Short Telomeres, Telomeropathy, and Subclinical Extrapulmonary Organ Damage in Patients With Interstitial Lung Disease

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e-Table 1: Criteria for telomere testing

22 patients tested for short telomeres							Family history		
	Macrocytosis	Anemia	Thrombocytopenia	Abnormal coagulation profile	HSM	Fatty liver/ abnormal LFT	FIP	Early graying	Cirrhosis/AA
Tested for telomere length (N)	19	5	4	2	0	6	10	10	1
Confirmed short telomeres (N)	13	5	2	1	0	4	7	8	1

AA = aplastic anemia

FIP = familial interstitial pneumonia

LFT = liver function test

HSM= Hepatosplenomegaly

e-Table 2: Summary of demographic and laboratory data.

	Not suspected of short telomere N=97	Suspected of short telomere N=30
Age	62.46 ± 8.73	63.39 ± 7.11
Female (%)	36 (36.7)	11 (36.6)
Diagnosis (biopsy proven) IPF Unclassified NSIP CPFE HP LCH	60 (44) 12 (8) 10 (7) 11 (4) 3 (2) 1 (1)	16 (11) 7 (4) 2 (2) 3 (2) 2 (2) -
Mean corpuscular volume* Hemoglobin Platelets^	90.5 ± 5.04 14.24 ± 3.34 272 ± 79	97.8 ± 5.68 13.5 ± 2.98 225 ± 59

*p<0.0001; ^p<0.001

CPFE: Combined pulmonary fibrosis emphysema

HP: hypersensitivity pneumonitis

IPF: idiopathic pulmonary fibrosis

LCH: Langerhans cell histiocytosis

NSIP: Non-specific interstitial pneumonia

e-Table 3: Demographic and clinical data of patients tested for short telomeres

Subject	Sex, Age	Biopsy	Familial	Other familial traits	Hematologic abnormality	Hepatic abnormality	Telomere length
Idiopathic pulmonary fibrosis							
1	M, 48	Yes	No	Early graying	M	N	Short
2	M, 58	No	Yes	Early graying	N	N	Short
3	F, 45	Yes	Yes	No	N	Fatty liver	Normal
4	F, 68	Yes	No	No	A, M	N	Short
5	M, 72	No	No	No	M	N	Short
6	M, 63	Yes	Yes	Early graying	M	N	Normal
7	M, 71	Yes	No	No	A, M	N	Short
8	M, 58	No	No	No	A, M,T	N	Short
9	M, 58	No	Yes	Cirrhosis, aplastic anemia	A, M,T	Low albumin	Short
10	F, 59	No	Yes	Early graying	N	Elevated AST/ALT/ALK P	Short

11	F,56	Yes	Yes	No	M	N	Short
Non specific interstitial pneumonia							
12	F, 54	Yes	No	No	M	N	Normal
Hypersensitivity pneumonitis							
13	F, 51	Yes	No	Early graying	M	Low protein	Short
14	F, 66	Yes	No	Early graying	A, M	N	Short
Unclassified interstitial pneumonia 5							
15	M, 69	No	No	No	M	N	Normal
16	M, 65	Yes	Yes	Early graying	M	N	Normal
17	M, 54	Yes	Yes	Early graying	M	N	Short
18	M, 70	No	No	No	M	N	Normal
19	M, 57	No	N	N	M, T	N	Short
Combined Pulmonary Fibrosis Emphysema 3							
20	M, 54	No	Yes	Early graying	M	N	Short

| 21 | M, 68 | Yes | No | No | M, T | Low protein and globulin | Normal |
| 22 | M, 60 | Yes | Yes | Early graying | M | Fatty | Short |

A = anemia
ALT= Alanine Aminotransferase
ALK P= Alkaline Phosphatase
AST= Aspartate Aminotransferase
M = macrocytosis
N = normal
T = thrombocytopenia

e-Table 4: Absolute and age-adjusted telomere length in leukocyte subtypes in patients with normal and abnormal bone marrow biopsies

|	Lymphocytes	Granulocytes	Naïve T-cells	Memory T-cells	B-Lymphocytes	Natural Killers
Absolute telomere length						
Normal marrow (n=5)	4.38 ± 0.54	4.92 ± 0.33	4.40 ± 0.39#	4.37 ± 0.40#	6.00 ± 0.80#	3.85 ± 0.57#
Hypocellular marrow (n=8)	4.02 ± 0.64	4.98 ± 0.50	4.27 ± 0.82	3.78 ± 0.60	5.42 ± 0.71^	3.68 ± 0.55^
Age adjusted telomere length to the 50th percentile for age						
Normal marrow (n=5)	-1.74 ± 0.54	-2.98 ± 0.29	-2.12 ± 0.31#	-1.13 ± 0.39#	-1.55 ± 0.82#	-1.77 ± 0.63#
Hypocellular marrow (n=8)	-2.08 ± 0.72	-2.90 ± 0.57	-2.30 ± 1.00	-1.71 ± 0.67	-2.11 ± 0.76^	-1.91 ± 0.63^

#n=4; ^n=7
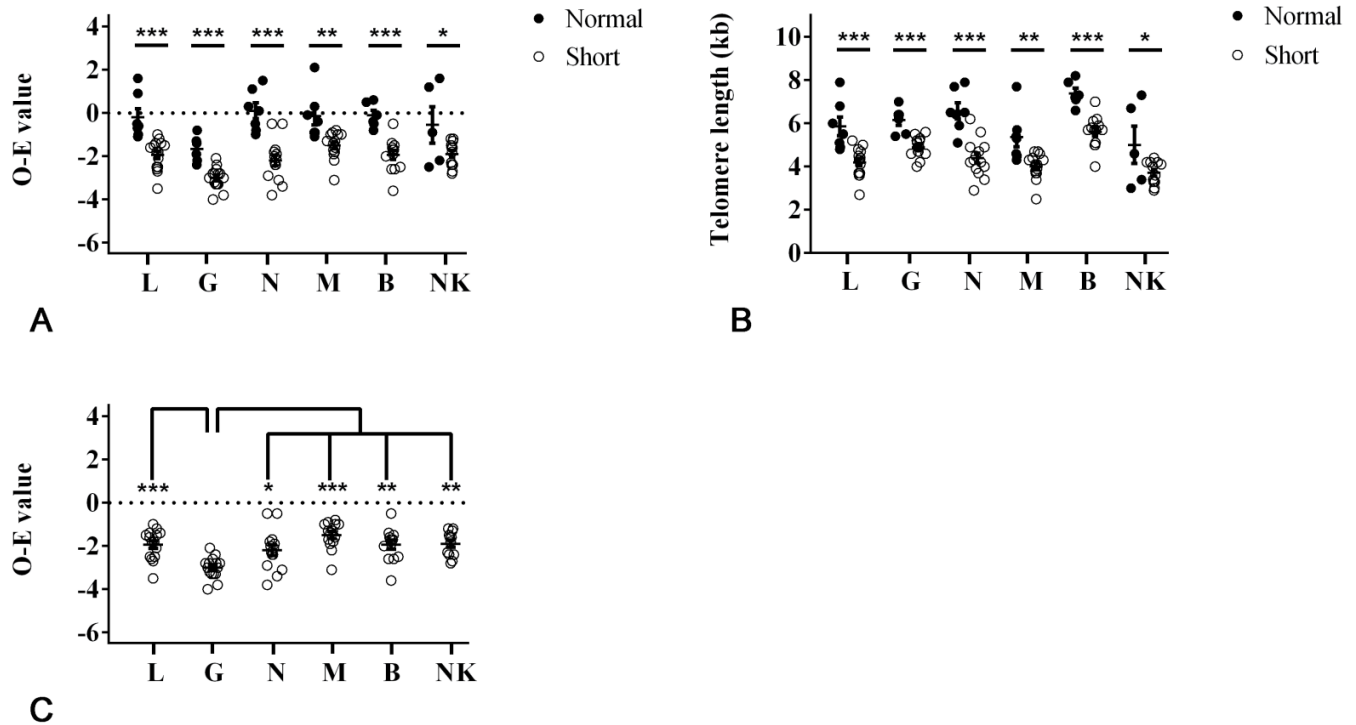
Supplemental e-Figure 1: Telomere length measurements in leukocyte subsets from patients with normal telomere length (black circle) versus patients with short telomeres (open circle). Leukocyte subtypes that were tested: lymphocytes (L) (n=7●, 15○), granulocytes (G) (n=6●, 15○), "naïve" T lymphocytes (CD45RA+) (N) (n=7●,14○), memory T lymphocytes (CD45RA−) (M) (n=6●,13○), B lymphocytes (CD20+) (B) (n=6●,13○), and natural killer (CD57+) (NK) (n=5●,12○). Shown are samples represented by a circle as well as mean and standard deviation of each subtype. In A) difference in telomere length between observed − expected value at the 50th percentile, with dotted line representing no difference between expected and observed telomere length. In B) absolute telomere length is shown. (Differences assessed using student t-test; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). In C) Age-adjusted telomere length measurements in leukocyte subsets from patients with short telomeres. Granulocytes age-adjusted telomere length is significantly different than all other leukocytes subtypes. (*p<0.05, ** p<0.01, *** p<0.001 by ANOVA).
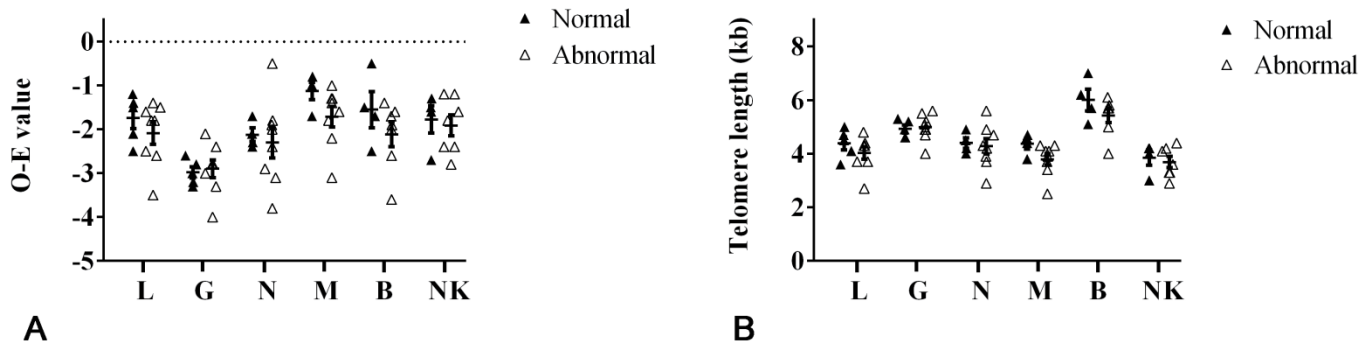
Supplemental e-Figure 2: Telomere length measurements in leukocyte subsets from patients with short telomeres with (open triangle) and without (black triangle) bone marrow abnormalities. Leukocyte subtypes that were tested: lymphocytes (L) (n= 5▲ , 8 △), granulocytes (G) (n= 5▲, 8 △), "naïve" T lymphocytes (CD45RA+) (N) (n= 4▲ ,8△), memory T lymphocytes (CD45RA−) (M) (n= 4▲, 8△), B lymphocytes (CD20+) (B) (n= 4▲ , 7△), and natural killer (CD57+) (NK) (n= 4▲ ,7△). Shown are samples represented by a triangle as well as mean and standard deviation of each subtype. In A) difference in telomere length between observed – expected value at the 50th percentile. Dotted line represents no difference between observed and expected telomere-length. In B) absolute telomere length is shown. No statistically significant difference was observed between normal and abnormal bone marrow groups in all subtypes.