Accelerated DNA aging in familial pulmonary fibrosis

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BACKGROUND

Age is an important risk factor for progressive pulmonary fibrosis (PF) and familial pulmonary fibrosis (FPF). Changes to DNA methylation at specific CpG sites can be used to estimate the biological age of cells/tissues. We determined if accelerated epigenetic aging is present in FPF by measuring DNA methylation ("DNAge") levels.

METHODS

We studied aging in healthy controls (n=23, 66±9.7y), at-risk, first-degree family members in FPF kindreds (termed "at-risk for FPF"; n=54, 53±7y), and proband with FPF (n=56, 65±8.5y). DNAge® based on the Horvath epigenetic clock (methylation at 353 CpG sites) and telomere length (TL) were measured in whole blood-isolated DNA (Southern blot). Persons at-risk for FPF are enrolled in a longitudinal study that uses high-resolution CT scan (HRCT) to screen for subclinical FPF, termed interstitial lung abnormalities (ILA). Telomere length (adjusted for age) among those at-risk for FPF was 33% and among FPF participants was 14%. The ΔDNAge® is the difference between DNAge and chronological age.

1- DNA isolation (Whole blood sample)

2- Quality control and Bisulfite conversion

3- High throughput methylation Simplified Whole-panel Amplification Reaction Method

4- Data Analysis/Report (Elastic net regression of DNA methylation levels)

5- Delta DNA methylation=predicted – chronological age)

RESULTS

	Control (N=23)	*At-Risk (N=54)*	*FPF (N=56)*
Age (y)	66 ±9.7	53 ±7*	65 ±8.5
Sex, F (%)	52%	72%	47%
RV (%)	n.d.	20%	59%
Telomere length Mean ±SD (% adjusted for age)	n.d.	33% ±33%	14%±21%

Table 1: Participant's demographics: Individuals selected for DNA-associated aging analysis are part of a longitudinal study of FPF families and a sub-cohort called "at-risk" for FPF (no clinical disease). Telomerase mutations are among genetic mechanism of pulmonary fibrosis. Participants were selected based on the existence of these mutation. Continuous variables among the three groups were compared with a one-way Anova, and categorical variables by chi-square test. FPF= familial pulmonary fibrosis, RV=Rare variant, (y) = years, n.d. = not determined.

Accelerated epigenetic age and telomere length are independent aging phenomena in FPF

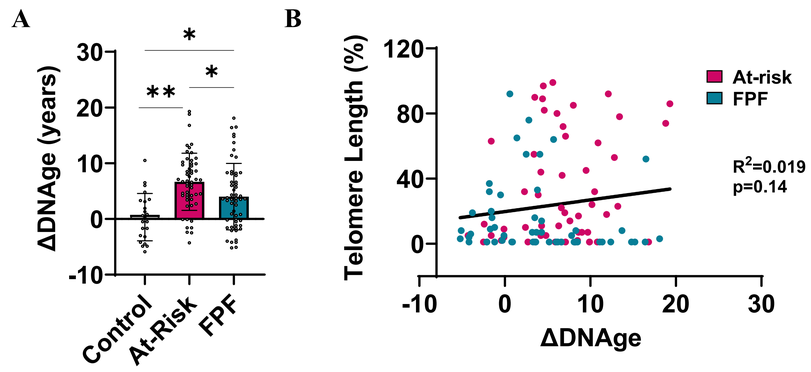

Figure 1: (A) Epigenetic age (DNA age minus chronological age) calculated in healthy controls, at-risk (pre-clinical) and FPF members. (B) Simple linear regression analysis of ΔDNAge® and telomere length (TL) (circles are color code measures for at-risk and FPF individuals).

Accelerated epigenetic age is independent on RV mutation and lung abnormality

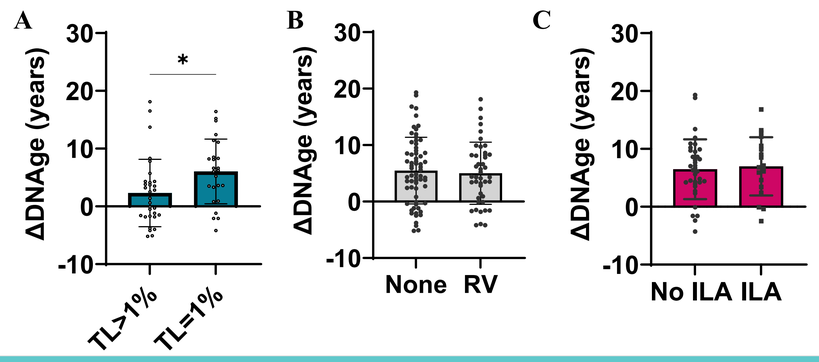

Figure 2: (A) ΔDNAge of FPF subjects with TL=1% or TL>1%. (B) ΔDNAge of participants with and without heterozygous rare variants (RV) in telomerase pathway genes. (C) ΔDNAge of at-risk family members with and without interstitial lung abnormalities (ILA) on CT scan. Mean ± SD are shown. *p < 0.05.

RESULTS

Accelerated epigenetic age was observed in at-risk for FPF kindreds (6.6±5y) and proband with clinical FPF (4.4±5y) compared to control subjects (0.3±4y).

Using a linear regression analysis, we found little correlation (R^2=0.019, p=0.14) between ΔDNAge and telomere length (percentile corrected for chronological age) in peripheral blood cells among members of families with FPF.

FPF patients with very short telomeres (at the 1st percentile for age) had greater ΔDNAge compared to those with longer telomeres.

There was no difference in ΔDNAge among FPF family members with or without a pathogenic telomere-related gene RV.

There was no difference in ΔDNAge between those with or without evidence of subclinical FPF (i.e., interstitial lung abnormalities) on CT scan.

CONCLUSIONS

- In this study, we compared epigenic aging with the presence of telomerase pathway gene RVs and peripheral blood cell telomere length and showed that exaggerated epigenetic aging is a feature of FPF.
- There was little correlation between ΔDNAge and telomere length or the presence of telomerase pathway RVs
- Accelerated epigenetic age may be present in at-risk family members prior the onset of pathologic lung changes detectable by CT imaging.
- FPF might be influenced by biological aging processes other than telomere shortening.

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