Short lung transplant donor telomere length is associated with decreased CLAD-free survival

ABSTRACT

Telomere length (TL) decreases with cellular ageing and biological stressors. As advanced donor and recipient ages are risk factors for chronic lung allograft dysfunction (CLAD), we hypothesised that decreased age-adjusted donor TL would predict earlier onset of CLAD. Shorter donor TL was associated with increased risk of CLAD or death (HR 1.26 per 1 kb TL decrease, 95% CI 1.03 to 1.54), particularly for young donors. Recipient TL was associated with cytopenias but not CLAD. Shorter TL was also seen in airway epithelium for subjects progressing to CLAD (p=0.02). Allograft TL may contribute to CLAD pathogenesis and facilitate risk stratification.

INTRODUCTION

Chronic lung allograft dysfunction (CLAD) reflects progressive fibrosis and constitutes the most common cause of death after the first year following transplant.[1] However, time to development of CLAD varies substantially across lung transplant recipients. While donor and recipient ages are risk factors for decreased CLAD-free survival, they do not fully capture the extent of molecular ageing.[2]

Telomeres are nucleoprotein caps on the terminal region of chromosomes that provide protection from chromosomal shortening during cell replication. Telomere lengths (TLs) vary inversely with age but are widely distributed within cells and cell populations. TL shortening past a critical length triggers cellular senescence. Accelerated telomere attrition is associated with increased cell turnover and stress, while inherited or sporadic mutations in telomerase-associated genes cause syndromes mimicking early ageing.[3]

Short allograft TL has been associated with delayed graft function and chronic allograft dysfunction following renal transplantation[4] and poor graft survival following stem-cell transplant for aplastic anaemia.[5] Case series of recipients with known telomerase mutations following lung transplantation suggest an increased risk of leucopenia but did not evaluate CLAD.[6] A small retrospective lung transplantation cohort study found no association between donor or recipient peripheral blood TL and survival.[7]

Telomere dysfunction in lung disease has been increasingly appreciated, as telomere-related mutations are implicated in familial pulmonary fibrosis and COPD.[8] Experimentally, telomere dysfunction induced by selective deletion of the shelterin complex proteins in alveolar

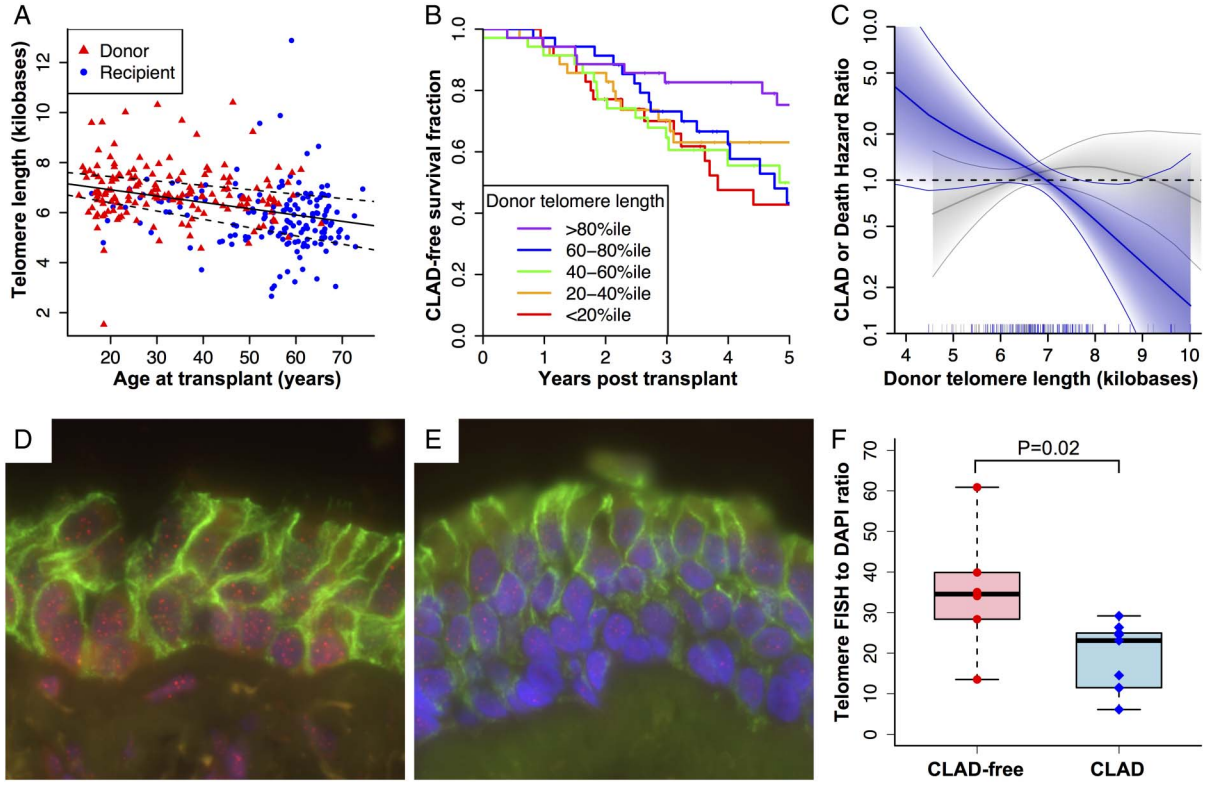

Figure 1 Short donor telomere length in peripheral blood and airway biopsies is associated with decreased chronic lung allograft dysfunction (CLAD)-free survival. (A) Telomere length in kilobases versus age in years for donors (red triangles) and recipients (blue circles). Linear regression of telomere length versus age across both cohorts resulted in a slope of −25 (95% CI −17 to −32) base pairs per year. (B) Kaplan-Meier plot showing CLAD-free survival stratified by quintiles of donor telomere length. Improved CLAD-free survival was seen across increasing quintiles of donor telomere length (p=0.04), with improved survival in the >80th percentile group compared with the <20th percentile group (p=0.02). Increasing quintile of recipient telomere length was not associated with CLAD-free survival (p=0.59). (C) HRs for CLAD or death per 1 kb decrease in telomere length stratified by donor age under 30 (blue) or over 30 (grey) and adjusted for subject characteristics in online supplementary table S2 are plotted against donor telomere length. Solid line shows spline fit of data with shaded area indicating 95% CIs. (D–E) Quantification of telomere length in airway epithelial cells from lung transplant recipients who would either develop CLAD or remain CLAD-free. Endobronchial biopsies collected during surveillance bronchoscopy within the first 90 days post transplant (median 31 days) were stained for telomeric DNA (red), E-cadherin (green) and total DNA (DAPI, blue). Shown are representative images from (D) a CLAD-free subject with a telomere to DAPI ratio of 40 and (E) a CLAD subject with a telomere to DAPI ratio of 15. As shown in (F), telomere length was higher in CLAD-free subjects than in subjects with CLAD (p=0.02 by Mann-Whitney test). FISH, fluorescence in situ hybridisation. DAPI, 4′,6-diamidino-2-phenylindole.

type II cells results in epithelial cell failure, remodelling and fibrosis.[9]

These observations motivated the hypothesis that shortened donor peripheral blood telomeres would identify lung allografts at increased risk for early CLAD or death following transplantation.

METHODS

See online supplement for detailed methods. Briefly, lung allograft recipients at University of California, San Francisco (UCSF) were included if they provided informed consent, donor and recipient DNA samples were available and had at least 18 months of follow-up data (see online supplementary table S1). We measured TL by quantitative PCR on DNA isolated from peripheral blood mononuclear cells (PBMC) or spleen. For the subcohort described in online supplementary table S4, TL in endobronchial biopsies collected within 90 days following transplant was measured by quantitative fluorescence in situ hybridisation.

Table 1 Cox proportional hazards models for CLAD-free survival as a function of donor and recipient telomere length

	HR	95% CI	p Value
Multivariable risk of CLAD or death model†*			
Telomere length (per 1 kb decrease)			
Donor	1.25	1.03 to 1.52	0.02
Recipient	1.02	0.87 to 1.2	0.79
Age (per decade)			
Donor	1.03	0.86 to 1.22	0.76
Recipient	1.06	0.8 to 1.41	0.67
*Multivariable risk of CLAD or death interaction model**			
Telomere length (per 1 kb decrease)			
Donor	2.39	1.4 to 4.07	0.001
Age (per decade)			
Donor	0.29	0.11 to 0.79	0.02
Donor age donor telomere length*			
Interaction	0.83	0.71 to 0.96	0.01
*Donor age stratified, multivariable-adjusted risk of CLAD or death models**			
Donor telomere length (per 1 kb decrease)			
Donor age <30	1.65	1.18 to 2.31	0.004
Donor age ≥30	1	0.79 to 1.27	0.97

*Includes donor and recipient telomere length, age, diagnosis group, lung allocation score, donor gender, recipient gender, non-Hispanic white donor, non-Hispanic white recipient, transplant type and donor 'ever smoker' status.
†Univariable and multivariable models including all covariates are included as online supplementary table S3.
CLAD, chronic lung allograft dysfunction.

RESULTS

Demographic features of the 175 included subjects are summarised in online supplementary table S2. Mean donor TL exceeded that of recipients by 1.0 kb (95% CI 0.8 to 1.2, p<0.001) and by 0.5 kb (95% CI 0.2 to 0.7, p<0.001) after adjusting for age. Forty-five per cent of recipients developed CLAD with a median time to CLAD of 3.8 years, and 28% of subjects died. Median follow-up time was 4.9 years (IQR 4.0 years). TL was inversely correlated to age for the entire cohort (figure 1A).

Shorter donor TL was associated with an increased risk of CLAD or death with an adjusted HR of 1.25 per 1 kb decrease in TL (95% CI 1.03 to 1.52, 88 events, p=0.02, figure 1B, C). Recipient TL and donor age were not associated with CLAD-free survival (table 1). Interaction modelling suggested that short donor telomeres are most hazardous in younger donors (p=0.01). In a competing risk analysis adjusted for subject characteristics, decreasing donor TL was associated with both CLAD censored on death (subdistribution HR 1.25, 95% CI 1.00 to 1.56, 72 events, p=0.045) and death alone (subdistribution HR 1.43, 95% CI 1.06 to 1.93, 40 events, p=0.02).

An increased OR of mild (OR 2.5, 95% CI 1.1 to 5.5) and severe leucopenia (OR 4.8, 95% CI 1.8 to 13.0) was found among recipients in the lowest 20th percentile of PBMC TL (see online supplementary table S5).

Endobronchial TL was measured in subjects with extremes of time to CLAD to determine the association with allograft TL. The populations are described in online supplementary table S4. Endobronchial TL was significantly greater in the >8 year CLAD-free group, relative to those that developed CLAD at a median of 2.9 years (p=0.02, figure 1D).

DISCUSSION

In summary, short donor PBMC TL was associated with worse CLAD-free survival, independent of donor age, in univariable and multivariable models, while recipient TL was associated with increased incidence of post-transplant leucopenia. TL was also shorter in airway epithelial cells from lung allografts that went on to develop CLAD, suggesting that decreased TL within the lung may directly contribute to the development of CLAD.

Genetic predisposition or environmental insults affecting the lung may contribute to short telomeres. Telomere shortening beyond what is expected with ageing might signify systemic or inherited telomere dysfunction, which may explain the observed interaction with donor age. Cell turnover that is either homeostatic or in response to injury is impaired in the context of critically short telomeres. Allografts with short telomeres may have a higher frequency of senescent cells and thus be at increased risk for the airway-centric or parenchymal lung fibrosis that are pathological correlates of CLAD.[1] Lung allografts may be particularly susceptible to telomere attrition because of repeated environmental insults or high rates of cell turnover relative to other solid organs.[10]

Recipient PBMC TL was associated with clinically significant leucopenia, consistent with previous reports of increased leucopenia in lung allograft recipients with telomerase mutations.[6] Short PBMC TL may reflect a larger population of senescent bone marrow cells unable to increase leucocyte production, particularly under the influence of immunosuppressive medications. Assessment of recipient TL has the potential to guide post-transplantation immunosuppressive and antiviral dosing.

Strengths of this study include the large sample size relative to other investigations of TL in lung transplantation,[7] length of follow-up, uniform assessment of TL with quality controls and confirmation with measurement of TL in airway tissue. The study is from a single centre, limiting differences in clinical practice; however, external validation would strengthen the generalisability of the findings. In particular, relative to International Society for Heart and Lung Transplantation (ISHLT) registry reports, this cohort included more recipients transplanted for pulmonary fibrosis.[2] Also, we did not evaluate TL relevance for subjects with <18 months follow-up.

Our findings, if replicated, may assist with improved stratification of donor risk, and facilitate the use of lungs from donors of advanced age if TL is found to be

adequate. Telomerase activation may be of interest in allografts with short TL.[3]

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