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Early pregnancy intrauterine fetal exposure to maternal smoking and impact on fetal telomere length

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ABSTRACT

Background: Reduced telomere length, or its accelerated attrition, has been implicated in aging, mortality, and several human diseases, including respiratory diseases. Age dependent manifestation of telomere-mediated disease during life span indicates the role of developmental stage in these diseases and highlights the importance of fetal developmental process *in utero* and at earlier life stages. Environmental determinants during developmental and later stages of life could affect telomere length. Smoke exposure as one of these significant determinants have been investigated in association with telomere length in neonates at time of delivery, children and adults.

Objective: We sought to investigate whether intrauterine fetal exposure to tobacco smoking characterized by placenta cotinine levels during early weeks of pregnancy might be associated with shorter relative telomere length (T/S ratio) as compared to fetuses without exposure to tobacco smoking.

Study design: 207 Human placenta and epithelial lung samples were used for both fetal lung telomere length assessment and measurement of placental cotinine levels. Tissues were obtained from two NICHD–supported tissue retrieval programs with registries for elective abortions, the University of Washington Center for Birth Defects Research (Seattle, WA) and the University of Maryland Brain and Tissue Bank for Developmental Disorders (Baltimore, MD). Cotinine levels (ng/g total placental tissue) were determined in whole cell extracts prepared from human placenta samples to characterize and confirm the cotinine exposure status associated with maternal smoking. Relative telomere length (T/S ratio) in genomic DNA extracted from fetal lung tissue was measured by use of quantitative real-time polymerase chain reaction. Multivariable linear regression was used to investigate the relationship between fetal Telomere-to-Single Copy (T/S) ratio and tobacco exposure.

Results: The estimated post-conception ages for included samples in the study ranged from 54 to 137 days (7–19 weeks of gestation); 47.37% of fetal samples had female sex. Of the samples included in the analysis 96 and 111 fetal samples with and without intrauterine tobacco smoking exposure were distinguished. While T/S ratio was not different between those with and without smoking exposure (1.24 ± 0.41 and 1.27 ± 0.48, respectively; $P = 0.70$), a significant effect modification of post-conception age on the relationship of intrauterine smoke exposure on fetal T/S ratio was observed (adjusted coefficient = -0.008, 95% CI: -0.016, -0.0004). The smoke exposure status was associated with T/S ratio after 93-day post conception (adjusted coefficient = -0.29, 95% CI: -0.53, -0.052).

Conclusions: Our results demonstrate a significant association of smoke exposure *in utero* at early pregnancy with shortened fetal relative telomere length in the developing lung and suggest that the detrimental effect of smoking exposure on future disease sequelae may start at the early stages of pregnancy.

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1 Introduction

The prevalence of women who are active smokers is estimated to be 23.2% and 10.7% in pre-pregnancy and during pregnancy, respectively in the United States [1]. Intrauterine fetal exposure to maternal smoking might impose detrimental consequences on fetal lung development and pulmonary function such that respiratory disease predisposition could be likely during postnatal life. These potential effects could be subtle and undetectable at early developmental stages, resulting in later alterations as well as long-term adverse pulmonary outcomes [2]. For example, shorter telomere length has been linked to decreased lung function and increased risk of chronic obstructive pulmonary disease (COPD) and interstitial lung diseases [3–5].

Telomeres, repetitive DNA sequences capping the chromosomal ends, prevent the loss of coding genetic material during chromosomal replication and in different cell type have been linked to the maintenance of genomic integrity [6]. Reduced telomere length, or its accelerated attrition, has been implicated in several diseases and syndromes, including respiratory diseases [7,8]. Age dependent manifestations of telomere-mediated syndromes such as Hoyeraal–Hreidarsson syndrome, dyskeratotis, congenital aplastic anemia, and pulmonary fibrosis indicate the role of developmental and early life stage [9]. Recent longitudinal studies have provided substantial evidence that telomere length contribute to the onset of diseases such as asthma that highlights the importance of the fetal developmental process *in utero* and in early life stages [10,11]. Twin studies have shown that environmental encounters, in addition to genetic factors, have a substantial role in the observed association of telomere length with morbidity and mortality [12]. Accordingly, time-dependent exposure to various deleterious environmental factors may alter the telomere biology and cause cellular dysfunction, senescence and disease susceptibility during life.

The effect of smoking on leukocyte telomere length in neonates at time of delivery, and on the rate of its shortening in children and adults have been examined in a few studies [13,14]. These findings are suggestive of the possibility of early intrauterine programming for accelerated aging and earlier predisposition to morbidities. To our knowledge, there has been no study to investigate the effect of early pregnancy *in utero* tobacco smoke exposure on fetal telomere length of the developing pulmonary system. Therefore, we aimed to investigate whether fetal exposure to tobacco smoking in early pregnancy might be associated with shorter fetal telomere length as compared to fetuses without exposure to tobacco smoking and whether this effect might be dependent on post conception age.

Table 1
Fetal sample's characteristics.

	Intrauterine Smoke Exposure Status		P-value
	Negative (n = 111)	Positive (n = 96)	
Sex (n, %)			
Female	52 (46.85)	47 (48.96)	0.76
Male	59 (53.15)	49 (51.04)	
Post-Conception Age (days)			
Mean (SD)	85.5 (15.3)	85.6 (16.9)	0.95
Range	57–127	54–137	
T/S ratio			
Mean (SD)	1.27 (0.48)	1.24 (0.41)	0.70
Range	0.58–2.82	0.6–2.82	
Placenta Cotinine Level (ng/g)			
Median	0	57.83	<0.001
Range	0–5.27	8.42–149.99	
0 (n, %)	88 (79.28)	–	–
>0 and ≤7.53 (n, %)	23 (20.72)	–	

2 Materials and methods

2.1 Tissue samples

As previously detailed [15,16], human fetal lung and placental tissues were obtained from two NICHD–supported tissue retrieval programs, the University of Washington Center for Birth Defects Research (Seattle, WA) and the University of Maryland Brain and Tissue Bank for Developmental Disorders (Baltimore, MD). The samples were from elective fetal abortions and the post-mortem interval was <2 h and <6 h for samples from the University of Washington and the University of Maryland, respectively. Samples were anonymized, flash frozen and maintained at −80 °C prior to preparation of whole cell extracts. The University of Missouri-Kansas City Pediatric Health Sciences Review Board declared the use of these tissues non–human subject research as approved by Institutional Board Review at Brigham and Women's Hospital. 207 samples had qualified data for both fetal lung telomere length assessment and measurement of placental cotinine levels. The samples' fetal sexes were identified by determination of X chromosome heterozygosity rates using available GWAS data on samples. Placenta cotinine level was used to determine the mothers' smoking exposure status.

2.2 Smoking exposure status

Maternal smoking status was unknown due to the anonymity of samples. Therefore, to characterize the exposure status associated with maternal cigarette smoking, cotinine levels (ng/g total placental tissue) were determined in whole cell extracts prepared from placenta samples. Crude placenta tissue extracts were prepared from 100 to 150 mg placenta tissue by homogenization in 200 ml homogenization buffer (50 mM Tris-HCl, 150 mM KCl, 2 mM EDTA, pH 7.5). Homogenates were centrifuged at 1000*g* for 15 min at 4 °C. Cotinine, the major metabolite of nicotine, was detected using 10 μl placenta extract (diluted 1:2 in tissue homogenization buffer) with the Cotinine Direct ELISA kit (Calbiotech, Spring Valley, CA) according to the manufacturer's guidelines. Standard curves were prepared from serial dilutions of cotinine. Cotinine concentrations in placenta extracts were calculated from a 4-parameter log analysis of the standard curves as previously described [15]. We applied the cotinine cut-off of 7.53 ng/g (0 or any value less than 7.53 ng/g) demonstrating highest sensitivity (0.8) and specificity (1.0) in reflecting the maternal tobacco smoke status (either environmental tobacco smoke exposure or active smoking) during early pregnancy [15]. This cut-off was determined by examining cotinine placenta level of pregnant women with known smoking status [15]. Nevertheless, to examine any potential effect of low placenta cotinine concentrations (0 < placenta cotinine level ≤ 7.53 ng/g) on T/S ratio, a post-hoc sensitivity analysis was conducted to investigate the relationship of T/S ratios with post conception day after exclusion of subjects with low placenta cotinine. Additionally, the T/S ratio in samples with placenta cotinine levels of ≤7.53 ng/g but more than 0 was compared with those with 0 ng/g placenta cotinine levels.

2.3 Telomere length

Telomere length in genomic DNA extracted from fetal lung tissue was measured by use of quantitative real-time polymerase chain reaction using a 7900HT Thermocycler (Applied Biosystems) and determined as previously described [17]. Although the assay provides a relative measurement of telomere length, ratios of