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Differences in placental telomere length suggest a link between racial disparities in birth outcomes and cellular aging

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Conflicts of Interest

The authors report no conflict of interest

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Abstract

BACKGROUND—Health disparities begin early in life and persist across the life course. Despite current efforts Black women exhibit greater risk for pregnancy complications and negative perinatal outcomes compared to White women. The placenta, a complex multi-tissue organ, serves as the primary transducer of bidirectional information between the mother and fetus. Altered placental function is linked to multiple racially disparate pregnancy complications, however little is known about racial differences in molecular factors within the placenta. Several pregnancy complications, including preeclampsia and fetal growth restriction, exhibit racial disparities and are associated with shorter placental telomere length, an indicator of cellular stress and aging. Cellular senescence and telomere dynamics are linked to the molecular mechanisms associated with the onset of labor and parturition. Further, racial differences in telomere length are found in a range of different peripheral tissues. Together these factors suggest that exploration of racial differences in telomere length of the placenta may provide novel mechanistic insight into racial disparities in birth outcomes.

OBJECTIVE—This study examined whether telomere length measured in four distinct fetally-derived tissues were significantly different between Blacks and Whites. The study had two hypotheses: (1) that telomere length measured in different placental tissue types would be correlated and (2) that across all sampled tissues telomere length would differ by race.

STUDY DESIGN—In a prospective study, placental tissue samples were collected from the amnion, chorion, villus, and umbilical cord from Black and White singleton pregnancies (N=46). Telomere length was determined using monochrome multiplex quantitative real-time polymerase chain reaction in each placental tissue. Demographic and pregnancy-related data were also collected. Descriptive statistics characterized the sample overall and among Black and White women separately. The overall impact of race was assessed by multilevel mixed-effects linear regression models that included empirically relevant covariates.

RESULTS—Telomere length was significantly correlated across all placental tissues. Pairwise analyses of placental tissue telomere length revealed significantly longer telomere length in the amnion compared to the chorion ($t=-2.06$, $p=0.043$). Overall telomere length measured in placenta samples from Black mothers were significantly shorter than those from White mothers ($\beta=-0.09$, $p=0.04$). Controlling for relevant maternal and infant characteristics strengthened the significance of the observed racial differences ($\beta=-0.12$, $p=0.02$). Within tissue analyses revealed that the greatest difference by race was found in chorionic telomere length ($t=-2.81$, $p=0.007$).

CONCLUSION—These findings provide the first evidence of racial differences in placental telomere length. Telomere length was significantly shorter in placental samples derived from Black mothers compared to White. Given previous studies reporting that telomere length, cellular senescence, and telomere dynamics are molecular factors contributing to the rupture of the amniotic sac, onset of labor, and parturition, our findings of shorter telomere length in placentas from Black mothers suggests that accelerated cellular aging across placental tissues may be

relevant to the increased risk of preterm delivery in Blacks. Our results suggest that racial differences in cellular aging in the placenta contribute to the earliest roots of health disparities.

Keywords

Cellular aging; Health disparities; Placenta; Pregnancy complications; Race; Telomere length

Introduction

Health disparities are well documented, beginning early in life and persisting over the life course.¹ Non-Hispanic Black women have higher rates of preterm delivery, low birthweight infants and infant mortality relative to non-Hispanic Whites.^{2–7} In addition, racial differences in pregnancy complications, including preeclampsia, gestational diabetes (GDM), and fetal growth restriction (FGR) also exist,^{5,7,8} likely contributing to other infant health disparities across the first year of life. Despite substantial efforts and heightened awareness these disparities persist. Increased understanding of the underlying mechanisms and how they contribute to perinatal outcomes are needed.⁹

The placenta is a critical organ at the interface between the fetus and mother, coordinating maternal physiology and fetal development. The human placenta is comprised of maternally and fetally-derived tissues with interdigitated vascular flow. The fetal portion includes four anatomically distinct tissues: amnion, chorion, villus, and umbilical cord, that exhibit unique gene expression profiles and different time points of differentiation during fetal development.^{10–13} The vascularized villus is the main site of oxygen and nutrient exchange while the umbilical cord enables fetoplacental circulation.¹⁴ The amnion and chorion protect the developing fetus and facilitate nutrient and hormone transfer between the mother and fetus.^{10,11,13} Pregnancy complications that exhibit racial disparities, including preeclampsia, GDM, preterm birth, and premature rupture of membranes are all associated with altered placental physiology, metabolism, and function.^{15–20} As such exploration of racial differences in placental function may provide insight into the mechanisms underlying early health disparities.

One biological marker associated with altered placental function, as well as these pregnancy complications, is telomere length (TL). Telomeres are nucleoprotein complexes that cap chromosomes in eukaryotic cells and are essential for cell survival, chromosome stability, and influence cellular differentiation, senescence, and apoptosis.^{21–23} Shortening of TL has been associated with cellular aging and TL is influenced by DNA repair mechanisms, oxidative stress, and inflammation.²⁴ TL tends to be highly correlated across tissues at birth, but this correlation lessens as an individual ages.^{25–27} Shorter placental TL has been associated with racially disparate pregnancy complications such as FGR, GDM, and preeclampsia.^{5,8,28–32} Correlations between placental TL and gestational age, socioeconomic status, and parity have also been reported.^{30,33,34} To date no previous studies have addressed racial differences in placental TL. Previous studies of placental TL have utilized DNA extracted from an array of sites, with inconsistent attention to confounding maternal tissue, inclusion of multiple cell tissue types, or failing to define the specific

sampling site altogether.^{28–31,34,35} Given the complexity of placenta these methodological variations curtail their generalizability, warranting further investigation.

Racial differences in TL have also been observed, where Black newborns and adolescents exhibit longer TL than Whites.^{36–38} Longer TL and associated greater TL attrition across the life course has been reported in Black adults, although some debate exists.^{37,39–41} Longer initial TL is a predictor of increased TL attrition over time, and consistent with this, an aged cohort of Blacks displayed shorter TL than Whites.^{42,43} From an aging and health disparities perspective, the placenta represents a unique opportunity to examine racial differences in cellular aging given its definitive lifespan and the molecular evidence pointing to a role of cellular aging and telomeres in parturition.^{44–47}

To better understand how placental factors may contribute to persistent racial disparities in perinatal outcomes this study examined both the correlation of TL across fetally-derived tissues and racial differences.

Materials and Methods

Subjects

Subjects were a subset of mothers (N=46) recruited beginning in January 2015 from a larger longitudinal study in New Orleans, Louisiana who consented to placental collection. Recruitment of pregnant women, aged 18–41 years, took place in prenatal and Women, Infant, and Children (WIC) clinics and from other ongoing studies involving pregnant women at Tulane University. Women were at least 18 years of age, English-speaking, and pregnant with a singleton fetus. Women provided information via a face-to-face interview-assisted computer survey (Questionnaire Development System; Nova Research, Bethesda, Maryland) conducted by trained interviewers. This study was approved by the Tulane University Institutional Review Board.

Demographic (e.g., maternal and infant characteristics) and pregnancy-related data were collected by maternal report and medical record abstraction. Data collected by maternal report included: maternal age at conception, race, and education level. Data collected from medical records included: gestational age, infant birthweight, infant sex, delivery mode, parity, and pregnancy complications. Pregnancy complications included preeclampsia, FGR, GDM, gestational hypertension, and eclampsia/preeclampsia. Given the low prevalence of individual pregnancy complications in the sample, a composite categorical variable (yes/no) was created.⁴⁸

Placental tissue sample collection

Placenta collection and dissection occurred within approximately one hour of delivery. The reflected fetal membranes (e.g., amnion and chorion) were separated manually, isolated, and excised >4cm from the fusion to the placental disk. For fetal villus tissue sampling, the chorionic plate was removed, and ~2cm of fetal villus tissue was excised within ~4cm of the umbilical cord insertion site. Fetal villus samples were collected just below the chorionic plate to avoid sampling maternal villi and visible vasculature was excised. Umbilical cord samples were collected within ~4cm of the insertion site after removing any fused

membranes. Tissues were washed thoroughly in 1M PBS to minimize contamination from blood or atrophied villi. Samples were flash frozen in liquid nitrogen and stored at -80°C . DNA was extracted from placental tissues using QIAamp DNA mini kit protocol for tissues (Qiagen, Valencia, CA). Samples were evaluated for double-stranded DNA integrity and concentration by Qubit dsDNA BR assay kit (Invitrogen, Carlsbad, CA) and purity by NanoDrop-2000 (Thermo Fisher Scientific, Waltham, MA). DNA was stored at -35°C .

Telomere length (TL) measurement

The average relative TL, as represented by the T/S ratio, was determined by monochrome multiplex quantitative real-time polymerase chain reaction (MMqPCR) and standard methods in our laboratory.^{36,49} All tissue samples from each individual placenta were run on the sample duplicate plates and all samples were run with the same control purchased genomic placental DNA from a single donor (BioChain). Coefficient of variance (CV) for the whole sample was 2.12% for all plates. Samples (N=4) with unacceptably high CV were repeated.

Statistical analysis

Descriptive statistics characterized the sample overall and among Black and White women with chi-square and t-tests. One Black infant birth weight exceeded three standard deviations above the mean and was winsorized for analyses. The duration between delivery and sample collection was missing for N=4 deliveries and the mean duration by delivery type was imputed. Spearman correlation coefficients compared crude relationships between TL across the four placental tissue types. The normality of TL was examined using visual inspection of plots, skewness, and kurtosis. TL was normally distributed.

Multilevel analysis using mixed-effects linear regression models were used to produce intraclass correlation coefficients (ICC) to estimate the degree of within individual correlation of TL across the four tissue types. First, we examined an empty model – regressing TL without covariates – to determine the degree of association among TLs within individuals and allowing for decomposition of the variation in TL into between- and within-individual variation (Model 1). Next, we added maternal race and a tissue type indicator variable in a model with randomly distributed individual-specific intercepts (Model 2). We tested for heterogeneity in the relationship between tissue type and TL between individuals by fitting this model with tissue type as a random effect in addition to the random intercept. Due to statistical power considerations, clustering TL at both the level of the individual and tissue type was not feasible. The study hypotheses explored differences between race at the level individual above tissue type and therefore clustered TL within an individual. Model 2 was used to test for an interaction between race and tissue type to determine whether the relationship between TLs across tissues differed between racial groups. Finally, we fit a model including empirically-relevant covariates with a fixed slope value for each predictor variable and random individual-specific intercepts (Model 3). We used this fully-adjusted model to test for an interaction between race and tissue type to determine whether the relationship between TL across tissues differed between racial groups.

In a sensitivity analysis, we repeated the modeling after excluding the chorion – the tissue type with the largest racial difference in TL – to assess the degree to which the chorion drove the race effect. All analyses were conducted using SAS version 9.4 (SAS Institute, Inc. Cary, NC).

Results

Placental tissue telomere length (TL) was available from 46 pregnancies (n=34 Black, n=12 White). Mean age at conception was 27 years and 63% of women attained at least some college education (Table 1). The majority of deliveries were vaginal (56.5%) and all infants were born at term. Collection of placental tissue samples occurred on average 45 minutes after delivery.

There were no racial differences in maternal age at conception, delivery mode, duration between delivery and sample collection, infant birth weight, composite maternal pregnancy complications, parity, or infant sex (Table 1). A greater proportion of White women attained a college degree or higher ($p=0.001$) and infants born to Black women had earlier gestational age ($p=0.029$).

The rank order of placental tissue TLs from longest to shortest was Amnion exhibiting the longest TL with a mean of 0.877 ± 0.15 (Black = 0.862 ± 0.16 ; White = 0.919 ± 0.09), then cord with a mean of 0.845 ± 0.15 (Black = 0.829 ± 0.16 ; White = 0.891 ± 0.10), then villus with a mean of 0.831 ± 0.16 (Black = 0.806 ± 0.16 ; White = 0.900 ± 0.15), and the shortest was the chorion with a mean of 0.812 ± 0.16 (Black = 0.776 ± 0.15 ; White = 0.912 ± 0.12). Chorionic TL was significantly shorter than TL from the amnion ($t=-2.06$; $p=0.043$); no other pairwise comparisons between tissues were significant (Figure 1). Crude racial differences were observed in chorionic TL ($t=-2.81$; $p=0.007$) and villus TL approached significance ($t=-1.80$; $p=0.079$), where placentas from Black pregnancies exhibited shorter TL relative to White (Figure 2).

TL was positively correlated across placental tissues (Table 2). Correlation coefficients between placental tissue types ranged from 0.67 to 0.53. A high degree of correlation across placental tissue types from within the same individual was evident in the mixed effects empty model (Model 1; ICC=60.6%), indicating that the majority of the variance was accounted for by differences between individuals (Table 3).

Estimates from Model 2 indicated that independent of the tissue type, placental TL was shorter among Black pregnancies ($\beta=-0.09$, $p=0.04$). Positive β estimates for amnion, villus, and cord tissues indicated longer TLs relative to chorion with the difference between amnion and chorion being largest ($\beta=0.07$, $p=0.0011$). Allowing the relationship between TL to vary across tissues randomly between individuals did not change the estimate associated with race. Race did not significantly interact with placental tissue type indicating that the relationship between TL and race was consistent across tissue types.

The main effect of race remained significant when accounting for empirically relevant covariates: infant sex, maternal education and age at conception, gestational age, and duration to sample collection (Table 3; Model 3). The ICC derived from Model 3

(ICC=61.4%) was higher than Model 1 (ICC=60.6%) given the addition covariates which captured a greater proportion of the variance in TL between individuals. None of the empirically relevant covariates in Model 3 were associated with placental TL. Delivery mode, parity, and composite pregnancy complications were not associated with placental TL and not included in final model.

Sensitivity analysis excluding chorion resulted in an attenuated racial difference in Model 2 (Black vs. White $\beta=-0.07$, $p=0.10$) but remained significant in Model 3 (Black vs. White $\beta=-0.10$, $p=0.045$).

Comment

TL from fetally-derived placental tissues was significantly shorter in placentas from pregnancies of Black compared to White mothers. This finding is the inverse of racial differences in TL reported in newborns, adolescents, and adults, where Blacks exhibited longer TL than Whites.^{36–38,40,41} However, it is consistent with reports of accelerated TL decline in Blacks in adulthood.⁴² Although racial differences in maternal education and gestational age existed these were not significantly associated with TL and their inclusion did not alter results, nor did the inclusion of pregnancy complications or parity. Crude placental TL pairwise comparison revealed the greatest racial difference in TL was in the chorion.

In this study, Black mothers had shorter gestational periods relative to Whites, which was evident only in vaginal deliveries; there was no racial difference in the gestational period for C-sections. However, along with a shorter gestational period, Black mothers also exhibited shorter placental TL. As earlier gestational age is associated with longer TL the results indicate that larger studies with matched gestational ages may reveal larger racial differences.³⁰ Shorter TL is an indicator of accelerated aging and cellular stress. Shorter TL in placentas from Black pregnancies may reflect accelerated cellular aging. This is consistent with the “Weathering Hypothesis” proposed by Dr. Arline Geronimus whereby health disparities in Black women are presumed to be the consequence of cumulative socioeconomic disadvantage across generations resulting in accelerated aging and earlier health decline.⁵⁰ This model is supported by data finding greater allostatic load in Blacks relative to Whites beyond differences in socioeconomic status.⁵¹ If gestational age is considered the “age” of the placenta, the findings of shorter TL *with earlier gestational age* in Blacks offer novel evidence that the molecular processes related to racial differences in TL attrition and cellular aging found in older adults may be programed *in utero*.

The placenta represents a unique organ system where the aging process is accelerated, occurring within gestation and ending at delivery. Across a variety of different cell types, TL shortening triggers cellular senescence and activation of apoptotic pathways. Within placental trophoblasts decreased TL has been reported to trigger apoptosis, increase pro-inflammatory cytokines, and result in the release of cell-free fetal DNA (cffDNA).^{45,46} Increased inflammation and cffDNA contribute to the onset of labor.^{45,46} Increases in cellular senescence and inflammatory markers in the chorioamniotic membrane are reported in women in active labor at term compared to women delivered at term but not in active

labor.^{44–46,52} Preterm premature rupture of membranes has been hypothesized to be linked to increased cellular senescence due to oxidative stress and, consistent with this pathway, exhibited shorter placental membrane TL relative term births.^{47,53,54} In term births, telomere fragments from the amniochorion that are shed into the amniotic fluid elicit oxidative stress and contribute to the onset of parturition.⁵⁵ The chorion contains the most trophoblast cells of the membranes and trophoblast dysfunction and senescence are relevant for rupture of the amniotic sac. Our findings that chorionic TL is both the shortest across placental tissues and has the greatest racial difference, when combined with a molecular mechanism linked to parturition and rupture of amniotic membranes provide intriguing initial evidence that accelerated cellular aging and TL decline, specifically in the chorion, may mechanistically contribute to the higher incidence of preterm births in Black women.

Placental collection is fraught with methodological challenges beginning with issues related to the time of collection. Placentas for this study were collected approximately 45 minutes following partition (range 6–128 minutes). Practical issues related to placenta collection underlie inconsistencies in the duration between parturition and sample collection in previous placental TL studies where durations ranged from 20 minutes to 24 hours.^{29,30,33} In this study, the duration between parturition and placenta collection was not associated with TL suggesting this window of time (e.g. less than 2 hours) is an acceptable interval for telomere studies, however caution is warranted before extending this interval to other epigenetic or RNA studies. Controlled time course studies of TL, RNA expression, and other epigenetic markers are needed to determine the optimal collection parameters.

Despite the strengths of this study, limitations exist. The number of individual placentas was small, although similar to previous epigenetic and molecular studies in placentas. Analyses leveraging samples across four tissue types partially mitigates the concern of sample size; however larger studies are needed. Racial groups were unbalanced as a result of initial study design and demographic differences existed, but did not alter the findings. Despite population data showing racial differences in pregnancy complications and studies associating shorter placental TL with pregnancy complications, composite pregnancy complications did not significantly predict placental TL (data not shown). Inclusion of composite pregnancy complications in the final model (Model 3) did not alter significant findings and was not an independent predictor of placental TL. This is likely a function of sample size or the low incidence of pregnancy complications. Although placental TL has been inversely associated with gestational age, no association was found in this study.³⁰ This may be due to the inclusion of only term deliveries (>37 weeks gestational age) or that the previous study was racially homogeneous.³⁰ Concerns about inter and intra-assay variability for the MMqPCR method also exist. To address this concern, we integrated several protocol adaptations. First, we used commercially available reference genomic DNA from a single placenta. Second, to minimize the potential for plate to plate variability influencing the findings, all samples from the same placenta were run on matched duplicate plates. Third, a stringent quality control algorithm for PCR efficiencies and CVs was used; the resultant CV across all samples was 2.12%. Blood contamination remains a challenge for all molecular studies of the placenta. While all samples were thoroughly washed to reduce blood contamination minimal contamination is probable. The chorion contains microscopic maternal vasculature that may also contribute to measurement of TL in the chorion. Future

studies including maternal TL as a covariate or other histopathological measurements of TL (i.e., quantitative fluorescence in situ hybridization) can address concerns of blood and maternal contamination. However, these techniques are time intensive and a balance between contamination and feasibility is needed for larger studies.

This is the first study to identify racial differences in placental TL representing the earliest developmental time point when differences have been reported in TL. The low prevalence of pregnancy complications in this cohort limits the ability to make direct inferences in this sample. However, the findings of shorter placental TL in Black women suggests that accelerated cellular aging is one mechanism contributing to persistent racial disparities in birth outcomes, particularly preterm birth and preterm rupture of membranes. Future studies examining placental TL in larger sample sizes and in preterm deliveries in both races are needed. The findings also highlight that while correlated, differences between placental tissue types exist. Careful examination of both similarities and differences in placental tissues is an important area of future placental research that may enhance the understanding of typical and atypical placental function. Larger studies are needed to better define the implications of shorter chorionic TL in placentas from pregnancies of Black women and their potential contribution to racial differences in preterm birth. From a clinical perspective, confirmation of accelerated placental aging in Black pregnancies may lead to novel approaches to decreasing preterm birth focused on telomere dynamics, oxidative stress, and apoptosis-related inflammation. Decreasing the rate of preterm birth in Black pregnancies represents a critical starting point to truly shifting the needle on the earliest roots of racial health disparities.

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Figure 1. Placental tissue TL across all tissue types

Bar graph of TL by placental tissue type for all subjects. The mean and SEM are presented for each group. T-tests examined crude differences between placental TL between tissue types. Chorionic TL was significantly shorter than amnionic TL ($t=-2.06$, $p=0.043$). * $p<0.05$

Figure 2. Crude racial differences in placental tissue TL

Bar graph of TL by all placental tissues and by placental tissue type stratified by race, where placental tissues from Black pregnancies are grey and tissues from White pregnancies are blue. The mean and SEM are presented for each group. Numbers represent total number of samples for individual bars. Chorionic TL exhibited the greatest difference between racial groups ($t=-2.81$, $p=0.007$) while villus TL showed a marginal difference between races ($t=-1.80$; $p=0.079$). ns, not significant; # $p<0.1$, ** $p<0.01$

Table 1

Demographic characteristics and pregnancy-related outcomes of study participants

Descriptive outcome, mean±SD	Total (N=46)	Black (N=34)	White (N=12)	p-value
Maternal conception age (yrs)	26.94±6.73	26.50±6.94	28.17±6.19	0.467
Gestational age (wks)	38.94±1.14	38.76±1.23	39.42±0.67	0.029
Infant birth weight (kg)	3.28±0.56	3.21±0.54	3.49±0.58	0.140
Duration to sample collection (min)	44.52±29.56	44.78±29.24	43.77±31.76	0.920
Parity	1.11±1.35	1.21±1.50	0.83±0.84	0.293
Infant sex, % (n)				0.883
Male	56.5 (26)	55.9 (19)	58.3 (7)	
Female	43.5 (20)	44.1 (15)	41.7 (5)	
Delivery mode, % (n)				0.410
Vaginal	56.5 (26)	52.9 (18)	66.7 (8)	
C-section	43.5 (20)	47.1 (16)	33.3 (4)	
Maternal education level, % (n)				0.001
<high school	37.0 (17)	44.1 (15)	16.7 (2)	
<some college	32.6 (15)	41.2 (14)	8.3 (1)	
>=college degree	30.4 (14)	14.7 (5)	75.0 (9)	
Pregnancy complications, % (n)				0.300
No	73.9 (34)	67.7 (23)	83.3 (10)	
Yes	26.1 (12)	32.4 (11)	16.7 (2)	

Groups were compared using *t* tests, chi-squared tests, or Fisher exact test where appropriate. Pregnancy complications included preeclampsia/eclampsia, FGR, GDM, and gestational hypertension.

Table 2

Spearman correlation between placental tissue TL and relevant covariates

Outcome	Cord TL	Amnion TL	Chorion TL	Villus TL	Duration to sample collection (min)	Gestational age (wks)
Cord TL	1					
Amnion TL	0.640 ***	1				
Chorion TL	0.543 ***	0.613 ***	1			
Villus TL	0.665 ***	0.526 ***	0.540 ***	1		
Duration to sample collection (min)	0.343*	0.233	0.190	0.094	1	
Gestational age (wks)	-0.016	-0.017	-0.011	-0.018	-0.108	1
Maternal conception age (yrs)	-0.011	-0.018	0.067	0.112	0.138	-0.165

Spearman rank correlation coefficient values presented.

p<0.001,***
p<0.0001

Table 3

Multilevel modeling results for estimates of association with placental tissue TL.

	Model 1: Empty model	Model 2: Race and tissue type	Model 3: Full model
ICC	60.6%	60.4%	61.4%
Race			
White	Ref	Ref	Ref
Black	-0.09	0.04	-0.12 0.02
Placental tissue type			
Chorion	Ref	Ref	Ref
Amnion	0.07	0.001	0.07 0.001
Villus	0.02	0.35	0.02 0.35
Cord	0.03	0.10	0.03 0.10
Infant sex			-0.008 0.86
Maternal education			-0.02 0.44
Maternal age			0.0001 0.96
Gestational age			-0.02 0.52
Duration to sample collection			0.001 0.13

ICC, intraclass correlation coefficient