Psychoneuroendocrinology 102 (2019) 189–195

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Early childhood family instability and immune system dysregulation in adolescence

Kammi K. Schmeer[a,*], Jodi L. Ford[b], Christopher R. Browning[a]

[a] Department of Sociology, The Ohio State University, United States
[b] College of Nursing, The Ohio State University, United States

ARTICLE INFO

Keywords:
Epstein-Barr virus
Virus reactivation
Childhood stress
Adolescent health

ABSTRACT

Exposure to stress is one way in which social disadvantages during childhood may alter biological and psychological systems with long-term consequences. Family social and economic conditions are critical for early childhood development and exposure to difficult family conditions may have lasting physiological effects. However, there is little research linking early childhood conditions with physiological indicators of stress and system dysregulation in adolescence. In this study, we assess how family social and economic instability that occurred in early childhood (birth to age 5) is associated with immune system dysregulation in adolescence, as indicated by DNA shedding of the Epstein-Barr virus (EBV). We utilize a biomarker of EBV obtained through saliva, a non-invasive method of collecting immune-system biomarkers, in 674 adolescents 11–17 years old. Multivariable regression results indicated that experiences of moving into a new parent/caregiver household or moving in with a grandparent during early childhood was associated with an estimated 100% increase in EBV DNA shedding among prior EBV-infected adolescents. Other measures of early childhood family instability, total number of family structure changes and economic insecurity, were marginally significant. Contemporaneous family conditions were not associated with adolescents' EBV DNA shedding.

1. Introduction

Family contexts are important for children's health and development, particularly during the critical stage of early childhood (Bronfenbrenner, 2001). Young children need supportive, nurturing and stable family environments to support their physical, mental, and emotional health. Unstable family environments have been associated with a number of poor health and developmental outcomes among children (Bzostek, 2008; Lee and McLanahan, 2015; Schmeer, 2011) and adolescents (Brown, 2006; Cavanagh, 2008). Family instability may also increase children's exposure to chronic stress, as is evidenced in early life adversity research (Berens et al., 2017; Elwenspoek et al., 2017b; Shirtcliff et al., 2009; Shonkoff et al., 2012). This research suggests traumatic family conditions, such as abuse or child abandonment, can alter children's physiological systems with long-term effects. However, there is little evidence of whether more common unstable or insecure family environments, such as changes in parental marital status, create levels of stress in children that alter their biological systems in the long-term.

In this study, we consider how family instability in early childhood, including number and type of family structure changes and economic insecurity, may result in long-term dysregulation of the immune system as indicated by viral reactivation of the latent herpes virus, Epstein Barr virus (EBV), in a population-based sample of adolescents. Family instability may cause stress in children due to less consistent and supportive parenting (Beck et al., 2010), higher levels of caregiver stress and depression (Williams and Cheadle, 2016), or children's increased perceptions of family insecurity when changes occur in the family environment (Forman and Davies, 2003). Higher stress during early childhood, in turn, may alter physiological processes, including immune system dysregulation, with potentially lasting effects (Berens et al., 2017).

We test this idea by assessing how family structure changes and economic instability during early childhood are associated EBV reactivation during adolescence. Biosocial research has long supported the idea that one way individuals respond physiologically to stress is through reactivation of latent viruses (Glaser et al., 1991). Reactivation can occur in individuals with prior exposure to viruses, such as herpes simplex virus (HSV), cytomegalovirus (CMV), or EBV. These viruses are often acquired during childhood and remain latent with few symptoms in the body unless a physical or psychosocial threat activates the immune system.

* Corresponding author at: Department of Sociology, The Ohio State University, 238 Townshend Hall, 1885 Neil Ave. Mall, Columbus, Ohio 43210, United States.
E-mail address: schmeer.1@osu.edu (K.K. Schmeer).

https://doi.org/10.1016/j.psyneuen.2018.12.014
Received 14 September 2018; Received in revised form 11 December 2018; Accepted 11 December 2018

Among adults, adverse childhood experiences (child abuse in the first study; children separated from parents and adopted in the other studies) have been associated with elevated EBV antibody titers suggestive of EBV reactivation (Slopen et al., 2013a, 2013b) as well as CMV (Elwenspoek et al., 2017c), and EBV seropositive status (Elwenspoek et al., 2017a). One study of less severe family experiences found that childhood family relationships (less family warmth, less harmony, greater dysfunction, and suboptimal parental bonding), but not parental divorce, were significantly associated with CMV reactivation in adulthood (Janicki-Deverts et al., 2014). Childhood economic conditions, assessed as the number of years of parents owning a home, was not significantly associated with CMV reactivation when accounting for the family interpersonal environment (Janicki-Deverts et al., 2014). It should be noted that, though the study collected childhood family information with references to ages 5, 10 and 15 years, the measures were averaged across the three time periods, thus, potential effects of early childhood conditions were not estimated separately.

There has been less research considering early childhood family environments and physiological dysregulation in adolescence. One study of early childhood adversity (child abandoned/institutionalized) indicated an association between these experiences and physiological dysregulation, as indicated by DNA methylation, in adolescence (Esposito et al., 2016). In another study, adverse childhood experiences of institutionalization or abuse were associated with increased herpes simplex virus (HSV) titers in adolescence (Shirtcliff et al., 2009). However, the selectivity of the sample (international orphans and adolescents with a history of abuse) limit generalizability. A recent Dutch study found no association between total life events score in childhood and multiple measures of viral reactivation at age 16 (Jonker et al., 2017). Although the Jonker et al. study included family separation experiences (the closest to family instability assessed here), these family experiences were included in a total life events score and not analyzed separately.

There is little evidence of whether early childhood economic instability, considered here to be another aspect of family instability, is associated with viral reactivation in adolescence. Studies that have considered viral reactivation and children's economic conditions have focused on concurrent income, with mixed results. For example, studies of children ages 6–19 years indicated no association between quartile household income (Dowd et al., 2013) or income to poverty ratio (Ford and Stowe, 2013) and level of EBV antibodies in youth who had evidence of prior EBV infection (EBV seropositive). However, another study found household poverty to be significantly associated with CMV titers in CMV seropositive children ages 11–16 but not those ages 6–10 years (Dowd et al., 2012). These studies, however, do not capture economic instability nor how early childhood economic conditions may be associated with later viral reactivation in adolescence.

Our study examines Epstein-Barr virus (EBV) reactivation in a population-based sample of adolescents in and around Columbus, Ohio from the Adolescent Health and Development in Context (AHDC) study. Biomarkers of EBV were obtained through saliva samples from a representative subsample of the youth (N = 674) who participated in the Linking Biological and Social Pathways to Adolescent Health and Well-Being sub-study.

EBV is ubiquitous in the U.S.; an estimated 80% of youth have been exposed to EBV by age 19 years (Dowd et al., 2013). Consistent with other latent viruses, EBV remains dormant in the body after primary exposure but the virus can be reactivated due to chronic stress as indicated by increases in replication of the viral DNA and antiviral antibody titers in the blood (Ford and Stowe, 2017, 2013; Stowe et al., 2010). We focused on EBV over other latent viruses due to its high prevalence among adolescents, as we are most interested in reactivation of the virus among those with prior EBV exposure rather than whether adolescents are positive vs negative for prior EBV exposure. Thus, in this study, we utilize the level of salivary EBV DNA shedding to indicate the extent of viral reactivation in the total sample of adolescents as well as those who had evidence of prior EBV infection.

Building on family and biosocial research, we assess how family instability during early childhood may be associated with EBV DNA shedding during adolescence. We assess various types of instability, including total number of family structure changes, type of family structure chance, and family economic instability experienced when the youth were ages 0–5 years. We focus on early childhood family conditions since it is a sensitive developmental period, a time when children are highly dependent on their family environments (Bronfenbrenner, 2001; Shonkoff and Garner, 2012), and because research suggests that adversity that occurs during the early years of childhood may be particularly salient for later EBV reactivation (Slopen et al., 2013a, 2013b). We further consider whether current family disadvantages (during adolescence) account for the effects of early childhood family instability on salivary shedding of EBV DNA.

2. Methods

2.1. Data

The AHDC study and the Linking Biological and Social Pathways to Adolescent Health and Well-Being sub-study emphasize the interplay of social, psychological, and biological processes in shaping youth developmental outcomes such as health risk and pro-social behavior, mental and physical health, and educational outcomes. During 2014–2016 the study collected data on multiple contexts of youth development from a representative sample of households with adolescents ages 11–17 residing in an urbanized area of Franklin County, OH (containing a majority of the city of Columbus and several suburban municipalities) using a prospective cohort design. Informed consent was obtained from all individual participants included in the study. The data were collected over a week, beginning with entrance surveys with the focal youth and his/her caregiver, followed by a 7-day period when smartphones were used to obtain GPS and ecological momentary assessment (EMA) data. The data collection was concluded with an end of the week in-home exit survey, which included the collection of saliva, later analyzed for presence of EBV VCA IgG antibodies and EBV DNA in a representative sub-sample of the youth (N = 674).

2.2. Sample

Our analytical sample consists of those adolescents with EBV and independent variable data. Of the 674 youth who participated in the biomarker study, 667 had EBV data and 630 had both EBV and independent variable data. Given our interest in reactivation of the previously acquired EBV, we assess associations between childhood family instability and salivary EBV DNA shedding for the total sample of adolescents with and without evidence of prior EBV infection (N = 630) as well as for the subsample of youth with evidence of prior EBV infection as indicated by salivary EBV VCA IgG antibody titer > .02 (N = 508). Although the measurement of EBV VCA IgG has been most commonly assessed using serum, researchers have successfully employed salivary ELISA methods in a large sample of children (Crowcroft et al., 1998). In addition, pilot work for the Linking Biological and Social Pathways to Adolescent Health and Well-Being sub-study compared salivary EBV VCA IgG antibodies determined through an adapted ELISA method (Stowe et al., 2014) to those in blood (N = 50 young adults, unpublished). Findings indicated a 0.92 correlation coefficient between the two sources and an antibody titer greater than 0.02 was suggestive of prior EBV infection (Ford and Stowe, 2017). After missing data for family conditions were taken into account, the final analytic sample sizes are 601 (full sample) and 482 (prior EBV infection).

2.3. Measures

Our dependent variable is a linear measure of salivary EBV DNA shedding. Salivary shedding of the virus indicates replication of the DNA of the virus and is suggestive of viral activity, which, among those with a prior EBV infection indicates EBV reactivation. A full description of the analytical procedures for extracting and quantifying the EBV DNA can be found in a previously published study (Ford and Stowe, 2017).

Our independent variables of interest were assessed based on a series of questions asked of the caregiver about the family conditions of the adolescent during childhood, with particular reference to early childhood (birth to age 5). Our measures of family structure instability included whether the child experienced the following: parents' divorce, parent's partner moving into the household; parent's partner moving out of the household; the child moved into a grandparent household; the child went to live with new caregiver; the child moved into a different parent's household; and, the child moved out of a grandparent's household. We constructed a total instability score summing the instability measures, capped at four to reduce the influence of outliers. To test for potential differences in the effects of types of changes in family structure, we assessed each of these changes as dummy variables. We also created a dummy variable of whether the child had ever lived with only one adult in the household during this period, to separate out single-parent from family instability effects.[1]

To answer our second research question, of whether early family economic instability has long-term physiological effects, we assessed caregiver reports of economic loss between birth and age five, including: whether the family experienced bankruptcy, received government assistance, or a parent lost a job. We created a dummy variable indicating exposure to any of these family economic conditions during the early childhood period.

Finally, we assessed family conditions during adolescence, including: caregiver marital status (married, cohabiting, never married, divorced, separated, or widowed), number of children in the household, and total household income during the past year (defined as a categorical variable of below $30,000, $30-60,000, and above $60,000).

Our covariates included in all models were adolescent and caregiver demographic characteristics. We assessed adolescents' age, sex, and race and ethnicity (white, black, Latino, other); and caregiver age, sex and education level (defined as high school degree or less, some college, and college degree or more).

2.4. Statistical analysis

Given the distribution of the dependent variable, where a significant portion of the data was below detection level (censored), we used Tobit regression models in STATA. Youth with missing data were dropped (n = 29 or 4.8% for the total sample of EBV positive and negative adolescents and n = 26 of 5.1% for the EBV positive sample). We logged the linear EBV DNA measure to reduce its skewed distribution. With a logged dependent variable, the regression coefficients can be interpreted as the percent increase in EBV DNA with a one unit change in the independent variable. We assessed each model twice, once with the full sample and then for the subsample of youth who had evidence of prior EBV infection. For all models, we tested gender and race interactions with family instability and found no significant differences. We report significant levels up to $p < 0.1$.

[1] Due to collinearity between total family instability and whether the child lived with only one parent ($r > 0.44$), we excluded the variable "child lived with only one parent" from the models that included total family instability.

3. Results

Table 1 provides descriptive results of EBV shedding and evidence of prior infection for the analytical sample of youth ages 11–17 years (N = 630). EBV DNA shedding was found in 66% of adolescents in the full sample. Table 2 also indicates that in 80% of the adolescents' there was evidence of prior EBV infection. Among those with prior infection, 65% had evidence of current shedding of EBV, which suggests viral reactivation.

Table 2 shows the sample characteristics for the full sample and those with prior EBV infection. The mean total number of family structure changes between birth and age 5 years was less than 1 for both the full sample and the subsample of adolescents with prior EBV infection, reflecting that most had no family instability events during this time (72% of the sample experienced no family structure change). Of the types of family change experienced in the full sample of youth, 6% of youth had a parental divorce, 10% had a parent's new partner move in, 8% had a parental partner move out, 9% moved into a new household (grandparent, new caregiver, or different parent household), and 3% moved out of a grandparent household. In addition to family structure instability, 34% of the youth experienced one or more family economic difficulty during these early years. The descriptive statistics for the subsample of adolescents with prior EBV infection indicate means and standard deviations similar to the full sample (see Table 2).

We next turn to regression results that assess the associations among various family instability measures during early childhood and EBV DNA shedding in adolescence. The results of the multiple regression models are shown in Tables 3 and 4 . Table 3 includes the results from the main models estimating associations between early childhood total family structure instability (Model 1), type of family structure change (Model 2), and economic instability (Model 3). Table 4 presents the results when adolescent family conditions are included as possible explanations for early childhood family instability effects.

Model 1 in Table 3 shows that each additional family structure change is associated with a 24% increase in EBV DNA shedding in adolescence, controlling for adolescent and caregiver demographic characteristics. Among those with prior EBV infection, each family structure change is associated with a 26% increase in EBV DNA shedding.

In Model 2 we separate out the family structure instability measure into various types of changes. The results indicate that moving into a new family household (grandparent, new caregiver or new parent) during early childhood is associated with a 94% increase in EBV DNA shedding during adolescence. We see a similar result for the prior EBV infection subsample, where this family structure change is associated with over 100% increase in EBV DNA shedding. The other types of changes do not appear to be associated with later EBV DNA shedding. In order to rule out collinearity problems, models were tested with each type of change entered separately and the findings were the same; of the types of change assessed, moving into a new family household was the only family instability event associated with later EBV DNA shedding. These changes were estimated net of whether the child ever lived in a one-parent household during early childhood, as well as the adolescent and caregiver demographic variables (see Model 2, Table 3).

Model 3 shows the results of estimating the association between family economic instability and EBV DNA shedding. The results indicate that adolescents who experienced one or more economic difficulty during early childhood have, on average, 51% higher EBV DNA shedding. Again, the association is similar for those who had prior EBV infection, and are net of early childhood single-parent family status and adolescent/caregiver demographic characteristics.

Of the covariates included, only African American race is significant. Across the models, African American youth have approximately 50% higher EBV DNA shedding than white youth.

Table 4 shows the results when we account for current adolescent family characteristics. We included all control variables from Table 3 in

Table 1
Descriptive results of Epstein-Barr Virus (EBV) DNA shedding and prior infection. AHDC Study adolescents with EBV data, ages 11–17 years.

	Total Sample (N = 630)		Sample with Prior EBV Infection (N = 508)	
EBV Variables	Mean or %	Std. Dev. or N	Mean or %	Std. Dev. or N
EBV DNA copy number	14502.24	114836.40	17338.30	127480.80
Presence of EBV DNA Shedding	66%	415	65%	328
Prior EBV infection (EBV VCA IgG antibody titer > .02)	80%	508	100%	508

Note: Mean and standard deviation presented for continuous variables, percent and N presented for categorical variables.

Table 2
Sample characteristics for Adolescent Health and Development in Context (AHDC) Study adolescents with EBV data, ages 11–17 years.

	Full Sample (N = 630)		With prior EBV (N = 508)	
Variable	Mean or %	Std. Dev. or N	Mean or %	Std. Dev. or N
Birth to Age 5 Family Conditions				
Total number of family changes (capped at 4)	0.44	0.87	0.46	0.91
Child's parents divorced	6%	40	6%	31
Parent's partner moved in	10%	62	10%	50
Parent's partner moved out	8%	53	9%	44
Child moved into new family/caregiver household	9%	58	10%	51
Child moved out of grandparent household	3%	19	4%	18
Ever lived with only 1 adult	23%	147	24%	121
Family experienced economic instability	34%	218	38%	192
Control Variables				
Adolescent age	14	1.8	14	1.8
Adolescent male	50%	313	51%	259
Adolescent white	55%	345	51%	261
Adolescent black	37%	231	39%	198
Adolescent Latino	6%	35	6%	32
Adolescent other race	3%	19	3%	17
Caregiver age	46	7.6	46	7.6
Caregiver male	14%	88	14%	69
Caregiver education				
High school or less	17%	111	18%	92
Some college	33%	206	34%	172
Bachelor's degree or more	50%	313	48%	244
Family Conditions in Adolescence[a]				
Caregiver married	62%	372	60%	289
Caregiver cohabiting	8%	46	8%	37
Caregiver single (never married, divorced, separated)	30%	178	32%	152
Caregiver widowed	1%	5	1%	4
Number of children in household	2.1	1.2	2.1	1.2
Household income under $30K	29%	175	31%	149
Household income $30-$60K	22%	134	23%	113
Household income over $60K	49%	292	46%	220

Note: Mean and standard deviation presented for continuous variables, percent and N presented for categorical variables.
[a] Due to missing adolescent family conditions, full sample N = 601, prior EBV infected N = 482.

these models, but do not report them in Table 4 for brevity. As Table 4 shows, none of the current family condition variables are associated with EBV DNA shedding among adolescents.

Model 4 indicates that the coefficients on early childhood family instability for both the full sample and the prior EBV infection sample change little when including adolescent family variables. Similarly, Model 5 shows that the effect of changing caregiver households in early childhood is not altered by controlling for family conditions in adolescence. Though the coefficient on the economic instability in early childhood remained similar in size, it is insignificant in the model with adolescent family conditions included (Model 6, Table 4).

4. Discussion

The goal of this study was to assess the associations of early childhood (birth to age 5) family conditions with immune-system dysregulation in adolescence. We focused on EBV reactivation, as this virus is often acquired during childhood/adolescence and may be reactivated by a physical or psychosocial threat.

We found that 80% of our sample of adolescents had evidence of prior EBV infection. This is consistent with national estimates that 80% of children in the U.S. have been infected by EBV by age 19 as measured in blood antibody titers levels (Dowd et al., 2013). EBV DNA shedding levels indicated viral reactivation in 65% of the AHDC adolescents with prior EBV infection.

Our regression results provide support for the hypothesis that early childhood family instability is associated with increased DNA shedding of EBV in the full sample of adolescents, as well as among the subsample who had evidence of prior EBV infection. Total number of family structure changes during early childhood was marginally associated with increased EBV DNA shedding in adolescence ($p < .1$). When analyzing specific types of family change, youth who had

Table 3
Tobit regression results of the associations between early childhood family instability and EBV DNA shedding in adolescence. Coefficients shown with standard errors in parentheses.

VARIABLES	Model 1		Model 2		Model 3	
	Full sample	Prior EBV Infection	Full sample	Prior EBV Infection	Full sample	Prior EBV Infection
Birth to Age 5 Family Instability						
Total number of family structure changes	0.24*	0.26*				
	(0.13)	(0.15)				
Parents divorced			0.085	−0.042		
			(0.50)	(0.61)		
Parent had a partner move into the household			0.48	0.57		
			(0.47)	(0.54)		
Parent had a partner move out of the household			−0.44	−0.26		
			(0.48)	(0.54)		
Child moved into new family/caregiver household			0.94**	1.04**		
			(0.44)	(0.49)		
Child moved out of grandparent household			0.18	−0.27		
			(0.75)	(0.83)		
Child ever lived with only 1 parent			−0.093	0.010	0.011	0.11
			(0.32)	(0.37)	(0.29)	(0.34)
Family experienced economic insecurity					0.51*	0.49*
					(0.26)	(0.30)
Controls						
Adolescent age	−0.11	−0.12	−0.10	−0.11	−0.10	−0.12
	(0.066)	(0.076)	(0.066)	(0.076)	(0.066)	(0.076)
Adolescent male	−0.25	−0.30	−0.26	−0.31	−0.24	−0.28
	(0.23)	(0.27)	(0.23)	(0.27)	(0.23)	(0.27)
Adolescent black1	0.47*	0.53*	0.48*	0.51	0.48*	0.53*
	(0.27)	(0.31)	(0.28)	(0.32)	(0.28)	(0.32)
Adolescent Latino1	0.32	0.048	0.33	0.058	0.36	0.11
	(0.52)	(0.57)	(0.52)	(0.57)	(0.52)	(0.57)
Adolescent other race1	0.072	0.12	0.13	0.16	0.026	0.047
	(0.68)	(0.75)	(0.68)	(0.75)	(0.68)	(0.75)
Caregiver age	0.013	0.017	0.0088	0.012	0.017	0.022
	(0.017)	(0.019)	(0.017)	(0.020)	(0.017)	(0.020)
Caregiver male	−0.15	−0.16	−0.17	−0.15	−0.17	−0.18
	(0.34)	(0.40)	(0.34)	(0.40)	(0.34)	(0.40)
Caregiver some college2	−0.17	−0.13	−0.13	−0.085	−0.12	−0.081
	(0.34)	(0.39)	(0.34)	(0.39)	(0.34)	(0.39)
Caregiver bachelor's degree or more2	−0.48	−0.26	−0.41	−0.18	−0.35	−0.12
	(0.34)	(0.39)	(0.34)	(0.39)	(0.34)	(0.40)
N	630	508	630	508	630	508
Log likelihood	−1556	−1274	−1553	−1273	−1555	−1274

Robust SE in parentheses. ** $p < 0.05$, * $p < 0.1$. [a]Ref.: adolescent white; [b]Ref: caregiver high school degree or less.

experienced a move into a new caregiver/parent households during early childhood had higher levels of EBV DNA shedding in the full sample and the subsample with prior infection ($p < .05$). Other types of family structure changes were not associated with EBV shedding in adolescence. There was some evidence that family economic difficulty during early childhood was also marginally associated with increased EBV shedding in both samples ($p < .1$). When adding adolescent (current) family structure and economic conditions to the model, we saw little evidence that current family conditions affected adolescent EBV reactivation. This finding is consistent with other research indicating no association between concurrent family structure, household income or income to poverty ratio and EBV antibody titers in a national sample of children ages 6–19 years (Dowd et al., 2013; Ford and Stowe, 2013).

Our findings provide further input into the question of whether early childhood adverse conditions become embedded in individuals' immune systems. Research to date has found mixed evidence. For example, total number of childhood adverse life events assessed at ages 12, 14 and 16 had no association with multiple measures of viral reactivation (HSV, CMV and EBV) at age 16 in a Dutch sample (Jonker et al., 2017). Their null findings may be, in part, due to the measurement of adverse events in middle and later childhood. Similarly, a U.S. study of adults (N = 140) found no associations between childhood divorce or economic insecurity (measured as housing tenure) and later CMV reactivation, though the study also did not distinguish early childhood events (Janicki-Deverts et al., 2014). Our null findings for divorce is consistent with those findings (Janicki-Deverts et al., 2014). Interpersonal relationships in the family were associated with CMV reactivation (Janicki-Deverts et al., 2014), which, along with our result of a significant effect of the types of change suggestive of caregiver/parent and household instability, may indicate that family instability that alters interpersonal relationships may be particularly stressful for children. However, this hypothesis requires further study.

Other research suggests that timing may be important. Adverse family experiences of separation from parents (and institutionalization) and abuse occurring *early* in childhood in particular, have been associated with salivary herpes simplex virus (HSV) titers in adolescence (Shirtcliff et al., 2009) and EBV reactivation in young adults (Slopen et al., 2013a, 2013b). Our finding that some aspects of family instability between birth and age 5 (but not adolescent family conditions) were associated with adolescent EBV DNA shedding is consistent with this idea that early childhood events may be particularly important for long-term immune system dysregulation.

Regarding economic instability, most U.S. studies do not find contemporary low income to be associated with EBV seroprevalence or titers in children or adolescents (Condon et al., 2014; Dowd et al., 2013; Ford and Stowe, 2013). Studies focused on economic insecurity rather than income level per say, are mixed. The Janicki-Deverts et al. (2014) study found no association of childhood housing tenure with CMV reactivation in adulthood. Another study, in the UK, did find that lower

Table 4
Tobit regression results of the associations between early childhood family instability, contemporary family conditions and EBV DNA shedding in adolescence. Coefficients shown with standard errors in parentheses.

	Model 4		Model 5		Model 6	
	Full sample	Prior EBV infection	Full sample	Prior EBV infection	Full sample	Prior EBV infection
Birth to Age 5 Family Instability						
Total number of family structure changes	0.24*	0.27*				
	(0.14)	(0.15)				
Parents divorced			0.34	0.25		
			(0.52)	(0.62)		
Parent had a partner move into the household			0.53	0.63		
			(0.49)	(0.56)		
Parent had a partner move out of the household			−0.69	−0.53		
			(0.49)	(0.55)		
Child moved into new family/caregiver household			0.98**	1.05**		
			(0.45)	(0.50)		
Child moved out of grandparent household			−0.056	−0.25		
			(0.78)	(0.84)		
Child lived with only 1 parent			−0.001	0.068	0.13	0.24
			(0.35)	(0.40)	(0.32)	(0.36)
Family experienced economic insecurity					0.44	0.41
					(0.27)	(0.31)
Family Conditions in Adolescence						
Caregiver cohabiting[a]	0.28	0.44	0.32	0.45	0.29	0.40
	(0.48)	(0.55)	(0.48)	(0.56)	(0.48)	(0.56)
Caregiver single[a]	−0.34	−0.42	−0.37	−0.44	−0.33	−0.45
	(0.31)	(0.35)	(0.32)	(0.36)	(0.32)	(0.36)
Caregiver widowed[a]	−1.40	−1.83	−1.66	−2.03	−1.34	−1.88
	(1.31)	(1.54)	(1.32)	(1.54)	(1.31)	(1.54)
Number of children in household	0.17	0.17	0.18	0.18	0.18	0.17
	(0.11)	(0.13)	(0.11)	(0.12)	(0.11)	(0.13)
Household income under $30,000[b]	0.32	0.29	0.31	0.25	0.27	0.27
	(0.40)	(0.46)	(0.40)	(0.45)	(0.40)	(0.46)
Household income $30,000-$60,000[b]	0.041	0.20	0.025	0.16	−0.0080	0.13
	(0.36)	(0.41)	(0.36)	(0.41)	(0.36)	(0.41)
Observations	601	482	601	482	601	482
Log Likelihood	−1480	−1206	−1478	−1204	−1480	−1207

Control Variables: All controls from Table 3 included but not reported for brevity. Robust SE in parentheses. ** $p < 0.05$, * $p < 0.1$.
[a] Ref: Caregiver married.
[b] Ref: Household annual income over $60,000.

socioeconomic position and overcrowding at 9 months of age were associated with EBV infections in 3-year old children (Gares et al., 2017). This provides further support for early childhood conditions being particularly influential. However, given the lack of sufficient research on economic insecurity (beyond family socioeconomic status), future research should be further explore economic insecurity as an aspect of early childhood that has the potential to affect long-term changes in children's immune systems.

Our finding that African American adolescents had higher EBV DNA shedding than white adolescents is consistent with findings from national-level studies of adolescents ages 6–18 using blood EBV titers (Dowd et al., 2014, 2013; Ford and Stowe, 2013).

We recognize several limitations to this study. First, using retrospective data on family conditions that occurred possibly 14 years before the survey reports (for the average adolescent where the family condition occurred at birth) is not ideal. It may be that more stressful family changes during this early childhood period are more likely to be remembered by caregivers and have enduring effects. However, we cannot rule out that other types of family instability might have contributed to these associations and went unreported or were inaccurately recalled. Related to this issue is the small number of cases in some of the family change categories, suggesting that lack of significance of some of these family changes may be due to insufficient sample size.

Second, we have not assessed the mechanisms through which early childhood family conditions might affect adolescent viral reactivation. Although we considered the role of adolescent family structure and household income, other types of changes in adolescents' lives between early childhood and adolescence may be what is driving our evidence of long-term effects of early childhood environments on adolescent EBV shedding.

Third, we did not directly assessed stress during childhood. Instead, we evaluated potentially stressful family circumstances by their associations with later immune system dysregulation. Thus, we did not assess early childhood stress, but rather how early environments may shape later physiological dysregulation.

Finally, despite the growing evidence that childhood conditions may result in later viral reactivation, we have relatively little prior evidence of family instability and economic conditions during childhood and their associations with later viral activation. Given the lack of prior research in this area, the null findings of our study for many of the family structure changes, and the potential for the $p < .1$ findings to be due to chance, further studies need to replicate and expand on these findings with other samples.

Nonetheless, the findings here are consistent with broader research indicating the importance of early childhood for conditioning the stress response and immune system dysregulation in later stages of the life course (Cohen et al., 2004; Elwenspoek et al., 2017b; McEwen and McEwen, 2017). Family structure changes during early childhood, while not necessarily traumatic, may produce physiological changes that result in later reactivation of EBV, and possibly other viruses, during adolescence. We provide this initial evidence in a diverse and population-based sample of youth in an urban/suburban area. More research is needed to further delineate the implications of family instability and other stressors occurring in early childhood and to

understand the long-term consequences of EBV reactivation (and other indicators of immune-system dysregulation) during childhood and adolescence.

Declarations of interest

None.

Acknowledgements

This research was supported by the National Institute on Drug Abuse (R01DA032371 awarded to Browning and R21DA034960 awarded to Ford), the Eunice Kennedy Shriver National Institute of Child Health and Human Development (K01HD077063 awarded to Schmeer; P2CHD058484 awarded to the Ohio State University Institute for Population Research), and the William T. Grant Foundation.

References

Beck, A.N., Cooper, C.E., McLanahan, S., Brooks-Gunn, J., 2010. Partnership transitions and maternal parenting. J. Marriage Fam. 72, 219–233. https://doi.org/10.1111/j.1741-3737.2010.00695.x.

Berens, A.E., Jensen, S.K.G., Nelson, C.A., 2017. Biological embedding of childhood adversity: from physiological mechanisms to clinical implications. BMC Med. 15, 135. https://doi.org/10.1186/s12916-017-0895-4.

Bronfenbrenner, U., 2001. The bioecological theory of human development. Int. Encycl. Soc. Behav. Sci.

Brown, S.L., 2006. Family structure transitions and adolescent well-being. Demography 43, 447–461. https://doi.org/10.1353/dem.2006.0021.

Bzostek, S.H., 2008. Social fathers and child well-being. J. Marriage Fam. 70, 950–961.

Cavanagh, S.E., 2008. Family structure history and adolescent adjustment. J. Fam. Issues 29, 944–980. https://doi.org/10.1177/0192513X07311232.

Cohen, S., Doyle, W.J., Turner, R.B., Alper, C.M., Skoner, D.P., 2004. Childhood socioeconomic status and host resistance to infectious illness in adulthood. Psychosom. Med. 66, 553–558. https://doi.org/10.1097/01.psy.0000126200.05189.d3.

Condon, L.M., Cederberg, L.E., Rabinovitch, M.D., Liebo, R.V., Go, J.C., Delaney, A.S., Schmeling, D.O., Thomas, W., Balfour, H.H., 2014. Age-specific prevalence of Epstein-Barr virus infection among Minnesota children: effects of race/ethnicity and family environment. Clin. Infect. Dis. 59, 501–508. https://doi.org/10.1093/cid/ciu342.

Dowd, J.B., Palermo, T.M., Aiello, A.E., 2012. Family poverty Is associated with cytomegalovirus antibody titers in US children. Health Psychol. 31, 5–10. https://doi.org/10.1037/a0025337.

Dowd, J.B., Palermo, T., Brite, J., McDade, T.W., Aiello, A., 2013. Seroprevalence of epstein-Barr virus infection in US children ages 6-19, 2003-2010. PLoS One 8, e64921. https://doi.org/10.1371/journal.pone.0064921.

Dowd, J.B., Palermo, T., Chyu, L., Adam, E., McDade, T.W., 2014. Race/ethnic and socioeconomic differences in stress and immune function in the national longitudinal study of adolescent health. Soc. Sci. Med. 115, 49–55. https://doi.org/10.1016/j.socscimed.2014.06.011.

Elwenspoek, M.M.C., Hengesch, X., Leenen, F.A.D., Schritz, A., Sias, K., Schaan, V.K., Meriaux, S.B., Schmitz, S., Bonnemberger, F., Schaechinger, H., Voegele, C., Turner, J.D., Muller, C.P., 2017a. Proinflammatory T cell status associated with early life adversity. J. Immunol. 199, 4046–4055. https://doi.org/10.4049/jimmunol.1701082.

Elwenspoek, M.M.C., Kuehn, A., Muller, C.P., Turner, J.D., 2017b. The effects of early life adversity on the immune system. Psychoneuroendocrinology 82, 140–154. https://doi.org/10.1016/j.psyneuen.2017.05.012.

Elwenspoek, M.M.C., Sias, K., Hengesch, X., Schaan, V.K., Leenen, F.A.D., Adams, P., Meriaux, S.B., Schmitz, S., Bonnemberger, F., Ewen, A., Schaechinger, H., Voegele, C., Muller, C.P., Turner, J.D., 2017c. T cell immunosenescence after early life adversity: association with cytomegalovirus infection. Front. Immunol. 8, 1263. https://doi.org/10.3389/fimmu.2017.01263.

Esposito, E.A., Jones, M.J., Doom, J.R., MacIsaac, J.L., Gunnar, M.R., Kobor, M.S., 2016. Differential DNA methylation in peripheral blood mononuclear cells in adolescents exposed to significant early but not later childhood adversity. Dev. Psychopathol. 28, 1385–1399. https://doi.org/10.1017/S0954579416000055.

Ford, J.L., Stowe, R.P., 2013. Racial-ethnic differences in Epstein-Barr virus antibody titers among US children and adolescents. Ann. Epidemiol. 23, 275–280. https://doi.org/10.1016/j.annepidem.2013.02.008.

Ford, J.L., Stowe, R.P., 2017. Depressive symptoms are associated with salivary shedding of Epstein-Barr virus in female adolescents: the role of sex differences. Psychoneuroendocrinology 86, 128–133. https://doi.org/10.1016/j.psyneuen.2017.09.009.

Forman, E.M., Davies, P.T., 2003. Family instability and young adolescent maladjustment: the mediating effects of parenting quality and adolescent appraisals of family security. J. Clin. Child Adolesc. Psychol. 32, 94–105. https://doi.org/10.1207/S15374424JCCP3201_09.

Gares, V., Panico, L., Castagne, R., Delpierre, C., Kelly-Irving, M., 2017. The role of the early social environment on epstein Barr virus infection: a prospective observational design using the millennium cohort study. Epidemiol. Infect. 145, 3405–3412. https://doi.org/10.1017/S0950268817002515.

Glaser, R., Pearson, G., Jones, J., Hillhouse, J., Kennedy, S., Mao, H., Kiecolt-Glaser, J., 1991. Stress-related activation of Epstein-Barr virus. Brain Behav. Immun. 5, 219–232.

Janicki-Deverts, D., Cohen, S., Doyle, W.J., Marsland, A.L., Bosch, J., 2014. Childhood environments and cytomegalovirus serostatus and reactivation in adults. Brain Behav. Immun. 40, 174–181. https://doi.org/10.1016/j.bbi.2014.03.010.

Jonker, I., Rosmalen, J.G.M., Schoevers, R.A., 2017. Childhood life events, immune activation and the development of mood and anxiety disorders: the TRAILS study. Transl. Psychiatr. 7, e1112. https://doi.org/10.1038/tp.2017.62.

Lee, D., McLanahan, S., 2015. Family structure transitions and child development: instability, selection, and population heterogeneity. Am. Soc. Rev. 80, 738–763. https://doi.org/10.1177/0003122415592129.

McEwen, C.A., McEwen, B.S., 2017. Social structure, adversity, toxic stress, and intergenerational poverty: an early childhood model. Ann. Rev. Soc. 43.

Schmeer, K.K., 2011. The child health disadvantage of parental cohabitation. J. Marriage Fam. 73, 181–193. https://doi.org/10.1111/j.1741-3737.2010.00797.x.

Shirtcliff, E.A., Coe, C.L., Pollak, S.D., 2009. Early childhood stress is associated with elevated antibody levels to herpes simplex virus type 1. Proc. Natl. Acad. Sci. U. S. A. 106, 2963–2967. https://doi.org/10.1073/pnas.0806660106.

Shonkoff, J.P., Garner, A.S., 2012. The lifelong effects of early childhood adversity and toxic stress. Pediatrics 129, E232–E246. https://doi.org/10.1542/peds.2011-2663.

Shonkoff, J.P., Garner, A.S., Comm Psychosocial Aspects Child, F, Comm Early Childhood Adoption, D, Sect Dev Behav, P, 2012. The lifelong effects of early childhood adversity and toxic stress. Pediatrics 129, E232–E246. https://doi.org/10.1542/peds.2011-2663.

Slopen, N., McLaughlin, K.A., Dunn, E.C., Koenen, K.C., 2013a. Childhood adversity and cell-mediated immunity in young adulthood: does type and timing matter? Brain Behav. Immun. 28, 63–71. https://doi.org/10.1016/j.bbi.2012.10.018.

Slopen, N., McLaughlin, K.A., Dunn, E.C., Koenen, K.C., 2013b. Reply to letter Re: childhood adversity and cell-mediated immunity in young adulthood. Brain Behav. Immun. 34, 177–179. https://doi.org/10.1016/j.bbi.2013.08.002.

Stowe, R.P., Peek, M.K., Perez, N.A., Yetman, D.L., Cutchin, M.P., Goodwin, J.S., 2010. Herpesvirus reactivation and socioeconomic position: a community-based study. J. Epidemiol. Community Health 64, 666–671. https://doi.org/10.1136/jech.2008.078808.

Williams, D.T., Cheadle, J.E., 2016. Economic hardship, parents' depression, and relationship distress among couples with young children. Soc. Ment. Health 6, 73–89. https://doi.org/10.1177/2156869315616258.