EUROPEAN HEMATOLOGY ASSOCIATION
haematologica
Journal of the European Hematology Association

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Haematologica 2017 [Epub ahead of print]

Citation: Cardoso SR, Ellison ACM, Walne AJ, Cassiman D, Raghavan M, Kishore B, Ancliff P, Rodríguez-Vigil C, Dobbels B, Rio-Machin A, Al Seraihi AFH, Pontikos N, Tummala H, Vulliamy T, and Dokal I. Myelodysplasia and liver disease extend the spectrum of RTEL1 related telomeropathies. Haematologica. 2017; 102:xxx
doi:10.3324/haematol.2017.167056

Myelodysplasia and liver disease extend the spectrum of RTEL1 related telomeropathies

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Running title: **Expanding the RTEL1 clinical spectrum**

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Word count main text: 1489

2 Tables, 1 Figure and 2 Supplemental tables

Regulator of telomere elongation helicase 1 (RTEL1) is a DNA helicase involved in telomere maintenance.[1,2] Germline biallelic *RTEL1* variants have been previously reported in a subset of patients with dyskeratosis congenita (DC) and its severe variant Hoyeraal-Hreidarsson syndrome (HH).[3-6] Furthermore, germline heterozygous *RTEL1* variants have been linked to a subset of patients with pulmonary fibrosis.[2,7,8] We have undertaken sequencing analysis (whole exome and targeted[9]) of *RTEL1*, using genomic DNA extracted from peripheral blood of 429 patients from our international bone marrow failure registry which includes DC, HH, aplastic anemia (AA), and familial myelodysplasia/leukemia (MDS/AML). This has revealed that 35 out of the 429 patients have *RTEL1* variants (Table 1). Based on the minor allele frequency in the population reported on Exome Aggregation Consortium database (ExAC – http://exac.broadinstitute.org/), the type of variant (missense, nonsense and indels), telomere length, the Combined Annotation Depletion (CADD) score[10], and segregation as well as information found in the literature, we classified these variants into four different groups: (1) biallelic variants, (2) heterozygous loss of function (LOF) variants, (3) heterozygous missense variants of unknown significance (VUS) and (4) heterozygous missense bystander variants.

As a result, we have further defined the relationship between variants in the *RTEL1* gene and this spectrum of disease. The initial disease association was made when biallelic *RTEL1* variants were shown to cause early onset of a severe form of DC and HH.[3,5,6,11] Here, we describe five new biallelic families (Figure 1A and 1B), where the variants are believed to be disease causing in four (Families 1-4). Two families presented with AA, two with DC and one with HH (Table 1 and Table S1). Interestingly, in one of these the index case presented with AA in adulthood. In the HH family (Family 5), we believe the homozygous *RTEL1* variant is a bystander as it did not segregate with disease, being homozygous in both the index case, with severe disease in infancy, and in the asymptomatic 25 year old mother.

Three disease causing heterozygous LOF *RTEL1* variants (nonsense and frameshift deletion) were found in patients from four unrelated families (Figure 1B and 1C), one of whom presented with DC and the others with MDS and/or liver disease. Therefore, this extends the phenotypes associated with heterozygous loss of function *RTEL1* variants to include late onset of MDS and liver disease (Figure 1C and Table 2). This combination of hematological and liver disease is very

reminiscent of that established for heterozygous variants in another telomere related gene, TERT[12], which can also present with a severe early onset disease when the variants are biallelic.[13]

The families we present clearly illustrate the variable penetrance of heterozygous *RTEL1* variants. This is exemplified by Family 6 (Figure 1C, p.R1010*) where the index case had DC features, which did not become apparent until age 77 years. His daughter had liver disease at age of 52 years, and segregation analysis identified four asymptomatic carriers aged below 50 years. This family highlights not only variable penetrance of heterozygous LOF variants but also suggests a late onset disease predisposition. The same *RTEL1* variant was identified in Family 7 (Figure 1C and Table 2), where it was associated with MDS and nail dystrophy in the 45 year old index case. Interestingly, this same variant is reported by Ballew et al[6] in a heterozygous state as being the cause of HH in two siblings (aged three and one years) with very short telomeres. In that family, the mother also harboured the variant, had short telomeres but was asymptomatic. Indeed, in most of the families where the index case has disease due to biallelic *RTEL1* variants, both here and in previous reports, the heterozygous parents are generally asymptomatic. However, we now note that these individuals may nevertheless be predisposed to developing disease in their later years. This is suggested by Family 2 (Figure 1A, p.G1096W) where there is a history of pulmonary disease in the grandmother in her 70s and for the p.R998* variant, which has been seen in both severe recessive[3,5,6] and late onset dominant settings (Families 4 and 8 and Cogan et al[7]). Thus, it is important to be careful when counselling families.

We have identified 14 unrelated patients (Table 1 and Table S2) with nine heterozygous missense variants we believe to be bystanders due to their occurrence at an allele frequency of more than 1 in 3,000 in the ExAC population. Additionally, 12 unrelated patients with DC (n=5), AA (n=5), MDS (n=1) and AML (n=1) were found to harbour rare heterozygous missense variants. We have classified these as variants of unknown significance (VUS) as they are either not seen in the ExAC population or are present at an allele frequency of less than 1 in 10,000 (Table 1 and Table S2). The average CADD score for these VUS (average 15.43, range 0.001 – 33), is lower compared to those that we believe to be disease-causing (average 30.13, range 12.9 – 37, Table 1). We also note that of the 15 heterozygous *RTEL1*

variants previously reported to be associated with pulmonary disease[2,7,8], eight of them are missense.

We have measured telomere lengths by monochrome multiplex quantitative PCR[14] in peripheral blood DNA from all patients bar one, which had poor DNA quality (Table 1 and Figure 1D). In agreement with previous studies reporting the impact of *RTEL1* variants on telomere length, we observed that patients with biallelic variants and those with heterozygous loss of function variants had significantly shorter telomeres than controls as determined by the age-adjusted T/S ratio (p=0.0005 and p=0.003 respectively, 1 way ANOVA with Dunn's multiple comparison test). The median age adjusted T/S ratio for the biallelic group is below the 1st centile (-0.6 compared with -0.54) and for the LOF group is below the 10th centile (-0.43 compared with -0.34). It is interesting to note that for the VUS variants, there appears to be two subgroups. The lower four points correspond to p.G664V, p.P908R, p.R981W and p.T1377A. Three of these variants affect key domains within the protein and may impact on the function of RTEL1 (Figure 1B). These are the helicase C domain (G644V) and the harmonin domain (P908R and R981W).

Previously we reported a recurrent missense variant p.R981W as a compound heterozygote in three young unrelated probands (under 12 years old) with HH.[3] Here, we observed the same variant in a heterozygous state in a 24 year old patient with AA from a consanguineous family (patient 14 in Table 1 and Table S2). In this case, there is no strong evidence that this variant p.R981W is the cause of AA on account of the relatively high frequency of this variant in the ExAC population (6 in 119930 alleles). However, we do note the short telomeres in this patient and the very high CADD score of this variant, indicating the possibility that it acts as a risk factor for disease.

When a patient presents with an *RTEL1* variant, a difficulty therefore arises as to whether or not it should be considered pathogenic, as there are a multitude of rare coding *RTEL1* variants in the population at large. Using the ExAC database, the sum of number of very rare heterozygous coding alleles (at a frequency of <0.0001) is 1,195 in an average of approximately 56,700 people. This is significantly lower than the number of very rare coding variants that we have identified in our cohort (22 in 429 patients, Fisher's exact test, P = 0.003), but on a case-by-case basis this background poses a problem. In addition to looking at the ExAC database for

population frequency there are several parameters that we have used to assign pathogenic status. The association of the rare variant with the pathology is a given, if the patient under review is presenting with one of the *RTEL1* related disease features. Telomere length measurement is now widely used and our experience here is that the heterozygotes, who are often more elderly, may have telomere lengths that are short, but not necessarily very short. We have also looked at T-circles[15], and shown that in some cases their presence is clearly increased where there is a loss of function variant compared to a common missense variant (Figure 1E). However, this test is not easy to perform and a normal range has not been established. The in silico prediction tools are helpful and improving, but remain a guide, and by no means a definitive test. Finally, the segregation of the variant with disease can be decisive. This is more often the case in exclusion rather than inclusion as we show in Family 5 (Figure 1A and Table S1).

In summary, our study reports on several important observations. Firstly, heterozygous LOF *RTEL1* variants are associated with myelodysplasia and liver disease in adulthood. Secondly, biallelic *RTEL1* variants can present with just bone marrow failure in adulthood. Thirdly, many heterozygous variants and even some biallelic *RTEL1* variants are bystanders. Therefore, in order to assign an accurate status to each *RTEL1* variant, detailed clinical and laboratory studies are necessary.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors would like to thank all the clinicians and patients who have helped us over the years, particularly Dr Kiliz. Financial support was provided by The Brazilian National Council for Scientific and Technological Development, Bloodwise, Children with Cancer and the Medical Research Council, UK.

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Table 1. *RTEL1* variants identified in 35 index cases

		Index	Diagnosis	Age (years)	Sex	Telomere length T/S ratio (percentile)	DNA change	Protein change	ExAC frequency[#]	CADD PHRED
Biallelic	**Likely pathogenic**	1	AA	36	F	0.46 (1st)	c.2942G>A (homozygous)	p.R981Q	1 in 119934	28.4
		2	AA	12	F	0.49 (<10th)	c.3286G>T (homozygous)	p.G1096W	1 in 118164	26.2
		3	DC	14	M	0.4 (<1st)	c.2300G>A (homozygous)	p.R767Q	NR	27.7
		4	DC	15	F	0.64 (<10th)	c.2785_2787delCAG (heterozygous)	p.Q929del	NR	12.9
							c.2992C>T (heterozygous)	p.R998*	2 in 119914 (1 in 59,957)	37
	Bystander	5	HH	2	F	0.45 (<1st)	c.1716C>G (homozygous)	p.I572M	NR	24.9
Heterozygous	**LOF Likely pathogenic**	6	DC	77	M	0.5 (<10th)	c.3028C>T	p.R1010*	10 in 119716 (1 in 11,972)	34
		7	MDS	45	F	0.47 (<10th)	c.3028C>T	p.R1010*	10 in 119716 (1 in 11,972)	34
		8	MDS	55	M	0.73 (<50th)	c.2992C>T	p.R998*	2 in 119914 (1 in 59,957)	37
		9	MDS	54	M	0.48 (<10th)	c.3012_3028del	p.Q1005Kfs*80	NR	34
	Unknown significance	10	AML	23	F	1.14 (>50th)	c.3464C>T	p.T1155M	5 in 117408 (1 in 23,482)	12.94
		11	MDS	20	F	0.95 (<50th)	c.2965G>C	p.E989Q	3 in 119938 (1 in 39,980)	22
		12	DC	10	M	0.39 (<1st)	c.2723C>G	p.P908R	1 in 119146	0.001
		13	DC	24	M	0.99 (>50th)	c.208C>T	p.R70C	10 in 120456 (1 in 12,046)	25.3
		14	AA	24	F	0.65 (<10th)	c.2941C>T	p.R981W	6 in 119930 (1 in 19.988)	33
		15	AA	6	F	1.04 (>50th)	c.2351C>T	p.A784V	6 in 118274 (1 in 19,712)	2.88
		16	AA	28	F	1.56 (>90th)	c.3595G>A	p.G1199R	4 in 107372 (1 in 26,843)	5.246
		17	DC	8	F	0.98 (>90th)	c.1603A>G	p.I535V	NR	9.212
		18	DC	18	M	0.95 (<50th)	c.3430G>A	p.V1144M	NR	23.6
		19	AA	28	M	0.56 (<10th)	c.4129A>G[†]	p.T1377A	NR	1.406
		20	AA	10	F	1.01 (>50th)	c.3608G>A	p.S1203N	NR	23.5
		21	DC	16	F	0.47 (<10th)	c.1991G>T	p.G664V	NR	26.1
	Bystander	22	DC	18	M	1.21 (>50th)	c.2618G>A	p.G873D	249 in 19124 (1 in 77)	10.29
		23	DC	37	M	0.64 (<10th)	c.2516G>T	p.S839I	126 in 71024 (1 in 564)	17.05
		24	AA	4	F	1 (>50th)	c.3047C>T	p.P1016L	184 in 119184 (1 in 648)	10.85
		25	DC	50	F	0.94 (<50th)	c.3047C>T	p.P1016L	184 in 119184 (1 in 648)	10.85
		26	DC	NA	M	NA	c.3047C>T	p.P1016L	184 in 119184 (1 in 648)	10.85
		27	MDS/AML	61	F	0.63 (<10th)	c.3128A>G	p.Q1043R	151 in 118626 (1 in 786)	0.276
		28	DC	4	M	0.85 (<50th)	c.3992G>A[†]	p.R1331Q	120 in 101400 (1 in 845)	12.7
		29	DC	3	M	1.51 (>90th)	c.3992G>A[†]	p.R1331Q	120 in 101400 (1 in 845)	12.7
		30	HH	0	M	0.69 (<50th)	c.2734G>C	p.V912L	85 in 117986 (1 in 1,388)	6.325
		31	DC	54	M	0.47 (<10th)	c.4159C>T[†]	p.P1387S	71 in 110950 (1 in 1,563)	24.7
		32	AA	7	M	1.34 (>50th)	c.4159C>T[†]	p.P1387S	71 in 110950 (1 in 1,563)	24.7
		33	DC	31	M	0.64 (<10th)	c.1261C>G	p.Q421E	71 in 120318 (1 in 1,695)	24.2
		34	AA	34	M	0.6 (<10th)	c.1261C>G	p.Q421E	71 in 120318 (1 in 1,695)	24.2
		35	DC	3	M	1.44 (>50th)	c.3121G>A	p.D1041N	43 in 118650 (1 in 2,759)	14.34

NR: not reported; CADD PHRED: combined annotation dependent depletion score; AA: aplastic anemia; AML: acute myeloid leukemia; DC: dyskeratosis congenita; HH: Hoyeraal Hreidarsson syndrome; MDS: myelodysplasia. [†]variant is not in the canonical transcript ENST00000508582 seen in ExAC, but is found in ENST00000482936. Centiles for T/S ratios, established from a healthy control population (n = 202) are as follows: 99th centile = 1.99, 90th centile = 1.47, 50th centile = 0.96, 10th centile = 0.68, 1st centile = 0.46. Telomeres are considered short if they are at or below the 10th centile, and very short if they are at or below the 1st centile.
[#]: For each of the rare variants reported (less than 10 heterozygotes), the ethnicity of our patient matched at least one reported on the ExAC.
NB: Six index cases harbour variants in other known disease genes: index cases 11, 15, 28, 31, 33 and 35 harbour variants in *TERT* (heterozygous c.3197C>T; p.P1066L and c.322C>T; p.R108C), *DNAJC21* (homozygous c.793G>T; p.Q265*), *TERT* (heterozygous c.1336_1337insC; p.R446Pfs93* and c.329G>C; p.G110A), *TERT* (homozygous c.3150G>C; p.K1050N) and *TERC* (heterozygous c.205C>T), *TINF2* (heterozygous c.838A>G; p.K280Q), and *DKC1* (hemizygous c.941A>C; p.K314T), respectively.

Table 2. Characteristics of families with *RTEL1* LOF variants

Family	Individuals	Age at study (years)	Gender	Clinical status	Nucleotide	Amino acid	Variant status	Clinical features/diagnosis
6	I-1	NA	F	asymptomatic	NA	NA	NA	None
	I-2	NA	M	asymptomatic	NA	NA	NA	None
	II-1	NA	F	asymptomatic	NA	NA	NA	None
	II-2	77	M	affected	c.3028C>T	p.R1010*	Heterozygous	DC, lacy skin pigmentation, pancytopenia, pulmonary fibrosis, cirrhosis
	II-3	NA	F	asymptomatic	NA	NA	NA	None
	III-1	NA	M	asymptomatic	NA	NA	NA	None
	III-2	54	F	asymptomatic	c.3028C>T	p.R1010*	Heterozygous	None
	III-3	52	F	affected	c.3028C>T	p.R1010*	Heterozygous	Liver disease (non-specific hepatitic changes)
	III-4	49	F	asymptomatic	Wild type	Wild type	Wild type	None
	III-5	47	M	asymptomatic	c.3028C>T	p.R1010*	Heterozygous	None
	III-6	NA	F	asymptomatic	NA	NA	NA	None
	IV-1	20	F	asymptomatic	Wild type	Wild type	Wild type	None
	IV-2	17	F	asymptomatic	Wild type	Wild type	Wild type	None
	IV-3	16	F	asymptomatic	c.3028C>T	p.R1010*	Heterozygous	None
	IV-4	23	F	asymptomatic	Wild type	Wild type	Wild type	None
	IV-5	21	F	asymptomatic	Wild type	Wild type	Wild type	None
	IV-6	7	F	asymptomatic	c.3028C>T	p.R1010*	Heterozygous	None
	IV-7	4	M	asymptomatic	Wild type	Wild type	Wild type	None
7	I-1	NA	F	NA	NA	NA	NA	NA
	I-2	NA	M	NA	NA	NA	NA	NA
	III-1	45	F	affected	c.3028C>T	p.R1010*	Heterozygous	MDS, nail dystrophy
8	I-1	NA	F	asymptomatic	NA	NA	NA	None
	I-2	NA	M	asymptomatic	NA	NA	NA	None
	II-1	55	M	affected	c.2992C>T	p.R998*	Heterozygous	MDS (low risk), cirrhosis
	II-2	47	M	affected	c.2992C>T	p.R998*	Heterozygous	MDS (low risk), cirrhosis
	II-3	NA	F	asymptomatic	NA	NA	NA	None
9	I-1	46	F	affected	NA	NA	NA	Liver and lung disease
	I-2	NA	M	asymptomatic	NA	NA	NA	None
	II-1	NA	M	asymptomatic	NA	NA	NA	None
	II-2	NA	F	asymptomatic	NA	NA	NA	None
	III-1	NA	F	asymptomatic	NA	NA	NA	None
	III-2	54	M	affected	c.3012_3028del	p.E1005Kfs*80	Heterozygous	MDS (low risk), interstitial lung disease, cirrhosis, osteoporosis, baldness and psoriatiform skin
	III-3	49	F	asymptomatic	Wild type	Wild type	Wild type	None
	IV-1	32	F	asymptomatic	c.3012_3028del	p.E1005Kfs*80	Heterozygous	None
	IV-2	30	F	asymptomatic	Wild type	Wild type	Wild type	None

DC: dyskeratosis congenita; MDS: myelodysplasia; NA: not available; F: female; M: male.

Figure legends

Figure 1. The segregation, location and impact of *RTEL1* variants. **(A)** Families with biallelic *RTEL1* variants and sequencing traces of index cases (homozygous, Families 1, 2, 3 and 5; compound heterozygous, Family 4). The genotyping is described as follows: wild-type (+/+), heterozygous (+/-) or biallelic (-/-). The age at study is given in years. Affected individuals are coloured in black. NA: not available. **(B)** RTEL1 protein (NP_116575.3) schematic showing in blue the location of the biallelic variants and in red the heterozygous LOF variants. Conserved protein domains include the P-loop NTPase (yellow); the Rad3 domain (green) that includes the DEAD2 domain (red) and the Helicase C-terminal domain (purple); Harmonin N-like domain (blue); PIP-box – the proliferating cell nuclear antigen interacting protein domain (black). **(C)** Families with heterozygous LOF *RTEL1* variants and sequencing traces of index cases, annotated as in panel A. **(D)** Age adjusted telomere length values (delta-tel) were measured by subtracting the observed T/S ratio from the expected T/S ratio, using the equation derived from the line of best fit through the plot of T/S ratios from healthy control samples against age. Patients with *TERC* variants are included as a group with known short telomeres. Centiles were calculated from the control delta-tel values as follows: 99^{th} centile = 0.95, 90^{th} centile = 0.42, 50^{th} centile = 0.06, 10^{th} centile = -0.34, 1^{st} centile = -0.54. The different genotypes are represented as follows, TERC-circles (n=44); biallelic-squares (n=5); loss of function (LOF)-triangles (n=6); variants of unknown significance (VUS)-diamonds (n=12); bystanders-inverted triangles (n=13); controls-grey squares (n=202). **(E)** T-circle amplification using Phi29 polymerase detected by Southern blot analysis. Samples: p.R70C - patient with sporadic DC carrying this variant of unknown significance (patient 13 in Table 1); p.R998* - proband of Family 8 carrying this LOF variant (Table 1); positive control - genomic DNA extracted from WI-38 VA-13 cells, known to produce T-circle.

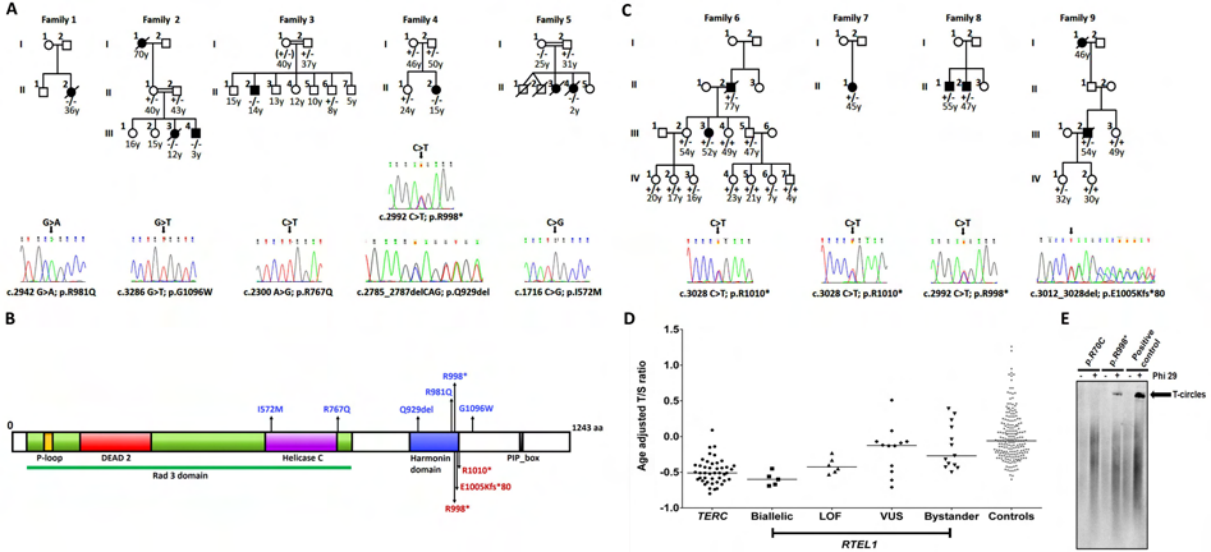
A
Family 1
Family 2
Family 3
Family 4
Family 5
C
Family 6
Family 7
Family 8
Family 9
c.2992 C>T; p.R998*
c.2942 G>A; p.R981Q
c.3286 G>T; p.G1096W
c.2300 A>G; p.R767Q
c.2785_2787delCAG; p.Q929del
c.1716 C>G; p.I572M
c.3028 C>T; p.R1010*
c.3012_3028del; p.E1005Kfs*80
B
R998*
R981Q
I572M
R767Q
Q929del
G1096W
0
1243 aa
P-loop
DEAD 2
Helicase C
Harmonin domain
PIP_box
Rad 3 domain
R1010*
E1005Kfs*80
R998*
D
Age adjusted T/S ratio
TERC
Biallelic
LOF
VUS
Bystander
Controls
RTEL1
E
p.R76C
p.R998*
Positive control
Phi 29
T-circles

Table S1. Characteristics of families with biallelic *RTEL1* variants

Family	Individuals	Age at study (years)	Gender	Clinical status	Nucleotide	Amino acid	Variant status	Clinical features/diagnosis
1	I-1	NA	F	asymptomatic	NA	NA	NA	None
	I-2	NA	M	asymptomatic	NA	NA	NA	None
	II-1	NA	M	asymptomatic	NA	NA	NA	None
	II-2	36	F	affected	c.2942G>A	p.R981Q	Homozygous	AA; short stature
2	I-1	70	F	affected	NA	NA	NA	Pulmonary fibrosis
	I-2	NA	M	NA	NA	NA	NA	NA
	II-1	40	F	asymptomatic	c.3286G>T	p.G1096W	Heterozygous	None
	II-2	43	M	asymptomatic	c.3286G>T	p.G1096W	Heterozygous	None
	III-1	16	F	asymptomatic	NA	NA	NA	None
	III-2	15	F	asymptomatic	NA	NA	NA	None
	III-3	12	F	affected	c.3286G>T	p.G1096W	Homozygous	AA, pulmonary fibrosis, corrugated tongue
	III-4	3	M	affected	c.3286G>T	p.G1096W	Homozygous	AA
3	I-1	40	F	asymptomatic	c.2300G>A	p.R767Q	Heterozygous	None
	I-2	37	M	asymptomatic	c.2300G>A	p.R767Q	Heterozygous	None
	II-1	15	M	asymptomatic	NA	NA	NA	None
	II-2	14	M	affected	c.2300G>A	p.R767Q	Homozygous	DC, blepharitis, conjunctivitis, pancytopenia, atrial septal defect, low birth weight, growth restriction
	II-3	13	M	asymptomatic	NA	NA	NA	None
	II-4	12	F	asymptomatic	NA	NA	NA	None
	II-5	10	M	asymptomatic	NA	NA	NA	None
	II-6	8	M	asymptomatic	c.2300G>A	p.R767Q	Heterozygous	None
	II-7	5	M	asymptomatic	NA	NA	NA	None
4	I-1	46	F	asymptomatic	c.2785_2787delCAG	p.Q929del	Heterozygous	None
	I-2	50	M	asymptomatic	c.2992C>T	p.R998*	Heterozygous	None
	II-1	24	F	asymptomatic	c.2992C>T	p.R998*	Heterozygous	None
	II-2	15	F	affected	c.2785_2787delCAG c.2992C>T	p.Q929del p.R998*	Compound heterozygous	DC
5	I-1	25	F	asymptomatic	c.1716C>T	p.I572M	Homozygous	None
	I-2	31	M	asymptomatic	c.1716C>T	p.I572M	Heterozygous	None
	II-1	still birth	M	NA	NA	NA	NA	NA
	II-2	Still birth	M	NA	NA	NA	NA	NA
	II-3	6 weeks	NA	affected	NA	NA	NA	Turner syndrome
	II-4	2	F	affected	c.1716C>T	p.I572M	Homozygous	HH
	II-5	NA	F	NA	NA	NA	NA	NA

AA: aplastic anemia; DC: dyskeratosis congenita; HH: Hoyeraal Hreidarsson syndrome; NA: not available; F: female; M: male.

Table S2. Characteristics of index cases with heterozygous VUS and bystander *RTEL1* variants

	Index	Age at study (years)	Gender	Diagnosis	Additional relevant clinical features
Unknown significance	10	23	F	AML	Short stature
	11	20	F	MDS	Skin pigmentation abnormality and squamous cell carcinoma of oesophagus. This patient harbours variants in *TERT* (heterozygous c.3197C>T; p.P1066L and c.322C>T; p.R108C)
	12	10	M	DC	Developmental delay, short stature, dysmorphic facial features, microcephaly, BMF and pulmonary disease
	13	24	M	DC	Skin pigmentation abnormality, leukoplakia, thin hair and BMF
	14	24	F	AA	
	15	6	F	AA	Short stature and oral ulceration with dysphagia. This patient harbours variant in *DNAJC21* (homozygous c.793G>T; p.Q265*)
	16	28	F	AA	
	17	8	F	DC	Nail dystrophy and leukoplakia
	18	18	M	DC	Skin pigmentation abnormality, thin hair, extensive dental caries and BMF
	19	28	M	AA	
	20	10	F	AA	
	21	16	F	DC	Skin pigmentation abnormality, nail dystrophy, leukoplakia, small teeth, sparse scalp hair, epiphora, microcephaly and BMF
Bystander	22	18	M	DC	Skin pigmentation abnormality, nail dystrophy, hair loss, extensive dental caries, developmental delay and short stature
	23	37	M	DC	Skin pigmentation abnormality, nail dystrophy, hair loss, frequent otitis, mild hearing loss and extensive caries/ dental loss
	24	4	F	AA	
	25	50	F	DC	Nail dystrophy, cirrhosis, duodenal ulcers, deafness and developmental delay
	26	NA	M	DC	Skin pigmentation abnormality, nail dystrophy, leucoplakia and leukemia
	27	61	F	MDS/AML	
	28	4	M	DC	Skin pigmentation abnormality, nail dystrophy, microcephaly, low birthweight, developmental delay and cerebellar atrophy. This patient harbours variants in *TERT* (heterozygous c.1336_1337insC; p.R446Pfs93* and c.329G>C; p.G110A)
	29	3	M	DC	Skin pigmentation abnormality, nail dystrophy, abnormal facies, microcephaly, ear abnormality and difficulty in swallowing
	30	0	M	HH	Congenital cytomegalovirus infection, microcephaly, generalized seizures, intracranial calcifications, growth restriction, low birth weight and BMF
	31	54	M	DC	Skin pigmentation abnormality, nail dystrophy, hair loss, tooth loss, renal failure and BMF. This patient harbours variants in *TERT* (homozygous c.3150G>C; p.K1050N) and *TERC* (heterozygous c.205C>T)
	32	7	M	AA	
	33	31	M	DC	Skin pigmentation abnormality, leukoplakia, epiphora, duodenal ulcers, cirrhosis, hepato-pulmonary syndrome and BMF. This patient harbours variant in *TINF2* (heterozygous c.838A>G; p.K280Q)
	34	34	M	AA	
	35	3	M	DC	Skin pigmentation abnormality, nail dystrophy, leucoplakia, hair loss, microcephaly, premature birth with intrauterine growth restriction, glaucoma, premature aging, malabsorption, developmental delay and BMF. This patient harbours variant in *DKC1* (hemizygous c.941A>C; p.K314T)

AML: acute myeloid leukemia; MDS: myelodysplasia; DC: dyskeratosis congenita; AA: aplastic anemia; HH: Hoyeraal Hreidarsson syndrome; BMF: bone marrow failure; NA: not available; F: female; M: male.