

### Clinical diagnostic value of telomere length measurement in inherited bone marrow failure syndromes

Bone marrow failure (BMF) is characterized by a hypocellular marrow and encompasses a diverse group of inherited and acquired disorders. Inherited bone marrow failure syndromes (IBMFS) occur in approximately 5%–30% of patients with BMF in pediatric cohorts and consist of more than 25 defined disease entities, including dyskeratosis congenita (DC), Fanconi anemia (FA), Diamond–Blackfan anemia (DBA), and Shwachman–Diamond syndrome (SDS).<sup>1</sup> IBMFS are a heterogeneous group of disorders in which BMF is usually associated with physical abnormalities. The diagnosis of IBMFS previously relied on the recognition of characteristic clinical features. Recent diagnostic advances using next-generation sequencing have revealed that some patients initially diagnosed with idiopathic aplastic anemia (AA) had cryptic presentations of IBMFS.<sup>2</sup> This issue is important as a more accurate diagnosis may improve treatment outcomes.

Telomeres are the end segments of chromosomes: they are composed of long DNA repeats and a protein complex, and are essential for genome integrity. Germline mutations in genes involved in telomere biology can result in significantly short telomere length (TL) in

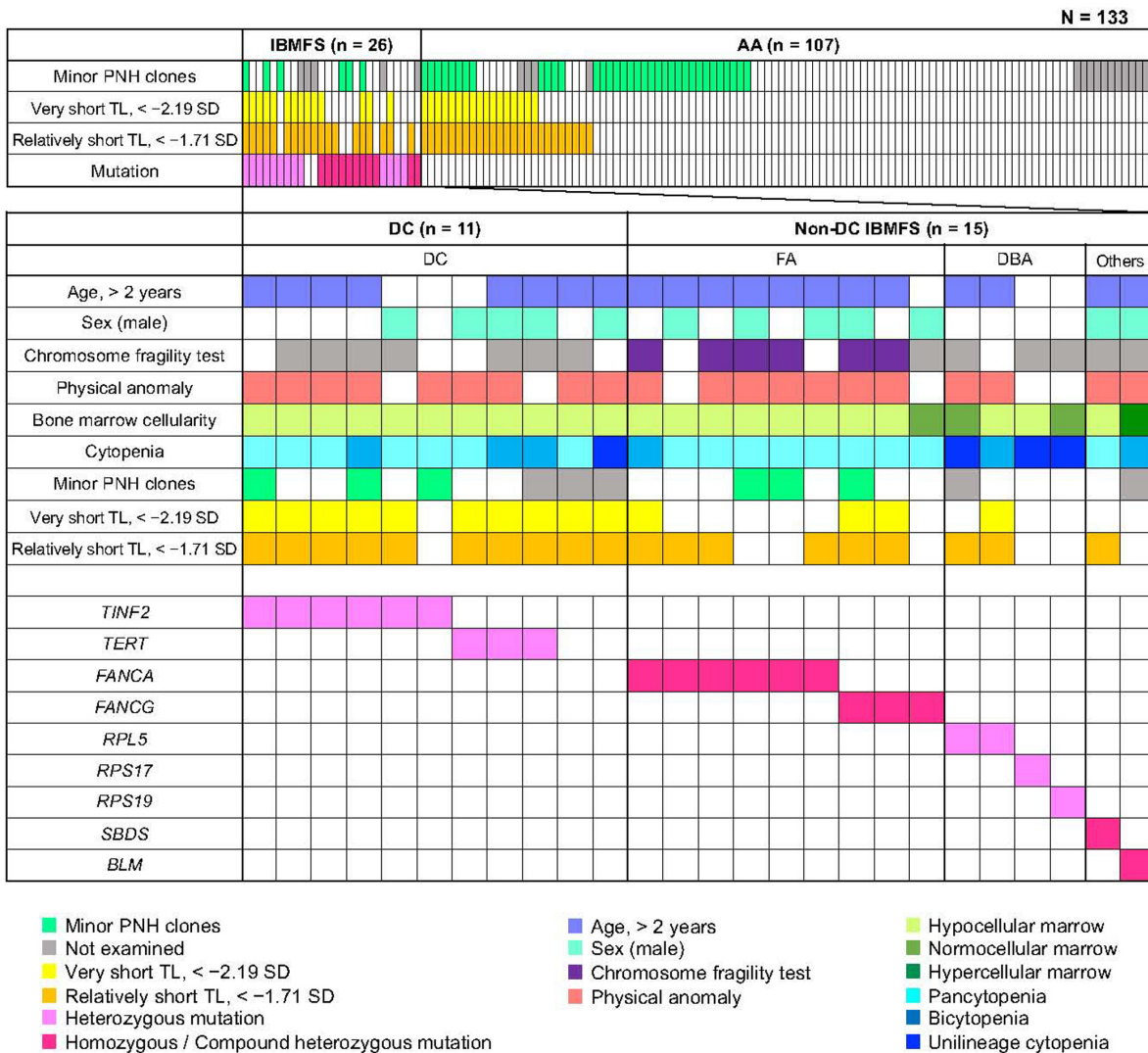
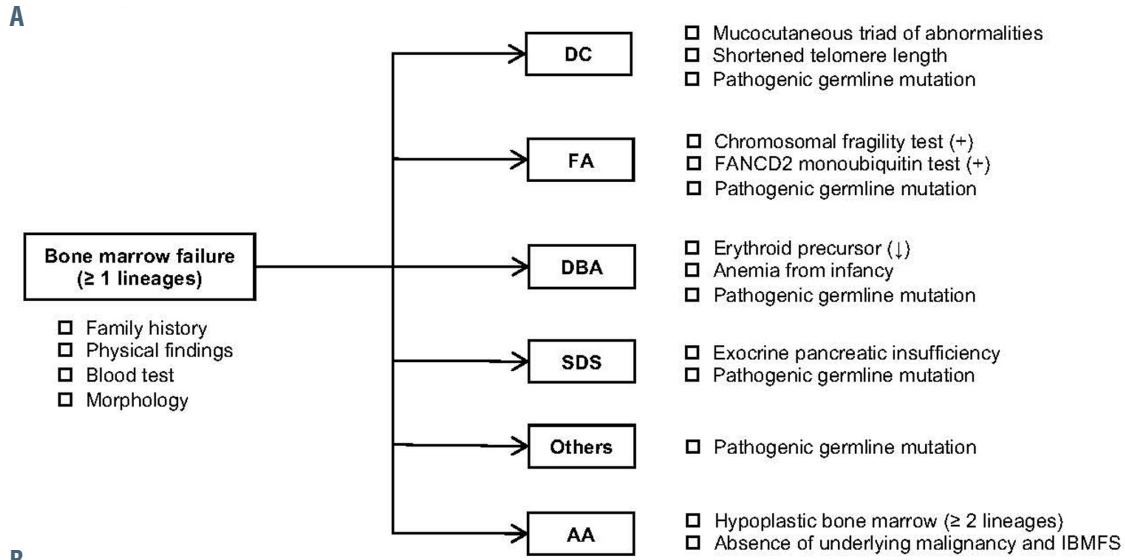
peripheral blood lymphocytes in patients with DC.<sup>3</sup> Although there is a consensus on the usefulness of TL for screening for DC, but not for other IBMFS, several investigators have demonstrated that TL is excessively short in patients with AA<sup>4</sup> and non-DC IBMFS,<sup>5</sup> including FA, SDS, and DBA. To assess the diagnostic value of TL, we measured TL in 133 patients with BMF and compared it to that in patients with DC, non-DC IBMFS, and AA.

We retrospectively studied 133 patients (68 male and 65 female) with BMF in Japan between 2013 and 2018. We collected peripheral blood samples at diagnosis from all patients, measured TL from peripheral blood lymphocytes, and performed targeted sequencing analysis covering 184 genes associated with IBMFS (*Online Supplementary Table S1*), as described in our previous studies.<sup>2,4</sup> TL was measured by flow-fluorescence *in situ* hybridization (flow-FISH) using a Telomere PNA Kit (Dako Cytomation, Glostrup, Denmark) according to the manufacturer's instructions. We calculated the age-adjusted relative TL in terms of the standard deviation (SD) from 71 normal, age-matched, healthy controls (median age, 29 years; range, 1–47 years) as previously described.<sup>4</sup> In 112 of 133 (84%) patients, paroxysmal nocturnal hemoglobinuria-type granulocytes and red blood cells were also evaluated by flow cytometry.<sup>4</sup> As thresholds for determining the presence of minor paroxysmal nocturnal hemoglobinuria clones, we defined >0.020% and >0.037% for CD11b<sup>+</sup> CD55<sup>-</sup>

**Table 1. Clinical characteristics and laboratory findings of patients with bone marrow failure.**

	All patients (N = 133)	DC (n = 11)	Non-DC IBMFS (n = 15)	AA (n = 107)	P
Age, years, median (range)	7 (0-22)	7 (1-19)	6 (0-15)	7 (0-22)	0.675
Gender, n (%)					0.851
Male	68 (51)	5 (45)	7 (47)	56 (52)	
Female	65 (49)	6 (55)	8 (53)	51 (48)	
Cytopenia, n (%)					<0.001
Unilineage cytopenia	4 (3)	1 (9)	3 (20)	0 (0)	
Bicytopenia	24 (18)	3 (27)	3 (20)	18 (17)	
Pancytopenia	105 (79)	7 (64)	9 (60)	89 (83)	
Severity, n (%)*					0.024
Moderate	67 (52)	7 (70)	10 (83)	50 (47)	
Severe	35 (27)	2 (20)	2 (17)	31 (29)	
Very severe	27 (21)	1 (10)	0 (0)	26 (24)	
WBC, ×10 <sup>9</sup> /L, median (range)	2.8 (0.3-12.8)	2.7 (1.7-5.3)	3.1 (1.7-7.8)	2.8 (0.3-12.8)	0.374
ANC, ×10 <sup>9</sup> /L, median (range)	0.6 (0.0-5.4)	0.8 (0.6-1.7)	0.9 (0.2-5.4)	0.5 (0-5.2)	0.011
ALC, ×10 <sup>9</sup> /L, median (range)	1.8 (0.1-8.2)	1.3 (0.5-4.3)	2.1 (1.2-5.9)	1.8 (0.1-8.2)	0.065
Hb, g/dL, median (range)	8.0 (2.7-14.3)	8.4 (3.1-14.3)	6.6 (3.7-11.3)	8.1 (2.7-14.0)	0.307
Platelets, ×10 <sup>9</sup> /L, median (range)	2.5 (0.2-40.9)	2.5 (0.3-7.5)	3.6 (0.2-40.9)	2.3 (0.2-38.0)	0.121
ARC, %, median (range)	11.5 (0.0-57.0)	15.5 (4.0-32.0)	12.7 (1.2-39.1)	10.0 (0.0-57.0)	0.106
Minor PNH clones, n (%)					0.632
Positive	42 (32)	3 (27)	3 (20)	36 (34)	
Negative	70 (53)	5 (46)	10 (67)	55 (51)	
Not done	21 (15)	3 (27)	2 (13)	16 (15)	
Very short TL, < -2.19 SD, n (%)	31 (23)	10 (91)	4 (25)	17 (16)	<0.001
Relatively short TL, < -1.71 SD, n (%)	44 (33)	10 (91)	9 (60)	25 (23)	<0.001
TL, SD, median (range)	-0.96 (-5.73 to +4.00)	-3.50 (-5.73 to +0.83)	-1.89 (-4.74 to +2.05)	-0.84 (-4.27 to +4.00)	<0.001

DC: dyskeratosis congenita; IBMFS: inherited bone marrow failure syndromes; AA: aplastic anemia; WBC: white blood cell count; ANC: absolute neutrophil count; ALC: absolute lymphocyte count; Hb: hemoglobin; ARC: absolute reticulocyte count; PNH: paroxysmal nocturnal hemoglobinuria; TL: telomere length; SD: standard deviation; \*excluding patients with unilineage cytopenia.



**Figure 1. Diagnostic flowchart and profiles of patients with bone marrow failure.** (A) Diagnostic flowchart for inherited bone marrow failure syndromes (IBMFS) and aplastic anemia (AA). The diagnoses were based on clinical criteria, syndrome-specific laboratory tests, and genetic analysis using targeted sequencing. (B) Clinical and genetic profiles of 133 patients with bone marrow failure (BMF). Each column indicates one patient. DC: dyskeratosis congenita; FA: Fanconi anemia; DBA: Diamond-Blackfan anemia; SDS: Shwachman-Diamond syndrome; AA: aplastic anemia; IBMFS: inherited bone marrow failure syndromes; PNH: paroxysmal nocturnal hemoglobinuria; TL: telomere length.

CD59<sup>+</sup> granulocytes and glycophorin A<sup>+</sup> CD55<sup>-</sup> CD59<sup>-</sup> erythrocytes, respectively.

We diagnosed patients on the basis of a diagnostic flow-chart (Figure 1A) developed using published diagnostic criteria for specific IBMFS and acquired AA.<sup>6,7</sup> The severity of cytopenia was determined according to the Camitta severity criteria for AA.<sup>8</sup> We divided the 133 patients into three groups: those with DC, those with non-DC IBMFS, and those with AA. All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan).<sup>9</sup> Written informed consent was obtained from patients or their legal guardians. This study was approved by the ethics committee of the Nagoya University Graduate School of Medicine.

Table 1 shows the clinical characteristics of patients included in this study. The median age at diagnosis of the total cohort was 7 years (range, 0-22 years). Of the 133 patients, 105, 24, and 4 were diagnosed with pancytopenia, bicytopenia, and unilineage cytopenia (3 anemia and 1 thrombocytopenia), respectively. In patients with pancytopenia or bicytopenia, severity was assessed as very severe, severe, and moderate in 27, 35, and 67 patients, respectively. The median TL in all 133 patients was -0.96 SD (range, -5.73 to +4.00 SD). Using targeted sequencing, in 24 patients (18%) we detected 35 pathogenic variants (5 nonsense, 13 missense, 5 frameshift, 7 splice site, and 5 deletions) of known causative IBMFS genes, including *TINF2* (n=6), *TERT* (n=3), *FANCA* (n=6),

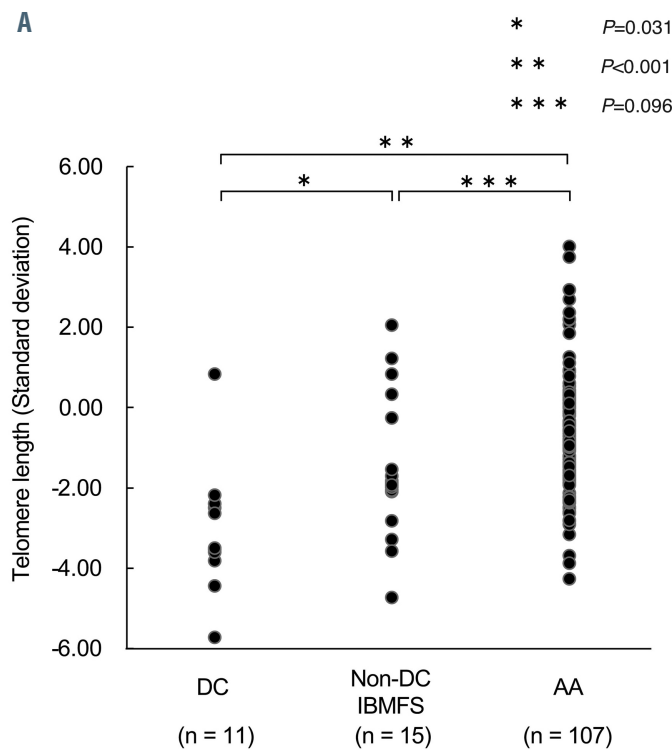
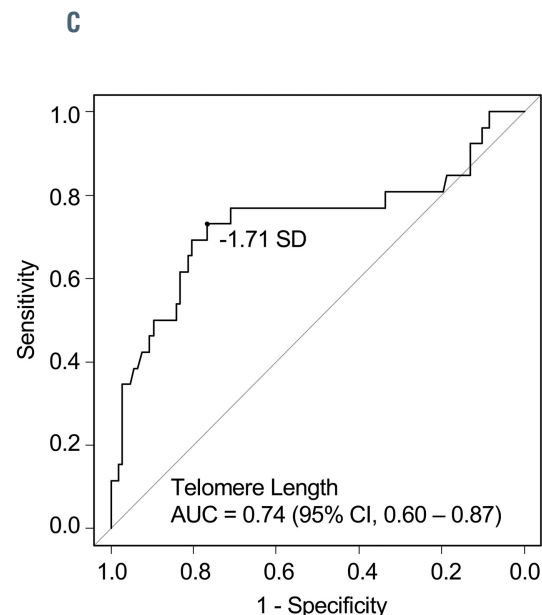
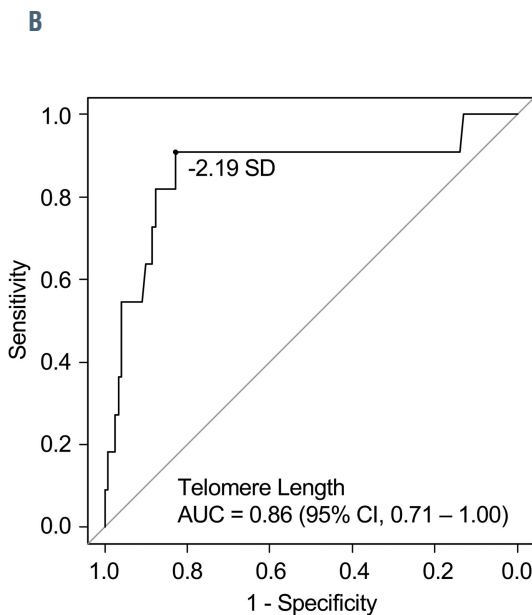


Figure 2. Comparison of peripheral blood lymphocyte telomere length in patients with bone marrow failure. (A) Comparison of standard deviations (SD) in telomere length (TL) in patients with dyskeratosis congenita (DC), non-DC inherited bone marrow failure syndromes (IBMFS), and aplastic anemia (AA). Kruskal-Wallis and Holm tests were used to investigate the relationships among the three groups. *P*-values < 0.05 were considered statistically significant. (B, C) The cut-off values for TL were set according to the optimal combination of sensitivity and false positive rate (1-specificity) derived from receiver operating characteristic curves, which determined <-2.19 SD (very short TL) as the optimal TL threshold for evaluating DC patients, and <-1.71 SD (relatively short TL) for evaluating IBMFS patients, including non-DC patients. AUC, area under the curve.



FANCG (n=3), RPL5 (n=2), RPS19 (n=1), RPS17 (n=1), SBDS (n=1), and BLM (n=1). Homozygous mutations were found in three patients (2 in FANCG and 1 in FANCA), compound heterozygous mutations in four patients (2 in FANCA and 1 each in FANCG and SBDS), hemizygous mutations in three patients in FANCA, and heterozygous mutations in 14 patients (6 in TINF2, 3 in TERT, 2 in RPL5, and 1 each in RPS17, RPS19, and BLM). Each patient's genetic variants are shown in *Online Supplementary Table S2*.

Out of the 133 patients and following the diagnostic flowchart (Figure 1A), 11 were diagnosed with DC (8%), 15 with non-DC IBMFS (11%), and 107 with AA (81%). The schematic representation of the results of gene analysis and the clinical features of IBMFS are shown in Figure 1B. Of the 11 patients with DC, nine were genetically diagnosed (6 with mutations in TINF2 and 3 with mutations in TERT), and those without diagnostic genetic mutations were diagnosed on the basis of clinical criteria. The 15 non-DC IBMFS cases consisted of nine FA, four DBA, one SDS, and one Bloom syndrome. All of these diagnoses were confirmed by the presence of germline mutations in IBMFS-related genes. Physical anomalies were observed in 11 of 15 (73%) patients. The individual clinical features and genetic results of the patients with IBMFS are shown in *Online Supplementary Table S2*.

We compared the clinical characteristics of patients with DC, non-DC IBMFS, and AA (Table 1). The median age and gender distribution did not show significant differences among the three groups. Severe or very severe cytopenia was significantly more frequent ( $P=0.024$ ) in AA cases (57/107, 53%) than in DC (3/10, 30%) and non-DC IBMFS cases (2/12, 17%).

The median TL in patients with DC, non-DC IBMFS, and AA were  $-3.50$  SD (range,  $-5.73$  to  $+0.83$  SD),  $-1.89$  SD (range,  $-4.74$  to  $+2.05$  SD), and  $-0.84$  SD (range,  $-4.27$  to  $+4.00$  SD), respectively (Figure 2A). Patients with DC had significantly shorter TL compared to those with non-DC IBMFS ( $P=0.031$ ) and AA ( $P<0.001$ ). Furthermore, patients with non-DC IBMFS tended to have shorter TL than those with AA ( $P=0.096$ ).

To validate the efficacy of TL measurement in diagnosing DC and IBMFS, receiver operating characteristic curves identified two cut-off values with the optimum sensitivity and false positive rate (1-specificity) combination,  $<-2.19$  SD (for patients with DC) (Figure 2B) and  $<-1.71$  SD (for patients with IBMFS) (Figure 2C), defined as "very short TL" and "relatively short TL," respectively. For the diagnosis of patients with IBMFS, the TL cut-off value at  $-1.71$  SD (relatively short TL) yielded a relatively high negative predictive value (0.921; 95% confidence interval [95% CI]: 0.873-0.958) and a moderately positive predictive value (0.432; 95% CI: 0.333-0.505). Of the total cohort, 44 patients (33%) were classified as having "relatively short TL", which was significantly more frequent ( $P<0.001$ ) in DC (10/11, 91%) and non-DC IBMFS (9/15, 60%) than in AA (25/107, 23%).

Although germline mutations in TL maintenance genes are known to cause very short TL in patients with DC,<sup>3</sup> several studies found that patients with AA also had shorter TL than healthy individuals.<sup>4</sup> Furthermore, several cases of short TL in patients with non-DC IBMFS have been reported (*Online Supplementary Table S3*).<sup>3,5,10-15</sup> Alter *et al.*<sup>5</sup> previously reported that very short telomeres ( $<1^{\text{st}}$  percentile of normal) were observed in five of 78 (6%) patients with non-DC IBMFS, although little overlap was seen in the distribution of TL between DC and non-DC IBMFS cases.

In this study we measured TL by standard flow-FISH in a cohort of BMF patients comprehensively and genetically evaluated by next-generation sequencing. We defined TL  $<-1.71$  SD of normal as a new criterion of "relatively short TL"; the proportions of patients who met this criterion were significantly higher in DC (91%) and non-DC cases (60%) than in AA cases (23%). These results suggest that TL measurement is useful as a screening test for DC and as a clinical diagnostic tool for non-DC IBMFS patients needing comprehensive genetic analysis.

One limitation of this study is its small sample size: we found that 73% (n=26) of IBMFS cases and 23% (n=107) of AA cases in this cohort had "relatively short TL." A power calculation to check "relatively short TL" effectiveness in diagnosing IBMFS concluded that the power of 0.998 was high enough to support the assumption of a sufficient number of cases in this study.

A second limitation of the present study is the small number of non-DC cases (n=15), which was insufficient to discuss the significance of TL measurements in each IBMFS subtype. However, "relatively short TL" ( $<-1.71$  SD) was observed in six of nine FA cases, two of four DBA cases, and one case of SDS (Figure 1B). The median TL were  $-1.84$  SD (range,  $-4.74$  to  $+2.05$  SD),  $-0.89$  SD (range,  $-2.83$  to  $+1.21$  SD), and  $-1.99$  for FA, DBA, and SDS cases, respectively (*Online Supplementary Table S2*). These results support those in previous case reports demonstrating relatively short TL in FA, DBA, and SDS (*Online Supplementary Table S3*). Nevertheless, future studies in larger cohorts of patients are warranted.

This study confirms that a relatively short TL was present in a significant proportion of patients with DC and non-DC IBMFS, indicating the clinical diagnostic value of TL measurement in identifying patients who need further testing, particularly comprehensive genetic analysis.

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**Supplementary Table 1. Target 184 gene list.**

Disease categories	Gene					
Aplastic anemia	<i>PRF1</i>	<i>TERF1</i>	<i>TERF2</i>			
Congenital amegakaryocytic thrombocytopenia	<i>MPL</i>					
Congenital dyserythropoietic anemia	<i>CDAN1</i>	<i>KLF1</i>	<i>SEC23B</i>			
Chromosome fragile syndromes	<i>ATM</i>	<i>BLM</i>	<i>DCLRE1C</i>	<i>LIG4</i>	<i>NBN</i>	<i>RAD50</i>
Diamond-Blackfan anemia	<i>GATA1</i>	<i>RPL31</i>	<i>RPS10</i>	<i>RPS19</i>	<i>RPS29</i>	
	<i>RPL11</i>	<i>RPL35A</i>	<i>RPS14</i>	<i>RPS24</i>	<i>RPS7</i>	
	<i>RPL26</i>	<i>RPL5</i>	<i>RPS17</i>	<i>RPS26</i>		
Dyskeratosis congenita	<i>C16orf57</i>	<i>DKC1</i>	<i>NOP10</i>	<i>TERC</i>	<i>TINF2</i>	<i>POT1</i>
	<i>CTC1</i>	<i>NHP2</i>	<i>RTEL1</i>	<i>TERT</i>	<i>WRAP53</i>	<i>TERF2IP</i>
Fanconi anemia	<i>BRCA2</i>	<i>FANCB</i>	<i>FANCE</i>	<i>FANCI</i>	<i>PALB2</i>	
	<i>BRIP1</i>	<i>FANCC</i>	<i>FANCF</i>	<i>FANCL</i>	<i>RAD51C</i>	
	<i>FANCA</i>	<i>FANCD2</i>	<i>FANCG</i>	<i>FANCM</i>	<i>SLX4</i>	
Hemolytic anemia	<i>ADA</i>	<i>ENO1</i>	<i>GPI</i>	<i>HBB</i>	<i>PGK1</i>	<i>SPTB</i>
	<i>ADD1</i>	<i>EPB41</i>	<i>GPX1</i>	<i>HK1</i>	<i>PIEZO1</i>	<i>TPI1</i>
	<i>AK1</i>	<i>EPB42</i>	<i>GSR</i>	<i>NT5C3</i>	<i>PKLR</i>	
	<i>ALDOA</i>	<i>G6PD</i>	<i>GSS</i>	<i>PFKM</i>	<i>SLC4A1</i>	
	<i>ANK1</i>	<i>GCLC</i>	<i>HBA1</i>	<i>PGD</i>	<i>SPTA1</i>	
Juvenile myelomonocytic leukemia	<i>ASXL1</i>	<i>FLT3</i>	<i>KRAS</i>	<i>NRAS</i>	<i>SETBP1</i>	
	<i>CBL</i>	<i>JAK3</i>	<i>NF1</i>	<i>PTPN11</i>		
Hematological Malignancies	<i>AEBP2</i>	<i>CSF3R</i>	<i>EZH2</i>	<i>KIT</i>	<i>SF3B1</i>	<i>U2AF1</i>
	<i>ATRX</i>	<i>CTCF</i>	<i>FBXW7</i>	<i>LAMB4</i>	<i>SH2B3</i>	<i>U2AF2</i>
	<i>B2M</i>	<i>CUX1</i>	<i>GNAS</i>	<i>LUC7L2</i>	<i>SMC1A</i>	<i>UMODL1</i>
	<i>BCOR</i>	<i>DAXX</i>	<i>GPRC5A</i>	<i>MAP3K4</i>	<i>SMC3</i>	<i>WT1</i>
	<i>BCORL1</i>	<i>DCAF7</i>	<i>IDH1</i>	<i>NCOR2</i>	<i>SRP72</i>	<i>ZRSR2</i>
	<i>BRAF</i>	<i>DIDO1</i>	<i>IDH2</i>	<i>NPM1</i>	<i>SRSF2</i>	<i>ZSWIM4</i>
	<i>BRCC3</i>	<i>DIS3</i>	<i>IRF1</i>	<i>PHF6</i>	<i>STAG2</i>	
	<i>CDH23</i>	<i>DNMT3A</i>	<i>JAK1</i>	<i>PRPF8</i>	<i>STAT3</i>	
	<i>CEBPA</i>	<i>EED</i>	<i>JARID2</i>	<i>RAD21</i>	<i>SUZ12</i>	
	<i>CREBBP</i>	<i>ETNK1</i>	<i>KANSL1</i>	<i>RB1</i>	<i>TET2</i>	
	<i>CSMD1</i>	<i>ETV6</i>	<i>KDM6A</i>	<i>RIT1</i>	<i>TP53</i>	
MonoMAC syndrome	<i>GATA2</i>					
Myeloproliferative disorder	<i>JAK2</i>					
Neuronal ceroid lipofuscinosis type 2	<i>TPP1</i>					
Pancytopenia	<i>AK2</i>	<i>IKZF1</i>				
Congenital thrombocytopenia	<i>ACTN1</i>	<i>GP1BA</i>	<i>ITGA2B</i>	<i>MYH9</i>	<i>TUBB1</i>	
	<i>FLI1</i>	<i>GP9</i>	<i>ITGB3</i>	<i>RUNX1</i>	<i>VWF</i>	
Paroximal nocturnal hemoglobinuria	<i>PIGA</i>					
Sideroblastic anemia	<i>ABCB7</i>	<i>GLRX5</i>	<i>SLC19A2</i>	<i>YARS2</i>		
	<i>ALAS2</i>	<i>PUS1</i>	<i>SLC25A38</i>			
Severe congenital neutropenia	<i>ELANE</i>	<i>G6PC3</i>	<i>GFI1</i>	<i>HAX1</i>	<i>VPS45</i>	
Shwachman-Diamond syndrome	<i>SBDS</i>					
Wiskott-Aldrich syndrome	<i>WAS</i>					
WHIM syndrome	<i>CXCR4</i>					
X-linked lymphoproliferative syndrome	<i>SH2D1A</i>	<i>XIAP</i>				

## Supplementary Table 2. Clinical characteristics of patients with inherited bone marrow failure syndromes (IBMFS).

UPN	Diagnosis	Age Sex	Triad of DC			Physical anomaly	Gene	Nucleotide change	Amino acid change	Zygosity	Allelic frequency (GnomAD)	Clinvar ID / Reference	TL (SD)
			Nail dystrophy	Skin pigmentation	Leuko-plakia								
1	DC	2 F	+	-	-	+	<i>TINF2</i>	c.845G>A	p.R282H	Hetero	Not reported	VCV000005625.1	0.83
2	DC	1 F	-	-	-	+	<i>TINF2</i>	c.845G>A	p.R282H	Hetero	Not reported	VCV000005625.1	-5.73
3	DC	5 F	+	-	+	+	<i>TINF2</i>	c.847C>T	p.P283S	Hetero	Not reported	VCV000038920.2	-2.40
4	DC	7 F	-	+	-	+	<i>TINF2</i>	c.851C>G	p.T284R	Hetero	Not reported	Alder <i>et al.</i> , 2015	-4.45
5	DC	11 F	-	-	-	+	<i>TINF2</i>	c.845G>A	p.R282H	Hetero	Not reported	VCV000005625.1	-3.55
6	DC	2 M	-	-	-	-	<i>TINF2</i>	c.844C>T	p.R282C	Hetero	Not reported	VCV000005627.3	-3.61
7	DC	17 M	-	+	-	+	<i>TERT</i>	c.1892G>A	p.R631Q	Hetero	Not reported	VCV000029899.1	-2.64
8	DC	6 M	-	-	-	+	<i>TERT</i>	c.2701C>T	p.R901W	Hetero	Not reported	VCV000029901.2	-2.19
9	DC	19 M	-	-	-	-	<i>TERT</i>	Deletion	-	Hetero	Not reported	-	-3.50
10	DC	12 F	-	+	-	+	-	-	-	-	-	-	-2.50
11	DC	11 M	+	+	-	+	-	-	-	-	-	-	-3.82
12	FA	10 M	-	-	-	-	<i>FANCA</i>	c.2546delC	p.S849fs*40	Homo	3.98E-06	VCV000408166.3	-1.84
13	FA	8 F	-	-	-	+	<i>FANCA</i>	c.2546delC	p.S849fs*40	Hemi	3.98E-06	VCV000408166.3	-1.89
							<i>FANCA</i>	Deletion	-		Not reported	-	
14	FA	13 M	-	-	-	+	<i>FANCA</i>	c.2470T>C	p.C824R	Hetero	Not reported	Novel	0.83
							<i>FANCA</i>	c.1418T>C	p.L473P	Hetero	Not reported	Novel	
15	FA	6 F	-	-	-	+	<i>FANCA</i>	c.2470T>C	p.C824R	Hemi	Not reported	Novel	-0.26
							<i>FANCA</i>	Deletion	-		Not reported	-	
16	FA	3 F	-	-	-	+	<i>FANCA</i>	c.2546delC	p.S849fs*40	Hemi	3.98E-06	VCV000408166.3	-3.29
							<i>FANCA</i>	Deletion	-		Not reported	-	
17	FA	15 M	-	-	-	+	<i>FANCA</i>	c.2778+1G>A	-	Hetero	Not reported	VCV000635518.5	-1.71
							<i>FANCA</i>	c.2210C>T	p.A737V	Hetero	Not reported	VCV000456093.1	
18	FA	2 M	-	-	-	-	<i>FANCG</i>	c.307+1G>C	-	Homo	Not reported	VCV000006714.5	2.05
19	FA	10 M	-	-	-	+	<i>FANCG</i>	c.307+1G>C	-	Homo	Not reported	VCV000006714.5	-4.74
20	FA	5 F	-	-	-	+	<i>FANCG</i>	c.1066C>T	p.Q356X	Hetero	Not reported	VCV000006715.6	-3.58
							<i>FANCG</i>	c.194delC	p.65fs*7	Hetero	Not reported	VCV000660043.2	
21	DBA	1 F	-	-	-	-	<i>RPS19</i>	c.301C>T	p.R101C	Hetero	3.99E-06	Ilenia <i>et al.</i> , 2010	0.32
22	DBA	0 F	-	-	-	-	<i>RPS17</i>	Deletion	-	Hetero	Not reported	-	1.21
23	DBA	6 F	-	-	-	+	<i>RPL5</i>	c.3+1G>A	-	Hetero	Not reported	Novel	-2.10
24	DBA	13 F	-	-	-	+	<i>RPL5</i>	c.657C>G	p.Y219X	Hetero	Not reported	Novel	-2.83
25	SDS	6 M	-	-	-	+	<i>SBDS</i>	c.184A>T	p.K62X	Hetero	0.000258	VCV000449095.5	-1.99
							<i>SBDS</i>	c.258+2T>C	-	Hetero	Not reported	VCV000003196.15	
26*	BS	13 M	-	-	-	+	<i>BLM</i>	c.557_559 delCAA	p.S186X	Homo	Not reported	VCV000005455.2	-1.55

\*UPN26: A 13-year-old boy presented with anemia and thrombocytopenia. He was receiving growth hormone replacement therapy for short stature associated with extremely low birth weight. General examination revealed a characteristic facial appearance including an erythematous rash on the nose and cheeks, cryptorchidism, urethral malformation, facial stenosis, and a small nose. Bone marrow aspiration showed hypercellularity with moderate dysplasia. Genetic analysis revealed a homozygous mutation in the *BLM* gene, leading to the diagnosis of Bloom's syndrome (BS). IBMFS, inherited bone marrow failure syndromes; UPN, unique patient number; DC, dyskeratosis congenita; TL, telomere length; SD, standard deviation; FA, Fanconi anemia; DBA, Diamond–Blackfan anemia; SDS, Shwachman–Diamond syndrome; BS, Bloom syndrome.



**Supplementary Table 3. Summary of reports analyzed on TL of inherited bone marrow failure syndromes (IBMFS).**

Reference	Age, Median (range)	Patients (N)	Patients of TL shortening (%)	Method	Threshold
<b>Fanconi anemia</b>					
Ball, 1998	ND (4–18)	6	4 (67)	Southern, TRF	<Normal range for age
Hanson, 2001	4.5 (0–11)	16	15 (94)	Q-FISH	Loss of telomere signal
Li, 2003	11 (2–48)	71	47 (66)	Southern	<200 bp/year
Pavessi, 2009	6 (2–33)	9	2 (22)	Q-PCR	<1st percentile
Alter, 2015	18 (3–56)	30	2 (7)	Flow-FISH	<1st percentile
Our data	8 (2–15)	9	6 (67)	Flow-FISH	<-1.71 SD
<b>Diamond–Blackfan anemia</b>					
Pavessi, 2009	2 (0–31)	45	1 (2)	qPCR	<1st percentile
Du, 2009	12 (0–62)	41	5 (12)	Flow-FISH	<1st percentile
Alter, 2015	14 (2–58)	34	1 (3)	Flow-FISH	<1st percentile
Our data	4 (0–13)	4	2 (50)	Flow-FISH	<-1.71 SD
<b>Shwachman–Diamond syndrome</b>					
Thornley, 2002	7 (0.5–18)	12	7 (58)	Southern	<Normal range for age
Du, 2009	20 (2–41)	5	1 (20)	Flow-FISH	<1st percentile
Alter, 2015	12 (5–42)	14	2 (14)	Flow-FISH	<1st percentile
Ong, 2018	19	1	1 (100)	qPCR	<1st percentile
Our data	6	1	1 (100)	Flow-FISH	<-1.71 SD

TL, telomere length; IBMFS, inherited bone marrow failure syndromes; ND, not described; SD, standard deviation; TRF, terminal restriction fragment; Flow-FISH, flow-fluorescence in situ hybridization; Q-FISH, quantitative fluorescent in situ hybridization; qPCR, quantitative polymerase chain reaction; Southern, Southern blotting.