

REGULAR ARTICLE

Telomere length was similar in school-age children with bronchopulmonary dysplasia and allergic asthma

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ABSTRACT

Aim: Inflammation is a major factor in the pathophysiology of bronchopulmonary dysplasia (BPD), and it contributes to accelerated telomere shortening and cellular ageing. This study aimed to determine its effect on telomere length and lung function in school-aged children born preterm with BPD.

Methods: We examined 29 children with BPD, born preterm in Stockholm county 1998–99, along with 28 children with allergic asthma born at term matched for age and gender. At 10 years of age, we measured relative telomere length (RTL) in blood by quantitative polymerase chain reaction, lung function by spirometry and inflammation by fractional exhaled nitric oxide and blood cytokines.

Results: RTL was not different in preterm born with BPD compared to term born children with asthma. The gender effect was strong in both groups; girls had significantly longer median RTL than boys (1.8 versus 1.5, $p < 0.01$). Short RTL was associated with low forced expiratory flow, also after adjusting for gender, but was not affected by severity of BPD or ongoing inflammation.

Conclusion: Telomere length was similar in 10-year-old children born preterm with a history of BPD and term born children with allergic asthma. However, impaired lung function and male gender were associated with short telomeres.

INTRODUCTION

Telomere length is a biomarker for accelerated cellular ageing (1). In most normal cells, telomeres shorten by each cell division and telomere length is negatively correlated to cellular age (2). Adult population-based studies have reported that females have longer blood telomere length than males, but gender differences are less studied in childhood (3,4). Accelerated shortening of blood cell telomeres has been linked to inflammation and oxidative stress (1), cardiovascular disease and chronic obstructive lung disease (5,6).

Survival after preterm birth has dramatically increased over the last few decades but long-term consequences remain unclear (7). The foetal origins hypothesis claims that intrauterine factors, low birth weight and prematurity have

a causal relationship to disease later in life. Increasing epidemiological evidence supports this hypothesis and preterm birth is emerging as a risk factor for arterial hypertension, chronic lung disease and type 2 diabetes (8,9), all of which may contribute to accelerated ageing, cardiovascular disease and early death (10).

Lung disease is still the major cause of mortality and morbidity in preterm born infants (11). Bronchopulmonary dysplasia (BPD) is defined as a prolonged oxygen

Abbreviations

BPD, Bronchopulmonary dysplasia; FEF, Forced expiratory flow; FeNO, Fraction of exhaled nitric oxide; FEV1, Forced expiratory volume in one-second; FVC, Forced vital capacity; PULMM, PREMATURE follow-up with Lung function Mannitol and Methacholine; RTL, Relative telomere length.

Key notes

- Cellular ageing, measured as telomere length, could be accelerated by inflammatory conditions such as bronchopulmonary dysplasia (BPD) or childhood asthma.
- Telomere length was similar in 10-year-old children, irrespective of a history of preterm birth, BPD or childhood asthma, but longer in girls and shorter with low forced expiratory capacity.
- In children growing up with lung disease, lung function impairment and male gender were independently associated with shorter telomeres.

requirement, and in its more severe forms, it is characterised by arrested alveolar development and a simplified lung structure. The pathogenesis of BPD is complex and contributing factors are persistent bronchial inflammation (12) and oxidative stress (13) in association with physiological and structural lung immaturity. The long-term consequences of preterm birth and BPD are reflected in impaired lung function at school age and young adulthood (7). Furthermore, there are concerns that children with BPD will grow up and develop a phenotype similar to that of chronic obstructive pulmonary disease (14). A study by Hadchouel et al. (15) showed a significant association between telomere length measured in saliva and lung function in preterm born adolescents.

Hence, several mechanisms that can potentially influence telomere biology are at play in the neonatal period. In this case-controlled cohort study of preterm children with BPD born in Stockholm, we measured telomere length in blood and lung function at 10 years of age in comparison with term born children diagnosed with allergic asthma. The aim was to investigate blood telomere length in relation to perinatal factors such as preterm birth and neonatal lung disease.

METHODS

Study design

Our study population was part of a larger follow-up study of preterm born children with BPD, the Premature follow-up with Lung function Mannitol and Methacholine (PULMM) study, subjects identified from all children born in Stockholm County between 1998 and 1999 (16). BPD was defined as the need for oxygen for 28 days postpartum and was graded into mild, moderate or severe at 36 weeks

corrected gestational age, according to the definitions by Jobe and Bancalari (17). Mild BPD was defined as no breathing support, moderate BPD as the need for supplementary oxygen of <30% and severe BPD as the need for supplementary oxygen of 30% or more and, or, ventilatory support. Children with BPD were invited to a follow-up examination at 10 years of age and a control group was recruited at the outpatient clinic of the Pediatric Asthma and Allergy department at Karolinska University Hospital, Stockholm. The control group consisted of gender- and age-matched children with allergic asthma, born at term and without respiratory symptoms in the neonatal period. The follow-up investigations included blood sampling and lung function testing. All individuals available for follow-up, who had a blood sample collected, were included in this study (Fig. 1). As the study cohort was predefined, sample size calculations were not performed and the sample number was limited. Furthermore, no control group of healthy individuals was available.

Perinatal information was retrieved from the Medical Birth Registry and neonatal morbidity parameters were extracted from hospital records. Parental written informed consent was obtained. The Regional Ethical Review Board in Stockholm granted approval for the PULMM study (Dnr 2008/1677-31/4 and Dnr 2009/684-32) and the add-on protocol for telomere analyses (Dnr 2012/679-32 2012-05-07).

Measurement of telomere length

Whole blood, 2–3 mL, was collected in ethylenediaminetetraacetic acid (EDTA) tubes and frozen at -80°C within one hour. Genomic deoxyribonucleic acid (DNA) was extracted using the MagNA Pure LC instrument (Roche Diagnostics Scandinavia AB, Stockholm, Sweden), and

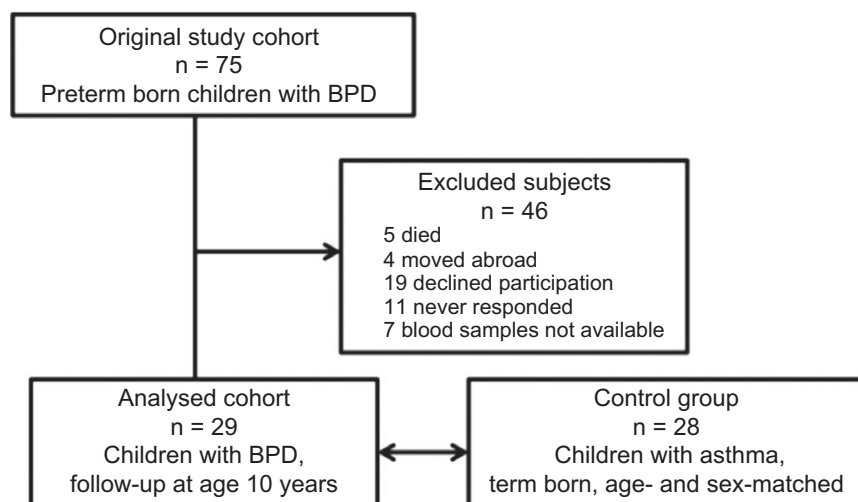


Figure 1 Flow chart of eligible patients from the original cohort of children with bronchopulmonary dysplasia (BPD) born in Stockholm and prospectively identified over 22 months from 1998 to 1999. Ten years later, a letter was sent out asking them to participate in the Premature follow-up with Lung function Mannitol and Methacholine (PULMM) study. The final cohort included in the analyses of telomere length consisted of 57 children, the former preterm born children with BPD and a term born matched control group of children with allergic asthma recruited through the patient register at the Pediatric Pulmonology Outpatient Clinic, Karolinska University Hospital, Stockholm.

DNA yield and purity were determined spectrophotometrically using the NanoDrop (Thermo Fisher Scientific Inc., Wilmington, DE, USA). Relative telomere length (RTL) was determined by the quantitative polymerase chain reaction method described by Cawthon et al. (18), with minor modifications (19), with an inter-assay coefficient of variation between 4 and 8%. The telomere/haemoglobin subunit beta, gene ID:3043 (TEL/HBB) and telomere/single copy gene (T/S) values were calculated by the $2^{-\Delta C_t}$ method, where $\Delta C_t = C_{t_{TEL}} - C_{t_{HBB}}$. C_t refers to the threshold cycle. The RTL value for each sample was generated by dividing the samples T/S value with the T/S value of a T-lymphocyte cell line called CCRF-CEM, which was used as a reference and included in all runs.

Lung function testing

Dynamic spirometry performed using the Vitalograph 2120 (Ennis of Ireland, County Clare, Ireland) was used to assess pulmonary function prebronchodilation, including forced vital capacity (FVC), forced expiratory volume in one-second (FEV₁) and forced expiratory flow (FEF), using the European Respiratory Society and Polgar reference values (20). A positive reversibility test was defined as an increase of at least 10% in FEV₁ after 0.5 mg inhaled terbutaline. Plethysmography (CareFusion, Bavaria, Germany) was used to measure lung volumes: vital capacity, total lung capacity, fractionated residual volume and residual lung volumes. Diffusion capacity for carbon monoxide was evaluated using a single breath technique. Reference values of Hedenström Solymar were applied for static spirometry values and diffusion capacity (21). A more detailed description of lung function in this cohort was published by Nordlund et al. in 2017 (16) and in accordance with that we have reported lung function values as percentages of predicted values.

Inflammatory markers

Exhaled fractional nitric oxide (FeNO), reported as parts per billion, was performed using Niox equipment (Aerocrine AB, Stockholm, Sweden) according to published methodological guidelines (22). Blood cytokines, interleukin-6, interleukin-8, tumour necrosis factor- α and interleukin-1 β , were analysed by the electro-chemiluminescence immunoassay routinely used by the Karolinska University Hospital Laboratory.

Statistical analysis

The main outcome measure was RTL. Crude and adjusted linear regression was used to investigate group differences in RTL, lung function, inflammatory markers and background characteristics. Associations were assessed using t-test and chi-square test for continuous and categorical variables, respectively. In the preterm group, crude and adjusted linear regression was used to analyse differences in RTL in relation to different predictor variables. An F-test was performed for categorical predictors with more than two categories. Data with normal distribution are reported as means and standard deviations and data with

non-normal distribution as medians and interquartile ranges. Statistical significance was set to $p < 0.05$. Analyses were performed using STATA Version 13 (StataCorp LLC, College Station, TX, USA) and IBM SPSS Statistics version 24 (IBM Corporation, Armonk, NY, USA).

RESULTS

There were 57 children enrolled in the telomere length study, 29 preterm infants with BPD and 28 term controls with allergic asthma (Fig. 1). The preterm group had a mean gestational age of 27 weeks and a birth weight of 1070 g; the basic perinatal characteristics are described further in Table S1. More than 90% were Caucasians and ethnicity, parental age and smoking habits at birth did not differ between groups. Neonatal respiratory disease and outcomes are also described in Table S1.

The characteristics of the cohort at the age of 10 are summarised in Table 1. Both groups had impaired lung function compared with normal reference values. Preterm children with BPD showed significantly lower results than

Table 1 Basic physiology, lung function and inflammatory markers at 10 years of age

	BPD/preterm (n = 29)	Asthma/term (n = 28)	p
Gender, male/female (% male)	20/9 (69)	19/9 (68)	0.93
Age at study start, years	10.4 $\pm$ 0.9	10.6 $\pm$ 0.6	0.26
Weight, kilograms	36 $\pm$ 7	40 $\pm$ 7	0.03
Length, centimetres	142 $\pm$ 10	147 $\pm$ 7	0.02
Body mass index, BMI	18 $\pm$ 2	18 $\pm$ 2	0.23
Systolic blood pressure, mmHg	102 $\pm$ 7	106 $\pm$ 6	0.07
FEV ₁	77 $\pm$ 12	84 $\pm$ 11	0.01
FVC	95 $\pm$ 14	99 $\pm$ 11	0.32
FEF ₅₀	61 $\pm$ 15	79 $\pm$ 21	<0.01
FEF _{25–75}	55 $\pm$ 14	69 $\pm$ 19	<0.01
Vital capacity	95 $\pm$ 13	99 $\pm$ 12	0.25
Total lung capacity	103 $\pm$ 12	104 $\pm$ 23	0.80
FRC	112 $\pm$ 20	109 $\pm$ 19	0.67
RV	160 $\pm$ 51	167 $\pm$ 40	0.58
DLCO adjusted, mmol/min/kPa	81 $\pm$ 13	88 $\pm$ 10	0.03
Positive reversibility test (%)	5 (20)*	7 (25)	
Asthma medication (%)	11 (39) [†]	28 (100)	
Interleukin 1 beta, IL-1 β	1.0 $\pm$ 1.4	1.2 $\pm$ 1.2	0.50
Interleukin 6, IL-6	0.1 $\pm$ 0.7	0.0 $\pm$ 0.0	0.33
Interleukin 8, IL-8	9.8 $\pm$ 11.3	6.4 $\pm$ 4.3	0.14
Tumour necrosis factor, TNF- α	12.2 $\pm$ 2.6	11.3 $\pm$ 3.5	0.14
Fractional exhaled nitric oxide, ppb	12.2 $\pm$ 9.3 [‡]	23.7 $\pm$ 21.1 [†]	0.01

DLCO = Diffusing capacity for carbon monoxide adjusted for age.

Mean $\pm$ SD unless otherwise stated. BMI calculated from <http://www.cdc.gov/healthyweight/assessing/bmi/index.html>. The following are reported as per cent of expected value, corrected for height: FEV₁ = forced expiratory volume in one-second; FVC = Forced vital capacity; FEF = Forced expiratory flow, 50% or 25–75%; FRC = Fractionated residual capacity; RV = Residual volume. Asthma medication defined as inhaled steroids, long-acting beta-agonist, montelukast in the six months period preceding the study.

*Missing data n = 4.

[†]Missing data n = 1.

[‡]Missing data n = 3.

term children with asthma for both dynamic and static spirometry, which was most pronounced in the smaller airways with reduced $FEF_{50\%}$ ($p < 0.01$) and impaired diffusion capacity ($p = 0.03$; Table 1). Lung function parameters expressed as Z-scores are given in Table S3.

Airway inflammation, measured as FeNO, was more prominent in the group of children with asthma than the BPD group ($p < 0.01$). Other blood cytokine levels were similar (Table 1).

RTL showed no significant difference at the age of 10 years between the two groups as shown in Table 2 and in Figures S1 and S2.

RTL was significantly affected by gender, with females having longer telomeres than males in the total cohort as well as in the BPD and the asthma groups, respectively (Table 2, Fig. 2, Fig. S2). The difference was also significant when adjusted for different background variables in the neonatal period, such as gestational age, birth weight, small for gestational age, mode of delivery, parental age and parity and descriptive variables at the 10-year follow-up, such as exact age at the study start, weight, height, body mass index and blood pressure.

RTL did not correlate to perinatal factors such as gestational age, birth weight, small for gestational age, mode of delivery, parental age or parity (Table 2). Nor did RTL correlate to basic physiological parameters at the age of 10 years, such as age, weight, length, body mass index or blood pressure (Table S2). Morbidity during the neonatal period, described by ventilatory support requirements, surfactant administration, duration of supplemental oxygen, neonatal sepsis, intraventricular haemorrhage, retinopathy

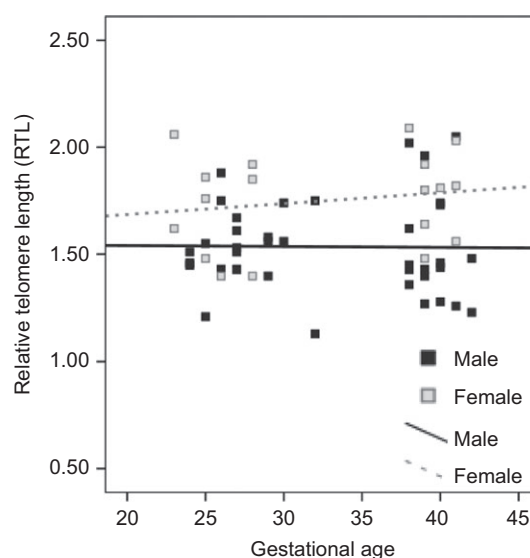


Figure 2 Relative telomere length (RTL) in 10-year-old children in relation to gestational age and gender.

of prematurity, patent ductus arteriosus and the severity of BPD, did not affect RTL at the age of 10 years (data not shown).

RTL showed a positive association to dynamic spirometry indices, FEV_1/FVC , $FEF_{50\%}$ and $FEF_{25\%}$ in the crude linear regression (Table 3). When adjusted for gender, this

Table 2 Relative telomere length at 10 years of age

	N	RTL
Group		
Preterm/BPD	29	1.56 [1.45;1.74]
Term/asthma	28	1.52 [1.43;1.82]
Gender		
Female	18	1.80 [1.56;1.92]*
Male	39	1.49 [1.42;1.66]
Preterm female	9	1.76 [1.48;1.86]*
Preterm male	20	1.54 [1.44;1.64]
Term female	9	1.81 [1.63;1.92]*
Term male	19	1.45 [1.36;1.73]
Mode of delivery [†]		
Partus normalis	32	1.53 [1.41;1.82]
Secio	22	1.57 [1.45;1.75]
Small for gestational age [‡]		
Yes	6	1.59 [1.42;1.66]
No	50	1.56 [1.43;1.80]

RTL = Relative telomere length; BPD = Bronchopulmonary dysplasia.

Median and interquartile range (IQR).

*Statistical significant difference: Female versus male $p < 0.01$, preterm female versus preterm male $p < 0.05$ and term female versus term male $p < 0.05$.

[†]Missing data $n = 3$.

[‡]Missing data $n = 1$.

Table 3 Relative telomere length (RTL) predicted by lung function and inflammation – crude regression analysis

	N	Coefficient (95%CI)
Lung function		
FEV_1/FVC		
All	55	0.01 [0.005 to 0.02]**
Preterm	27	0.006 [−0.002 to 0.01]
Term	28	0.015 [0.006 to 0.02]**
FEF_{50}		
All	55	0.003 [0.0004 to 0.006]*
Preterm	27	0.0005 [−0.005 to 0.006]
Term	28	0.006 [0.001 to 0.01]*
Inflammation		
FeNO		
All	53	0.001 [−0.003 to 0.005]
Preterm	26	0.004 [−0.005 to 0.012]
Term	27	0.0005 [−0.005 to 0.006]

FEV_1 = Forced expiratory volume one second; FVC = Forced vital capacity; FEF = Forced expiratory fraction, 50% or 25–75%; $DLCO$ = Diffusing capacity for carbon monoxide adjusted for age; FeNO = Fraction of exhaled nitric oxide, parts per billion.

Coefficient for change in relative telomere length in relation to lung function and inflammation, for example, with every percentage of increase in FEV_1/FVC , RTL is 0.01 units longer with a significance of $p < 0.01$.

*Significance $p < 0.05$.

**Significance $p < 0.01$.

association only remained statistically significant for FEF_{50%} in the total cohort (regression coefficient = 0.003, $p = 0.03$) and the asthma group alone (regression coefficient = 0.004, $p = 0.02$). In the preterm BPD group alone, no association between RTL and dynamic spirometry indices was found. When RTL was analysed in relation to the inflammatory markers, it did not reveal any associations (Table 3, Table S2).

DISCUSSION

To the best of our knowledge, this is the first study to analyse blood telomere length in relation to lung function in 10-year-old children born preterm with BPD. A previous study by Hadchouel et al. (15) measured salivary telomere length in adolescents born preterm and showed an association between lung function and telomere length in this age group. Our main findings were that RTL was not shorter in 10-year-old children with a history of preterm birth and BPD when compared to term born children with allergic asthma. In addition, female gender was a strong determinant for longer RTL in childhood, impaired lung function in childhood was associated to shorter RTL and the severity of neonatal lung disease did not impact RTL at the age of 10.

RTL has almost exclusively been studied in adults, but data are emerging on RTL in relation to prematurity. Previous studies have shown that extremely preterm and very low birth weight infants have longer telomeres at birth (23,24). In contrast, leucocyte RTL in young adulthood was shorter in individuals born preterm with a mean gestational age of 32 weeks (25). In our study, we could not detect a difference in RTL due to prematurity at the age of 10 years, although our cohort was more immature, with a mean gestational age of 27 weeks and had greater neonatal morbidity. Our controls were term born and healthy at birth, but they later developed childhood asthma. The fact that both groups were affected by lung disease may have attenuated the differences in RTL related to gestational age. Previous studies have shown that body size at birth had no association to RTL in adulthood (26) and children born small for gestational age did not have short telomeres at birth (27). This was in line with our findings that there were no associations between RTL and body size at the age of 10 years or between RTL and weight or small for gestational age at birth.

Our finding that females had longer telomeres than males was consistent with previous reports (25,28). The follow-up of the French national Epidemiological Study on Small Gestational Ages cohort of children born extremely preterm showed no association between perinatal events and RTL in buccal cells at the age of 15 years and female gender was the only factor significantly associated with RTL (15). Whether gender differences are already present at birth is unclear (3). In addition, hereditary factors and ethnicity have been shown to play a role in telomere biology (4). To explain the gender difference, the hypothesis that oestrogen might reduce telomere attrition rate by its anti-oxidant effect has been postulated but that remains to be proven

(28). Our results confirm that gender needs to be accounted for in studies of RTL and that the size effect may be equal or greater to that of other potential risk factors for accelerated telomere attrition.

The association between lung function and telomere length in circulating leucocytes was recently elucidated in a large meta-analysis of 3768 cases and 44 059 controls. This showed significant negative associations for both asthma and chronic obstructive pulmonary disease. Spirometric indices in 12 595 individuals showed a positive association between RTL and FEV₁, FVC and FEV₁/FVC (6). In our material, the crude analysis also revealed a positive association between RTL and FEV₁/FVC, but this was no longer significant when adjusting for gender. For spirometry indices, the only remaining significant association was between RTL and FEF_{50%}. This was in line with the only other study reporting on lung function in relation to RTL in children growing up following extreme preterm birth (15). However, in our material, this was only significant when we looked at all children or just the term born asthma group, but not in children born preterm. As our sample size was limited, with only 29 individuals in the preterm group and the RTL changes were relatively small, the clinical relevance remains to be determined. Chronic asthma has been related to shorter telomeres in adulthood, possibly via systemic eosinophilic inflammation, but childhood onset of asthma without persistence did not affect RTL in mid-life (29). In our cohort, the children with asthma had significantly more ongoing airway inflammation than the children with a history of BPD and this was consistent with Baraldi et al. (30), which also showed lower FeNO levels in children with BPD compared with matched healthy controls. On the other hand, Filippone et al. (13) showed evidence of persistent oxidative stress measured as 8-isoprostane in the exhaled breath condensate of preterm born adolescents, but levels of FeNO did not differ in the groups born preterm, with or without BPD, or the healthy term born controls. However, low levels of FeNO in our cohort were not reflected in blood cytokines, which may suggest that inflammation occurs very locally in the lung and do not affect RTL in blood cells. The PULMM study concluded that reduced lung function characterised by hyper-responsiveness towards direct acting methacholine and impaired diffusion capacity were respiratory hallmarks of BPD in the same children that we included in our cohort (16).

The major limitations of our study were the lack of a term born control group with full health and a preterm born control group without BPD. The choice of children with asthma as controls was pragmatic, as these were individuals that would routinely undergo lung function testing at age 10 years. However, it limited the possibility to fully disentangle the relationship between neonatal events and RTL later in life, as the impact of childhood lung disease cannot be overlooked. In addition, it did not allow us to clearly evaluate the effect on RTL by impaired lung function, as the RTL in children with normal lung function was unknown. Our limited sample size might have precluded the detection

of RTL differences between the groups and did not allow us to analyse relative telomere length in relation to the degrees of severity of BPD. Furthermore, we have only measured RTL at one time point at the age of 10 years and that has prevented any conclusions on telomere attrition rate over time, which may be an important predictor for cellular ageing and future disease. With these limitations, this study adds new knowledge of blood RTL in relation to neonatal lung disease and lung function in childhood. In our cohort, children with BPD were prospectively identified and details about treatment and disease severity were available. Furthermore, our results are of importance as the mean gestational age was only 27 weeks and data on RTL in children born extremely preterm are scarce.

CONCLUSION

Blood telomere length at the age of 10 years was not shorter in children with a history of preterm birth and BPD, compared with term born children with allergic asthma. Impaired lung function with low forced expiratory flows and male gender were independently associated with shorter telomere length. Larger and prospective longitudinal studies with healthy controls are needed to further elucidate the importance of cellular ageing and telomere biology in children growing up with lung disease related to lung immaturity and inflammation.

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CONFLICT OF INTERESTS

The authors have no conflict of interests to declare.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

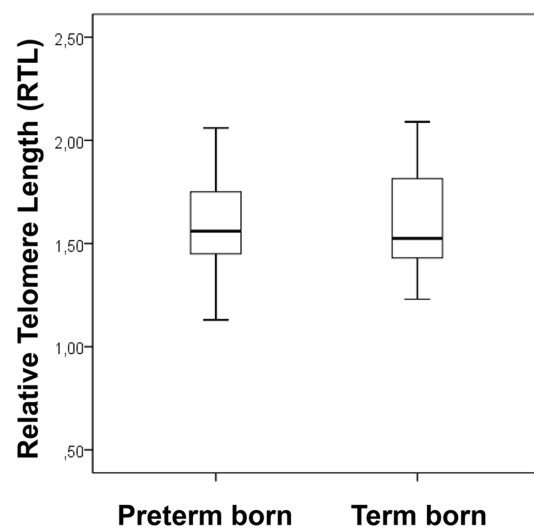
Figure S1 Relative telomere length (RTL) in 10-year-old children in blood in relation to preterm birth.

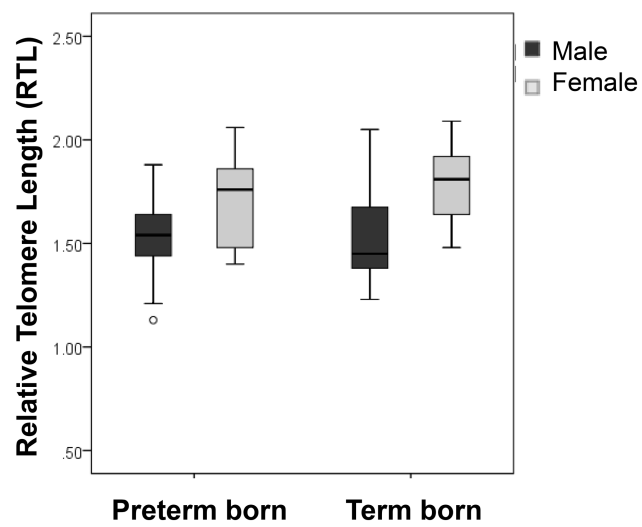
Figure S2 Relative telomere length (RTL) in 10-year-old children in relation to preterm birth and gender.

Table S1 Neonatal characteristics and morbidity.

Table S2 Crude analysis of relative telomere length in relation to perinatal factors, physiological parameters and inflammation at 10 years of age.

Table S3 Lung function in Z-score and relative telomere length (RTL) in school age children with lung disease.





SUPPORTING INFORMATION

Table S1. Neonatal characteristics and morbidity

	BPD/preterm (n=29)	Asthma/term (n=28)
Gestational age, weeks ^{+days} (range)	27 ⁺¹ (23 ⁺² -32 ⁺⁵)	40 ⁺⁰ (38 ⁺¹ -42 ⁺¹)
Birth weight, grams	1070 ±423	3591 ±356
Caesarean section, n (%)	17 (59)	5 (20) [#]
Antenatal steroids, n (%)	25 (85)	-
PROM, n (%)	5 (17)	-
Surfactant treatment, n (%)	18 (69) [¶]	-
Mechanical ventilation, days	10 ±12	-
Duration of oxygen treatment, days	76 ±38	-
Postnatal steroid treatment, n (%)	25 (86)	-
BPD grade, n (%) – mild	12 (41)	-
- moderate	10 (35)	-
- severe	7 (24)	-
PDA treatment, n (%)	19 (66)	-
Neonatal sepsis, n (%)	16 (55)	-

Mean ±SD if not otherwise specified. [#]Missing data n=2, [¶]missing data n=3.

BPD=bronchopulmonary dysplasia, PROM=premature rupture of membrane, PDA=patent ductus arteriosus

SUPPORTING INFORMATION

Table S2. Crude analysis of relative telomere length in relation to perinatal factors, physiological parameters and inflammation at 10 years of age

	N	Coefficient (95%CI)	P
<i>Perinatal factors</i>			
Birth weight	56	0.003 [-0.046 - 0.05]	0.90
Mothers age	57	-0.0007 [-0.016 - 0.01]	0.92
Fathers age	55	0.003 [-0.007 - 0.01]	0.54
Parity, multipara	31	0.03 [-0.09 - 0.16]	0.59
<i>Physiological parameters at age 10</i>			
Age at study start	57	-0.0002 [-0.08 – 0.08]	1.0
BMI	57	-0.005 [-0.03 - 0.02]	0.69
Weight at 10 years	57	0.0009 [-0.008 - 0.01]	0.84
Length at 10 years	57	0.004 [-0.003 - 0.01]	0.30
Blood pressure systolic	56	0.003 [-0.006 - 0.13]	0.49
Blood pressure diastolic	56	0.003 [-0.006 - 0.01]	0.57
<i>Inflammation</i>			
Interleukin 1 beta (IL-1 β)	57	0.045 [-0.002 - 0.093]	0.06
Interleukin 8 (IL-8)	56	-0.005 [-0.12 - 0.002]	0.18
Tumour necrosis factor (TNF- α)	57	-0.011 [-0.03 - 0.009]	0.26
<i>Lung function at age 10</i>			
FEF₂₅₋₇₅			
All	55	0.003 [-0.00003 - 0.007]	0.05
Preterm	27	0.002 [-0.004 - 0.007]	0.51
Term	28	0.005 [-0.0004 - 0.01]	0.07
FEF₂₅			
All	55	0.004 [0.000 - 0.008]	0.04

Preterm	27	0.002 [-0.003 - 0.007]	0.47
Term	28	0.006 [-0.0001 - 0.01]	0.05
DLCO adjust			
All	49	-0.025 [-0.10 - 0.05]	0.50
Preterm	22	0.03 [-0.06 - 0.12]	0.54
Term	27	-0.07 [-0.20 - 0.05]	0.24

SUPPORTING INFORMATION

Table S3. Lung function in Z-score and relative telomere length (RTL) in school age children with lung disease

	Mild BPD N=13	Moderate BPD N=14	Preterm / BPD N=27	Term / Asthma N=27
Z-score FEV ₁	-0.83 [-1.36; 0.39]	-2.32 [-2.72; -1.34]	-1.35 [-2.39; -0.71]	-0.7 [-1.88; 0.35]
Z-score FVC	0.16 [-0.77; 1.52]	-1.30 [-1.91; -0.80]	-0.87 [-1.41; 0.16]	-0.06 [-0.67; 0.43]
Z-score FEV ₁ /FVC	-1.49 [-1.88; -0.99]	-1.38 [-2.46; -0.96]	-1.43 [-1.92; -1.02]	-0.83 [-1.71; 0.97]
Z-score FEF _{25-75%}	-1.25 [-2.29; -0.82]	-2.34 [-3.07; -1.36]	-1.53 [-2.47; -0.97]	-1.18 [-1.99; -0.04]
Z-score FEF _{75%}	-0.91 [-1.61; -0.30]	-1.65 [-2.61; -0.87]	-1.30 [-1.77; -0.55]	-0.82 [-1.49; 0.24]
RTL	1.51 [1.41; 1.65]	1.61 [1.47; 1.76]	1.55 [1.44; 1.75]	1.52 [1.43; 1.82]

Median and interquartile range [IQR].

Z-score corrected for age, length, gender and ethnicity.

Calculations according to Global Lung Initiative, European respiratory society, Philip H.

Quanjer, Sanja Stanoievic, Tim J Cole, Janet Stocks. Individual calculations performed using

GLI-2012 Desktop Software, <http://www.ers-education.org/guidelines/global-lung-function-initiative/spirometry-tools/desktop-individual-calculator.aspx>

Missing values for lung function from two children in the preterm group and one child in the term group

BPD=bronchopulmonary dysplasia, RTL=relative telomere length, FEV₁=Forced Expiratory Volume in one second, FVC=Forced Vital Capacity, FEF=Forced Expiratory Flow, 75% or 25-75%