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ORIGINAL ARTICLE

Children being Reared by their Grandparents in Rural Appalachia: A Pilot Study of Relations Between Psychosocial Stress and Changes in Salivary Markers of Inflammation Over Time

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Abstract
Grandparents in rural Appalachia with primary caregiving responsibilities for their grandchildren often struggle with high levels of stress, inadequate resources, and poor physical and mental health. However, implications for children of being raised by grandparents rarely have been examined, particularly in terms of stress biomarkers. The present study investigated salivary C-reactive protein, interleukin-6, and tumor necrosis factor alpha in a small sample of children ($N = 20$) aged 5 to 18 years being reared by grandparents in two rural counties in Kentucky, a region well known for its resource scarcity. Saliva samples were collected from children 30 min after waking at two time points spaced one year apart. Grandparents and children completed a series of questionnaires via interview. Children's internalizing symptoms were related to greater markers of inflammation over time. Grandparent stress and poor mental health were also related to greater inflammation, while grandparent positive parenting and religiosity were associated with lower inflammation.

Keywords Inflammation · custodial grandparents · mental health

Like all families, those in rural Appalachia are characterized by strengths and challenges. Strengths include strong family ties, supportive social networks, and membership in trusted organizations such as faith groups (Schoenberg et al. 2008). Challenges include high rates of poverty, parental addiction, and parental crime and incarceration (e.g., Collins et al. 2011). Recent increases in these challenges has led to a greater prevalence of grandparents rearing their grandchildren ("grandfamilies"). There are an estimated 3 million US children being reared by their grandparents, but rates are especially high in Appalachia (Appalachian Regional Commission 2014). Caregiving grandparents face additional challenges such as increased stress and the physical limitations of age (Segerstrom et al. 2008). However, there has been little research on the health and well-being of children in this context. The present study addresses this research gap through examination of associations between grandfamily strengths and challenges and salivary markers of inflammation in a small sample of children over time.

Salivary Markers of Inflammation

Inflammation is an immune response to infection or injury that is designed to eliminate the source of infection or injury and repair damaged tissue (Delves et al. 2017). Inflammation involves increased blood flow to the affected site (resulting in heat and redness), increased permeability of blood vessels that leaks plasma into the area (resulting in swelling), and the release of molecules that stimulate pain nerves (Delves et al. 2017). Although inflammation is an adaptive response to infection and injury, chronic inflammation can itself cause damage to the body (Chrousos 2000).

Inflammatory processes are highly complex and involve multiple cell types and cellular mediators. Thus,

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examination of only one inflammatory marker would provide a very limited picture of inflammation (Slavish et al. 2015). Three markers of inflammation are examined in the present study: (1) C-Reactive Protein (CRP); (2) Interleukin-6 (IL-6); and (3) Tumor Necrosis Factor α (TNF-α). The CRP binds to dead or dying cells and some types of bacteria in order to activate inflammation; it is considered a marker of systemic inflammation because it is produced by the liver (Pepys and Hirschfield 2003). C-Reactive Protein (CRP) is involved in the production of fever, tiredness, increased blood pressure, and loss of appetite; at chronic high levels it can lead to kidney and heart damage. The IL-6 is a pro-inflammatory cytokine that is produced by several types of immune and other cells and plays an important role in chronic inflammation (Gabay 2006). It can induce fever and is often involved in autoimmune responses that lead to conditions such as arthritis, diabetes, lupus, and cancer. The TNF-α is a pro-inflammatory cytokine, produced primarily by macrophages, that induces fever, kills unhealthy cells, and prevents cell division in tumors and replication of viruses (Gaur and Aggarwal 2003). Chronically elevated TNF-α is associated with Alzheimer's disease, psoriasis, cancer, and irritable bowel syndrome (Popa et al. 2007).

There are multiple sources of increased inflammatory markers in human saliva. First, periodontal disease and injuries may prompt an immune response that includes inflammation (Miller et al. 2010). Second, systemic inflammation can cause increases in salivary markers of inflammation when blood enters the oral cavity through injured tissue or when they are secreted from serum into saliva via the salivary glands (Johnson 2001). Systemic inflammation may be caused by bodily infection or disease (Delves et al. 2017), physical exertion (Woods et al. 2009), acute and chronic psychosocial stress (Morey et al. 2015; Slavish et al. 2015).

The mechanisms through which stress promotes inflammation includes the hypothalamic-pituitary-adrenal (HPA) axis (Carlsson et al. 2014). The primary effector molecules of the HPA axis are glucocorticoids, and almost all immune cells have receptors for these molecules. When glucocorticoids attach to receptors on immune cells, the result is removal of inflammatory mediators from affected sites (Bellavance and Rivest 2014). Paradoxically, heightened HPA activity in the context of psychosocial stress is instead linked to chronic high inflammation. Although this process is not well-understood, it has been well-established in rodent and in vitro models (e.g., Espinosa-Oliva et al. 2011) and is consistent with a model of allostatic load (McEwen 1998). Allostatic load refers to the wear and tear on the body that occurs when stress response systems are repeatedly activated. HPA activation results in increased cardiovascular workload that can lead to high blood pressure and heart disease. The damage done to the cardiovascular system may prompt an inflammatory response.

Inflammation in the Context of Rural Appalachian Grandfamilies

The current study focuses on relations between psychosocial stress and inflammation in a small sample of grandfamilies in two rural Appalachian counties in Kentucky. This part of Appalachia is economically depressed, with high concentrations of poverty and low levels of education (Jenkins-Howard et al. 2013). Nearly one in five households is led by grandparents in the study counties (Appalachian Regional Commission 2014). Grandparents in this region often take over the care of their grandchildren when parents suffer from addiction (Collins et al. 2011), become incarcerated (Walters 2013), or are abusive or neglectful parents (Administration for Children and Families, 2014). Our goal is to examine possible chronic stressors present in this population (such as low socioeconomic status) in relation to children's inflammation. We focus on changes in salivary markers of inflammation over a one year period. According to the allostatic load model, it takes time and repeated exposure for a significant stressor to produce systemic inflammation.

We adapt the Family Stress Model (Conger and Donnellan 2007) to understand how the chronic stress experienced by grandfamilies in rural Appalachia may lead to allostatic load and increased inflammation in children. This model proposes that the stresses of making ends meet, dealing with deteriorating or crime-ridden neighborhoods, and other poverty-related stressors are disruptive to family relationships (Conger and Donnellan 2007). In particular, parents experience increased mental health issues in response to these stressors, including depression and anxiety, which impair their ability to act as consistent and sensitive caregivers (Goodman et al. 2011). Such parents often resort to harsh and punitive parenting that exacerbates child emotional and behavioral issues and contributes to academic underachievement (Conger et al. 2012). Specifically, we examine multiple features of socioeconomic status and family demographics (education, perceived financial status, grandparent age, custody arrangements, and marital status) as well as aspects of grandparent functioning highlighted by the Family Stress Model (grandparent mental health, physical health, stress, and parenting) in relation to change in children's salivary markers of inflammation. Notably, these grandfamily stressors have been associated with child maladaptation in other regions (e.g., Poehlmann et al. 2008; Smith and Hancock 2010).

In addition to exploring the role of stressful experiences in Appalachian grandfamilies, a secondary goal is to identify possible protective factors against inflammation in this population. Despite their many challenges, grandfamilies in rural Appalachia are also characterized by multiple strengths. Sociologists have identified several key values that are

common to Appalachian culture that are likely to foster resilience, including hospitality, commitment to religion, and closely knit families (Jones 1994).

Thus, research on Appalachian culture, the allostatic load model of stress, and the Family Stress Model of the effects of poverty on child development led to the selection of several potential covariates of changes in children's inflammation. It is hypothesized that low grandparent education, single marital status, low financial status, lack of formal custody of children, high grandparent stress, greater grandparent depression and mental health issues, poor grandparent health, poor child health, child depression, anxiety, and aggressive behavior will be associated with increases in child salivary CRP, IL-6, and TNF-α over time. Conversely, it is hypothesized that grandparent religiosity and social support will be related to decreases in child salivary CRP, IL-6, and TNF-α over time.

Method

Participants

Participants included 20 children (10 girls) between the ages of 5 and 18 years of age ($M = 12.30$ years, $SD = 4.33$) and one of their grandparents (age 55 or older; $M = 66.53$; $SD = 4.62$; 15 women; 7 single) who served as a primary caregiver for the child. Families were recruited at grandparent coalition meetings or other community gatherings, and through snowball sampling. Only one grandparent and one child (chosen by the grandparent) per family participated. Fourteen grandparents had formal legal custody of the grandchild and 6 grandparents did not have formal legal custody but provided care for their grandchild most of the time. All of the participants were white, only 9 grandparents had a high school diploma or more education, and most were low income, with half indicating that they struggle to make ends meet.

Procedure

This study was conducted with the approval of the university Institutional Review Board; informed consent was obtained from grandparents and informed assent was obtained from grandchildren. Data were collected at two time points, spaced approximately one year apart (18 out of 20 families participated at T2), and procedures were identical at each time point. Research staff visited the homes of the grandfamilies. One staff person interviewed the grandchild while another interviewed the grandparent in a separate room. All questions were asked via interview. Grandparents were also provided with vials to collect saliva from the children. Grandparents were instructed to select a typical weekday morning. Upon waking children were to brush their teeth and rinse their mouths with water. Thirty minutes after waking, children passively drooled their saliva into the vial for no more than 5 min or until 5 mL had been collected. This saliva collection method is considered highly reliable because it can produce large amounts of saliva and avoids the contamination that can occur when devices or products are used to collect saliva or stimulate the flow of saliva; it has also been shown to work well with children over the age of 6, adolescents, and adults (Granger et al. 2007). Children were not permitted to eat, drink, or use tobacco before or during saliva collection. Samples were immediately frozen in the family freezer. Research staff picked up the samples, packed them in dry ice, and brought them to a university laboratory for assay.

Measures

Determination of Salivary Markers of Inflammation. The CRP levels were determined using a commercially available ELISA kit designed specifically for saliva following manufacturer instructions (www.salimetrics.com). The intra-assay and inter-assay coefficients of variance (CV) were 5.9 and 11%, respectively. Tests were run in duplicate. Values were detectable for all samples at T1, and for 15 samples at T2. The IL-6 and TNF-alpha levels were determined using a commercially available magnetic bead panel kit following manufacturer instructions (www.emdmillipore.com). The intra-assay CVs were < 5% and < 5% for IL-6 and TNF-alpha, respectively. The inter-assay CVs were < 20% and < 15%, respectively. The Il-6 values were detected for 18 samples at T1 and 17 samples at T2. TNF-alpha values were detected for 16 samples at both T1 and T2.

Child Anxiety Children completed the Penn State Worry Questionnaire adapted for use with children (PSWQ; Chorpita et al. 1997). The 14 items are rated on a scale from 1 to 4, with higher scores indicating greater worry, Cronbach's $\alpha = .92$.

Child depression Children completed the Center for Epidemiologic Studies Depression scale for Children (CESD-C; Fendrich et al. 1990). The 20 items are rated on a 4 point scale, with higher scores indicating greater depression, Cronbach's $\alpha = .85$. Item scores are summed to provide a total depression score; 6 children (33.3%) were above the cut-off for potential clinical depression.

Child Stress Children completed the first portion of the Responses to Stress Scale (Family Stress Version) as an index of how much family-related stress they felt (Connor-Smith et al. 2000). This section of the measure includes 11 items representing different family stress experiences (e.g., arguing with grandparents, arguing with siblings) rated on a scale from 1 to 4 in terms of how much each of the experiences stresses them. Scores were computed by averaging responses across

the 11 items, Cronbach's $\alpha = .77$. Due to evidence that grandparent-related stress and sibling-related stress function differently in this sample, separate scores were also computed for these two types of stress.

Child aggression Children completed the Proactive/Reactive Aggression Questionnaire (Raine et al. 2006). There are 12 items on the Proactive Aggression scale (aggression without provocation) and 11 items on the Reactive Aggression scale (aggression in response to provocation). Items are rated on a three-point scale, with higher scores indicating greater aggression. The reliability was low for Proactive Aggression, Cronbach's $\alpha = .60$, but good for Reactive Aggression, Cronbach's $\alpha = .80$. Therefore, only the Reactive Aggression scale is used in analyses.

Child Physical Health Grandparents completed the Child Health Questionnaire-Parent Form 28 (CHQ28; Landgraf et al. 1996). A physical health score was computed by summing items assessing physical functioning, the impact of physical health on role functioning, bodily pain, general health perceptions, and change in health over the past year. Higher scores indicate better child physical health. Reliability was good, Cronbach's $\alpha = .86$.

Grandparent Health Grandparents completed the Short-Form 36 (SF-36) Health Survey (Ware et al. 1994). Two composite scores were calculated: Physical Health Scores and Mental Health Scores, with higher scores indicating better health.

Grandparent Depression Grandparents completed the Patient Health Questionnaire-9 (PHQ9; Martin et al. 2006). The 9 items are rated on a 4 point scale, with higher scores indicating greater depression, Cronbach's $\alpha = .80$. Among elderly patients a score of 6 or higher is indicative of any depressive disorder (Lamers et al. 2008), and 8 grandparents (30.0%) met this criterion.

Grandparent Parenting Stress Grandparent parenting stress was assessed via a modified version of the Parenting Stress Scale (PSS; Berry and Jones 1995). The modified version substituted "grandparent" for "parent" in all items. Items are rated on a scale from 1 to 5, with higher scores indicating higher stress, Cronbach's $\alpha = .79$.

Grandparent Positive Parenting Children completed the Acceptance subscale of the Child Report of Parental Behavior Inventory (CRPBI; Schaefer 1965). Children are asked how much their grandparents are people who make them feel accepted in 10 different ways. Each of the 10 items was rated on a scale from 1 to 3, with higher scores indicating greater grandparent acceptance of the child, Cronbach's $\alpha = .91$,

Grandparent Religiosity Grandparents answered two questions about religiosity: (1) How often do you attend church or other religious meetings? And (2) How often do you spend time in private religious activities, such as prayer, meditation, or Bible study? Both questions were rated on a 6 point scale, which higher scores indicating greater frequency of religious activity. Given that public and private religious behaviors are conceptually distinct (e.g., Nonnemaker et al. 2003), the two questions were treated separately for analysis.

Grandparent Social Support Grandparents completed the Medical Outcomes Study Social Support Survey (MOS-SSS; Sherbourne and Stewart 1991). This survey includes four subscales for the different types of social support grandparents may experience: emotional/informational, tangible, affectionate, and positive social interaction. There are 18 items, each rated on a five point scale in terms of how frequently a person is available to provide social support. Cronbach's α ranged from .93 to .98. Scores were computed by averaging item responses for each scale. Rather than aggregating the different scales into a single measure of social support, the individual scales were retained for analyses to be consistent with established theory (Schumaker and Brownell 1984).

Grandfamily Demographics We considered the following demographic variables: Grandparent age, child age, child sex, marital status (partnered or single grandparent), number of children in the household, highest level of grandparent education, and perceived financial status. Perceived financial status was rated on a scale from 1 to 3, with 1 reflecting "I have more than I need to live well", 2 indicating "I have just about enough to get by", and 3 indicating "I sometimes struggle to make ends meet."

Results

Salivary Markers of Inflammation

Table 1 provides the means and standard deviations for CRP, IL-6, and TNF-α at T1, T2, and for the change from T1 to T2. Also shown in Table 1 are the results of t tests evaluating the degree of change in salivary markers of inflammation over time. Of the three biomarkers, CRP and IL-6 showed no significant mean change over time, and TNF-α showed a significant average increase over time.

Evaluation of the means and standard deviations indicates that values at T1 and T2 are skewed (the standard deviation is often larger than the mean). Further examination indicated that the skewness statistic divided by its standard error was greater than 3.00 in all but two cases. To normalize the variables, natural log, square root, and inverse transformations were

Table 1 Means and standard deviations for salivary markers of inflammation in children

T1	*M*	*SD*	*t*
C-Reactive Protein (*N* = 19)	1123.03	1666.45	
Interleukin-6 (*N* = 18)	5.64	12.62	
Tumor Necrosis Factor-α (*N* = 17)	5.06	6.24	
T2			
C-Reactive Protein (*N* = 15)	918.22	1289.32	
Interleukin-6 (N = 18)	9.25	9.50	
Tumor Necrosis Factor-α (N = 18)	10.39	8.91	
Change from T1 to T2			
C-Reactive Protein (*N* = 14)	410.82	1041.58	1.48
Interleukin-6 (N = 17)	3.79	16.44	0.95
Tumor Necrosis Factor-α (*N* = 16)	5.88	8.22	2.86*

Change from T1 to T2 was computed by subtracting the T1 value from the T2 value, thus positive values indicate increases and negative values indicate decreases

All salivary parameters are measured in micrograms/deciliter (μg/dL)

*$p < .05$

conducted on the change variables (the variables of primary interest in analyses). However, each of these transformations increased the skewness. Therefore, the gold standard nonparametric approach (bootstrapping; see Efron and Tibshirani 1994) was employed for all additional analyses with these variables. Specifically, bias corrected and accelerated 95% CIs were computed using SPSS for all measures of association based on 5000 bootstrapped samples. Bootstrapping makes no assumptions about the distributions of variables or statistics and provides more accurate tests of significance than other approaches.

Characteristics of Grandfamilies and Children

Table 2 provides the means and standard deviations for grandparent and child characteristics. Descriptive statistics indicate a range of both strengths and challenges in the participating grandfamilies. For example, grandparents indicated poor physical and mental health compared to population norms; grandparents' physical health was on average 1.71 standard deviations below the mean for the general population (50). Grandparents' mental health was on average 0.44 standard deviations below the mean for the general population (also 50).

Consistent with reported cultural advantages, mean levels of all types of social support were near 4.00, indicating that persons were available to provide multiple forms of social support "most of the time." In addition, children reported high levels of positive parenting by grandparents; the mean score was 2.59, which is halfway between "somewhat like" the grandparents and "a lot like" the grandparents to show warmth and acceptance to children.

Table 2 Means and standard deviations for study variables

Variable	*M*	*SD*
Child Depression (scale = 0–60)	36.26	12.63
Child Anxiety (scale = 14–56)	27.26	10.76
Child Overall Stress (scale = 1–4)	1.92	0.60
Child Grandparent-Related Stress (scale = 1–4)	1.83	0.63
Child Sibling-Related Stress (scale = 1–4)	2.13	0.90
Child Reactive Aggression (scale = 1–3)	1.59	0.40
Child Physical Health (scale = 9–45)	35.20	6.91
Grandparent Age (years)	67.15	5.29
Child Age (years)	12.30	4.33
Child Female	47.62%	
Grandparent Single	38.10%	
Number of Children <18 years in Home	1.76	1.14
Grandparent Has Formal Custody	66.67%	
Grandparent Education (grade level attained)	10.71	2.28
Poor Perceived Financial Status (scale = 1–3)	2.43	0.68
Religious Service Attendance (scale = 1–6)	3.67	1.91
Private Religious Activity (scale = 1–6)	4.05	1.72
Grandparent Physical Health	32.92	12.08
Grandparent Mental Health	45.57	10.47
Grandparent Depression (scale = 0–27)	4.95	4.93
Grandparent Parenting Stress (scale = 18–90)	31.14	9.43
Grandparent Social Support (scale = 1–5)		
Emotional/Informational Support	3.74	1.31
Tangible Support	4.05	1.14
Affectionate Support	4.23	1.14
Positive Social Interaction	3.81	1.45
Grandparent Positive Parenting (scale = 1–3)	2.59	0.56

Associations Between T1 Grandfamily Characteristics and Change in Child Inflammation

Partial correlations between T1 child and grandfamily characteristics and change in salivary markers of inflammation from T1 to T2 are provided in Table 3, along with the 95% bias corrected and accelerated CIs. Given the large age range of the children and the focus on longitudinal associations that may be confounded by child age, child age was partialed for all correlations. Higher stress about sibling relationships at T1 was associated with increases in CRP. Older grandparent age was related to decreases in CRP and TNF-α. Grandparent reception of emotional/information and affectionate social support were both related to increases in TNF- α. Poor financial status at T1 was associated with increases in IL-6 while better grandparent mental health and more grandparent positive parenting were both related to decreases in IL-6.

Multiple regression models were then fit to determine unique associations among the covariates. Each change

Table 3 Bootstrapped partial correlations (With 95% CIs) between child and grandfamily characteristics and change in salivary markers of inflammation from T1 to T2

Variable	Δ C-Reactive Protein	Δ Interleukin-6	Δ Tumor Necrosis Factor-α
Child Depression	.28 (−.68, .88)	.35 (−.42, .94)	−.09 (−.66, .57)
Child Anxiety	.55 (−.09, .92)	.16 (−.71, .83)	−.02 (−.55, .46)
Child Overall Stress	.59 (−.69, .98)	.36 (−.59, .90)	−.24 (−.83, .28)
Child Grandparent-Related Stress	.19 (−.85, .85)	.48 (−.67, .95)	−.42 (−.89, .11)
Child Sibling-Related Stress	**.91 (.17, .99)**	.04 (−.77, .76)	.01 (−.61, .65)
Child Reactive Aggression	.09 (−.80, .76)	.30 (−.72, .87)	−.18 (−.79, .52)
Child Physical Health	−.19 (−.79, .43)	.07 (−.40, .46)	.09 (−.44, .50)
Grandparent Age	**−.39 (−.68, −.25)**	.08 (−.64, .59)	**−.44 (−.78, −.10)**
Child Female	.24 (−.58, .69)	.22 (−.45, .63)	−.22 (−.73, .44)
Grandparent Single	.34 (−.37, .70)	.14 (−.48, .51)	−.17 (−.60, .43)
Number of Children <18	.12 (−.37, .97)	.20 (−.17, .69)	.35 (−.18, .96)
Grandparent Has Formal Custody	−.36 (−.98, .68)	−.13 (−.63, .66)	.39 (−.04, .82)
Grandparent Education	.16 (−.39, .51)	−.03 (−.55, .65)	.03 (−.51, .45)
Poor Perceived Financial Status	.11 (−.56, .61)	**.44 (.02, .99)**	.07 (−.47, .68)
Religious Service Attendance	.21 (−.72, .69)	−.05 (−.67, .68)	−.08 (−.81, .57)
Private Religious Activity	.18 (−.53, .55)	.16 (−.48, .58)	−.37 (−.79, .09)
Grandparent Physical Health	.17 (−.72, .92)	−.08 (−.75, .81)	.35 (−.38, .81)
Grandparent Mental Health	−.49 (−.90, .55)	**−.56 (−.82, −.34)**	−.05 (−.51, .64)
Grandparent Depression	.66 (−.66, .96)	.39 (−.49, .82)	−.22 (−.64, .37)
Grandparent Parenting Stress	−.31 (−.78, .05)	.48 (−.10, .78)	−.30 (−.82, .36)
Grandparent Social Support			
Emotional/Informational	.39 (−.04, .84)	.01 (−.37, .44)	**.61 (.07, .89)**
Tangible	.25 (−.41, .74)	−.19 (−.43, .48)	.28 (−.37, .72)
Affectionate	.11 (−.87, .83)	−.01 (−.57, .34)	.54 (−.48, .87)
Positive Social Interaction	.12 (−.71, .90)	−.33 (−.74, .06)	**.61 (.06, .86)**
Grandparent Positive Parenting	−.10 (−.95, .92)	**−.56 (−.90, −.22)**	.26 (−.34, .66)

Correlations partialed for child age;

Change scores were computed as the T2 value – the T1 value, thus higher change scores indicate increases over time while lower changes scores indicate decreases over time;

Values in italics are $p < .10$; values in bold are $p < .05$.

variable was predicted by child age and the variables that were significantly associated with it. Regression coefficients were bootstrapped following the procedures described above. The model predicting change in CRP accounted for a significant amount of variance, Adjusted $R^2 = .77$, $F(3, 7) = 12.31$, $p < .01$. However, none of the predictors included in the model (child age, grandparent age, and child sibling-related stress) were uniquely related to change in CRP. The model predicting change in IL-6 did not predict a significant amount of variance, Adjusted $R^2 = .16$, $F(4, 11) = 3.45$, $p = .05$. Thus, model coefficients were not examined. The model predicting change in TNF-α accounted for a significant amount of variance, Adjusted $R^2 = .41$, $F(4, 10) = 3.45$, $p = .05$. However, none of the predictors included in the model (child age, grandparent age, emotional/information social support, and affectionate social support) was independently related to change in TNF-α.

Discussion and Limitations

Findings indicated that studied grandfamilies experienced a range of strengths and challenges. Of the different factors examined, children's sibling-related stress, poor family financial status, and grandparent reception of social support emerged as possible risk factors for child inflammation. In contrast, evidence was found that older grandparent age, better grandparent mental health, and grandparent positive parenting may serve as protective factors against child inflammation.

Children's stress and family financial issues may are consistent with the types of factors that can induce allostatic load (McEwen 1998). It is interesting that social support served as a risk factor rather than a protective factor. This finding does need to be replicated, but it is possible that grandparents receiving greater social support do so because they are under greater pressure and are experiencing greater stress. Allostatic load in the form of systemic inflammation has been observed

in similar contexts (e.g., Blair et al. 2011; Matthews et al. 2014; Slopen et al. 2013a). The immune system responds to stress in much the same way it does to injury or infection; this response is mediated by close interaction between the immune system and stress response systems such as the HPA axis (Carlsson et al. 2014; Morey et al. 2015). It is believed that this response was adaptive to our early ancestors since stress often signaled danger and a high risk for injury (Raison et al. 2006). However, the immune reaction to stress is less adaptive in modern life as it contributes to mental and physical health problems (Black and Garbutt 2002).

To the extent that these stressors are more common in rural Appalachian grandfamilies, they may contribute to the noted health disparities of this region. People of the Appalachian region have elevated levels of inflammation compared to other regions, a difference that is not explained by traditional demographic characteristics or life-style behaviors (Clark et al. 2011). There are Appalachian disparities in mental health issues such as depression (Hendryx and Innes-Wimsatt 2013), oral disease (Martin et al., 2008), respiratory and kidney disease (Hendryx 2009), coronary heart disease and heart attack (Hendryx and Zullig 2009), cancer (Behringer and Friedell 2006) and overall mortality (Hendryx 2010). A greater focus on psychosocial risk factors that activate stress response systems and promote inflammation may help to explain these disparities.

As anticipated, some grandfamily strengths emerged as potentially protective against children's inflammation in this population. It is interesting that older grandparent age was related to decreases in inflammation over time. It is possible that older grandparent age is an indication that parents of the children were not teen parents (younger parents would generally be associated with younger grandparents). According to CDC data, Kentucky has higher rates of teen mothers than the national average, and rates in Appalachian counties are the highest in the state. Infants of teen mothers are more likely to be pre-term, have lower birth weights and worse physical health (Chen et al. 2007), possibly because teen mothers are less likely to receive prenatal care, more likely to smoke during pregnancy, and more likely to have sexually transmitted diseases during their pregnancy (Hueston et al. 2008). Teen mothers are also less likely to initiate breastfeeding and breastfeed for a shorter period of time (Spear 2006); breastfeeding promotes healthy development of the HPA axis (Beijers et al. 2013). Thus, children born to adolescent mothers face a number of risk factors for poor physical health beyond what might be experienced in other rural Appalachian families.

Another possible protective factor was grandparent positive parenting. Warm family relationships are a welcome characteristic of grandfamilies for many children (Downie et al. 2010) and these warm relationships may reduce children's stress. They may also contribute to enhanced attachment security. Attachment refers to the emotional bond between caregiver and child, and it serves the purpose of helping the child maintain proximity to the caregiver during periods of vulnerability and encouraging confident exploration of the environment under normal conditions (Cassidy and Shaver 2002). Attachment security is a fundamental goal for healthy socioemotional development (Madigan et al. 2013), and is also recognized as supporting physical health (Pietromonaco and Powers 2015). For example, insecure attachment during childhood is a risk factor of inflammation-related diseases in adulthood (Puig et al. 2013). Secure attachment develops as a result of consistent and sensitive parenting (Wolff and Ijzendoorn 1997), and insecure attachment is likely to develop in the context of neglect and abuse (Stronach et al. 2011). Attachment can change if there are corresponding changes in the caregiver environment, such as when children are removed from abusive homes and placed into foster care (Smyke et al. 2010). There has been relatively little research on improvements in attachment security in the context of grandfamilies, and future research may explore attachment as a mechanism through which grandparent positive parenting may reduce inflammation in children.

Findings should be interpreted in light of study limitations. First, although longitudinal research designs have an advantage over cross-sectional designs, they do not permit causal inferences. An important avenue for future research is the introduction of stress-reducing interventions in randomized clinical trials as a way of testing causal relations between stress and salivary markers of inflammation in children. Second, the sample size is small, possibly inflating both Type I and Type II error rates. Unfortunately, this is quite common among studies of inflammatory markers (e.g., Izawa et al. 2013; Slavish et al. 2015). One reason for the small sample sizes is the cost involved in the research.

Although representative of rural Appalachian grandfamilies, it is unclear whether findings might generalize to other populations, including those of different race/ethnic backgrounds. Third, levels of pro-inflammatory complement in saliva may not be related to systemic inflammation (Miller et al. 2010). As noted, salivary markers of inflammation are due to systemic inflammation and to immune activity related to periodontal disease and injury, that is, local inflammation (Miller et al. 2010). Nevertheless, salivary measures are a far more feasible and appropriate collection method for children, remote rural communities, and populations that may be wary of outsiders. The wide age range of the children is also a limitation. The immune system undergoes development throughout childhood and adolescence (Goenka and Kollmann 2015). Although certain immune parameters (T, B, and NK cells) decline from infancy, childhood, and adulthood (Valiathan et al. 2016). However, very little is known about how stress may impact the immune systems differently at different period of development and this is an important direction for future research.

Despite these limitations, the current study contributes to knowledge of stress and inflammation in children, particularly among rural Appalachian children being raised by their grandparents. Findings suggest that the stressors these children face may contribute to risk for chronic inflammation over time. However, strengths of grandfamilies such as the positive parenting of grandparents may counteract these challenges.

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Compliance with Ethical Standards

Disclosure of Interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical Standards and Informed Consent This study was conducted with the approval of the university Institutional Review Board; informed consent was obtained from grandparents and informed assent was obtained from grandchildren.

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