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Risk as a First Derivative: Using Intensive Repeated Measures and Molecular Approaches to Studying Families

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Risk as a first derivative: Using intensive repeated measures and  
molecular approaches to studying families

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## Abstract

This chapter highlights developments in using intensive repeated measures of family environments through daily diaries to shed light on neuroendocrine and immune processes linking family functioning and health. I review data from the UCLA Families and Health Study, which included a 2-month daily diary, 8 days of diurnal cortisol sampling, and a blood draw to obtain DNA and RNA in immune cells. Frequent sampling of family conflict and warmth over weeks to months allowed for examining how changes over time were related to changes in multiple indicators of hypothalamic-pituitary-adrenal axis function. Multiple daily measures of child-reported parent-parent and parent-child interactions, as well as negative and positive mood, allowed for computing individual differences in children's mood "reactivity" to parent-parent and parent-child interactions over two months. Greater child negative mood reactivity to parent-parent conflict was related to shorter leukocyte (immune cell) telomere length. Finally, for both parents and children, greater family conflict (combined across parent- and child-reports) was related to greater expression of genes regulated by nuclear factor - kappa B, a transcription factor that plays a key role in promoting inflammation in immune cells that are first responders to infection and injury. Using intensive repeated measures can shed light on the kinds of outcomes that may be responsive to family-based interventions, and potential treatment targets (e.g., emotional reactivity to family conflict). Combined with cutting-edge biomarker assessment, such approaches to exposures may also help identify markers of risk and resilience with high translational potential.

Keywords: family conflict, middle childhood, daily diaries, emotional reactivity, HPA axis, cortisol, telomeres, inflammation, gene expression

Risk as a first derivative: Using intensive repeated measures and molecular approaches to studying families (Total length should be approximately 24 pages) (6000 words)

### **Introduction**

In calculus, the first derivative of a point is not its position in space, but its propensity to change its position; not where an object *is*, but how it *moves* in space and time (Mukherjee, 2015, p. 355).

The ups and downs of family life act through biobehavioral mechanisms to influence physical health (Miller & Chen, 2010; Repetti, Robles, & Reynolds, 2011). This chapter describes how multiple repeated measures of those “ups and downs,” when combined with measures of biologically plausible mechanisms like the hypothalamic-pituitary-adrenal axis and immune system function (Miller, Chen, & Cole, 2009), can yield insights into how individuals respond to challenges in the family and in the body, which can ultimately inform understanding both risk and resilience.

The quote that opened this chapter refers to a “first derivative” in mathematics, which is the instantaneous slope of a function, reflecting the change in Y per unit of X. This is illustrated in Figure 1 with a scatterplot of hypothetical data, with variable X on the x-axis and variable Y on the y-axis, and a best-fit line running through the points. With intensive repeated measures of parent-child conflict as the Y, over X repeated days, researchers can derive between-child or family differences in change in parent-child conflict, often described as slopes of change over time. This traditional way of thinking about intensive repeated measures can be extended to measuring two variables over time, such as negative mood as the Y, and parent-child conflict as X; importantly, each point on the

scatterplot in Figure 1 would then represent a given day in the study. The best-fit line then represents the person-level correlation between X and Y during a particular time period, which I describe as within-person associations. In these examples both types of derivatives, change over time and within-person associations, can be viewed as “potential” for change over time or over within-person changes in parent-child conflict. In addition to using intensive repeated psychosocial measures to evaluate “potential” to respond, this paper also describes ways to possibly infer how the immune system might respond to threats. Thus, the purpose of this chapter is to highlight the benefit of using intensive repeated measures approaches to studying families to conceptualize risk for poor health. Rather than providing “snapshots” of family functioning, maximizing the use of intensive repeated measures may provide more precise estimates of risk and resilience in families. The chapter also describes the value of genomic approaches to illuminate common immune pathways that may be markers of risk or resilience.

## **Literature Review**

### **Foundational points regarding research design**

Much of this volume focuses on biological measures as outcomes and potential risk/resilience markers. Any time biological measures are incorporated in biobehavioral research two key points should be kept in mind. First, while all biological measures are “objective,” some are more relevant for health than others; some are surrogate endpoints that based on empirical evidence can substitute for “hard” clinical endpoints in clinical trials (Biomarker Definitions Working Group, 2001). For instance, across numerous studies, levels of so-called “bad” cholesterol predict cardiovascular disease onset as well as progression of disease, including cardiac events like having a second heart attack (Vasan, 2006). Thus, many clinical

trials have examined “bad” cholesterol as a primary outcome. Other markers indicate potential explanations of how psychosocial factors can influence health, but are not specifically diagnostic or prognostic for specific health outcomes. Many of those *biological mediators* that reflect allostatic processes (responses to environmental demands) that may be common across health conditions (Robles & Carroll, 2011), and the vast majority of biomarkers described in this volume and all the markers in this chapter refer to biological mediators.

Second, biomarkers that reflect allostatic processes are often described as potential measures of stress exposure (Harkness & Monroe, 2016), or resilience to such exposure. However, allostatic processes like the hypothalamic-pituitary-adrenal axis and immune function respond to a variety of stimuli, ranging from stressful life events to infections. Accordingly, inferring stress exposures from biological responses, or more specifically, exposures to conflict or support in the family environment from biological responses, is not sound practice (Cacioppo & Tassinary, 1990; Harkness & Monroe, 2016). Put another way, if low levels of inflammation are viewed as resilience to stressful circumstances, that inference cannot be made without knowing whether such individuals were actually exposed to such circumstances. The ability to infer “psychological significance from physiological signals” (Cacioppo & Tassinary, 1990) is an aspirational goal, but peripheral biological markers that reflect allostatic processes are the output of multiple layers of processing at multiple levels of the brain (Ulrich-Lai & Herman, 2009), and because of that, simply knowing that an individual has an average of 3.19 µg/dl of cortisol in the evening cannot tell us about the type, duration, or course of stressful life events and circumstances the person is exposed to. Accordingly, both stress exposures and allostatic processes must be measured in

the same study (Harkness & Monroe, 2016). In the context of family environments, understanding both stressful and supportive aspects of the family become critical for inferring how families impact health through direct biological influences.

Everyday family circumstances are primarily assessed using self-report, in part because of the difficulty inherent in conducting systematic behavioral observations in the home (Repetti, Reynolds, & Sears, 2015). That said, several means exist for objective systematic observations of family experiences, such as the Electronically Activated Recorder (Slatcher & Robles, 2012), and in-person observations (reviewed in Repetti et al., 2015). This paper focuses on self-report measures because of their ease of use relative to objective methods, as well as their value in assessing social-cognitive and affective responses to events in the home. Using intensive repeated measures of the family environment, relative to single, retrospective and infrequently administered self-report measures (i.e., “how often in the last month did you argue with your spouse?”), offers several advantages: reduced recall and retrospection bias, increased relevance for interventions, and greater ability to use sophisticated quantitative approaches (see Repetti et al., 2015 for a comprehensive review). To illustrate these advantages, I describe two applications of intensive repeated measures from the UCLA Families and Health study, a prospective daily diary study of 47 families with children between 8 – 13 years of age (Robles, Reynolds, Repetti, & Chung, 2013). Both applications involve moving beyond static snapshots of exposure to “first derivative” conceptualizations of how family environments change over time, and how children respond to such changes.

### **UCLA Families and Health Study participants and procedures**



Briefly, families with children 8 - 13 years old were recruited in the Los Angeles area from 2009 - 2012 to participate in a study during the fall and winter months, which corresponds to the cold and flu season in Los Angeles county (Robles et al., in press). Children had to be free of medical conditions that could confound endocrine and immune measures, including chronic lung conditions, endocrine and metabolic disorders, immunodeficiency, and cardiovascular disease. Of 60 families that were eligible to participate, 47 were enrolled in the study, and include 47 mothers, 39 fathers, 47 target children (28 female), and 12 siblings (7 female) who were in the target age range (for more details, see Robles et al., in press). Families were majority-minority (55% of parents were people of color), average parent age was mid-40's, just over 50% of the sample had a 4-year college degree or higher, and 80% of fathers and 45% of mothers worked full-time.

A timeline of the study procedures is shown in Figure 2. Participating parents and target children completed the UCLA Life Stress Interview, which assesses stressful life events and circumstances, as well as questionnaire measures of the family environment during a home visit. During a subsequent visit, parents and children were trained on how to complete online daily diary questionnaires and provide saliva samples. The following Saturday, participants completed the 8-week daily diary portion of the study (More details regarding compliance and measurement issues can be found in Reynolds, Robles, & Repetti, 2016). During weeks 3 and 6, four saliva samples were collected on four consecutive days (Saturday - Tuesday) for salivary cortisol assays (Kuhlman, Repetti, Reynolds, & Robles, 2016). At the end of the study, parents and children that opted to provide a blood sample did so, and children completed a brief laboratory stressor in our laboratory.

## **“First derivative” approaches to characterizing exposures**

**Change in family functioning over time and HPA axis function.** The unique saliva sampling protocol in the study allowed for examining how changes in the family environment may be related to changes in HPA axis function and regulation over short timeframes (weeks). Identifying the timeframe over which family environments and changes in those environments are related to HPA axis function has direct implications for psychosocial interventions that examine HPA axis measures as outcomes (Slopen, McLaughlin, & Shonkoff, 2014). For instance, if the HPA axis is insensitive to week-to-week or month-to-month changes in the family environment, such as improving parent-child relationships during a family intervention, then pre- to post-measures of cortisol may not be an appropriate outcome; more long-term post-intervention follow-up may be needed to observe biologically plausible and relevant changes.

On the other hand, if the HPA axis is sensitive to short-term fluctuations in the family environment, measuring cortisol changes over shorter intervals, like pre- to post-intervention, may provide a relevant window for understanding the role of biological processes in mediating intervention effects. The existing literature to date has examined day-to-day changes in cortisol as a function of family environments over a week or so (e.g., Lippold, McHale, Davis, Almeida, & King, 2016), but not over longer timeframes. Accordingly, our study design allowed for examining changes in HPA-axis regulation from Week 3 to Week 6, as a function of changes in child-reported parent-child conflict from Week 1 to Week 2, and from Week 4 to Week 6. We hypothesized that increasing parent-child conflict over time would be related to upregulated HPA axis activity, reflected in larger cortisol awakening responses,

higher daily cortisol output (area under the curve ground, AUCg), flatter diurnal cortisol slopes, and higher bedtime cortisol (Kuhlman et al., 2016).

Daily parent-child conflict was assessed by asking children six items from the Youth Everyday Social Interaction and Mood scales (Lehman & Repetti, 2007; Repetti, 1996), including “My mom/dad got mad at me today,” “My mom/dad punished me today,” and “I was angry at mom/dad today.” Week 1 to Week 2 change was characterized by computing individual slopes of change in parent-child conflict reports from day 1 – 16, and Week 4 – 6 change was characterized by computing slopes of change from day 22 – 37 (Kuhlman et al., 2016). Notably, conflict was an occasional event, reported on 33% of study days. On average, conflict levels decreased over the course of the study from 1.20 ( $SD = 0.31$ ) on a 1 (not at all) to 3 (a lot) scale after averaging across the six items, by a rate of -0.005 units per day. While conflict was occasional, 48% of children showed an increase in parent-child conflict from Week 1 – Week 2, and 60% showed an increase from Week 4 – Week 6.

The primary finding was that increased parent-child conflict from Week 4 – Week 6 was related to increases in daily cortisol output and a flattened diurnal cortisol slope from Week 3 to Week 6, all of which were likely accounted for by increases in bedtime cortisol. Children who showed a 1  $SD$  increase in parent-child conflict showed an increase in bedtime cortisol from Week 3 to Week 6, whereas children who showed a decrease in parent-child conflict showed no change in bedtime cortisol. In additional analyses, we found that longer periods of daily diary sampling (16 days vs. 14, 9, or 3 days) were needed to observe associations between change in parent-child conflict and daily cortisol output and bedtime cortisol, whereas shorter periods of sampling (particular 3 days) were needed to

observe associations between changes in parent-child conflict and diurnal cortisol slope. Overall, these findings suggested that certain parameters of HPA axis function (daily output and bedtime cortisol) were sensitive to changes in the family environment over several weeks. Our findings also imply that changes in bedtime cortisol levels, when the HPA axis is expected to be the least “active,” may be the most response to variations in the family environment, including variations that may be introduced through family-based interventions. The intensive repeated measures of parent-child conflict, combined with the unique two-stage sampling of cortisol on Weeks 3 and 6 allowed for testing such questions.

**Using intensive repeated measures to model mood “reactivity” to marital and parent-child conflict and warmth and leukocyte telomere length.** Intensive repeated measures provide multiple occasions of measurement over time, and when *multiple measures* over multiple occasions are obtained, can provide additional “first derivative” insights into how children respond or react to their environments. Such an approach was initially pioneered by research on adults from the National Study of Daily Experiences, in which stressful event exposures were assessed daily for 8 days, along with daily measures of negative mood. The study team computed the difference in negative mood between the day with the fewest exposures and the day with the most exposures, and used that difference as an index of participants’ “emotional reactivity” to daily stressful events (e.g., Charles, Piazza, Mogle, Sliwinski, & Almeida, 2013). With 56 days in the Families and Health study, we were able to compute correlations between exposures (conflict and warmth in the family) and negative mood within each child over two months of sampling. Put another way, we could compute slopes of the association between exposures and negative mood for individual children in our study.

Emotional reactivity to the family environment, particularly interparental conflict, is implicated as a key mechanism linking stressful family environments to children's emotional and physical well-being (Repetti et al., 2011; Troxel & Matthews, 2004). Emotional security theory posits that children's repeated exposure to interparental conflict over time contributes to emotional insecurity, and subsequent difficulties with regulating emotions that manifest in greater affective reactivity, behavior problems (i.e., externalizing symptoms), and social-cognitive dysregulation such as persistent distrust of others (Davies & Cummings, 1994; Davies & Martin, 2013). Greater emotional reactivity is then implicated as a contributor to risky health behaviors (e.g., substance use), dysregulated allostatic processes, with eventual deleterious effects on health and well-being (Troxel & Matthews, 2004).

Using data from the Families and Health study, we examined links between emotional reactivity to the family environment and a potential indicator of dysregulated allostatic processes: accelerated immune cell aging (Robles et al., 2016). Conceptually, cells of the immune system have a finite capacity to divide (e.g., 50 - 70 cell divisions), and markers of immune cell aging provide a window into how impacted immune cells are by infectious threats, normal cellular damage, and perhaps even exposure to stressful events (Puterman & Epel, 2012). Moreover, "older" immune cells may actually contribute to poorer health by promoting elevated inflammation (Campisi & di Fagagna, 2007). One marker of immune cell aging that has gained significant interest over the past two decades is telomere length; telomeres are nucleotide structures that cap the ends of chromosomes in a manner analogous to how plastic "aglets" at the end of shoelaces prevent shoelaces from fraying (Blackburn, 2000). Normal cell division results in the loss of

genetic material at the end of chromosomes, and the genetic material in telomeres is sacrificed to prevent loss of genetic material that we need for survival. While telomeres can be lengthened or shortened, the general view is that shorter telomeres indicate older cells.

Emotional reactivity is more broadly implicated in models linking psychological stress to premature cellular aging, and shorter immune cell telomere length in older adults is associated with poorer health outcomes (Puterman & Epel, 2012). Such observations have led developmental researchers to explore whether stressful life event exposures are systematically related to cellular aging in children (Shalev, 2012). In several studies examining telomere length in cells that line the inside of the cheek (buccal cells), exposure to major life events involving loss, as well as longitudinal changes in parent-reported exposure to violence from ages 5 – 15 were related to shorter buccal cell telomere length (Drury et al., 2014; Shalev et al., 2013). Both aforementioned studies examined cumulative exposure to major life events, and the Families and Health data provided an opportunity to extend this line of inquiry to mild-to-moderate daily family stress exposures, emotional reactivity to those stressors, and telomere length in immune cells.

With two months of daily data on child-reported parent-child conflict (described above) we could compute an individual's average parent-child conflict exposure over a two-month period. Additionally, we had measures of child-reported parent-child warmth (e.g., "My mom/dad and I got along well today"), marital conflict (e.g., "My Mom and Dad argued today"), and marital affection ("My Mom and Dad kissed or hugged today"). Thus, we could assess exposure to conflict and warmth in the family environment. With daily reports of negative and positive mood (example items are: sad, on edge; relaxed, happy, respectively), we computed

associations between exposures (conflict, warmth) and mood for each child across two months by generating empirical Bayes' estimates in multilevel modeling (Cohen, Doyle, & Skoner, 1999; Mohr et al., 2013). These "reactivity scores," represented conceptually on the left side of Figure 3 reflected the association between an exposure measure (e.g., parent-child warmth) and mood (positive mood). We then tested two different models of how family environments might impact telomere length, where the dependent variable of interest was children's immune cell telomere length at the end of the study: an "exposure" model that used average levels of parent-child and marital conflict and warmth as predictors, and a "reactivity" model that used reactivity scores as predictors. A conceptual description of the analytic approach is shown at the right portion of Figure 3, and the primary finding is shown in bold. Specifically, children who tended to report greater negative affect on days that they also reported greater marital conflict showed lower immune cell telomere length, even after controlling for average levels of conflict and warmth. Our findings are consistent with models of biological embedding of childhood adversity (Miller, Chen, & Parker, 2011), as well as models linking family environments to emotional reactivity and health (Repetti et al., 2011; Troxel & Matthews, 2004), and were made possible by intensive repeated measures over multiple days.

In this "first derivative" application, the inference is that intensive repeated measures provide a metric of a child's potential to respond to marital conflict with negative mood. However, our daily diary approach places some key boundary conditions on that metric. A stronger association between marital conflict and negative mood in some children compared to others may also indicate that some children are more likely to recall marital conflict when they are in a negative mood

(mood-congruent recall), or that certain children that report more daily negative affect may promote interparental conflict (Kihlstrom, Eich, Sandbrand, & Tobias, 2000; Schermerhorn, Chow, & Cummings, 2010). Disentangling “reactivity” from mood-congruent recall or stress generation requires frequent sampling used in ecological momentary assessment approaches, and is a key direction for future work.

### **Using genomic approaches to conceptualize the potential to respond to infectious threats.**

Thus far, “first derivative” has been represented by within-person changes in a variable over time, or within-person changes in one variable (mood) as a function of within-person changes in another psychological variable (conflict and warmth). Both approaches were made possible through intensive repeated measures over time, which could be extended to allostatic biological processes, such as cardiovascular, neuroendocrine, or immune function. However, intensive repeated measures of those processes burdensome, highly invasive, and intrusive. Thus, biobehavioral researchers have been exploring “snapshot” measures that can provide more than just a snapshot – that is, single-occasion measures that may provide a window into first derivatives of biological functioning. Importantly, while those snapshot measures can provide inferences about how biological systems normally function, they are not used to infer anything about psychological states or stress exposures. One area of significant interest in biobehavioral research, with applications across a number of psychological phenomena and health problems that includes assessing “potential” to respond, is the body’s rapid immune response to infection and injury: inflammation (Kiecolt-Glaser, McGuire, Robles, & Glaser, 2002).



The immune system's primary job is to recognize threats to the organism like viruses and bacteria, and respond to those threats by eliminating them (for accessible reviews, see Repetti et al., 2011; Robles, Glaser, & Kiecolt-Glaser, 2005). The initial immune response to threat, whether that be bacteria invading through a cut, or viruses infecting the cells that line one's nasal cavity, involves a rapid response from immune cells which produce chemical messengers that can disrupt the ability of viruses and bacteria to function, recruit additional help by increasing blood flow to the affected site and attracting other immune cells, and activate other immune cells to respond. Immune cells also "eat" and break down foreign particles when possible. Taken together, inflammation is the rapid immune response to infection and injury, and has taken on significant prominence in biobehavioral research because inflammation: 1) plays a key role in the pathophysiology of chronic conditions considered major public health threats including cardiovascular disease and Alzheimer's disease; 2) is a key player in ubiquitous health conditions like upper respiratory infections; and importantly, 3) is influenced by and influences social and emotional functioning (Kiecolt-Glaser et al., 2002; Robles et al., 2005).

Multiple cells drive the inflammatory response, and for simplicity I focus on a particular immune cell: the macrophage, which is also called a monocyte when it circulates in blood (Owen, Punt, & Stranford, 2013). Macrophages/monocytes inhabit most tissues in the body, and are important sentinels in tissues that interface with the outside environment, like the skin, lungs, and gut (Geissmann et al., 2010). Within a single macrophage (shown in Figure 4), the inflammatory response is initiated when the macrophage detects a threat through specially designed "detectors" known as toll-like receptors. When the detector is activated, such as through binding to a bacteria, this sets off a cascade of signals within the

macrophage that ultimately lead to the activation of nuclear factor - kappa B, which is a “transcription factor” molecule that migrates into the nucleus of the macrophage (Cole, Yan, Galic, Arevalo, & Zack, 2005). Transcription factors are generally responsible for activating specific genes within a cell, leading to the transcription of those genes into messenger RNA (mRNA), and the eventual translation of that mRNA into protein. In the context of inflammation, NF-κB migrates to the cell nucleus, leading to transcription of genes that code the chemical messengers that are involved in the inflammatory response. Thus, conceptually speaking indicators of greater NF-κB activation also indicate that the inflammatory response is either turned on or has greater to potential to be turned on. Figure 4 also indicates that other intracellular processes that are involved in turning off inflammation, and highlights the glucocorticoid receptor. When cortisol binds to the glucocorticoid receptor, the complex travels to the nucleus and inhibits the transcription of inflammation-related genes. In sum, NF-κB is widely viewed as a key *pro*-inflammatory transcription factor, and the glucocorticoid receptor is viewed as a key *anti*-inflammatory transcription factor.

Figure 4 also describes the multiple methods (described in *italics*) that exist for measuring the inflammatory response. The primary methods are measuring circulating levels of inflammatory biomarkers from blood; or removing immune cells from blood, stimulating those cells with molecules that initiate an inflammatory response (i.e., lipopolysaccharide, which is the main component of the cell wall of certain types of bacteria), and measuring inflammatory biomarkers produced inside immune cells or secreted outside the immune cells (Vedhara & Wang, 2005). Each measure has strengths and limitations, and the goal here is to focus on the degree to which such measures can be used as measures of potential to respond to

infectious threats. Conceptually, measuring potential to respond to threats requires knowing measuring “the threat” (exposure) and the response to the threat. Circulating measures of inflammatory biomarkers provide a window into responses, but not exposures. Stimulating immune cells involves deliberately exposing cells to a threat and measuring the response, making stimulated measures the most ideal. However, stimulated inflammatory responses pose logistical challenges that are particularly problematic to family and developmental researchers. Namely, blood must be transported immediately to the laboratory for processing (isolating cells) and stimulation, and immunology laboratories often operate according to normal business hours (i.e., 8 am to 5 pm on weekdays). Thus, drawing blood from children and families during times that are convenient and minimally intrusive, such as evenings and weekends, may not be feasible for an immunology laboratory. Thus, the most ideal method for assessing potential to respond may not be logistically possible in family research.

Genomic approaches to studying the inflammatory response, with a focus on the transcription factor control pathways described above, may provide a feasible but somewhat imperfect window into first derivative approaches to the inflammatory response. Such methods involve determining gene expression through sequencing mRNA in immune cells, and making inferences about what genes are being expressed, the function of those genes, and potential themes inherent in the patterns of gene expression (Cole, 2010, 2014). Transcription factors are one possible theme; researchers can ask whether genes regulated by NF- $\kappa$ B or GR appear to be differentially active in people with differing levels of exposure to a psychosocial factor of interest. Notably, exposure to stressful events that including caregiving for brain cancer patients (Miller et al., 2014), chronic interpersonal stress

(Miller, Rohleder, & Cole, 2009), and exposure to the combination of low SES and low levels of maternal warmth in childhood (Chen, Miller, Kobor, & Cole, 2011) are all related to greater expression of pro-inflammatory genes regulated by NF- $\kappa$ B and lower expression of anti-inflammatory genes regulated by GR.

We sought to extend prior work on social adversity and inflammation-related gene expression to conflict and warmth in the family environment, and importantly, examine patterns in children and their parents (Robles et al., in press). In addition, to take advantage of the multi-method (interview, questionnaire, and daily diary) and multi-reporter (parents and children) approach to assessing the family environment, we combined measures of family conflict across methods and reporters (see Robles et al., in press for more details). Similar to the telomere work described above, whole blood was obtained from parents and children that elected to provide samples. Following RNA extraction from immune cells, we used gene microarray technology to quantify expression of over 30,000 genes in immune cells.

For parents and children separately, we compared patterns of gene expression between participants in high vs. low conflict families, which yielded lists of several hundred differentially (over- and under-) expressed genes. The gene lists were analyzed by a bioinformatics tool that allowed for inferring whether genes regulated by the transcription factors NF- $\kappa$ B and GR were relatively over- or under-expressed in high conflict/low warmth families (Cole et al., 2005). For both children and parents, living in a high conflict family was associated with greater expression of genes regulated by NF- $\kappa$ B, consistent with the proinflammatory phenotype observed in prior work. Parents, but not children, in high-conflict families also showed lower expression of genes regulated by GR, consistent with the idea that chronic stress may lead to immune cells becoming insensitive to the effects of

cortisol. Interestingly, for children, greater family conflict was related to elevated upper respiratory infection symptoms, both on days when children were not verifiably “sick” with the cold or flu, and on days when children had verifiable illness. The latter provides an example of a potential clinical consequence of a proinflammatory phenotype. In sum, our gene expression data provide a potential “first derivative” window into how the immune system might respond to threats as a function of the family environment. Both parents and children in higher conflict family environments may have more amplified inflammatory responses to threats that activate the immune system, and there may be clinical consequences as well, such as more severe upper respiratory infection symptoms.

## **Implications and Conclusions**

### **Implications for Practice and Policy**

Our work highlights the importance of including intensive repeated measures to monitor mechanisms of change (e.g., preventing increases in conflict over time, reducing negative mood reactivity to conflict). For instance, repeated assessments of family functioning over time can provide a window into the speed of progress in family-based treatments. Moreover, repeated assessments of family functioning and mood may provide insight into the degree to which family interventions can “loosen” the ties between stressors at home and mood. For instance, a plausible treatment target in family therapy may be reducing the covariation between interparental conflict and children’s mood, so that interparental conflict becomes less distressing over time and children understand that disagreements between spouses/partners are normative (assuming a healthy level of conflict exists in the interparental relationship). Our work also provides insight into biomarkers that may be responsive to interventions (and downstream health outcomes plausibly linked to

those downstream mediators), such as bedtime cortisol; such biomarkers may be useful secondary outcomes for use in efficacy and effectiveness trials. However, the value of collecting biomarkers for the purpose of monitoring children and families during interventions in everyday clinical practice has not been demonstrated; this remains a key issue for future effectiveness down the road.

Research on intensive repeated measures and genomic markers of risk, such as pro-inflammatory gene expression, is still in very early phases. Thus, translating research from the initial studies described in this chapter may be a bit premature. That said, work on biobehavioral research on families and resilience more generally suggests several implications for policy and program evaluation. Programs and policies that are specifically designed to intervene at the level of family, including financial or instrumental assistance, or increasing access to family-based interventions for families at risk, should consider including measures of the quality of the family environment as primary outcomes, and health outcomes as secondary outcomes. In addition, recent efforts to include assessments of the social environment, including experiencing recent stressors, and degree of social integration into electronic health records may be extended to assessments of conflict and warmth in the family environment. Finally, given the implications of chronic inflammation for current and long-term health, systemic levels of circulating inflammatory biomarkers or perhaps even “first derivative” measures of inflammatory response potential may be considered as exploratory outcomes in family-based interventions to reduce risk and increase resilience.

### **Implications for Understanding Family Resilience**

This chapter described several examples of how understanding exposures using intensive repeated measures provides insight into the biobehavioral

mechanisms that may be sensitive to the family environment, including HPA axis regulation, immune cell aging, and inflammation, and that have biologically plausible health implications. Risk and resilience can be conceptualized based on how intensive repeated measures change over time, such as increases in conflict over several weeks to months, or increases in support and involvement, respectively. Perhaps more intriguing is using relationships between frequently sampled measures, such as greater or lower emotional reactivity to daily interparental conflict, to conceptualize risk or resilience. On one hand, conceptualizations of risk/resilience that focus on individual differences in capacity to respond in better or worse ways to adversity may be interested in using the covariation between two measures as ways to identify individuals who are “at risk” (e.g., high, positive within-person correlation between conflict and negative mood) or “resilient” (e.g., high, positive within-person correlation between family support and positive mood). On the other hand, prevention and intervention research that focuses on reducing risk and increasing resilience may examine covariation between two measures as ways to evaluate treatment mechanisms or efficacy. For instance, if family therapy can reduce the within-person correlation between interparental conflict and children’s negative mood from pre- to post-treatment, this could be a mechanism of treatment effects or even an indicator of treatment outcomes.

On the biological side, incorporating minimally invasive and logistically feasible measures that indicate how a person may respond to physical threats like infection or other immune-stimulating substances like environmental pollutants, with sensitive measures of stress exposure in the family environment, are a key future direction for research on family risk and resilience. Individuals who have

known exposures to physical threats, like high frequency of infections or living in regions with high pollution exposure, may be key populations to explore the deleterious effects of stressful family environments. In addition, individuals who have high proinflammatory phenotype potential may be a key subgroup for whom family-based prevention and intervention research is indicated.

### **Conclusions**

I conclude this chapter by emphasizing the need for replication of the effects described in this chapter, particularly in samples with large sample sizes with longitudinal follow-up. At the same time, the research described herein provides “proof-of-concept” for the value of incorporating intensive sampling of the naturalistic family environment in future biobehavioral research. In addition, the work described in this chapter complements other theory and research that emphasizes the key role of inflammation and the regulation of inflammation in linking risk and resilience in families to current and future physical health (Kiecolt-Glaser et al., 2002; Miller, Chen, et al., 2009; Repetti et al., 2011). Family therapists and researchers recognized long ago that families are dynamic entities; research methodologies that capture the dynamic ebb and flow within families over time, as well as the internal dynamics of how the family environment regulates immunity, will be key tools in understanding family risk and resilience in the decades to come.



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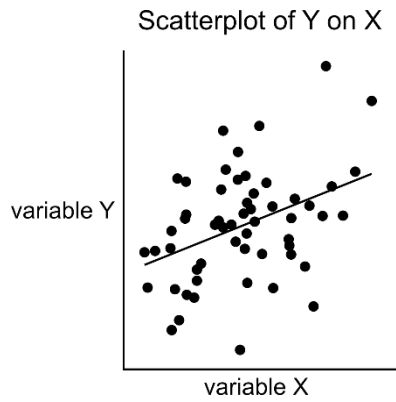


Figure 1. Scatterplot of two variables (X and Y), where the slope of the best fit line through the points represents a “first derivative.”



Figure 2. Timeline of procedures in the UCLA Families and Health Study



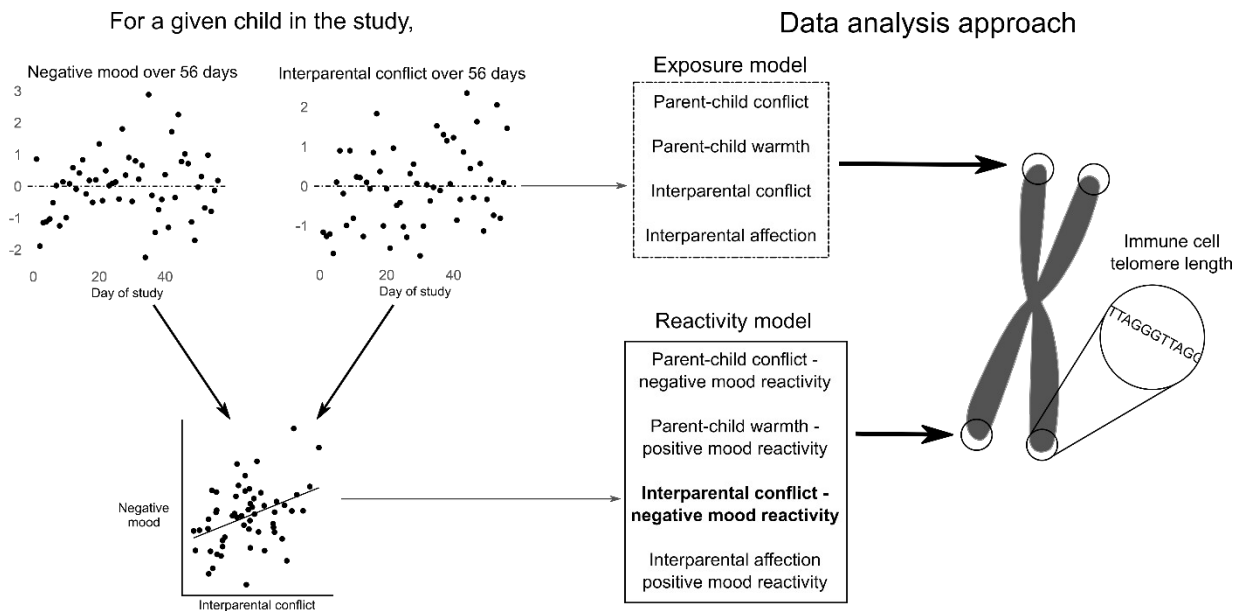


Figure 3. Conceptual depiction of reactivity scores (left), and data analyses linking daily conflict/warmth in the family environment to immune cell telomere length.

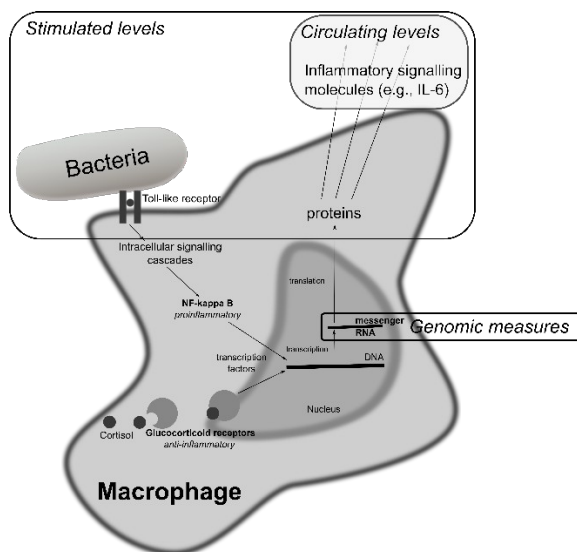


Figure 4. Diagram of the inflammatory response at the level of a single macrophage, with measures of inflammation shown in the rounded rectangles.

## **Questions for Thought and Discussion (1-2 pages)**

[Could use some help with this one!]

### **Index terms**

Allostatic processes

Cortisol

Gene expression

Genomic measures

Glucocorticoid receptor

Hypothalamic-pituitary-adrenal axis

Immune system

Inflammation

Macrophage

Monocyte

nuclear factor - kappa B

Telomere

Transcription factor

### **Glossary of Terms**

Allostatic processes – Biological changes designed to help organisms adapt to changes in the environment, including changes in the cardiovascular, neuroendocrine, and immune systems.

Biological mediators – Biological systems and processes that can explain links between psychosocial factors and health.

Cortisol – a glucocorticoid hormone produced in the adrenal cortex, in response to signals from the brain as part of the hypothalamic-pituitary-adrenal axis;

frequently measured in naturalistic settings in saliva; plays a critical role in regulating metabolism and immune function.

Genomics - studying biological processes using genetic material, including messenger ribonucleic acid (mRNA), and the patterns of gene expression from that material

Hypothalamic-pituitary-adrenal axis - a major neuroendocrine system that plays a key role in allostatic processes

Immune system - responsible for recognizing and responding to threats inside the organism; composed of numerous cells, tissues, and organs, as well as signaling molecules

Inflammation - the body's first line of immune defense against infection and injury; also described as innate immunity

Intensive repeated measures - psychosocial assessments that are administered with a high degree of frequency over long periods of time

Macrophage/Monocyte - key cell involved in innate immunity that produces inflammatory responses

nuclear factor - kappa B - a transcription factor that regulates the activation of genes within immune cells that promote inflammation

Telomere - nucleotide structures at the end of chromosomes that protect genetic material from being damaged during the process of normal cell division; shorten with each cell division

Transcription - the process by which genetic code in deoxyribonucleic acid (DNA) is read and coded into messenger RNA; the first part of the central dogma of molecular biology

Translation - the process by which mRNA is read and coded into amino acids, which are then assembled into proteins; the second part of the central dogma of molecular biology