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TELOMERE LENGTH AND LUNG FUNCTION IN A POPULATION-BASED COHORT OF CHILDREN AND MID-LIFE ADULTS

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RUNNING TITLE

Telomere Length and Lung Function

ABSTRACT

Objective: Telomere length is associated with poorer lung health in older adults, possibly from cumulative risk factor exposure, but data are lacking in pediatric and population-based cohorts. We examined associations of telomere length with lung function in children and mid-life adults.

Methods: Data were drawn from a population-based cross-sectional study of 11-12 year-olds and mid-life adults. Lung function was assessed by spirometric FEV_1, FVC, FEV_1/FVC ratio and $MMEF_{25-75}$. Telomere length was measured by quantitative polymerase chain reaction from blood and expressed as the amount of telomeric genomic DNA to the beta-globin gene (T/S ratio). Associations of telomere length with spirometric parameters were tested by linear and logistic regression models, adjusting for potential confounders of sex, age, body mass index, socioeconomic position, physical activity, inflammation, asthma, pubertal status and smoking.

Results: Mean T/S ratio was 1.09 (n=1,206, SD 0.55) in children and 0.81 (n=1,343, SD 0.38) in adults. In adults, for every additional unit in T/S ratio, FEV_1/FVC and $MMEF_{25-75}$ z-scores were higher (β 0.21 [95% CI 0.06-0.36] and 0.23 [95% CI 0.08-0.38] respectively), and the likelihood of being in the lowest quartile for FEV_1/FVC and $MMEF_{25-75}$ z-scores was lower (odds ratios 0.59 [95% CI 0.39-0.89] and 0.64 [95% CI 0.41-0.99] respectively). No evidence of association was seen for adult FEV_1 or FVC, or any childhood spirometric index after adjustments.

Conclusion: Shorter telomere length showed moderate associations with poorer airflow parameters, but not vital capacity (lung volume) in mid-life adults. However, there was no convincing evidence of associations in children.

INTRODUCTION

Cell senescence may play an important role in the lifecourse pathways of lung health. Incidences of respiratory diseases increase with age, suggesting that age-related processes may be common to these diseases.[1] For example, chronic obstructive pulmonary disease (COPD) is strongly related with frailty as early as mid-life.[2] Pathophysiological mechanisms thought to underlie respiratory diseases and continuous lung function decline include chronic inflammation, oxidative stress and mitochondrial dysfunction,[3] all of which are closely related to cell senescence.[4] One molecular mechanism strongly implicated in the induction of cellular senescence is telomere shortening.[5]

Telomeres are nucleoprotein structures that preserve the ends of linear chromosomes,[6] and their shortening is linked to mortality, cancer, and cardiovascular disease.[7] Short telomeres drive cell senescence, resulting in inhibited tissue repair capacity and function.[6] The role of telomere shortening in lung health has been examined in adults but studies are lacking in children.

Changes in lung function provide a snapshot of overall lung health developing over the lifecourse. Increasing evidence in adults suggests that shortened telomeres are associated with reduced lung health.[8,9] For instance, shortened telomeres are found in pulmonary vascular endothelial cells of COPD patients[8] and in fibrotic areas of adults with pulmonary fibrosis.[10] Further, a study of 46,396 adults aged between 43 and 73 years showed a modest association of shorter telomere length with decreased lung function.[11] However, the generalizability of these findings across the lifecourse is uncertain as most studies were conducted in older patients already exhibiting lung disease. Few studies have explored the relationship between telomere length and lung function in younger general populations, despite the fact that pathways to ill health in adulthood begin much earlier in life.[12] Telomere-linked cell senescence could be on the pathway to decreased lung function and

increased respiratory disease risk. Understanding this association could enhance our understanding of the pathogenesis of respiratory disease and identify novel mechanisms for intervention.

Exploring the relationships between telomeres and lung function across the lifecourse could elucidate the role of telomere length-driven cell senescence in the development of healthy lungs. In a population-based study of children and mid-life adults, we investigated the associations of telomere length with lung function and specifically telomeres in those with the lowest quartile lung function. Given the progressive nature of the proposed association across the lifecourse, we predicted that the magnitude of associations between telomere length and lung function would be stronger in adults relative to their children.

MATERIALS AND METHODS

Study design, subjects and procedure

In 2004, the Longitudinal Study of Australian Children (LSAC) recruited a nationally-representative birth cohort (n=5,107), which has since been followed at seven biennial waves. The Child Health CheckPoint (CheckPoint) study was an additional physical health and biomarker module at child age 11-12 years, nested between LSAC's sixth and seventh waves. All families who completed the LSAC wave 6 assessment were eligible. Ultimately, 1,874 families took part in the CheckPoint. Most non-participation was due to inability to attend or to reschedule a visit during the short period CheckPoint was in each location. Details of the LSAC and CheckPoint study are previously described.[13-15]

CheckPoint's data collection ran from Feb-2015 to Mar-2016. Each child was invited to attend an assessment center with one parent/caregiver. No more than one child from each family participated. The main assessment center operated across Australia in major and

regional centers. However, a small number of families attended mini assessments or opted for home visits, and were therefore excluded from the current analysis as these condensed assessments did not include venous blood samples, used to measure telomere length. Children and their attending parent rotated through a series of 15 to 30-minute stations where different aspects of health were assessed and biological samples collected. Child and parent protocols and equipment were identical. The attending parent provided written informed consent for themselves and their child, and the child provided assent.

DNA isolation and telomere length measurement

At the assessment center, medically-trained researchers or phlebotomists collected venous blood at a 15-minute station. Blood was processed within 2 hours at an on-site laboratory and stored at -80 degrees Celsius. For the first two months, this included blood clots from plasma tubes; for logistical reasons, clots were then discontinued and replaced with a whole blood sample for the remaining centers. At the Murdoch Children's Research Institute (MCRI), genomic DNA was isolated from available blood (e.g. whole blood or blood clot) using the Qiaamp 96 DNA Blood Kit (Qiagen, Venlo, Netherlands). Purity and integrity of DNA were confirmed using spectrophotometry (NanoDrop 2000, NanoDrop Technologies, USA), fluorimetry (Qubit 2.0, Thermo Fisher Scientific, USA) and gel electrophoresis. Telomere length was measured by quantitative polymerase chain reaction, originally described by Cawthon.[16] This method measures the amount of telomeric DNA (T) and a single copy gene beta-globin (S) for each sample. A ratio (T/S ratio) was then calculated by comparing the relative amount of 'T' and 'S' for each participant. The mean intra-assay variability was 1.7 % (standard deviation (SD) 0.3, range 0.9 to 2.6). The inter-assay variability was 1.7 % (SD 1.4, range 0.3 to 6.2). Further details on the telomere procedure and assay are described in the online Supporting Information, and epidemiological findings have been published.[17]

Lung function assessed by spirometry

Trained researchers conducted spirometry testing at a 30-minute station. Spirometry was performed between 3-8 repeats on each participant using a spirometer (Vyntus Pneumo, Vyntus, USA) running SentrySuite software (Care Fusion, Germany) with a bacterial filter and nose clip, in accordance with international guidelines.[18] Further details of the spirometry method and epidemiology are described elsewhere.[14,19]

Spirometric indices: Forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), their ratio (FEV_1/FVC) and maximal mid-expiratory flow ($MMEF_{25-75}$) were extracted from the SentrySuite platform and converted to z-scores using the Global Lung Initiative equations. [20] The quality of flow-volume loops were assessed by trained spirometry experts and was included in analyses if guidelines were met (Supporting Table S1).[18]

Spirometry trace reliability: Two experienced raters scored the same 21 loops from a random sample of 21 children and the same 19 loops from adults. Cohen's Kappa statistic was used to measure the agreement between waveform classifications. Overall agreement between waveform classifications was substantial (kappa=0.79, p-value<0.001) with a high percentage of agreement (95%), indicating a high level of agreement by the two raters. Further details are in the online Supporting Information.

Potential confounders

Several variables were considered *a priori* as potential confounders taking published literature into account, including body mass index (BMI), socioeconomic status, puberty, smoking, physical activity and inflammation. Each of these variables has been associated with telomere length,[4,21-24] as well as being commonly known factors that influence lung growth.[25-29] BMI was calculated from height (Stadiometer, Invicta IP0955, UK) and weight (InBody230, Biospace, South Korea). For children, an age- and sex-adjusted BMI z-score was

calculated using growth reference charts.[30] A standardized score that summarizes the social and economic conditions of Australian neighborhoods was calculated using the Socio-Economic Indexes for Areas Index of Relative Disadvantage score (Disadvantage Score); it has a national mean of 1,000 and SD of 100 (higher values represent less disadvantage). For family socioeconomic position we used LSAC's wave 6 composite measure representing parent-reported household income, occupation and education level, standardized nationally to have a mean of 0 and SD of 1 (higher scores represent more advantage). Parental cigarette smoking behavior and Indigenous status (Aboriginal or Torres Strait Islander) were collected at LSAC wave 6. Child second-hand smoking was determined when at least one parent was a smoker. Asthma was self-reported in a questionnaire at CheckPoint. The novel inflammation marker of glycoprotein acetyls (GlycA) was measured using the Nightingale nuclear magnetic resonance metabolomics platform (Helsinki, Finland) from blood serum. Physical activity was calculated as the average duration of moderate-to-vigorous physical activity (MVPA) measured using a wrist-worn accelerometer (GENEActiv, Activinsights, Cambridgeshire, UK). Further details of these measures are extensively described elsewhere.[14]

Statistical analysis

Stata 14.2 (StataCorp, College Station, TX) was used for all analyses, with children and adults considered separately. Linear regression models were fitted where continuous telomere length was the independent variable, and continuous spirometric values were the dependent variables for each model. Assumptions for linear regression were examined using histograms and quantile-quantile plots. Spirometric indices in children and adults followed approximately normal distributions with no discernible outliers. There was minimal right-skewing for child's and adult's telomere length. Logistic regression models were also fitted

using continuous telomere length as the independent variable to assess the odds of having poor lung function. The lowest quartile for each spirometric index was compared to the highest quartile (i.e. the reference group), given that being in the lowest quartile has been shown to infer future risk of lung disease and increased respiratory morbidity.[31]

For both children and adults, model 1 included adjustments for the potential lifelong confounders of sex and age, as well as *a priori* potential confounders, including BMI and socioeconomic position. Adjusted analyses included the covariates as independent variables. Model 2 additionally included adjustments for the additional potential confounders physical activity (i.e. MVPA), inflammation (i.e. GlycA), asthma, pubertal status and second-hand smoking status for children, and smoking status for adults. In addition, sensitivity analyses were done in adults by adjusting for adults' non-linear age (i.e. centered age squared and centered age cubed) instead of linear age, as well as, stratifying results by sex. Further, sensitivity analyses were done by stratifying by original blood sample type (e.g. blood clot or whole blood) and excluding participants without complete covariate data in unadjusted regression models to examine the effect of missing data. Key baseline characteristics of LSAC families who did and did not participate in the current analyses were reported to consider the representativeness of the maintained analytic sample in relation to the total CheckPoint cohort and the preceding LSAC waves.

RESULTS

Figure 1 shows the CheckPoint study participant flow. Both telomere length and spirometry data were available for 1,153 children and 1,293 adults.

Sample characteristics

Participant characteristics are displayed in Table 1, and sex stratified results are in Supporting Table S2. Mean ages of children and adults were 11 years (SD 0.5) and 44 years (SD 5.1), respectively. Most adults were mothers (87%) as they typically accompanied children to the assessment center, whereas the numbers of boys and girls were similar. Both children and adults' BMI scores and prevalence of asthma were similar to the population for similar ages.[32] Adult self-report of diabetes (2.4% vs. 4% for the general population) and being a current smoker (8% vs. 16%) were lower than the general population for similar ages.[32] Our sample came from relatively less disadvantaged areas (Disadvantage Score 1,026, SD 61) than the national average (mean 1,000, SD 100).

The nationally-representative LSAC cohort had 57% uptake at wave 1 in 2004, with 74% retention at wave 6 in 2014. Baseline characteristics (LSAC wave 1) indicate that retained families were from slightly less disadvantaged areas, and fewer were from Aboriginal or Torres Strait Islander backgrounds, than those lost to follow-up; however, proportions of males and females were similar (see Supporting Table S3). Detailed comparisons of the CheckPoint and LSAC samples to the Australian population have been published previously.[14]

Children had greater T/S ratios than adults (T/S ratio 1.09 vs. 0.81, $p<0.001$). Further details on telomere length epidemiology have been previously published,[17] showing that our cohort's telomere lengths were comparable to those of other populations of similar ages, size and structure. As expected, compared to children, adults had overall greater raw FEV_1, FVC and $MMEF_{25-75}$, but lower FEV_1/FVC ratio. Children and adults' spirometric z-scores followed similar distributions and were within normal limits, but with overall FEV_1 and FVC z-scores slightly higher, and FEV_1/FVC and $MMEF_{25-75}$ z-scores slightly lower than the international reference populations.[20]

Association of telomere length with lung function

Linear and logistic regression results are shown in Table 2 and Table 3, respectively. In adults, for every unit increase in T/S ratio, FEV_1/FVC z-score and $MMEF_{25-75}$ z-score was 0.21 (95% CI 0.06-0.36, p=0.008) and 0.23 (95% CI 0.08-0.38, p=0.003) higher, respectively (Table 2, model 2). This equated to z-scores approximately one-fifth of a SD higher (i.e. better) for FEV_1/FVC and $MMEF_{25-75}$ z-scores for each one unit T/S increase (equivalent to approximately two SD T/S ratio).

On average, adults with the lowest T/S ratio (0.02) had 0.49 FEV_1/FVC z-score lower and 0.58 $MMEF_{25-75}$ z-score lower than those with the highest T/S ratio (2.9). These adjusted associations were only slightly smaller than in the unadjusted linear regression models. A similar pattern was observed when lung function outcomes were considered categorically. For every unit increase in T/S ratio in adults, there was a 0.59 (95% CI 0.39-0.89, p=0.01) and 0.64 (95% CI 0.41-0.99, p=0.04) reduced odds of being in the lowest quartile of FEV_1/FVC and $MMEF_{25-75}$ z-scores, respectively (Table 3, model 2). In contrast, telomere length was not associated with FEV_1 or FVC in adults.

Sensitivity analyses showed similar patterns between men and women, and between non-linear age and linear age, although imprecise results were observed for blood clot (Supporting Tables S4-S6). Moreover, the exclusion of participants without complete covariate data did not alter the unadjusted associations (Supporting Table S7).

For children, we saw an unexpected inverse association between higher telomere length and lower FEV_1 and FVC in unadjusted linear regression models. However, this attenuated to null in the adjusted models (Table 2, model 2). When each potential confounder was considered, child's age and BMI were most likely masked confounders in the unadjusted model. Logistic regression models showed little evidence of an association between telomere length and any spirometric index in children (Table 3).

DISCUSSION

Main findings

In a population-based cohort, we found a moderate association between shorter telomeres and reduced lung function in adults relating to airflow limitation (FEV_1/FVC ratio and $MMEF_{25-75}$), but not with lung volumes (FEV_1 and FVC). These associations were not seen in children, in whom we saw little evidence to suggest that telomere length was associated with lung function.

Strengths and limitations

Strengths of our study include the objective examination of spirometry, conducted according to international standards, and T/S ratios with low replicate variability. Our study further benefits from the large sample sizes at two generations, children and mid-life adults. For telomere length, we employed the widely used quantitative polymerase chain reaction method which has previously been validated against the gold-standard Southern blot.[16] This method is well-suited for large epidemiological studies but does not quantify absolute telomere length. The informativeness of blood telomere length as a surrogate for telomere length in the lungs remains unknown, but good telomere length correlation between different tissues,[33] and between blood and lung tissue are reported.[34] We acknowledge that our cohort somewhat under-represents adult males, Australian families from disadvantaged neighbourhoods and those of Indigenous/non-Caucasian background. While we adjusted for these factors, our models might not generalise to such individuals/families if they showed different associations between lung function and telomere length. Finally, despite our use of spirometry to assess various lung function parameters, we acknowledge that definitive

conclusions on lung size should ideally include measures of total lung capacity, which were not assessed in our study.

Interpretation in light of other studies

In adults, shorter telomeres (lower T/S ratio) was moderately associated with reduced lung function relating to airflow limitation (lower FEV_1/FVC and $MMEF_{25-75}$ z-score), but not with FEV_1 and FVC. This suggests that if there are any biological impacts these act on airway relative to lung size, rather than on lung growth itself. This is congruent with the observation that, after peaking in childhood, lung size in healthy individuals plateaus with little change in FEV_1 and FVC.[35] Others have reported stronger associations in individuals with lung pathologies such as COPD, idiopathic pulmonary fibrosis, asthma and lung cancer.[8,10,36] General population studies in adults have generally found weak to moderate associations between telomere length and lung function.[11,37] For example, two large studies in healthy adults (aged 28 to 73 years) reported weaker associations between telomere length and lung function (n=44,041 controls), compared to COPD patients (n=934) or asthmatics (n=2,834).[8] Interestingly, a recent small study (n=280, mean age 62 years) examined several markers of ageing (including sirtuin 1, p16/21 and Ku70/80) and found telomere length to be the only marker consistently associated with lung function in COPD patients.[38] Compared to other adult studies, the relatively smaller association we observed may be explained by the fact that our adult cohort was relatively healthier and younger with lower rates of smokers and was slightly less disadvantaged.

To our knowledge, this study is the first to examine the associations of telomere length and lung function in a general cohort of children, where we found no evidence of an association with any spirometric index after adjustments. Possibly, associations become more pronounced later in life when the contributions of cumulative environmental burden have

taken effect, which would explain the lack of association in our children but emerging associations in our mid-life adults. Alternatively, it is possible that factors implicated in senescence impact airway and lung health during adult life, rather than childhood. The underlying mechanism is unclear but might include the accumulation of adverse environmental influences that impact both telomeres and lung health,[3,4] or the loss of stem cell regenerative capacity in association with decreased telomerase activity of the lungs (i.e. pulmonary endothelial cells).[39] Ideally, longitudinal studies extending from childhood into adult life are required to investigate this further. Finally, the unexpected inverse association between telomere length and FEV_1 and FVC in children was not present after adjusting for sex, age, BMI and socioeconomic position. This adjustment may not necessarily be appropriate when testing some of the hypotheses that might explain an association between lung function and telomere length and we are, therefore, cautious about ruling out this relationship.

Clinical implications, unanswered questions and future direction

Our study found an association that was present by mid-life, but not at 11 to 12 years. Exactly when it first develops between these two ages is unknown and would be of interest. Given that children's lung physiology at 11 to 12 years is still rapidly developing, we hypothesise that clinically meaningful associations might develop by the end of puberty or appear during early adulthood when lung function capacity typically peaks.

The inter-subject variability of forced expiratory flows is larger than that for forced expiratory volumes, which is why the latter is more often used to monitor lung function changes over time. However, in studies of airway disease, mid-expiratory flows have been reported to be more sensitive than forced expiratory volumes in detecting airway obstruction. Our study did not detect evidence of airway obstruction, which is likely due to our relatively good health of our cohort.

If replicated in other settings, our findings suggest that telomere length may have some clinical utility in that, at least in adults, these markers of cell senescence appear to correlate with respiratory health. Telomere length may have a role in the pathways to lung diseases. As reported in other studies, our observations are likely to be of greater clinical relevance in association with more pronounced respiratory pathology, such as those with COPD, idiopathic pulmonary fibrosis, asthma and lung cancer.[8,10,36]

We cannot exclude the possibility that our findings may have arisen by chance, and might be explained by factors beyond the scope of this study. Evidently, there is notable telomere length inter-individual variability at birth and across the lifecourse. Telomere length at any point in life represents the integration of multiple risk factors, both inherited and acquired, including the effects of inherited telomerase genes, telomere length attrition with age and early life environmental contributions. Consequently, telomere's measure of molecular age might better complement chronological age as a predictor of overall survival and health. This was observed in COPD patients where telomere shortening was associated with all-cause mortality.[40]

Conclusion

In a healthy cohort of mid-life adults, we report a moderate association between shorter telomere length and reduced lung function, specifically with measures of airflow relative to lung volume, FEV_1/FVC ratio and $MMEF_{25-75}$. In contrast, no convincing association was observed in children after adjustments. The interaction between telomere dynamics and lung health over the lifecourse is multifaceted with contributions from genetic variation and environmental exposures and warrants further study. Telomere length may have a role in chronic lung conditions but its role in early life in general populations remains unclear. This represents the first study to investigate these associations in a general cohort of children. Further studies are needed to reproduce our findings in other settings.

CONTRIBUTORS

MW, DB, SR and RS conceptualized and developed the CheckPoint study with other investigators. MTN assisted with sample collection, isolated genomic DNA, quantified telomere length, analyzed the data and wrote the first draft of the manuscript. MW is the lead investigator of the Child Health CheckPoint study. RS supervised laboratory work and protocol optimization. SR supervised spirometry testing and quality control of flow-volume loops. All authors commented on the first and subsequent drafts and approved the final version of the manuscript.

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ETHICS APPROVAL

The study protocol was approved by the Royal Children's Hospital Human Research Ethics Committee (33225D) and the Australian Institute of Family Studies Ethics Committee (14-26).

COMPETING INTERESTS

None declared.

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TABLE 1 Summary characteristics of children and adults

Participant characteristic	**Children**	**Adults**
N	1,206	1,343
Age (years)	11.4 (0.5)	43.9 (5.1)
Female, %	51	87
Height (cm)	154 (8)	167 (8)
Body mass index (kg/m^2)	19 (3)	28 (6)
Body mass index z-score	0.3 (1)	-
Current smoking, %	-	8.2
Cigarettes smoked per day	-	2.6 (1.4)
Second-hand smoke, %	13.9	-
Prepubertal, %	10	-
SEIFA Disadvantage Score	1026 (62)	1026 (61)
Socioeconomic position	0.3 (0.9)	0.2 (1.0)
Aboriginal or Torres Strait Islander, %	1.3	0.7
Glycoprotein acetylation (mmol/l)	0.99 (0.13)	1.04 (0.17)
MVPA duration (min)	34 (30)	121 (56)
Asthma, %	12	10
Telomere length (T/S ratio)	1.09 (0.55)	0.81 (0.38)
Spirometric indices raw		
FEV_1 (liters)	2.5 (0.4)	3.1 (0.6)
FVC (liters)	3.0 (0.5)	4.0 (0.8)
FEV_1/FVC ratio (%)	82.9 (7.2)	76.5 (6.7)
$MMEF_{25-75}$ (liters/second)	2.6 (0.7)	2.7 (0.9)
Spirometric indices z-score		
FEV_1	0.32 (1.0)	0.32 (1.1)
FVC	0.84 (1.1)	0.91 (1.1)
FEV_1/FVC ratio	-0.78 (1.1)	-0.90 (1.0)
$MMEF_{25-75}$	-0.51 (1.1)	-0.46 (1.1)

Data are means (standard deviation) except where indicated as %. SEIFA: Socio-Economic Indexes for Areas Index of Relative Socioeconomic; MVPA: moderate-to-vigorous physical activity; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; $MMEF_{25-75}$: maximal mid-expiratory flow.

TABLE 2 Changes in each spirometric index z-score per unit increase in T/S ratio in children and adults

	Children				Adults			
Outcome z-score	**N**	**RC (95% CI)**	**R^2**	**p-value**	**N**	**RC (95% CI)**	**R^2**	**p-value**
Unadjusted								
FEV_1	1,153	-0.18 (-0.28 to -0.07)	0.01	0.001	1,293	-0.16 (0.01 to 0.3)	0.004	0.03
FVC	1,153	-0.22 (-0.32 to -0.11)	0.01	0.0001	1,293	-0.05 (-0.09 to 0.2)	0.0004	0.49
FEV_1/FVC	1,153	-0.07 (-0.04 to 0.18)	0.002	0.18	1,293	-0.19 (0.05 to 0.33)	0.005	0.008
$MMEF_{25-75}$	1,153	-0.06 (-0.17 to 0.04)	0.001	0.25	1,293	-0.27 (0.12 to 0.41)	0.01	0.0003
Model 1*								
FEV_1	1,147	-0.06 (-0.17 to 0.04)	0.14	0.21	1,283	0.05 (-0.07 to 0.16)	0.37	0.44
FVC	1,147	-0.10 (-0.19 to 0.004)	0.17	0.06	1,283	-0.05 (-0.17 to 0.06)	0.39	0.37
FEV_1/FVC	1,147	0.06 (-0.05 to 0.16)	0.08	0.28	1,283	0.17 (0.03 to 0.31)	0.03	0.02
$MMEF_{25-75}$	1,147	-0.001 (-0.11 to 0.10)	0.08	0.97	1,283	0.20 (0.06 to 0.34)	0.12	0.005
Model 2†								
FEV_1	788	-0.05 (-0.17 to 0.06)	0.20	0.36	1,005	0.06 (-0.07 to 0.19)	0.39	0.39
FVC	788	-0.08 (-0.20 to 0.04)	0.23	0.19	1,005	-0.06 (-0.19 to 0.07)	0.41	0.36
FEV_1/FVC	788	0.04 (-0.08 to 0.17)	0.13	0.49	1,005	0.21 (0.06 to 0.36)	0.08	0.008
$MMEF_{25-75}$	788	0.01 (-0.11 to 0.14)	0.12	0.87	1,005	0.23 (0.08 to 0.38)	0.15	0.003

CI: confidence interval; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; $MMEF_{25-75}$: maximal mid-expiratory flow; RC: regression coefficient; R-squared for the linear regression model.

* Model 1 adjusted for sex, age, body mass index, socioeconomic position.

† Model 2 additionally adjusted for physical activity, glycoprotein acetylation, asthma, pubertal status and second-hand smoking status for children, and smoking status for adults.

TABLE 3 Odds ratios for the lowest quartile of spirometric indices per one unit increase in T/S ratio in children and adults

	Children			**Adults**		
Outcome z-score	**N**	**Odds ratio (95% CI)**	**p-value**	**N**	**Odds ratio (95% CI)**	**p-value**
Unadjusted						
FEV_1	1,153	1.23 (0.97 to 1.56)	0.08	1,293	0.73 (0.50 to 1.07)	0.11
FVC	1,153	1.27 (1.00 to 1.61)	0.05	1,293	0.85 (0.58 to 1.25)	0.42
FEV_1/FVC	1,153	0.91 (0.71 to 1.17)	0.47	1,293	0.59 (0.41 to 0.86)	0.005
$MMEF_{25-75}$	1,153	1.10 (0.86 to 1.41)	0.43	1,293	0.63 (0.43 to 0.92)	0.02
Model 1*						
FEV_1	1,147	1.02 (0.79 to 1.32)	0.86	1,283	0.73 (0.50 to 1.07)	0.11
FVC	1,147	1.04 (0.80 to 1.35)	0.76	1,283	0.85 (0.58 to 1.25)	0.42
FEV_1/FVC	1,147	0.93 (0.71 to 1.21)	0.57	1,283	0.59 (0.41 to 0.86)	0.005
$MMEF_{25-75}$	1,147	1.03 (0.80 to 1.33)	0.82	1,283	0.63 (0.43 to 0.92)	0.02
Model 2†						
FEV_1	788	1.10 (0.80 to 1.51)	0.58	1,005	0.82 (0.53 to 1.26)	0.36
FVC	788	1.11 (0.79 to	0.56	1,00	0.97 (0.63 to	0.88

		1.54)		5	1.49)	
FEV_1/FVC	788	1.00 (0.72 to 1.40)	0.98	1,005	0.59 (0.39 to 0.89)	0.01
$MMEF_{25-75}$	788	1.10 (0.79 to 1.52)	0.57	1,005	0.64 (0.41 to 0.99)	0.04

CI: confidence interval; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; $MMEF_{25-75}$: maximal mid-expiratory flow.

* Model 1 adjusted for sex, age, body mass index, socioeconomic position.

† Model 2 additionally adjusted for physical activity, glycoprotein acetylation, asthma, pubertal status and second-hand smoking status for children, and smoking status for adults.

FIGURE 1 Longitudinal Study of Australian Children (LSAC) and Child Health CheckPoint (CheckPoint) participant flow.