SUPPLEMENTARY APPENDIX

Peripheral blood lymphocyte telomere length as a predictor of response to immunosuppressive therapy in childhood aplastic anemia

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Supplemental file for an original article entitled as;

Peripheral blood lymphocyte telomere length as a predictor for the response to immunosuppressive therapy in childhood aplastic anemia.

This file consisted of supplemental methods, 1 supplemental table and 1 supplemental figure.

Supplemental Methods

Patients

Patients with acquired aplastic anemia (AA) were eligible if the following criteria were satisfied: age under 18 years, newly diagnosed disease without specific prior treatment, moderate to very severe AA, and no genetic mutation in *DKC1*, *TERC*, *TERT*, *NOP10*, *TINF2*, and *TCAB1* by Sanger sequencing method despite of shorter telomere than -2 standard deviation (SD) of healthy cohort. Primers for each gene were listed in supplemental table 1.

Measurements of telomere length and population of PNH clones

The analysis was performed using the FACS Calibur flow cytometer (Becton Dickinson Biosciences, Mississauga, Canada). After gating of diploid cells based on staining with propidium iodine, lymphocytes were isolated on the basis of size and granularity. Relative telomere length (RTL) was calculated as the ratio between the telomere signal of each sample and the telomere signal of the control cell line (cell line 1301) using the following formula: (mean FL1 of

sample cells with probe – mean FL1 of sample cells without probe) × DNA index of control cells / (mean FL1 of control cells with probe – mean FL1 control cells without probe) × DNA index of sample cells. Because telomeres shorten in accordance with age, we measured RTL of peripheral blood lymphocytes from 71 healthy individuals (median age, 29years; range, 1 – 47 years). The RTLs of controls decreased significantly with age ($r=-0.405$, $p<0.001$) and the regression line for healthy individuals is shown as a solid line ($Y= -0.0907X + 14.751$, Supplemental Figure 1A). Because telomeres shorten in accordance with age, we measured RTL of peripheral blood lymphocytes from 71 healthy individuals (median age, 29years; range, 1 – 47 years). The RTLs of controls decreased significantly with age ($r=-0.405$, $p<0.001$) and the regression line for healthy individuals is shown as a solid line ($Y= -0.0907X + 14.751$, Supplemental Figure 1A). Delta RTLs were expressed as distances from the age-appropriate point on the normal regression line of RTL versus age (Supplemental Figure 1B). The standard deviation (SD) for the delta RTLs in healthy cohort was 0.23.

A minor population of paroxysmal nocturnal hemoglobinuria (PNH)-type granulocytes and red blood cells were also evaluated by flow cytometry according to the previously described method[10]. The presence of >0.020% $CD11b^{+}$ $CD55^{-}$ $CD59^{-}$ granulocytes and >0.037% $glycophorinA^{+}$ $CD55^{-}$ $CD59^{-}$ erythrocytes were defined as abnormal. When the number of PNH-type cells was elevated in granulocytes and/or erythrocytes, the patient was defined as having a minor PNH clone.

Supplemental Table 1. Primers for sequence analyses of the genes causing dyskeratosis congenita.

Gene	Exon	Forward	Reverse
DKC1	1	TCGGCTGTGGACCGGGCG	CGGGAACCAGAGGGAGGC
	2	AATCCATTTCCTACCTGCCC	CAAAGCTGGCCCATTCCTTG
	3	AAAGGCATACATTTCCATGG	CAAGGATGCCAGCAGTAAG
	4	GCCACATAGTGGTACTGACTC	CCTGAATAGCTGATGTGAAAG
	5	GATTTGTTGTTTCACTGGAGC	TTCACTCTAGCCAGTCCTTC
	6	GGAGTGACTGAGCATATAAG	AACCCATCTCCAGATGTTTAG
	7	GCTGCAGCCAGCCTGGACC	AGTCTTCAACTTCAAGGCATC
	8	GTGGCTCAGATGAAGGATAAC	GAGTGCCATCAAAGGCTGGC
	9	GGTCTGATGGGCTGAGATAC	GAGCAAGCGTCATCTTTGGAG
	10	CACTCCCTTGTTGTCCTCC	TATATACACCTAGTATGTAAC
	11	TAAAGTGGCATACAACAGTAG	ACCTGGCAGGGCACGCAAC
	12	ATTCTTTGTAGTCACCATGCC	AGCAAGTGTGCCGTCTCTACC
	13	CTACATAACATCAGTACTGCC	TAAGACGAATGCCAGTGCC
	14	TACCTTTTGACTCACTGAACC	GGTACCACCTGGGTAATT
	15	GAACTTTGTGTCACATGCAGC	AACATGTTTTCTCAATAAGGC
TERC		TCATGGCCGGAAATGGAACT	GGGTGACGGATGCGCACGAT
TERT	1	AGCCCCTCCCCTTCCTTT	CTCCTTCAGGCAGGACACCT
	2a*	ACCAGCGACATGCGGAGA	GTCGCCTGAGGAGTAGAGGAA
	2a IS1	ACGCTAGTGGACCCCGAAG	
	2a IS2	CCCTGACGCTATGGTTCCAG	
	2b	GGTGTACGCCGAGACCAAG	CGTTCGTTGTGCCTGGAG
	2c	CCGAGGAGGAGGACACAGAC	AAACCGCGTGTCCATCAA
	3	CCTTGGTGAGCTGGATGTG	CACAGAATCCACTTGGACCAG
	4	GTGAGCTTCCCCCTAGTCTGT	ATACCAAATGTGGGGCTCAA
	5	TTTCAAACAGGGTCTGAGGAA	TGTGTCCTCAACAGTGACAGG
	6	GGCAGAGGTGATGTCTGAGTT	AGATACATGCACCACGACACA
	7	GGAGTCCCAGGTGTGTCTGTA	CAAGGCACACAGCTCATCAT

	8	CGCACTTCATCACAAACACTG	CCAGAAAAGGAGACTCTGGTG
	9	GCTGAATGGTAGACGTGTCGT	CACTGAATGCATCAAAAGCAA
	10a	AGAATTGCACAAGCTGATGGT	GAGAGGACTTGGCAGAGACAA
	10b	AGTCCCTCGTAGACAGATACTA	GCCTCACCTGAGGAAGGTTT
	11	TGCTCCAAATCACCACTTCTC	CCTCACTCCCACAGAAAGATG
	12	GATGGCATGTAGCATTTGGAG	CCATGCCTTCATGTACACACA
	13	TCTCCTGGTTCCTTCCTGTCT	AGACATTCCTTGCCCCTAAAA
	14	TGCGTGTTCATACAGATGGTG	TCCTAAGCCCAGATTCACTCA
	15	GGAAATTTCACCTGGAGAAGC	CCAGCGTTTAATCACATAGGG
	16	GTCCTAGGAGGGTTGGAGGAT	ATTCCTATGTGGGGAGTGGAA
TINF2	6	GGCTCCGGGCATAAGAAAC	TGAGGTGAGAGCAAG CAAAG
NOP10	1	ATTGACGAACACGTGACGC	GCGAACTCCTGAGCTAAG
	2	GACCATTGCATCTTCTATTTTC	CCTTTATGGGTGATGCCAC
TCAB1	1-3	GAGTCTTCTGCCTACTCCCA	CAGTCCCGTTTGTGAACAGG
	4	CTGTGCTCCATATATACACCC	GACAGAGGGAGACTCAATCTA
	5-6	CACACCCAGCCTCATTTTTG	GTACAGAGGACGGCGTGAAC
	7-10	ATGTTGAGTCCAAGCATGTT	CAATATGCCATACCCACTGC
	7-10 IS	TCCCAAGCTGAAGGAGTGC	

Abbreviations; IS, internal sequencing.

Supplemental Figure 1. Relative telomere length in 71 healthy individuals.

A) Scatter plot of relative telomere length (RTL) versus age in normal individuals (n=71; gray diamonds), showing a progressive shortening of RTL with age. The regression line for healthy individuals is shown as a solid line ($y = -0.0907x + 14.751$).

B) Delta RTLs were expressed as distances from the age-appropriate point on the normal regression line of RTL versus age. The standard deviation for the delta RTLs in healthy cohort was 0.23.