Pt 8 | c.1913>T    | ex. 12   | c.2419ins5  | ex. 14 [8] |
| OS (17y) |                  | RTS      | c.2232_3007del C<sup>a</sup> | ex.14-17 | c.2232_3007del<sup>b</sup> | ex.14-17 [33] |
| OS (9y)  |                  | RTS FCP-125 | c.2269C>T    | ex. 14   | c.2269C>T  | ex. 14 [8] |
| Lymphoma (2.5y) |          | BGS      | c.2492_2493del | ex. 15   | c.2506_2518del | ex. 15 [34] |
| OS (19y) |                  | RST FCP-191 | c.2492_2493del | ex.15-    | -          | [8]        |
| OS (13y) |                  | RTS FCP-114 | c.2547_2548del | ex. 15   | -          | [8]        |
| OS (3y)  |                  | RTS FCP-203 | c.3072_3073del | ex. 18   | c.3276del  | ex. 19 [8] |

Patients with malignancy carrying alterations downstream the helicase domain (exons 8-15)

a: alteration reported as c.1573del in the original paper; b:alteration reported as g.4428_5437del (exons 14-18) in the original paper; c: three different amino acid substitutions (p.Arg522Cys, p.Val799Met and p.Pro1170Leu), none proven to be pathogenic.

HL: Hodgkin’s lymphoma; OS: osteosarcoma; SCC: squamous cell carcinoma; BSC: basal cell carcinoma; MFH: malignant fibrous histiocytoma;

RTS: Rothmund-Thomson syndrome; RAPA: RAPADILINO syndrome; BGS: Baller-Gerold syndrome.

**Table S2. Primers used for amplification and sequencing of RECQL4 gene**

| RECQL4 AMPICONS | PRIMER (5' TO 3')            | LENGTH (bp) |
|-----------------|------------------------------|-------------|
| 5' UTR - IVS3   | F: TTGACGCCCTCCCCATGGCT      | 815         |
|                 | R: TTGGTGCCGAGCCCGATTCA      |             |
| IVS3 - Exon 5   | F: AGAACTTGGGAGGGGACTG       | 791         |
|                 | R: CACTGTGACATCGCTGTAACC     |             |
| Exon 5 - IVS5   | F: GCAGAAAAAGTCAGTGATGAGC    | 747         |
|                 | R: TGGGCGGGAATAACCGGAGG      |             |
| IVS5 - Exon 7   | F: CATTCCCTTTCCCTCCCTCA      | 644         |
|                 | R: CTGCTCAGCCTGGCACAACGCC    |             |
| IVS6 - IVS9     | F: CTCCTATCTACCTCTCTCCT      | 709         |
|                 | R: CTGCCCTTGACCCTTGCCCA      |             |
| Exon 9 - Exon 10| F: TCTCTCCCCCTGCTGTCATCCT    | 513         |
|                 | R: GATTCCCCCTTGGTCTCTGGT     |             |
| IVS9 - IVS11    | F: GGGCTGGGCTGGGCTATG        | 409         |
|                 | R: CCGCCCAACCCCAGTCCAAT      |             |
| Exon 11 - IVS12 | F: CAGTGGTCTTCGCCCTGATT      | 396         |
|                 | R: ACCTGGTCTGGTCTGCTGTC      |             |
| IVS12 - Exon 15 | F: CTCTCTACCCATGCTGTTG      | 850         |
|                 | Fseq: ATGAAGGCTCTGCTGACCT    |             |
|                 | R: GAGGACACAGAGGCCGATCG      |             |
|                 | Rseq: CCTCTTCACAGCCAGGAAGT   |             |
| IVS13 - IVS16   | F: CCCCATCTCACGCTGACCT      | 912         |
|                 | Fseq: AGCCCCAGTGCTGACCTC    |             |
|                 | R: CTCCAACCTCGCTGACAAGCTC   |             |
|                 | Rseq: AGGAAGAGGGGTGGCAAGT    |             |
| Exon 16 - IVS19 | F: GCCGACACCTATAACCCATATT   | 873         |
|                 | R: CATCCACAGGACGAGCCC        |             |

The authors have provided a detailed table (Table S2) listing the primers used for amplification and sequencing of the RECQL4 gene. The table includes information on the different amplicons, the primers used, and their lengths.
| FAMILY | RECLQ4 cDNA AMPLIFIED REGION | PRIMER (5’ to 3’) | LENGTH (bp) |
|--------|-----------------------------|-----------------|-------------|
| A      | exon 16-17 junction - exon 20-21 junction | F: CCCCACAGGTGTCCTCCCTTT \nR: GTAGCAGGGGCTTCCGATG | 639 |
| B      | exon 5 – exon 7 | F: AGCCCCCTCCAGTCAAGCTAG \nR: TGAAGGAACCAGTGCTGCTAG | 342 |
| C      | exon 7 – exon 12 | F: ACCGTGCTGCCACTCTACTC \nR: GAAGCAGTGCACGCCCAT | 569 |

Table S3. Primer used for amplification and sequencing of RECLQ4 transcript

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