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

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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.
Patients with malignancy carrying at least one alteration affecting the helicase domain (exons 8-15)						
Lymphoma (24y)	RAPA r704	c.806G>A	ex. 5	c.1390+2del	IVS7	[8]
HL (35y)	RTS 1	c.1048_1049del	ex. 5	c.1391-1G>A	IVS7	[27]
OS (23y) OS (19y)	III-1 III-2	c.1048_1049del	ex. 5	c.1878+32_1878+55del	IVS11	**This work**
SCC and BSC (≤39y)	RTS Pt 15	c.1078C>T	ex. 5	c.1222C>T	ex. 6	[26]
OS (15y)	RAPA r504	c.1390+2del	IVS7	c.1390+2del	IVS7	[8]
OS (10y)	RAPA Pt 7	c.1390+2del	IVS7	c.1390+2del	IVS7	[8]
Lymphoma (21y) Lymphoma (25y)	RAPA r903 RAPA r904	c.1390+2del	IVS7	c.1390+2del	IVS7	[8]
Lymphoma (33y)	RAPA Pt 6	c.1390+2del	IVS7	c.3599_3600del	ex. 21	[8]
OS (21y) OS (7y)	RTS II-1 RTS II-2	c.1391-1G>A	IVS7	c.[1568G>C;1573delT][a]	ex. 9	[8]
OS (20y) OS (9y)	RTS FCP-153 and sibling	c.1391-1G>A	IVS7	c.[1568G>C;1573delT][a]	ex. 9	[8]
OS (≤2y)	RTS Pt 12	c.1391-1G>A	IVS7	c.2085del	ex. 13	[26]
OS (11y) OS (12y)	RTS FCP-102 and sibling	c.1483+25del	IVS8	c.1483+25del	IVS8	[8]
OS (14y) MFH (15y)	RTS IV-4 RTS IV-5	c.1483+27del	IVS8	c.1483+27del	IVS8	[8]
OS (10y)	RTS	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) OS (15y)	RTS II-3 RTS II-6	c.1650del	ex. 10	c.2269C>T	ex. 14	[8]
Lymphoma (9y)	RTS	c.1704+1G>A	IVS10	c.1919_1924del	ex. 12	[32]

OS (14y) Leukemia (21y)						
OS (8y)	RTS FCP-210	c.1718delA	ex. 11	c.1878+32del	VS11	[8]
OS (7y)	RTS FCP-136	c.1878+5G>A	IVS11	c.2476C>T	ex. 15	[8]
Lymphoma (2y)	RTS Pt 8	c.1913T>C	ex. 12	c.2419ins5	ex. 14	[8]
OS (17y)	RTS	c.2232_3007del C[b]	ex14-17	c.2232_3007del[b]	ex14-17	[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	-[c]	-	[8]
Patients with malignancy carrying alterations downstream the helicase domain (exons 8-15)						
OS (3y)	RTS FCP-203	c.3072_3073del	ex. 18	c.3276del	ex. 19	[8]

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* AMPLICONS**	**PRIMER (5′ TO 3′)**	**LENGTH (bp)**
5′ UTR - IVS3	**F:** TTGACGCCTCCCATTGGCT **R:** TTGGTCGCAGCCCGATTCA	815
IVS3 - Exon 5	**F:** AGAACTTGGGAGGGGGACTG **R:** CACTGTGACATCGCTGTAACC	791
Exon 5 - IVS5	**F:** GCAGAAAAAGTCAGTGATGAGC **R:** TGGGCGGGAAATACGGGAGG	747
IVS5 - Exon 7	**F:** CATTCCCTTTCCCTCCCCTCA **R:** CTGCTCACCTGCCAACTGCCC	644
IVS6 - IVS9	**F:** CTCCCATTCTACCCTCTCCT **R:** CTGCCTTTGACCTGCTGCCA	709
Exon 9 - Exon 10	**F:** TCTCTCCCCTGCTGCTGTCACTC **R:** GATTCCCGTTGCTTCCTGGT	513
IVS9 - IVS11	**F:** GGGCTGGGCTGGCGTATG **R:** CCGCCCACCCCAGTTCACAT	409
Exon 11 - IVS12	**F:** CAGTTGCTTTTGCCTGCATT **R:** ACCTGGTCTGTGTCCCTGTC	396
IVS12 - Exon 15	**F:** CTCCTCATCAGGCACTGTTG **Fseq:** ATGAAGGCTCGCTGTACCC **R:** GAGGACACAGAGCGGATCG **Rseq:** CCTCTTCACAGCCAGGAAGT	850
IVS13 - IVS16	**F:** CCCATCCCACTGACCATCT **Fseq:** AGCCCCCAGTGGTCCACC **R:** CTCCAACCTCGTCTCCAACT **Rseq:** AGGAAGAGGTGGCAGTGGG	912
Exon 16 - IVS19	**F:** GCGACCACCTATACCCATTG **R:** CATCCACAGAGCAAGCCCC	873

IVS18 - Exon 21	**F:** TCCTCCCCACAGCGTAGCC **R:** ACTGCCCTAGCCTCTGACAA	709
IVS20 - 3′ UTR	**F:** AGGACCGACGCTTCTGGAG **R:** TGTGCCTGGAATATGTGATGTG	303

Table S3. Primer used for amplification and sequencing of *RECQL4* transcript

FAMILY	*RECQL4* cDNA AMPLIFIED REGION	PRIMER (5′ to 3′)	LENGTH (bp)
A	exon 16-17 junction - exon 20-21 junction	**F:** CCCACAGGTGTCCCCCTTT **R:** GGTAGCAGGGGCTTCCGATG	639
B	exon 5 – exon 7	**F:** AGCCCCTCCAGTCAAGCTAG **R:** TGAAGGAACCAGTGGCTCAG	342
	exon 9 – exon 12-13 junction	**F:** TCTCTCCCCTGCTGTCACTC **R:** AGCGTCAACAGTGCCTGGTC	486
C	exon 7 – exon 12	**F:** ACCGTGCTGCCACTCTACTC **R:** GAAGCAGTGCACGCCCAT	569
	exon 15 –exon 20	**F:** ACCGTACAGGCTTTGGACAT **R:** GAACTTCTCCTCTGGCCTCA	741

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