Telomere length and cortisol reactivity in children of depressed mothers

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A growing body of research demonstrates that individuals diagnosed with major depressive disorder (MDD) are characterized by shortened telomere length, which has been posited to underlie the association between depression and increased instances of medical illness. The temporal nature of the relation between MDD and shortened telomere length, however, is not clear. Importantly, both MDD and telomere length have been associated independently with high levels of stress, implicating dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and anomalous levels of cortisol secretion in this relation. Despite these associations, no study has assessed telomere length or its relation with HPA-axis activity in individuals at risk for depression, before the onset of disorder. In the present study, we assessed cortisol levels in response to a laboratory stressor and telomere length in 97 healthy young daughters of mothers either with recurrent episodes of depression (i.e., daughters at familial risk for depression) or with no history of psychopathology. We found that daughters of depressed mothers had shorter telomeres than did daughters of never-depressed mothers and, further, that shorter telomeres were associated with greater cortisol reactivity to stress. This study is the first to demonstrate that children at familial risk of developing MDD are characterized by accelerated biological aging, operationalized as shortened telomere length, before they had experienced an onset of depression; this may predispose them to develop not only MDD but also other age-related medical illnesses. It is critical, therefore, that we attempt to identify and distinguish genetic and environmental mechanisms that contribute to telomere shortening.

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INTRODUCTION

A growing body of research demonstrates that individuals diagnosed with major depressive disorder (MDD) are characterized by shortened telomere length, which has been posited to underlie the association between depression and increased rates of medical illness, including cardiovascular disease, diabetes, metabolic syndrome, osteoporosis and dementia (see Wolkowitz et al.14 for a review). Indeed, a number of studies have documented shorter telomere length in depressed than in nondepressed individuals (e.g., refs 2–7).

Telomeres are specialized nucleoprotein structures that cap the ends of linear chromosomes; they consist of double-stranded, repetitive TTAGGG DNA repeats and associated proteins known as the shelterin complex.6,9 Telomere structure is critical for maintaining chromosomal stability by protecting against premature replication termination. Telomeres may shorten as a result of degradation by oxidative stress or other genotoxic stimuli, or by multiple cycles of mitosis in the absence of sufficient telomerase activity. When telomeres shorten to a critical length, the telomere ends are unprotected and initiate classic DNA damage responses, which may lead to apoptosis or to genomic instability. Telomeres shorten with each cell division and, therefore, are believed to be a marker of cellular aging. Although variability in telomere length has been found to be attributable in large part to chronological age,10 telomere shortening has also been associated with the presence of stressors, such as childhood adversity and the stress of caring for an ill family member.11–13 Interestingly, Wolkowitz et al.14 found an inverse relation between telomere length and lifetime exposure to depression, a finding that suggests that shortened telomere length is a consequence of the stressfulness associated with experiencing depressive episodes. It is also possible, however, that exposure to stressors in childhood is associated with shortened telomeres, which may then contribute to the onset of depressive episodes. Indeed, Verhoven et al.15 recently found that both currently and remitted depressed individuals had shorter telomeres than did healthy controls, suggesting either that MDD episodes leave a lasting imprint on telomere length or that shortened telomere length antedates the onset of depression. To address this question, it is critical to assess telomere length in individuals who are at high risk of developing MDD but who have not yet experienced a depressive episode.

In this context, numerous studies have shown that stress has a central role in MDD. Researchers have documented a consistent association between stressful life events and the onset of depression.10,17 Moreover, a growing body of research is demonstrating dysregulated hypothalamic-pituitary-adrenal (HPA)-axis responses to stress in individuals diagnosed with MDD (e.g., Chopra et al.18 and Weinstein et al.19). In fact, dysregulation of the HPA axis, reflected in anomalous levels of cortisol secretion, has been posited to be a core neurobiological abnormality in depression.20,21 Although this depression-associated dysregulation of HPA-axis functioning may be a consequence of the disorder, recent evidence suggests that abnormalities in the HPA-axis system precede and even contribute to the onset of MDD. Thus, theorists have posited that dysregulation of the HPA axis contributes to the increase risk for MDD documented in children of depressed parents.22,23 Indeed, beginning as early as infancy, offspring of
depressed mothers, who are at a three- to fivefold increase in the risk of developing a depressive episode by virtue of having a depressed parent, exhibit higher levels of cortisol in response to a psychosocial stressor than do infants of mothers with no history of depression. Similar adverse effects of parental depression on children’s HPA-axis functioning have also been documented in older offspring. For example, Mackrell et al. 26 found that maternal and paternal depression predicted greater cortisol reactivity to stress in 9-year-old children at a 2-year follow-up assessment. Similarly, Lupien et al.27 recently reported that 10-year-old children who were exposed to maternal depression since birth exhibited higher levels of glucocorticoids than did their non-exposed peers. Moreover, longitudinal studies have found that higher levels of salivary cortisol in high-risk children predict the subsequent onset of depressive symptoms and MDD.28,29

Taken together, these studies indicate that depressed individuals and children of depressed parents are characterized by high levels of cortisol secretion in response to stress and that this disregulation of the HPA axis may place offspring of depressed parents at increased risk for MDD. Investigators have just begun to examine explicitly the association between HPA-axis functioning and telomere length (e.g., refs 30–33). Indeed, only one study has examined the relation between HPA-axis activity and telomere length in depressed individuals. In that study, Wikgren et al.27 not only found shorter telomeres in depressed individuals than in non-depressed controls but also observed an association between shorter telomeres and hypocortisolism in both the depressed and non-depressed groups (as measured by low postdexamethasone cortisol and high percentage of cortisol reduction after the dexamethasone suppression test).

No study has yet examined telomere length, or the relation between telomere length and HPA-axis activity, in individuals at risk for depression. Studying such a population is critical in assessing whether shortened telomere length is a pre-existing condition or risk factor for developing depression, or, alternatively, is a response to, or concomitant of, major depressive episodes. The current study was designed to examine the relation between these two central markers of stress in a sample of children at risk of developing MDD. We focused in this study on daughters of depressed mothers, given the high intergenerational transmission of risk for depression.29 We assessed both cortisol levels in response to a laboratory stressor and telomere length in never-divorced daughters of mothers with recurrent episodes of depression (girls at familial risk for depression, or ‘high-risk’ girls) and age-matched daughters of mothers with no history of Axis I disorders (‘low-risk’ girls). We expected that the daughters of depressed mothers would have shorter telomeres than would their low-risk peers. Further, based on data showing that cortisol diminishes the activity of telomerase, the major enzyme responsible for preserving telomere length,34 we also predicted that, across the low- and high-risk groups, shorter telomeres would be associated with greater cortisol reactivity to stress.

MATERIALS AND METHODS

Participants

Participants were 97 girls aged 10-14 years with no current or past Axis I disorder. Forty-seven girls had mothers with no current or past Axis I disorder (low risk (CLU)), and 50 girls had mothers with a history of recurrent episodes of depression during their daughter’s lifetime (high risk (RSD)). Participants were recruited through the Department of Psychiatry and Behavioral Sciences at Stanford University and advertisements were posted online and throughout the community. Interested individuals were screened for initial inclusion and exclusion criteria via a telephone interview. Participants who were likely to be eligible for the CTL or RSK group were invited to the laboratory for more extensive screening.

Clinical assessment

Interviews. Diagnoses were determined via in-person structured clinical interviews. The Kiddie Schedule for Affective Disorders and Schizophrenia (KID) was administered to daughters and mothers (about their daughters); both informants had to report an absence of current or past Axis I disorder in the daughter. The Structured Clinical Interview for DSM-IV (SCID) was administered to mothers; mothers who reported recurrent episodes of MDD in the daughter’s lifetime were included in the RSK group, and mothers who reported an absence of current or past Axis I disorder were included in the CTL group. An independent rater who was blind to group membership randomly evaluated 10% of the Structured Clinical Interview for DSM-IV and Kiddie Schedule for Affective Disorders and Schizophrenia interviews. In all cases, diagnosis of recurrent episodes of depression in RSK mothers, absence of an Axis I disorder in CTL mothers, and absence of an Axis I disorder in daughters matched the diagnosis made by the original interviewer (k = 1.00). Eligible participants returned to the laboratory within 1 week to provide a saliva sample for telomere measurement and complete the stress task.

Questionnaires. Daughters completed the 10-item version of the Children’s Depression Inventory (CDI-S*). The CDI is a self-report measure of depressive symptomatology for children aged 8-17 years. Pubertal status was assessed via self-report Tanner staging. Ratings were made on a five-point scale, with Tanner stage I representing an absence of secondary sexual characteristics and Tanner stage 5 representing physiologic sexual maturity.

Telomere assessment

Genomic DNA was purified from 500 μl of saliva collected in the Oragene DNA Kit (DNA Genotek, Kanata, ON, Canada) with the DNA Agencourt DNAAdvance Kit (cat. no. A48705; Beckman Coulter Genomics, Brea, CA, USA) according to the manufacturer’s instructions. Although telomere length is most commonly assessed in leukocytes, Mitchell et al. recently reported a significant positive correlation of 0.72 (P=0.002) between leukocyte telomere length and telomere length measured in saliva, and Daniali et al. found significant positive correlations between telomere length measured in leukocytes, skeletal muscle, skin and subcutaneous fat. DNA was quantified by Quant-IT PicoGreen dsDNA Assay Kit (cat. no. P7589; Life Technologies, Grand Island, NY, USA) and run on 0.8% agarose gels to check the integrity. DNA samples were stored at ~80 °C; any samples that were degraded were excluded from telomere length analysis.

Telomere length measurement assay was adapted from the method originally published by Cawthon.3 The cycling profile for T (telomeric) PCR consisted of: denaturation at 96 °C for 1 min; denaturation at 94 °C for 15 s, annealing at 54 °C for 60 s, with fluorescence data collection, 30 cycles. The cycling profile for S (single-copy gene) PCR consisted of: denaturation at 96 °C for 1 min; denaturation at 95 °C for 15 s, annealing at 58 °C for 1 s, extend at 72 °C for 20 s, 8 cycles, followed by denaturation at 96 °C for 1 s, anneal at 58 °C for 1 s, extend at 72 °C for 20 s, hold at 83 °C for 5 s with data collection, 35 cycles. The primers for the telomere PCR were tef1b (5'-GGGTTTGGTTGGT-3'), used at a final concentration of 100 μM, and tef2b (5'-GGGTCTGAGCACCCTTACTG-3'), used at a final concentration of 900 μM. The primers for the single-copy gene (human β-glucuronid PPCR were hbg1 (5'-GGTTTGTCTGAACACTTT GTTCCACTGAGG-3'), used at a final concentration of 100 μM, and hbg2 (5'-CAGCACTTCTCAGGTCACCAGG-3'), used at a final concentration of 700 μM. The final reaction mix contained 20 μl Tris-HEC (pH 8.4), 50 μM KCl, 200 μM each dNTP, 1% DMSO, 0.4× Syber Green I, 1.2 ng Escherichia coli DNA per reaction, 0.4 U of Platinum Taq DNA polymerase (Life Technologies, Carlsbad, CA, USA) per μl reaction and 7 ng of genomic DNA. Tubes containing 26, 875, 29, 0.97, 0.324 and 0.136 ng of a reference DNA (from HeLa cancer cells) were included in each PCR run so that the quantity of targeted templates in each research sample could be determined relative to the reference DNA sample by the standard curve method. The same reference DNA was used for all PCR runs.

To control for interassay variability, eight control DNA samples were included in each PCR to check the T/S ratio. An average T/S ratio was divided by the average T/S for the same DNA from 10 runs to get a normalizing factor. This was carried out for all eight samples and the average normalizing factor for all eight samples was used to correct the participant DNA samples to get the final T/S ratio. The T/S ratio for each sample was measured two times. When the duplicate T/S value and the initial value varied by more than 7%, the sample was run a third time and
the two closest values were reported. Using this method, the average CV for this study is 2.1%.

Stress task and cortisol collection
Daughters refrained from eating or drinking beginning 1 h before arriving at the laboratory for the stress task. The session began with a 30 min rest period, during which girls were allowed to listen to music or read magazines. Next, participants were given instructions about the upcoming stressor. The first part of the stressor was a 3-min serial subtraction task. Girls were given 3 min to count backward aloud from 400 to 0 in 7-steps increments. When an error was made, they were interrupted by the experimenter and asked to start again at 400. Girls who moved quickly through the task were stopped and were told to start over at 400 and count backwards in 7-step increments. Following the serial subtraction task, girls completed the 12-min Erwat Social Competence Interview,32 a semistructured interview designed to induce emotional stress in adolescents by having them talk about stressful life situations. Following the stressors, participants watched a neutral video about the Denali National Park (Denali Park, AK, USA).

Participants used Sarstedt Salivettes (Sarstedt, Numbrecht, Germany) to provide four saliva samples throughout the stress task protocol: immediately before the stressor onset, and at 15, 30, and 45 min after stressor onset. Cortisol collection times were selected based on meta-analytic findings that peak cortisol response to stress occurs 21–45 min after stressor onset and cortisol recovery to baseline occurs 41–60 min after stressor onset.64 On average, the first sample was collected at 14:37 hours (see Table 1 for Session Start Times); the low- and high-risk groups did not differ in their collection times, t(95) = 1.708, P = 0.080. Saliva samples were stored in a freezer chest until they could be transferred to a −20°C freezer located at the General Clinical Research Center at Stanford University, where they were maintained until radioimmunoassay. Cortisol levels were assayed by luminescence immunoassay reagents using a commercial kit from Immuno-Biological Laboratories (Hamburg, Germany), and the assay sensitivity was set at 0.015 ng ml⁻¹. Samples were assayed together in large batches to control for interassay error, and control samples were included to evaluate variability.

RESULTS
Participant characteristics
Demographic and clinical characteristics of participants in the RSK and CTL groups are presented in Table 1. The RSK and CTL groups did not differ in age, ethnicity or Tanner stage (all Ps > 0.05); participants in the RSK group had slightly but significantly higher scores on the CDI than did participants in the CTL group, t(95) = 3.615, P < 0.001. Importantly, however, CDI scores of both groups were far below the recommended clinical cutoff of 8 for the likely presence of depression.27

We examined the association between telomere length and demographic and clinical characteristics, both across the RSK and CTL groups and separately within each group. These correlations are presented in Table 2. Telomere length was not significantly correlated with age, Tanner stage or CDI scores, either across the RSK and CTL groups or within either group, all Ps > 0.05.

Telomere length and cortisol responses to stress
Mean telomere length of the RSK and CTL groups is presented in Figure 1. As we predicted, girls in the RSK group (M = 1.524 T/S, s.d. = 0.265) had significantly shorter telomeres than did girls in the CTL group (M = 1.754 T/S, s.d. = 0.361), t(95) = 3.582, P = 0.001. This result did not change significantly when age, Tanner stage and CDI were included as covariates.

Next, we examined whether cortisol reactivity to stress differed as a function of risk group, telomere length and their interaction. Because the salivary cortisol data were positively skewed, we Winsorized values 2 s.d. above the mean to the 2 s.d. value. Based on the nested design of the study (i.e., time nested within people), we used hierarchical linear modeling35 to analyze the cortisol data. We used a piecewise linear growth model to fit the quadratic nature of the data and to test specific predictions concerning whether the slope of cortisol reactivity to stress (times 1–2) and the slope of cortisol recovery from stress (times 2–4; level 1) were influenced by person-level characteristics (level 2). The results of this model are presented in Table 3. Baseline cortisol levels did not differ significantly as a function of telomere length, risk group or their interaction. As predicted, however, individuals with shorter telomeres exhibited significantly greater cortisol reactivity to stress. There was no main effect of risk group on cortisol reactivity, and the nature of the association between telomeres and cortisol reactivity was similar across groups. In contrast, cortisol recovery from stress was not affected by telomere length, risk group or their interaction. These cortisol findings did not differ significantly when controlling for participants’ age, Tanner stage, CDI scores or the time of day at which the stress task was administered. In Figure 2, we depict the relation between telomere length and cortisol reactivity to stress for participants in the upper (> 1.846 T/S; long) and lower (< 1.391 T/S; short) quartiles of telomere length. Cortisol reactivity to stress was significantly greater for participants in the short versus long telomere group, t (44) = 2.052, P = 0.046.

DISCUSSION
The present study was designed to address the formulation that shortened telomere length is a vulnerability marker for major depression that is present before illness onset. We addressed this hypothesis by examining whether shorter telomere length is evident in individuals who are at increased risk for the development of depression but have not yet experienced a depressive episode. This study is the first to examine telomere length, as well as the association between telomere length and cortisol reactivity to stress, in never-depressed children at risk for MDD. By investigating telomere length in healthy individuals at
risk for the development of depression, we can examine whether accelerated biologic aging begins before the onset of disorder. This is also the first study to test the association between telomere length and cortisol reactivity to stress in a high-risk sample, enabling us to gain a better understanding of the association between two central markers of stress in the context of risk for psychopathology.

The results of this study indicate that healthy children at familial risk for depression have shorter telomeres than do their non-risk peers. Thus, telomere shortening appears to be an antecedent to, and potentially a risk factor for, the onset of depression. Telomere shortening in these young high-risk girls has important health implications. Telomere shortening is not only a marker of stress, but is also a mechanism of biological aging. Insufficient telomere maintenance can accelerate biological aging and increase individuals' risk for experiencing age-related chronic diseases. Therefore, it is critical to identify individuals, such as the children of depressed mothers in the present study, who might be vulnerable to experience telomere shortening. Also, it is important to follow these girls longitudinally to examine whether telomere length influences the onset of MDD and whether developing depression, in turn, contributes to further shortening of telomeres.

It is important to note that both genetic and environmental mechanisms could have contributed to the shorter telomere length we documented in our high-risk participants; because the daughters in this study were all living at home with their biologic mothers, we cannot distinguish between these two types of mechanisms. A recent meta-analysis estimated a 70% heritability of telomere length, with figures from individual studies ranging from 34 to 82%. Although this analysis suggests that telomere length is strongly influenced by genetic factors, it is clear that a substantial amount of variability in telomere length is also explained by environmental factors. Indeed, there is growing evidence that shorter telomere length is associated with exposure to stress, including childhood adversity, stressful life events and chronic stressors. In the present study, we operationalized participants' risk for psychopathology on the basis of their mothers' history of recurrent depressive episodes during the daughters' lifetime. Maternal depression has been associated with a stressful early environment for children (see Goodman and Gottlieb and Lovejoy et al for reviews); the chronic exposure of these children to this stress as a function of living with mothers who have experienced recurrent episodes of depression could represent a mechanism of accelerated biologic aging, operationalized as having shorter telomere length. It will be important in future research to attempt to elucidate the relative contributions of genetic versus environmental factors to telomere length in high-risk children.

Importantly, we found that girls with shorter telomeres exhibited greater cortisol reactivity to stress. The fact that we found this association in both the high- and low-risk participants suggests a universal relation between telomere length and HPA-axis regulation. This possibility is consistent with findings of an association between shorter telomere length and hypercortisolism in healthy populations (for a review see Price et al), and across psychiatric and control groups. Interestingly, we found that telomere length was associated with cortisol reactivity to stress, but not with baseline levels of cortisol. This finding extends results of studies with other populations indicating that telomere length is associated more strongly with biologic responses to stress than with basal levels of cortisol (e.g., refs 30,55,56). Other investigators have also reported stronger relations between cortisol and telomere length when measuring dynamic than static aspects of cortisol secretion (e.g., waking-associated increases in cortisol or cortisol responses provoked by psychological stress). Although this pattern of results may be because of the greater stability of cortisol measures for dynamic (i.e., within individuals) than for cross-sectional (i.e., across individuals) assessment, it is also possible that telomere shortening is related more consistently to cortisol reactivity to stress than to resting cortisol levels. It will be important in future research to conduct a more systematic study of this issue.
examination of the relation of telomere length with basal levels of cortisol by sampling at various points in the diurnal cycle under the usual living conditions for participants, rather than in a laboratory setting.

Similarly, although an increasing number of studies have documented a relation between telomere length and cortisol secretion, the nature of this association is not well understood. For example, high levels of cortisol secretion may contribute to accelerated telomere shortening (e.g., Picard et al.57). In this context, as we noted earlier, Choi et al.34 presented in vitro data indicating that cortisol diminishes the activity of telomerase, the major enzyme responsible for preserving telomere length. It is also possible, however, that a third variable is responsible for both shorter telomere length and greater cortisol reactivity to stress. Oxidative stress, for instance, has been implicated as an important mechanism of telomere shortening (Epel et al.14 and Szebeni et al.58, but also see Bull et al.76). Clearly, additional longitudinal research is needed to gain a more comprehensive understanding of the temporal and causal relations between these central markers of chronic stress.

We should note three limitations of the current study. First, the participants in our study were 10 to 14 years of age. We selected this relatively narrow age range, in part, to increase the likelihood that girls in the high-risk group had not yet experienced a depressive episode. As a consequence, however, we cannot examine whether our findings extend to a broader age range of individuals at risk for depression. This is an important direction for future research. Second, girls in the high-risk group had slightly, but significantly, higher CDI scores than did girls in the low-risk group. We should note, however, that there was no association between this narrow range of CDI scores and telomere length, and that our findings did not change significantly when we included CDI scores as a covariate in the analyses. Finally, we used Tanner staging as our measure of pubertal status, and found no relation between pubertal status and telomere length. Tanner staging is a self-report measure, and although researchers have demonstrated that it correlates highly with physicians’ physical examinations of pubertal development (e.g., Shirkiff et al.79), it will be important in future research to conduct more extensive physical assessments of pubertal status.

In this study, we demonstrated that (1) daughters of mother with recurrent depression have shorter telomeres than do their low-risk counterparts and that (2) shorter telomere length is associated with greater cortisol reactivity to stress across the high- and low-risk groups. These findings suggest that healthy girls who are at risk of developing MDD are characterized by accelerated biologic aging before the onset of depression, which may predispose them to develop age-related medical illnesses. Indeed, MDD is associated with an increased risk of experiencing serious comorbid medical illnesses (see Freedland and Carney81 for a recent review of this literature). Interestingly, the specific medical illnesses that are observed most frequently in individuals with psychiatric conditions are those that are most commonly seen with advanced age (e.g., cardiovascular disease, stroke, dementia, diabetes, osteoporosis82), raising the possibility that psychiatric illnesses are associated with accelerated aging at the cellular or organismic level. Finally, our findings also contribute to biologic models of health and aging by documenting what appears to be a universal association between telomere length and cortisol reactivity to stress in children. Future work should examine the role of telomere length in risk for MDD and the subsequent effect of a depressive episode on telomere shortening.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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