# Setting the Trajectory: Racial Disparities in Newborn Telomere Length

Stacy S. Drury, MD, PhD[1], Kyle Esteves, BS[1], Virginia Hatch, BS[1], Margaret Woodbury, BS[2], Sophie Borne[1], Alys Adamski, MPH[3], and Katherine P. Theall, PhD[3]

**Objective** To explore racial differences in newborn telomere length (TL) and the effect moderation of the sex of the infant while establishing the methodology for the use of newborn blood spots for TL analyses.

**Study design** Pregnant mothers were recruited from the Greater New Orleans area. TL was determined via monochrome multiplex quantitative real-time polymerase chain reaction on DNA extracted from infant blood spots. Demographic data and other covariates were obtained via maternal report before the infant's birth. Birth outcome data were obtained from medical records and maternal report.

**Results** Black infants weighed significantly less than white infants at birth and had significantly longer TL than white infants ($P$ = .0134), with the strongest effect observed in black female infants. No significant differences in gestational age were present.

**Conclusions** Significant racial differences in TL were present at birth in this sample, even after we controlled for a range of birth outcomes and demographic factors. Because longer initial TL is predictive of more rapid TL attrition across the life course, these findings provide evidence that, even at birth, biological vulnerability to early life stress may differ by race and sex. *(J Pediatr 2015;166:1181-6)*.

Telomeres normally shorten with age in somatic tissues and, in general, telomere length (TL) generally is correlated between different tissues, particularly in youth.[1-3] Aging and multiple other factors influence TL, including oxidative stress, DNA damage, DNA repair mechanisms, and genetic factors.[4] TL also has been associated with both psychosocial stressors (exposure to violence, neighborhood disorder, racial discrimination), as well as chronic diseases (obesity, cardiovascular disease, diabetes).[5-9] These diseases, associated with both early adversity and the aging process,[10] have significantly greater rates in black patients, particularly women, than in other races.[11,12]

Racial differences in TL have been demonstrated in adults and adolescents.[13,14] Baseline TL is longer in black patients. TL attrition, an indicator of cellular aging predicted by initial TL, also is significantly greater in black patients. This finding suggests that biological processes associated with aging may underlie persistent health disparities.[6,11,13] Chae et al[5] reported that racism was a significant predictor of shorter TL in black adults, suggesting that not only do racial differences exist but that exposure to racism may contribute to part of the observed racial differences in TL. Thus, the determination of the earliest developmental time point at which racial differences in TL exist is salient.[15]

Shorter infant TL has been associated with a range of negative birth outcomes, including preterm rupture of membranes, gestational diabetes, prenatal exposure to antiretroviral therapy in HIV-exposed infants, and maternal stressors during pregnancy.[16-19] Studies also have demonstrated shorter placental TL of infants with intrauterine growth restriction.[20] Only 1 previous study, designed to examine cross tissue correlation of TL, presented any data on the association between race and TL at birth.[21] In that study, TL was not significantly different between black and white infants. Although newborn TL was significantly associated with both maternal age and infant birth weight, analyses did not account for these associations or potential moderation of racial differences by gestational age or infant sex. Given consistent evidence of both sex differences in TL,[22,23] and racial differences in birth weight, these factors are likely critical covariates when examining racial differences in newborn TL.

One study has demonstrated a significant correlation between TL from dried blood spots and whole blood obtained via venipuncture, suggesting that blood spot DNA is a practical alternative DNA source.[24] This study examined the feasibility of newborn blood spots as a source for newborn TL analyses and explored racial differences in newborn TL in a prospective cohort of infants. On the basis of existing data in adults, we hypothesized that racial differences would be found, with black infants having longer TL than white infants. Further, as our previous work and that of others has demonstrated sex differences,[7,22,23] we examined whether the sex of the infant moderated racial differences. The established evidence that the rate of TL attrition is proportional to baseline TL coupled with the

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| CV | Coefficients of variations |
| PCR | Polymerase chain reaction |
| TL | Telomere length |
| T/S | Telomere to single copy |

From the [1]Department of Psychiatry, Tulane University School of Medicine, New Orleans, LA; [2]University of Maryland School of Medicine, Baltimore, MD; and [3]Department of Global Community Health and Behavioral Sciences, Tulane School of Public Health and Tropical Medicine, New Orleans, LA

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increasing evidence linking TL to negative health outcomes, suggest that evaluation of racial differences in infant TL may provide novel insight into the early biological evidence of health disparities.

## Methods

Data were collected on 71 infants recruited from New Orleans, Louisiana, as part of a larger longitudinal study examining the effect of cumulative maternal life course and prenatal stress on infant TL and early childhood development. Pregnant mothers, ages 18-41 years, were recruited from prenatal and Women, Infant, and Children clinics, as well as from other ongoing studies of maternal health and pregnancy outcomes at Tulane University. Recruitment areas were identified by the use of the community identification process, a mapping method to record epidemiologic indicators of the prevalence and incidence of community stressors and other selected social and health conditions. Mothers were excluded if they were younger than 18 years of age or expecting multiple infants. Only English-speaking mothers were recruited. Mothers provided information about multiple levels of their and their infant's social ecology (ie, household and neighborhood) by using an interview-assisted computer survey administered face-to-face at the research site or at prenatal clinics (Questionnaire Development System; Nova Research, Bethesda, Maryland). Oral responses were recorded onto the computer by trained interviewers. This study was approved by the Tulane University Institutional Review Board.

Newborn DNA was extracted from newborn blood spots. Maternal consent was obtained during pregnancy. Genomic DNA was isolated according to the manufacturer's recommended protocol included in the Purelink Genomic DNA Mini kit (Invitrogen, Carlsbad, California) and eluted in 70 μL of nuclease-free water. The resulting DNA samples were then stored at −20°C. Control DNA used for TL assay was obtained from pooled dried blood spot extracted DNA.

All DNA samples were evaluated for double-stranded DNA integrity and concentration with Qubit (Invitrogen), for purity with Nanodrop-2000 (Thermo Scientific, Waltham, Massachusetts), and DNA integrity via agarose gel electrophoresis. The average relative TL as represented by the telomere repeat copy number to single gene (albumin) copy number telomere to single copy (T/S) ratio, was determined by the use of monochrome multiplex quantitative real-time polymerase chain reaction (PCR) and standard methods in our laboratory.[7,25] A 10-μL DNA sample, containing ~0.1-0.5 ng of DNA diluted in pure water, was briefly combined with 15 μL of PCR mixture, for a final volume of 25 μL per reaction. The PCR consisted of 0.75X Sybr Green I (Invitrogen), 1X Gene Amp Buffer II (Applied Biosystems, Foster City, California), 0.8 mM deoxyribonucleotide triphosphates, 10 mM $MgCl_2$, 3 mM dithiothreitol, 1 M betaine, 2.5U AmpliTaq Gold polymerase (Applied Biosystems), 0.9 μM telg primer (ACACTAAGGTTTGGG TTTGGGTTTGGGTTTGGGTTAGTGT), 0.9 μM telc primer (TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCC TAACA), 0.6 μM albd2 primer (GCGGGCCCGCGTGGC GGAGCGAGGCCGgaaaagcatggtcgcctgt), and 0.6 μM albu2 primer (GCCTCGCTCCGGGAGCGCCGCGCGGCCaaatgc tgcacagaatccttg). All samples are performed in triplicate with a 7-point standard curve (0.0313-2 ng) with DNA extracted from pooled control blood spot cards. Each plate was duplic ated with all samples in a different well position. All plates were run with a standard curve obtained from the same pooled control blood DNA sample to eliminate possible additional sources of interplate variability. Intraplate and interplate coefficients of variations (CVs) were calculated for uniformity of TL estimates (CV ≤5%). The slope of the standard curve for both the telomere and albumin reactions was used to calculate the T/S ratio for each sample. PCR efficiency criteria for telomere and albumin reactions are between 90% and 110%, and paired-plates were not allowed to differ by more than 10% or they were repeated. CVs were calculated within each triplicate (CV criteria ≤6%) and between plates (CV criteria ≤10%). Samples with unacceptably high CVs (>6% intra-assay or >10% interassay CV) were removed from analysis or repeated (N = 5) and did not differ on any demographic or other birth outcome factors from samples that amplified appropriately.

Bloodspot TL ratio was determined by the average T/S ratio of the triplicates from both plates (eg, the average of 7 different assays of each individual). Children without valid bloodspot TL data did not significantly differ from children with bloodspot TL data on study measures.

Infant birth outcome data were obtained from medical records: sex, birth date, birth weight, and gestational age. Additional covariates were obtained during the prenatal maternal interview-assisted computer survey and included parental age when the infant was conceived, maternal race, and mother's greatest level of education as a proxy for socioeconomic status.

### Statistical Analyses

Descriptive, bivariate, and multivariate analyses were conducted using SAS 9.3 (SAS Institute, Cary, North Carolina). Univariate analyses included examination of central tendency measures and frequencies. Bivariate analyses were performed to determine initial association between infant TL and race, as well as between race and TL potential confounders. Bivariate analyses included Spearman and Pearson correlations, likelihood ratio $\chi^2$, or *t*-test where appropriate, as well as nonparametric equivalents when necessary. Multiple linear regression analyses, in which we controlled for relevant covariates, were performed. A variable was considered a potential confounder and kept in the multivariate model if there was a 10% or more change in estimate between race and infant TL. Variables also were included if there were established evidence in previous studies of confounding in relation to TL and TL dynamics. Given the differential impact of stressors on TL among children in previous studies, we also examined effect modification by the sex of the infant in the race-TL relation.[7,22]

## Results

A total of 71 infant blood spots were obtained; however, TL was not determined in 5 infants because of a failure in quality