Prenatal stress and newborn telomere length

Article in American journal of obstetrics and gynecology · January 2016
DOI: 10.1016/j.ajog.2016.01.177

All in-text references underlined in blue are linked to publications on ResearchGate, letting you access and read them immediately.

Available from: Mark Haussmann
Retrieved on: 17 August 2016
Prenatal stress and newborn telomere length

Nicole M. Marchetto, MD; Rebecca A. Glynn, BS; Mackenzie L. Ferry, BS; Maja Ostojic, BS; Sandra M. Wolff, MD; Ruofan Yao, MD; Mark F. Haussmann, PhD

BACKGROUND: The developmental origin of the health and disease hypothesis is based on the premise that many chronic diseases have their roots in fetal development. Specifically, maternal stress during pregnancy is associated with altered fetal development and many adverse long-term health outcomes. Although the mechanisms underlying this effect are currently unclear, at the cellular level 1 possible mediator is the regulation of telomere length. Telomere dynamics appear to play a role in disease progression, and an adverse intrauterine environment may contribute to the establishment of short telomeres in newborns. In accordance with this, it was recently reported that prenatal stress is significantly associated with shorter mean newborn telomere length. However, this finding has yet to be replicated, and currently we know nothing about whether different size classes of telomeres within the telomere length distribution are differentially affected by prenatal stress. Examining telomere length frequency distributions is important, because the shortest telomeres in the distribution appear to be the most indicative of telomere dysfunction and thus the best predictors of mortality and morbidity in humans.

OBJECTIVE: We investigated the effects of intrauterine exposure to maternal stress over the whole course of gestation on newborn mean telomere length and telomere length frequency distributions.

STUDY DESIGN: We conducted a prospective cohort study of 24 mother-newborn dyads at an urban teaching hospital. Pregnant women with nonanomalous, uncomplicated pregnancies were recruited and assessed in the third trimester of gestation. Maternal psychosocial stress was quantified using the Holmes and Rahe Stress Scale and categorized as high stress (≥300 points) or low stress (<299 points) exposure. Newborn telomere length was measured from cord blood at delivery using the Telomere Restriction Fragment assay.

RESULTS: We found a significant negative association between maternal stress and newborn telomere length (β = −0.463, P = 0.04). Newborns whose mothers experienced a high level of stress during pregnancy had significantly shorter telomere length (6.98 ± 0.41 kb) compared to newborns of mothers with low stress (8.74 ± 0.24 kb; t = −3.99, P = .003). Moreover, the difference in newborn telomere length between high-stress and low-stress mothers was due to a shift in the telomere length distribution, with the high-stress group showing an underrepresentation of longer telomeres and an over-representation of shorter telomeres.

CONCLUSION: Our findings replicate those of other recent studies and also show, for the first time, that the prenatal stress-associated difference in newborn mean telomere length is due to a shift in the overall telomere distribution.

Key words: developmental origins of health and disease, fetal programming, maternal stress, prenatal stress, telomere length

Introduction
Converging evidence from epidemiological, clinical, and experimental studies suggests that exposure to suboptimal conditions in early life can produce long-term effects on health and disease susceptibility.1,2 The developing fetus is especially sensitive to intrauterine perturbations,3 and this has led to the developmental origins of the health and disease hypothesis, which posits that an individual’s long-term risk of disease is dependent, in part, on the quality of the intrauterine environment.4 Although a number of intrauterine factors may contribute to the long-term risk of disease, fetal exposure to maternal stress appears to represent a particularly salient insult.5,6 The mechanisms that link prenatal stress exposure to disease decades later are still unclear, but recent work suggests that at the cellular level, telomere dynamics may represent 1 such underlying mechanism.2,5 Telomeres are protective complexes of noncoding, repetitive DNA, and shelterin proteins that cap chromosomes and promote chromosomal stability. As cells divide, telomeres shorten both because of incomplete replication and through oxidative damage. Telomere length (TL) varies between chromosomes within each cell and among cells within a tissue because of different replicative histories and past levels of cellular damage. This results in a distribution of TLs within the tissue of an individual.8 Although there are mechanisms in place to rebuild the shortest telomeres, such as the enzyme telomerase,9-11 if a telomere has undergone a critical degree of shortening, it can lead to cellular senescence. Senescent cells lose the ability to replicate and cease to divide or undergo apoptosis, contributing to the aging phenotype and susceptibility to disease.12,13 Based on this, measuring the average TL of the TL distribution has gained traction as a biomarker for cellular function and aging, and can serve as an early predictor of disease onset for cardiovascular disease,14 stroke,15 Alzheimer’s disease,16 diabetes mellitus,17 childhood autism,18 as well as overall mortality risk.19,20 Given the role that telomere dynamics may play in disease progression, current efforts have turned to identifying the mechanisms that cause variation in TL.21 TL is a function of both the initial setting of TL22 and TL attrition over time.14,23 Little is currently known about the causes of variation in the initial setting of TL, but 1 possibility is that the
intrauterine environment plays an important role in the establishment of initial (newborn) TL. In accordance with this hypothesis, recent work by Entringer et al showed that prenatal stress was significantly associated with shorter mean newborn leukocyte TL. However, this finding has yet to be replicated. Moreover, although average TL is the most commonly used biomarker to describe TL variation among individuals in a population, it is not the mean TL but rather the shortest telomeres that lose function and initiate cellular senescence. Therefore, characterizing the entire distribution of TL within a sample from an individual may provide particularly valuable information about the relative increases in the number of short telomeres, which is more indicative of telomere dysfunction than measures of mean TL.

In this study, we investigated the effects of intrauterine exposure to maternal stress over the whole course of gestation on newborn mean TL and TL frequency distributions. Thus, our study builds on previous work exploring prenatal stress and TL to replicate and expand on these previous studies. The notable additional contributions of our study involve the characterization of maternal stress over the entirety of gestation and also its effect on the entire frequency distribution of telomeres. The use of TL frequency distributions provides a better and more meaningful resolution on putative prenatal stress effects, because the shortest telomeres are the most indicative of telomere dysfunction and the best predictors of mortality and morbidity in humans.

**Materials and Methods**

**Subject characteristics**

The study cohort of pregnant women was enrolled at an urban teaching hospital in Philadelphia, PA, between April and August 2013. Eligible women were between the ages of 18 and 35 years and delivered a single, viable, nonanomalous infant. Women were excluded if the pregnancy was complicated by medical conditions that could affect fetal growth, such as hypertension, diabetes, or smoking; if they developed any condition that required steroid therapy; or if they developed chorioamnionitis. Medical records were reviewed to obtain sociodemographic characteristics and medical history, and to determine eligibility pertaining to maternal age at birth, maternal prepregnancy weight, obstetric complications during pregnancy, length of gestation, weight, height, and birthweight adjusted for gestational age. Financial incentive or compensation was not offered. The result was a sample of 24 mother—newborn dyads. The sociodemographic and newborn characteristics of this population are displayed in Table 1. The study was approved by Drexel University Institutional Review Board and informed, written consent was obtained from all participants.

**Measures**

**Maternal stress**

When the mother presented at the hospital for delivery, maternal psychosocial stress was assessed using the Holmes and Rahe Stress Scale questionnaire (also referred to as the Social Readjustment Rating Scale). This measurement scale has been called the gold standard for stress assessment and has been shown to correlate with health outcomes in a variety of studies. The 43-item dichotomous survey objectively ascertained whether the mothers had experienced life events within the past year that were likely to initiate a physiological stress response (see Supplemental material). Each survey event is associated with a numerical value, and this scale was used to assess the cumulative stress the mother experienced over the course of her pregnancy. A score of ≥300 indicates the mother is at high risk of stress-induced illness, while scores ≤299 correlate with lower risk levels of stress-induced illness.

**Cord blood telomere length**

Telomeres were measured with the Telomere Restriction Fragment (TRF) assay, and the procedure was carried out according to previous studies. Briefly, DNA was extracted from newborn umbilical cord blood using the Puregene Blood Core Kit B following the manufacturer’s specifications (Qiagen, Hilden, Germany). DNA integrity was assessed through the use of integrity gels, and then cord blood TL was measured using the telomere restriction fragment assay. A 10-µg quantity of DNA was digested using 1.0 µL of RsaI (New England Biolabs, Ipswich, MA, R0167L) and 0.2 µL of HinfI (New England Biolabs, R0155M) in CutSmart Buffer (New England Biolabs, B7204S) overnight at 37°C. The digested DNA was separated using pulsed field gel electrophoresis (3 V/cm, 0.5- to 7.0-second switch times, 14°C) for 19 hours on a 0.8% nondenaturing agarose gel. The gel was then dried without

---

**TABLE 1**

**Maternal and newborn characteristics**

<table>
<thead>
<tr>
<th>Maternal characteristics</th>
<th>Mother–newborn dyads, N = 24</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sociodemographic</strong></td>
<td></td>
</tr>
<tr>
<td>Age, y, mean ± SD</td>
<td>25.1 ± 4.0</td>
</tr>
<tr>
<td>Body mass index</td>
<td>29.5 ± 8.5</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>13% (n = 3)</td>
</tr>
<tr>
<td>African American</td>
<td>75% (n = 18)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>13% (n = 3)</td>
</tr>
<tr>
<td><strong>Newborn characteristics</strong></td>
<td></td>
</tr>
<tr>
<td>Gestational age at birth, wk, mean ± SD</td>
<td>40.0 ± 1.1</td>
</tr>
<tr>
<td>Birthweight, g, mean ± SD</td>
<td>3298 ± 510</td>
</tr>
</tbody>
</table>

heating and hybridized overnight with a
$^{32}\text{P}$-labeled oligo (5'-CCCTAA-3') that binds to the 3' overhang of telomeres. Hybridized gels were placed on a phos-
phorscreen (Amersham Biosciences, Buckinghamshire, UK), which was scan-
ned on a Storm 540 Variable Mode Imager (Amersham Biosciences). We
used densitometry (ImageQuant 5.03v ImageJ 1.42q) to determine the po-
sition and strength of the radioactive
signal in each of the lanes compared to the
molecular marker (1 kb DNA Extension Ladder; Invitrogen, Carlsbad, CA).

A major advantage of the TRF assay is
that it provides frequency distributions
telomere length for each sample.27
The resulting plots allow visualization
of the relative abundances of TRFs at
each molecular weight (MW), providing
useful information on where differences
in TL occur among groups. For each
individual sample, the area under the
optical density curve was calculated in
1-kb intervals from 1 to 20 kb, and each
interval was divided by the area under
the curve for the entire distribution. The
background was fixed as the nadir of the
low-MW region on the gel (<1 kb).
Relative abundances of TRFs in each of
the MW classes were log transformed
and then fit by least-squares fourth-order
polynomial regression with the MW
classes (1–20 kb).

Statistical analysis
As determined by the Shapiro–Wilk test,
neonatal cord blood TL was normally
distributed ($W = 0.99, P = .97$). To
examine the association between
maternal psychosocial stress during
pregnancy and newborn cord blood TL
adjusted for the effects of other possible
determinants, a linear regression model
was used that included the effects of
maternal stress, maternal age, gestational
age at birth, and birthweight (adjusted
for gestational age). To further explore
how the magnitude of maternal stress
affects newborn cord blood TL, the study
population was divided into 2 groups
based on their stress score (high, $\geq 300$
points; low, $\leq 299$ points). De-
mographics for the stress groups were
compared using the $\chi^2$ test for dichoto-
mous variables. We used a $t$ test
accounting for unequal variances to
compare cord blood TL between the
stress groups. Means ± standard de-
viations are shown.

Frequency distributions of TL for
the high-stress and low-stress groups
were produced following Haussmann
et al.25 Following Jemielly et al.,35 we
compared skewness and kurtosis values
of the distribution that were not nor-
mally distributed and so were analyzed
All statistical analyses were performed in
JMP (v11.1.1).

Results
After accounting for the effects of other
potential factors that might influence
newborn cord blood TL (maternal age,
gestational age at birth, and birth-
weight), our linear regression model
showed a significant negative association
of maternal stress exposure on newborn
cord blood TL ($\beta = -0.463, P = .04$)
(Figure 1, Table 2). Furthermore, new-
borns whose mothers had experienced
high levels of stress within the past year
had significantly shorter average cord
blood TL ($6.98 \pm 0.89, n = 6$) compared
to mothers experiencing relatively lower
levels of stress during pregnancy ($8.74 \pm
1.05, n = 18; t = -3.99, P = .003$)
(Figure 1).

Interestingly, the difference in mean
cord blood TL between the high-stress
and low-stress groups was due to a shift
in the telomere distribution, with the high-
stress group showing an under-
representation of longer telomeres, and
an overrepresentation of relatively shorter
telomeres (Figure 2). These dif-
ferences in distribution shape were sig-
nificant, as indicated by a significantly
greater kurtosis value ($\chi^2 = 5.67, P = .01$)
and a skew trending toward shorter
telomeres ($\chi^2 = 2.82, P = .09$) in the
high-stress group.

Comment
Here we show that, after accounting
for the potential effects of maternal
age, gestational age, and birthweight,
maternal stress was significantly and
independently associated with newborn
mean cord blood TL. The offspring of
mothers who experienced high levels of
maternal stress had mean cord blood
telomeres that were on average $1.76 \pm
0.48$ kb shorter than the offspring of
mothers who experienced lower levels
of stress during gestation. To place the
differences between the stress groups
in context, routine smoking (23 pack-year)
resulted in a 1.5-kb difference in TL be-
between smokers and nonsmokers, sug-
uggesting that high levels of maternal stress
may have a similar cumulative affect on
TL as does daily smoking.36 Although
this type of comparison can help place
our results in context, we emphasize that
because of the modest sample size of our
study, comparison of absolute differ-
ences between the groups should be
made cautiously. High variability in TL
was present in this newborn population;
however, this variability is consistent
with previous findings.25,37,38

The number of human studies
reporting a relationship between TL and
survival are steadily growing.19,20,39-43
Interestingly, in conjunction with the
human epidemiology literature, there
are an increasing number of studies in
the field of evolutionary ecology that report significant correla-
tions between TL or telomere shortening rates and survival pros-
pects. Regardless of the taxa studied, the sensitivity of TL as a biomarker to pre-
dict age-related diseases and mortality appears to differ based on the subject’s age when TL is measured. Longitudinal studies have revealed that telomere loss occurs at a faster rate early in life, and it is believed that this early attrition is due to increased proliferation and growth during early development. A recent longitudinal study indicated an independent association between shorter telomeres in early childhood and arterial wall thickness later in life, suggesting that relatively short telomeres in early childhood are a biomarker for vascular disease risk later in life. In addition, a study in birds showed that TL measured in the first month of life was a stronger predictor of survival and overall health than TL measured in adulthood 5 years later. Another recent longitudinal study in birds reported that offspring TL was dependent in part on the infection status of their mother, suggesting that maternal condition plays a significant role in the initial setting of offspring TL. Taken together, these studies suggest that maternal condition plays a significant role in the initial setting of offspring TL. Taken together, these studies suggest that the human newborns in our study that experienced a more stressful intrauterine environment may have experienced a degree of cellular aging by the time of birth that could have significant effects on aging and subsequent health and disease susceptibility later in life. This association between the prenatal environment and health con-
sequences later in life is consistent with the DOHaD hypothesis and provides further motivation to investigate telo-
meres as a potential mechanism by which stress influences the prenatal environment and the development of chronic disease later in life.

Our study provides a detailed analysis showing that the difference in mean cord blood TL in our high-stress and low-stress mothers appears to be due to a shift in the overall TL frequency distribution, so that the high-stress group has an overrepresentation of shorter telo-
meres and an under representation of longer telomeres compared to the low-stress group. It is reasonable to expect that the shift toward shorter TLs in the high-stress group will place these offspring at a higher risk for telomere dysfunction and subsequent disease. The proportion of short telomeres in previous studies has been shown to reflect the level of genomic instability within a tissue. Generally, a distribution shift toward shorter telomeres may suggest that telomeres of all lengths in the distribution were equally affected; however, the significant effect on the kurtosis of the distribution suggests that the longer telomeres were preferentially shortened. Longer telomeres may be more likely shortened because their size makes them a larger target for shortening caused by oxidative damage. Future work could expand upon the relationships found here by using single-telomere length analysis (STELA) to detect whether prenatal stress increases the number of extremely short telomeres in the length ranges that trigger both cellular senescence and telomere dysfunction.

The effect of maternal stress on offspring TL may be mediated, in part, through maternal stress hormones. Although we were unable to measure circulating glucocorticoids in pregnant mothers in this study, it is possible that mothers with higher stress scores had higher glucocorticoid levels. The pio-
neering work of Epel et al has established that chronic stress in humans results in an increased rate of telomere shortening. Further work reported that elevated glucocorticoids produce negative effects on telomeres, suggesting that these hormones mediate the destructive effect of stress on telomere mainte-
nance. Experimental evidence is in accordance with these findings, as a recent study in chickens showed that experimentally elevating glucocorticoid levels during prenatal development resulted in shorter TL in young in-
dividuals. These higher levels of glu-
cocorticoids also appeared to increase levels of oxidative stress.
telomeres are particularly vulnerable to oxidative damage. It is possible that the effect of prenatal stress and elevated glucocorticoids on telomeres is mediated through oxidative stress. In addition, glucocorticoids may have an impact on telomere loss through their effects on telomerase. Further research is needed to understand how prenatal glucocorticoids affect telomere dynamics and their long-term consequences on overall health and survival. We also need to better understand how the developing fetus can regulate exposure to maternal glucocorticoids and thereby alter fetal programming. For example, recent work in an avian model suggests that a metabolic buffer can metabolize maternally derived glucocorticoids to alter the developmental endocrine environment and thereby temper prenatal stress effects.

In summary, we found that higher levels of maternal stress are associated with shorter average cord blood TL in newborns and a shift in the overall frequency distribution of telomeres, indicating that the high-stress group had an accumulation of short telomeres. More experimental work is needed to determine the molecular underpinnings linking maternal stress and telomere dynamics in offspring. In addition, future studies that incorporate information regarding the frequency of short telomere in human cell culture will help to determine the risks associated with prenatal stress on telomere dynamics and subsequent health and disease susceptibility.

Acknowledgments
We are grateful to Owen Montgomery and Renee Turchi for logistical support (Drexel University), and Ammar Shahid and Andy Piao (Drexel University) for assistance with data collection.

References
survival among the elderly and oldest old. No association between telomere length and morbidity or mortality in the population-based cohort study. J Gerontol A 2010;65:1206:130-42.


## Supplemental Material

Drexel University College of Medicine — Pregnancy Survey Social Readjustment Rating Scale

INSTRUCTIONS: Respond YES or NO to if you have or have not experienced any of these life events during the past year.

<table>
<thead>
<tr>
<th>Life Event</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Death of your spouse</td>
<td>Yes</td>
</tr>
<tr>
<td>2. Divorce</td>
<td>Yes</td>
</tr>
<tr>
<td>3. Marital separation from mate</td>
<td>Yes</td>
</tr>
<tr>
<td>4. Death of a close family member</td>
<td>Yes</td>
</tr>
<tr>
<td>5. Detention in jail or other institution</td>
<td>Yes</td>
</tr>
<tr>
<td>6. Major Personal injury or illness</td>
<td>Yes</td>
</tr>
<tr>
<td>7. Marriage</td>
<td>Yes</td>
</tr>
<tr>
<td>8. Being fired at work</td>
<td>Yes</td>
</tr>
<tr>
<td>9. Marital reconciliation with mate</td>
<td>Yes</td>
</tr>
<tr>
<td>10. Retirement from work</td>
<td>Yes</td>
</tr>
<tr>
<td>11. Major change in the health or behavior of a family member</td>
<td>Yes</td>
</tr>
<tr>
<td>12. Pregnancy</td>
<td>Yes</td>
</tr>
<tr>
<td>13. Sexual difficulties</td>
<td>Yes</td>
</tr>
<tr>
<td>14. Gaining a new family member (i.e., birth, adoption, older adult moving in, etc.)</td>
<td>Yes</td>
</tr>
<tr>
<td>15. Major business readjustment</td>
<td>Yes</td>
</tr>
<tr>
<td>16. Major change in financial state (i.e., a lot worse or better off than usual)</td>
<td>Yes</td>
</tr>
<tr>
<td>17. Death of a close friend</td>
<td>Yes</td>
</tr>
<tr>
<td>18. Changing to a different line of work</td>
<td>Yes</td>
</tr>
<tr>
<td>19. Major change in the number of arguments w/ spouse (i.e., either a lot more or a lot less than usual regarding child rearing, personal habits, etc.)</td>
<td>Yes</td>
</tr>
<tr>
<td>20. Taking on a mortgage (for home, business, etc.)</td>
<td>Yes</td>
</tr>
<tr>
<td>21. Foreclosure on a mortgage or loan</td>
<td>Yes</td>
</tr>
<tr>
<td>22. Major change in responsibilities at work (i.e., promotion, demotion, etc.)</td>
<td>Yes</td>
</tr>
<tr>
<td>23. Son or daughter leaving home (marriage, attending college, joining military)</td>
<td>Yes</td>
</tr>
<tr>
<td>24. In-law troubles</td>
<td>Yes</td>
</tr>
<tr>
<td>25. Outstanding personal achievement</td>
<td>Yes</td>
</tr>
<tr>
<td>26. Spouse beginning or ceasing work outside the home</td>
<td>Yes</td>
</tr>
<tr>
<td>27. Beginning or ceasing formal schooling</td>
<td>Yes</td>
</tr>
<tr>
<td>28. Major change in living condition (new home, remodeling, deterioration of neighborhood or home etc.)</td>
<td>Yes</td>
</tr>
<tr>
<td>29. Revision of personal habits (dress manners, associations, quitting smoking)</td>
<td>Yes</td>
</tr>
<tr>
<td>30. Troubles with the boss</td>
<td>Yes</td>
</tr>
<tr>
<td>31. Major changes in working hours or conditions</td>
<td>Yes</td>
</tr>
<tr>
<td>32. Changes in residence</td>
<td>Yes</td>
</tr>
<tr>
<td>33. Changing to a new school</td>
<td>Yes</td>
</tr>
<tr>
<td>34. Major change in usual type and/or amount of recreation</td>
<td>Yes</td>
</tr>
<tr>
<td>35. Major change in church activity (i.e., a lot more or less than usual)</td>
<td>Yes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Life Event</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>36. Major change in school activities (clubs, movies, visiting, etc.)</td>
<td>Yes________ No______</td>
</tr>
<tr>
<td>37. Taking on a loan (car, TV, freezer, etc.)</td>
<td>Yes________ No______</td>
</tr>
<tr>
<td>38. Major change in sleeping habits (a lot more or a lot less than usual)</td>
<td>Yes________ No______</td>
</tr>
<tr>
<td>39. Major changes in number of family get-togethers (a lot more or a lot less than usual)</td>
<td>Yes________ No______</td>
</tr>
<tr>
<td>40. Major change in eating habits (a lot more or less food intake, or very different meal hours or surroundings)</td>
<td>Yes________ No______</td>
</tr>
<tr>
<td>41. Vacation</td>
<td>Yes________ No______</td>
</tr>
<tr>
<td>42. Major holidays</td>
<td>Yes________ No______</td>
</tr>
<tr>
<td>43. Minor violations of the law (traffic tickets, jaywalking, disturbing the peace etc.)</td>
<td>Yes________ No______</td>
</tr>
</tbody>
</table>

