Shortened Telomeres in Families With a Propensity to Autism

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Objective: Shortened telomeres have been linked to poorer health outcomes. Exposure to psychological stress is associated with accelerated telomere shortening, and a well-established body of evidence indicates that families with a child with autism spectrum disorder (ASD) experience heightened levels of psychological stress. Also, alterations in a number of biological processes implicated in telomere length dynamics (i.e., oxidative stress, DNA methylation) have been linked to ASD susceptibility. We examined whether families of children with ASD who have an infant show shortened telomeres.

Method: Saliva samples were collected from infants, their older sibling (proband), and parents in families with or without a child with ASD. Infants and their families were designated as high-risk for ASD (HRA; n = 86) or low-risk for ASD (LRA; n = 118) according to the older siblings' diagnostic status. We used the real-time polymerase chain reaction (PCR) telomere assay to determine relative average telomere length for each participant.

Results: HRA families demonstrated significantly shorter telomere length relative to LRA families. This effect was observed at the individual family member level, with infants, probands, and mothers in HRA families showing reduced relative telomere length compared to individuals in LRA families; although not significant, fathers of high-risk infants showed a similar pattern of decreased telomere length.

Conclusion: Families of children with ASD who have an infant show shortened telomeres relative to families with no history of ASD. These results suggest that such "high-risk" families should be monitored for the physical and mental health consequences that are often associated with accelerated telomere shortening.

Key Words: autism spectrum disorder, telomeres, biomarker, psychological stress

J Am Acad Child Adolesc Psychiatry 2015;54(7):588–594.

Telomeres are DNA protein structures that cap the ends of linear chromosomes.[1] Telomere length represents a biological marker of cellular aging.[1] Telomere shortening occurs with each cell division; when telomeres shorten to a critical length, the cell enters a state of senescence and can no longer divide.[2] Shortened telomere lengths have been reported in a variety of medical conditions, including cardiovascular disease and cancer,[3-5] as well as psychiatric disorders, including mood disorders, schizophrenia, and, recently, autism spectrum disorder (ASD).[6-8]

Although telomere length is highly heritable,[9] a variety of demographic variables and health-related behaviors have been linked to telomere dynamics. For example, age, sex, body mass index, ethnicity, smoking status, and physical activity have demonstrated contributions to telomere length.[10-13] In addition, psychosocial factors have been linked to telomere length. Specifically, an abundance of evidence links psychological stress to accelerated telomere shortening. In particular, Epel *et al.*[14] identified an association between telomere length and psychological stress among 20- to 50-year-old women who served as primary caregivers to children with chronic health issues or disabilities (including ASD) and women with healthy children. The greatest telomere shortening was observed among those women with more years of caregiving and, importantly, among those with the greatest levels of perceived stress (notably, women whose children had a health issue or a disability). Thus, telomere length, at least partially, represents a biological marker of both genetic predisposition and environmental influence.

It is well established that familial caregivers of children with ASD experience considerable perceived and actual stress.[14-18] Indeed, caregivers of children with ASD often report experiencing levels of stress that are higher than both caregivers of typically developing children and caregivers of children with other developmental disabilities.[15] In addition to self-reported levels of stress, there is evidence that both mothers and fathers of children with ASD experience dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis.[19-21] Studies have also reported increased rates of psychiatric problems and poorer health outcomes in caregivers of children with neurodevelopmental disorders, including ASD.[22,23] Considering that telomere shortening has been linked to increased levels of perceived stress, poorer health outcomes, and psychiatric disorders, it is highly likely that shortened telomeres would be observed in caregivers of children with ASD and, importantly, in families with a new infant sibling.

Recent evidence suggests that shorter telomere length is present in children with ASD. Li *et al.*[8] recently reported that

This article is discussed in an editorial by Dr. Stacy S. Drury on page 539.

children with ASD (at approximately 4 to 5 years of age) show significantly shorter leukocyte telomere length than typically developing children of the same age. It remains to be delineated whether decreased telomere length in this clinical group plays a causal role in ASD or is the result of an unrelated variable that is independently associated with both ASD and telomere length.[8] Furthermore, a number of biological mechanisms and genes linked to telomere length dynamics have been implicated in ASD and ASD susceptibility, including increased oxidative stress,[24,25] abnormalities in DNA repair mechanisms,[26] DNA methylation,[25] and chromatin remodeling.[27] These lines of evidence, in association with links between perceived stress and telomere length, suggests that there may be multiple and variable genetic and environmental factors that contribute to telomere dynamics in families with a child with ASD who also have an infant. Establishing whether shortened telomere length is specific to children with ASD or also extends to family members (including the at-risk infant) has implications for how we conceptualize treatment in families who have a child with ASD.

As part of our prospective study of infant siblings of children with ASD, we collected saliva samples from parents, infant siblings, and the affected proband with ASD. We aimed to determine whether families with at least 1 child diagnosed with ASD and a younger infant (whose risk of developing autism is 20 times greater than that of the general population[28]) show reduced telomere length compared to a comparison sample of families with no affected family members. Based on Li *et al.*'s evidence for shortened telomeres in children with ASD,[8] we anticipated telomere reductions in older siblings with ASD compared to those without the disorder. We also predicted decreased telomere length in parents of children with ASD compared to those without a child with ASD, likely operating through a mechanism of heightened stress. It was less clear, however, as to what to expect of telomere length in infant siblings of children with ASD compared to the infant siblings of typically developing children; although at high risk for ASD, the majority of these infants will not develop the disorder (however, an additional 20%–30% will likely develop another disorder or be viewed as possessing the broader autism phenotype).[29] If shortened telomere length is specific to individuals with ASD, overall reductions in telomere length in infant siblings of children with ASD would not be expected. If, however, shortened telomeres in families with ASD reflects an inherited or acquired familial vulnerability, we would anticipate telomere reductions in infant siblings of children with ASD.

METHOD

Study Design

Participants in the current study formed part of an ongoing, longitudinal investigation of neurodevelopment in infant siblings of children with ASD over the first 3 years of life. Infants and their families were designated as high-risk for ASD (HRA) or low-risk for ASD (LRA) according to their older siblings' (proband) diagnostic status. For HRA families, infants had at least 1 older sibling with a community diagnosis of ASD that was not attributable to a known genetic disorder (e.g., Fragile X, tuberous sclerosis complex). Proband diagnoses of ASD were made according to expert community clinicians before the family's enrollment in the study. After enrollment, diagnoses were verified using the Autism Diagnostic Observation Schedule (ADOS)[30] (n = 12) or the Social Communication Questionnaire (SCQ)[31] (n = 5). For 1 HRA proband, grouping was made according to expert community diagnosis, as ADOS and SCQ information were missing. For LRA families, infants had an older sibling that that did not have ASD (confirmed using the SCQ [<12] and/or the ADOS) and no first-degree relatives with a diagnosis of ASD or other neurodevelopmental disorder. Inclusion criteria for infant siblings included a gestational age of at least 36 weeks, no known perinatal or prenatal complications, and no known genetic disorders (e.g., Fragile X syndrome, tuberous sclerosis complex).

For the current study, saliva samples were obtained from consenting families as an optional portion of the larger, longitudinal study. Participating families received $10 compensation for each individual providing a sample. Institutional review board (IRB) approval was obtained from Boston Children's Hospital (IRB# X10-02-0082). Written, informed consent was obtained from each parent before his or her and their children's participation in the study. Written assent was obtained for siblings more than 8 years of age.

Participants

Saliva samples were collected from 205 individuals from HRA or LRA families. Data from 1 infant (HRA) were excluded due to a methodological error (with relative telomere length more than 3 standard deviations above the mean of the entire sample). The final sample comprised data for 204 individuals from 66 families, of whom 37 provided samples for all family members (infant sibling, proband, mother, father), and 29 provided data for some family members. Table 1 outlines the sample size and descriptive characteristics for each group.

Age was calculated from each individual's birth date. Age at saliva sample collection was missing for 8 infant siblings (4 HRA; 4 LRA), 7 mothers (4 HRA; 3 LRA), and 3 fathers (2 HRA; 1 LRA). For 14 HRA infants, their older siblings were diagnosed with ASD before the infants' conception (mean length of ASD diagnosis until the infant's birth = 42.46 months, standard deviation [SD] = 20.95 months). Seven older siblings were diagnosed while their mother was pregnant with the infant (mean length of ASD diagnosis until the infant's birth = 4.37 months, SD = 2.07 months). Seven older siblings received an ASD diagnosis after the infant's birth (mean age of infant sibling at proband diagnosis = 13.06 months; SD = 9.21 months).

Telomere Length

Saliva was collected from participating families using Oragene-assisted DNA collection kits (OGR-575; DNA Genotek Inc., Ottawa, ON, Canada). For parents, 2 mL of saliva was collected. For infants and probands, Oragene saliva sponges (CS-2) were used in conjunction with DNA collection kits (OGR-575). Sponges were placed in the cheek pocket of the mouth and then transferred to the collection kit; 5 saliva sponges were taken for each infant/proband. All individuals were requested not to have eaten within 30 minutes of saliva collection.

We used the real-time polymerase chain reaction (PCR) telomere assay[32] for use in a high-throughput, 384-well format with an Applied Biosystems 7900HT PCR System to determine the relative average telomere length. Briefly, 5 ng of buffy coat–derived genomic DNA was dried down in a 384-well plate and resuspended in 10 μL of either the telomere or 36B4 PCR reaction mixture for 2 hours at 4°C. The telomere reaction mixture consisted of 1x QiagenQuantitectSybr Green Master