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Genetic Underpinnings of the Relationships Among Stress, Inflammation, and Depression

A dissertation submitted in partial satisfaction
of the requirements for the degree Doctor of Philosophy
in Psychology

by

Margaret Anne Tartter

2015

ABSTRACT OF THE DISSERTATION

Genetic Underpinnings of the Relationships Among Stress, Inflammation, and Depression

by

Margaret Anne Tartter

Doctor of Philosophy in Psychology

University of California, Los Angeles, 2015

Professor Constance L. Hammen, Chair

Increases in pro-inflammatory cytokines, the immunological response to pathogens, have been

observed in close to one third of patients with major depression. Further, interpersonal stressors,

the most potent and well-established risk for depression, have also been found to elicit pro-

inflammatory cytokines. Thus, inflammation may represent a biological response to stress that

heightens the risk of depression. A better understanding of the relationships among stress,

depression and inflammation has the potential to shed light on the cycle of stress and depression,

as well as the increased prevalence of inflammatory illness in depressed patients, which is of growing concern in our aging population.

This dissertation sought to confirm that among young, healthy adults, depressive symptoms tend to develop before, and contribute to elevations in pro-inflammatory cytokines, and to determine whether functional variation in immunological genes would create susceptibility to depression and inflammation in response to stress, and/or create susceptibility to inflammation following an elevation in depressive symptoms. The dissertation explored these questions in a pair of longitudinal cohort studies, the first of which followed Australian youth with a high prevalence of familial risk for depression from ages 15 to 20, and included assessments of depressive symptoms, stress exposure and genotypic information for a trio of immunological genes. Exposure to interpersonal stress was moderated by variation at *IL6* and *IL1β*, so that -174C allele carriers at *IL6,* and -511C carriers at *IL1β* had greater depressive symptoms in response to stress, while neither of these genotypes affected the prediction of depressive symptoms from exposure to non-interpersonal stressors, such as finances and work. *TNF* genotype was not a moderator of either type of stress exposure.

In the second dissertation study, the prediction of growth in depressive symptoms and C-reactive protein (CRP) from childhood stress exposure, and potential moderation of these paths by *IL6* genotype, was examined in 4,276 participants in the Coronary Artery Risk Development

Study of Young Adults. Stress exposure in the childhood home was predictive of depressive symptoms at age 30, which were in turn associated with slight, but significant growth in CRP between age 32 and age 50. African Americans in this sample showed a unique moderating effect of *IL6* genotype, so that the relationship between stress and CRP was significant among G allele carriers, but not among African American CC homozygotes, or among Caucasians, regardless of genotype. Taken together, the two dissertation studies suggest that genetic variation at *IL6* may contribute to increases in depressive symptoms and CRP following interpersonal stress exposure. Further, although a strong relationship from stress exposure to depression was observed in both study populations, a direct relationship between stress and CRP was not found. Instead, stress led to depression which in turn predicted CRP, and this effect was more pronounced in Caucasians.

The dissertation of Margaret Anne Tartter is approved.

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2015

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PUBLICATIONS

Tartter, M., Hammen, C., Bower J., & Cole S.W. (2015). Effects of chronic interpersonal stress exposure on depressive symptoms are moderated by variation at *IL6* and *IL1β* in youth. *Brain, Behavior and Immunity*, *46*, 104-111.

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SELECTED PRESENTATIONS

Molly Tartter, Constance Hammen, Julienne Bower & Patricia Brennan (2013, November). *Interaction of early adversity and* IL6 *genotype predicts depression in adolescents: evidence of antagonistic pleiotropy?* Poster presented at the annual meeting of the Association for Behavioral and Cognitive Therapies, Nashville, Tennessee.

George Slavich, **Molly Tartter** & Constance Hammen (2013, March). *Variation in the μ-opioid Receptor Gene* (OPRM1) *Moderates the Effects of Targeted Rejection on Depression in*

Adolescents. Abstract presented at the annual meeting of the American Psychosomatic Society, Miami, Florida.

Molly Tartter & Constance Hammen (2012, November). *Rejection Sensitivity and Adolescent Affective Disorders: Proposed Evocative Gene-Environment Correlation between* OPRM1 *and Interpersonal Stress.* Poster presented at the annual meeting of the Association for Behavioral and Cognitive Therapies, National Harbor, Maryland

Molly Tartter & Constance Hammen (2011, November). *Offspring Externalizing Disorders Mediate the Effect of Maternal Depression on Substance Dependence in Young Adulthood.* Poster presented at the annual meeting of the Association for Behavioral and Cognitive Therapies, Toronto, Ontario.

Molly Tartter & Lara Ray (2009, June). *Genetic polymorphisms of the corticotropin releasing hormone receptor 1* (CRHR1) *and binding protein* (CRH-BP) *and subjective levels of stress in heavy drinkers.* Abstract presented at the annual meeting of the Research Society on Alcoholism, San Antonio, TX.

Chapter 1: General Introduction

The potential for the defenses of the immune system to generate intense emotional suffering was first observed in patients treated with interferon-alpha (IFN-α) for hepatitis C. Interferon alpha is a potent immunotherapy that works to destroy the hepatitis C virus and simultaneously triggers a coordinated inflammatory response from the patient's innate immune system. The inflammatory process is initiated by pro-inflammatory cytokines, molecules that martial immune cells to attack and destroy invading pathogens. Capillaries loosen at the site of infection to allow attack cells to leak out and eliminate viruses. Patients undergoing IFN-α treatment are exposed to high levels of inflammation over six months or longer, and up to half develop a major depressive episode (Bonaccorso et al., 2002; Janssen, Brouwer, van der Mast, & Schalm, 1994; Valentine, Meyers, Kling, Richelson, & Hauser, 1998). In the decade since the depressogenic effects of IFN-α treatment were first described, scientific interest in inflammation as a precipitant of depression has flourished, revealing that close to one-third of depressed persons show chronic elevations in markers of inflammation, regardless of physical health (Dowlati et al., 2010; Howren, Lamkin, & Suls, 2009). These markers include high blood plasma concentrations of the pro-inflammatory cytokines interleukins 1 (IL-1) and 6 (IL-6), tumor-necrosis factor alpha (TNF-α), and C-reactive protein (CRP), a marker of chronic inflammation which is stimulated by the release of IL-6.

Inflammation levels observed in depression are low compared to IFN-α treatment and inflammatory diseases, but are significant nonetheless for their potential etiological role in depression and for their possible contribution to the association between depression and inflammatory disease, including cardiovascular disease and metabolic syndrome (Dowlati et al.,

2010; Kinder, Carnethon, Palaniappan, King, & Fortmann, 2004; Kuo et al., 2005; Miller & Blackwell, 2006; Raison & Miller, 2011).

In addition to the depression syndrome itself, many of the risks for depression are associated with heightened inflammatory markers (Shelton & Miller, 2011; Dickerson, Gable, Irwin, Aziz, & Kemeny, 2009; Dickerson, Kemeny, Aziz, Kim, & Fahey, 2004). Interpersonal stress, the most potent and well-studied precipitant of depression (Monroe, Rohde, Seeley, & Lewinsohn, 1999), has been found to increase levels of pro-inflammatory cytokines (Aschbacher et al., 2012; Chiang, Eisenberger, Seeman, & Taylor, 2012; Dickerson et al., 2009; Murphy, Slavich, Rohleder, & Miller, 2012). Although many different types of stress have been associated with depression onset, interpersonal stressors in childhood, including maternal rejection, neglect, and disruption of caregiver relationships are some of the most consistent predictors (Burge & Hammen, 1991; Crook, Raskin, & Eliot, 1981; Heim, Newport, Mletzko, Miller, & Nemeroff, 2008; Kendler, Neale, Kessler, Heath, & Eaves, 1992; Kessler & Magee, 1993; Monroe et al., 1999). Scores on the childhood trauma questionnaire, which measures parental abuse and neglect, have been associated with profound changes in stress-activated biological pathways, including diminished cortisol response and increased plasma levels of IL-6 in response to stress (Carpenter et al., 2007; Pace et al., 2006). Similarly, low levels of affection paired with high conflict in the childhood home are predictive of increased C-reactive protein (CRP) in adulthood (Taylor, Lehman, Kiefe, & Seeman, 2006). Illuminating the mechanisms by which early interpersonal stress increases liability for depression is vital, and biological mediators including inflammation and genetics have received increasing attention.

Raison and Miller (2013) propose that many of the genes linked to depression are immunological and have been conserved because they enhance the innate immune response,

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offering protection from infectious parasites, diseases and wounding. The search for depression genes began in earnest with the advent of genome-wide association studies in the mid-2000s, but has failed to uncover genetic variants that dependably predict depression (Garriock et al., 2010; Hodgson & McGuffin, 2013; Ising et al., 2009; Verbeek et al., 2012). Raison and Miller's (2013) re-examination of the few genes reliably linked to depression revealed that nearly all serve immunological functions. *DCNP1* has been confirmed by GWAS to be associated with depression and is involved in identifying pathogens for destruction (Bosker et al., 2011). A single nucleotide polymorphism (SNP) in *TNF*, which codes for the pro-inflammatory cytokine TNF-α has also been confirmed by GWAS (Raison & Miller, 2013). The inconsistent association between depression and inflammation suggests that interactive effects are likely at play between a biological diathesis and an environmental stressor (Raison & Miller, 2011). To date, no studies have examined genetics of the innate immune system as a vulnerability to depression in response to stress. However, the serotonin transporter polymorphism (*5-HTTLPR*), the most well understood genetic determinant of depressive responses to stress (Caspi, Hariri, Holmes, Uher, & Moffitt, 2010; Uher & McGuffin, 2010) has been associated with a greater ratio of pro-inflammatory to anti-inflammatory cytokines (Fredericks et al., 2010), and confers protection against sudden infant death syndrome (Narita et al., 2001; Opdal, Vege, & Rognum, 2008), suggesting that *5-HTTLPR* could be reframed as an immunological gene that is depressogenic under conditions of psychosocial stress (Raison & Miller, 2013).

A review of the current literature linking inflammation and depression is presented below, beginning with the proposed biological mechanisms of inflammation-induced depression. Second, evidence of a bi-directional relationship, in which depression also leads to elevations in pro-inflammatory cytokines, will be reviewed, and the impact of age and gender on the strength

of these relationships will be explored. The role of stress, particularly in the context of the childhood environment, which is known to disrupt immune functioning, will be examined as a liability for both inflammation and depression. Finally, evidence for a genetic contribution to the relationships among stress, inflammation, and depression will be reviewed, and preliminary evidence that genetic susceptibility changes with sexual maturity will be explored.

The present literature review and proposed pair of studies attempt to shed light on what is known about the relationship between inflammation and depression, and to expand the current knowledge regarding the contribution of genetic vulnerability and stress exposure to these relationships.

Inflammation-Induced Depression

The study of inflammation-induced depression has moved from observational studies in medical populations to elegant translational work on molecular mechanisms in a matter of years. Early work identified an increased prevalence of depression in patients with diseases that produce inflammation (cancer), evoke an inflammatory response from the host (cancer, heart disease) or are treated with antiviral drugs that mimic activation of the innate immune system (Hepatitis C) (Bonaccorso et al., 2002; Capuron et al., 2003; Irwin, Olmstead, Ganz, & Haque, 2013; Steptoe, Wikman, Molloy, Messerli-Bürgy, & Kaski, 2013).

Prior to the discovery of IFN-α-induced depression, a collection of symptoms known as sickness behaviors had been documented in animal models of inflammation (Dantzer & Kelley, 2007; Kelley et al., 2003). These symptoms are known to anyone who has had the flu, and include fever, hypoferremia (low iron levels), decreased appetite, social withdrawal and sleep disturbance (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008). Each of these symptoms serves an important function in the elimination of, and recovery from, infection by pathogens.

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Mounting a fever slows the replication of viruses and prompts the continued activation of the immune system. These functions are enhanced when iron levels are low, which helps to explain why hypoferremia occurs during infection (Kochan, Wagner, & Wasynczuk, 1984).

Other work has characterized the behavioral symptoms of inflammation as a "conservation-withdrawal" state, defined by the presence of anhedonia, psychomotor retardation, fatigue, social avoidance and anorexia. Conservation-withdrawal closely resembles depression, and is thought to allow the body to redirect metabolic resources to the healing process after infection or injury. Insomnia and hypervigilance are observed during IFN-α treatment, and have been explained from an evolutionary perspective as a way to remain alert to potential predators and other dangers during the vulnerable recovery process (Raison et al., 2010; Raison & Miller, 2013).

Several sickness behaviors correspond with clinically defined depression symptoms, while others (hypoferremia, fever) do not. Despite their omission from the clinical definition of depression, evidence exists to support increased temperature and decreased iron stores during a clinically depressed state (Daimon, Yamada, Tsujimoto, & Takahashi, 1992; Rausch et al., 2003). Evidence from mouse models of inflammation-induced depression, and from IFN-α patients, indicates that sickness behaviors develop immediately in response to increases in interleukin 1β (IL-1β) and TNF-α, while depression symptoms develop only after prolonged exposure to cytokines (Capuron, Ravaud, Miller, & Dantzer, 2004). While all patients experience some level of sickness symptoms in response to IFN-α treatment, around 30-50% go on to develop sadness, loss of pleasure, and suicidal thoughts. These findings suggest that sickness is a general response to inflammation, while depression manifests only in those who have a specific vulnerability (Bonaccorso et al., 2002; Janssen et al., 1994; Valentine et al., 1998). Both

psychological vulnerability, evidenced by elevated baseline depression scores, and biological vulnerability, characterized by an exaggerated pituitary-adrenal response to treatment, increase the likelihood that IFN-α patients will have a depressive episode during their treatment (Capuron & Ravaud, 1999; Capuron et al., 2004).

When depression occurs in response to inflammation, it is almost certainly the result of pro-inflammatory cytokines acting on the brain. IL-1β, the primary cytokine implicated in sickness behavior, cannot passively cross the blood-brain barrier, and may instead pass through unprotected areas of the blood-brain barrier via specific soluble transporters (these pathways are outlined in detail by Dantzer et al. (2008) and are beyond the scope of this literature review). Several hypotheses have been put forth regarding the causal component of inflammation's action on the brain. No one mechanism, however, has been confirmed as the cause of inflammation-induced depression. In mice, the progression from sickness behaviors to depressive behaviors is dependent upon the increased activity of the enzyme indoleamine 2,3 dioxygenase (IDO), which degrades tryptophan into kynurenine. Increased IDO activity decreases synthesis of serotonin (which depends on the availability of tryptophan), and has been hypothesized to account for cytokine-induced depression (Dantzer, O'Connor, Lawson, & Kelley, 2011). However, IDO only reduces tryptophan in the periphery, not in the cerebrospinal fluid (CSF), negating the possibility that its effects are via decreased serotonin in the brain (Hughes et al., 2012). Alternatively, kynurenine, a neurotoxin produced during tryptophan degradation, has recently been explored as a cause of depression. Kynurenine is in fact elevated in the CSF of depressed patients, when compared to controls, lending support to this theory (Myint et al., 2007).

Complementary findings have shown that the neurotoxic effects of inflammation decrease neurogenesis and also hippocampal volume, both of which are linked to depression

(Ekdahl, Claasen, Bonde, Kokaia, & Lindvall, 2003). The destruction of hippocampal cells and resulting decrease in hippocampal volume has been associated with major depression (MacQueen et al., 2003; Sheline, Wang, Gado, Csernansky, & Vannier, 1996; Videbech & Ravnkilde, 2004), and may contribute to the cognitive impairment observed in depressed patients (O'Brien, Lloyd, McKeith, Gholkar, & Ferrier, 2004). In animal models, TNF-α and IFN-α suspend hippocampal neurogenesis via their action on IL-1, which binds to highly concentrated receptors in the rat hippocampus (Iosif et al., 2006; Kaneko et al., 2006; Katsuura, Gottschall, & Arimura, 1988). Neurogenesis in the hippocampus resumes when inflammation is blocked (Monje, Toda, & Palmer, 2003), confirming that inflammation can cause decreased neurogenesis. Thus, inflammation may induce depression by suspending neuron growth.

Inflammation has additionally been found to directly increase the activity and expression of the serotonin transporter (an effect opposite to that of SSRIs) leading to less serotonin at the synapse, a state hypothesized to underlie depression (Owens & Nemeroff, 1994). The pro-inflammatory cytokines TNF-α and IL-1β have been found to increase serotonin transporter activity and the binding affinity of the 5-HT1A receptor in a mouse model (Abe et al., 1999), decreasing the available synaptic serotonin (Maes, Leonard, Myint, Kubera, & Verkerk, 2011). Although not confirmed to promote depression in humans, the action of cytokines on neuron growth and serotonergic function are promising as potentially causal pathways linking inflammation to depression.

These neurobiological findings are supported by research on the biological changes that occur in depression, and in response to inflammation. A number of parallel changes to the central nervous system have been observed between depression and inflammation, including resistance to glucocorticoids (Norbiato, Bevilacqua, & Vago, 1997). In healthy individuals, a negative

biological feedback loop controls the production of IL-1: IL-1 activates the release of cortisol, a glucocorticoid and the body's primary stress hormone, from the hypothalamic-pituitary-adrenal (HPA) axis, which in turn shuts off the production of cytokines, returning immune functioning to baseline (Sapolsky, Rivier, Yamamoto, Plotsky, & Vale, 1987). Chronic exposure to cytokines deadens the ability of the HPA axis to respond to cortisol, a condition known as glucocorticoid resistance (Pace & Miller, 2009). When this occurs, both cortisol and pro-inflammatory cytokines are produced unchecked. The same phenomenon has been noted in depression (Pace, Hu, & Miller, 2007), and appears to be necessary for inflammation-induced depression, as patients undergoing IFN-α treatment only develop depression after ceasing to respond to cortisol (Raison et al., 2010).

Further, the immune systems of depressed persons may be more sensitive to psychosocial stress. In a sample of depressed men, spikes in circulating IL-6 and NF-κβ, a protein responsible for cytokine production, were observed both at rest and in response to the Trier Social Stress Test (TSST), compared to healthy (male) controls (Pace et al., 2006). A similar study of depressed women identified stress-induced increases in IL-6 and TNF-α that failed to resolve after the termination of the TSST (Miller, Rohleder, Stetler, & Kirschbaum, 2005). Both studies found that resting inflammatory activity in the depressed group was equivalent to peak, stress-induced inflammation in healthy controls. Further, the sensitivity of IL-1β production to stress predicted increased depressive symptoms one year later, and this effect was mediated by negative cognitive appraisals of the task (Aschbacher et al., 2012). This finding suggests that negative cognitive appraisals maintained the psychological experience of stress long after the stressful event had concluded. Negative cognitions, which are characteristic of depression, may prolong the impact of stress on bodily systems, creating greater wear and tear than the impact of

the stressful event alone. These findings demonstrate greater immunological responsiveness to psychological stressors in depressed persons compared to healthy controls.

Given the many parallels between the effects of inflammation and a depressive state, along with evidence that inflammation is a cause of depression in some cases, we and others have hypothesized that the relative sensitivity of the inflammatory response to biological or psychological triggers is a diathesis for depression. It would follow that individuals with a more sensitive inflammatory response are more likely to experience depression following biological triggers (e.g., IFN-alpha treatment) or psychological triggers (e.g., interpersonal stress) of the inflammatory response. For example, an individual with a stress-sensitive inflammatory response would experience heightened cytokine levels in response to a chronic social stressor, which would put wear and tear on the negative feedback of the HPA axis, leading to further elevations in cytokine levels and eventually depressive symptoms or perhaps a depressive episode (Dantzer et al., 2008). An individual with a less sensitive immune response may avoid exhausting the negative feedback mechanisms of the HPA axis and as a result, never experience inflammatory levels high enough to induce depression. The present dissertation focuses on genetic variation that contributes to the stress-sensitivity of the inflammatory response as a diathesis for depression.

Elevated Inflammation in Depression

In addition to evidence that inflammatory medical treatments and diseases induce depression, inflammatory markers are positively correlated with depressive symptoms in community samples. Meta-analysis of 127 studies measuring IL-6, TNF-α and CRP levels as a function of depression symptoms and diagnosed depression revealed small but significant correlation and group difference effect sizes (Cohen's d from .22-.35), with structured interviews

producing larger effects than self-report measures (Dowlati et al., 2010; Howren et al., 2009). Findings for IL-6 and CRP appeared to be robust, as the failsafe *N's* were quite large (2343 and 1119, respectively). Of the two meta-analyses published to date, only Howren et al. (2009) controlled for confounding health factors including body mass index (BMI) and smoking status. Controlling for BMI, a factor linked to both inflammation and depression, resulted in attenuated but significant associations, while smoking status sometimes accounted for the associations, but did not provide a consistent explanation (Howren et al., 2009). Therefore, the rise in inflammation seen in a portion of depressed patients cannot be explained away by physical and lifestyle factors.

Longitudinal work has uncovered a complicated relationship across time between depression and inflammation that appears to be influenced by age and gender. In young, healthy persons, rises in CRP are more often a consequence of first-onset depression, rather than a precipitant (Copeland, Shanahan, Worthman, Angold, & Costello, 2012; Danese et al., 2009; Raison & Miller, 2013). In a large sample (n = 1,490) of young people ages 9-21, followed over nine waves of data collection, Copeland et al. (2012) found that CRP levels were not predictive of depression outcomes. Instead, depression, particularly multiple depressive episodes, led to increased circulating levels of CRP. Rates of depression and levels of inflammatory markers each naturally increase with age (Chung et al., 2009; Mirowsky & Ross, 1992). Further, the temporal relationship between inflammation and depression may reverse direction in older adults. With increasing age, inflammation has been found to precede depression more often than follow it (Matthews et al., 2010; Pasco et al., 2010; van den Biggelaar et al., 2007). Despite this roughly sketched picture of depression producing inflammation in young people, and *resulting from* inflammation in older persons, a single longitudinal study suggests that the young and old

may not be so different. Pasco et al. (2010) enrolled never-depressed adult women, ranging widely in age (20-84) and measured plasma CRP at baseline. The group identified a prospective relationship from plasma CRP concentrations at baseline to new cases of depression up to 10 years later. Pasco et al. (2010) measured CRP concentrations only at baseline, however, and might have found reciprocal effects if they had repeatedly taken CRP measurements. Further, their recruitment of never-depressed women, particularly those in their 70s and 80s, is likely to have affected the results. The relationship between inflammation and depressive symptoms in elderly women who have never experienced a major depressive episode (MDE) is likely of limited value to understanding the typical relationship between depression and inflammation, which appears to begin early in life. A meta-analysis of cross-sectional work has found cross-sectional relationships between depression symptoms/diagnoses and inflammatory markers (CRP, IL-1) to be stable across the lifespan (Howren et al., 2009). This meta-analysis additionally found IL-6 and depression to have a close association in young people, and a weak correlation in older samples.

Gender also plays a significant role in the co-occurrence of depression and inflammation. Meta-analysis finds IL-6 and depression to be associated only in women (Howren et al., 2009), and these cross-sectional findings are bolstered by evidence from a laboratory study of endotoxin administration, showing that women's (but not men's) chemically-induced increases in IL-6 are associated with acute increases in depressed mood (Eisenberger, Inagaki, Rameson, Mashal, & Irwin, 2009). The opposite appears to be true of CRP, however, which was associated with depression in samples of men, but not women (Howren et al., 2009). Oddly, in samples composed of both genders, the percentage of women did not weaken the relationship between CRP and depression. Although studies have begun to build a case for a specific effect of IL-6 on

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depression in women, gender effects have generally been inconsistent for individual markers and few studies have examined gender differences (Howren et al., 2009). Given that women bear the majority of the world's depression burden, it is imperative that future work examine the role of gender in the association between depression and inflammation (Nolen-Hoeksema & Girgus, 1994).

Despite what appear to be small, uniform elevations in markers of inflammation in large samples of depressed individuals, Raison & Miller (2011) have shown that greater pro-inflammatory elevations in one-third of depressed persons account for the small mean differences between depressed and non-depressed samples. As discussed earlier, inflammation is neither necessary nor sufficient to induce depression. Inflammation may thus characterize a subtype of the depression syndrome, a unique etiological pathway, or both (Raison & Miller, 2011). The co-occurrence of depression and inflammation in one patient (barring explanation by a third factor) may indicate that their depression is etiologically different from a depressed patient with no change in circulating inflammatory markers. Clinical trials of SSRIs indicate that patients with high levels of IL-6 and TNF-α are more likely to be treatment resistant, suggesting evidence of a "type" of depression that may involve etiological mechanisms different from typical presentations treatable with SSRIs (O'Brien, Scully, Fitzgerald, Scott, & Dinan, 2007). Raison & Miller (2011) reason that if inflammation plays an etiological role in a subset of patients, and these patients can be identified by increases in pro-inflammatory cytokines and/or CRP, then anti-inflammatory treatments should be helpful for this "inflammatory subtype" of depression. Patients with depression and normal immune activity, on the other hand, would not be expected to benefit from an anti-inflammatory treatment. Raison and colleagues (2013) have recently tested their hypothesis using a randomized, controlled trial of TNF-α antagonist

infusions for treatment-resistant depression (n = 60). In line with their hypotheses, patients with high baseline CRP benefitted more from the treatment than those with lower levels of circulating CRP. This supports the concept of an inflammatory subtype of depression that requires alternate treatment and may have a unique symptom profile. Further, in emotionally healthy individuals, aspirin, an anti-inflammatory agent, has been found to decrease feelings of social rejection, depression, anxiety, and guilt (DeWall et al., 2010; Ketterer, Brymer, Rhoads, Kraft, & Lovallo, 1996).

Childhood Interpersonal Stress Disrupts Immune Function

Mounting an immune response takes considerable energy and resources, and is best put on hold in the name of survival when the environment demands mobility (e.g., while being chased by a predator). In such a case, resources are best directed toward the fight or flight response, rather than healing. Because our ancestors faced multiple, and sometimes conflicting threats from the environment, our immune system responds not only to internal triggers, such as viruses and bacteria, but also to signals from the HPA axis and sympathetic nervous system (SNS) that alert us to the presence of an external menace (Eisenberger & Cole, 2012; Irwin & Cole, 2011; Cole et al., 2009; Nance & Sanders, 2007). The immune system, HPA axis and SNS respond individually to stress, and each exerts feedback on the others, allowing for complex optimization of responses to both the internal and external environment.

Interpersonal stressors preferentially elicit an inflammatory response, likely via their activation of the SNS, which *enhances* transcription of pro-inflammatory genes, including *IL6* (Cole et al., 2010, reviewed in Eisenberger & Cole, 2012). Equally challenging stressors that are not of an interpersonal nature do not elicit an inflammatory response. For example, a cold and rejecting audience is necessary for the inflammatory effects of the TSST, in which participants

prepare and deliver a speech and solve difficult math problems. During the TSST, production of IL-6, TNF-α and its soluble receptor (sTNFαRII) increase (Dickerson et al., 2009; Slavich, Way, Eisenberger, & Taylor, 2010). In the absence of an audience, subjects find the speech writing and math problems equally challenging and effortful, but experience no rises in inflammation (Dickerson et al., 2009). Thus, the experience of performing a cognitive task while being negatively evaluated leads to significant increases in inflammation, while performing the same task without social evaluation does not. In a similar study, levels of NF-κβ transcription were measured at regular intervals in a cohort of adolescent women. The experience of social rejection was uniquely linked to increased NF-κβ transcription, compared to a variety of other stressors (Murphy et al., 2012). Thus, interpersonally painful stress has been uniquely shown to elicit rises in inflammatory markers both in a laboratory setting, and in naturalistic assessments.

It is not surprising then, that exposure to extreme interpersonal stress in childhood, including abuse, rejection and disrupted relationships, is associated with chronically elevated CRP, glucocorticoid resistance (Carpenter et al., 2007), and elevated cortisol levels (Danese et al., 2008; Fries, Shirtcliff, & Pollak, 2008; MacMillan et al., 2009; van der Vegt, van der Ende, Kirschbaum, Verhulst, & Tiemeier, 2009), all indicative of maladaptive biological responses to chronic stress. Even less severe stressors that persist chronically, such as hostile, cold and unaffectionate parenting styles and family environment, have been associated with higher plasma CRP in adulthood.

Activating the immune system over long periods of time can be adaptive when exposure to pathogens is common, but in the absence of a bacterial infection or wound, chronic activation damages the feedback mechanisms of the stress and inflammatory responses (McEwen, 2000; Taylor, Way, et al., 2006; Wilkinson & Goodyer, 2011). Decreased efficiency of negative

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feedback, in turn, begins to change the checks and balances among the HPA axis, SNS and immune system. For example, after glucocorticoid resistance has occurred, activation of the HPA axis no longer suppresses the transcription of pro-inflammatory cytokine genes (Dhabhar & McEwen, 1999). Chronic or extreme interpersonal stress in childhood has been proposed as part of an allostatic model of depression, in which the frequent and prolonged activation of bodily systems that respond to stress eventually leads to psychopathology and disease (reviewed in McEwen, 2000; Wilkinson & Goodyer, 2011). The ability to identify childhood stressors that lead to inflammation is vital, given the detrimental effects of even small, chronic elevations in inflammatory markers over time, which can include depression, hypertension, and coronary artery disease (Seeman, McEwen, Rowe, & Singer, 2001).

Several researchers have begun to ask why socially painful stressors, particularly rejection or mistreatment of a child by the caregiver, elicit the body's defenses against infections and wounding. Nesse and Ellsworth (2009) and Dhabhar (2009) (summarized in Raison & Miller, 2013), have hypothesized that in our ancestry, feelings of disconnection and rejection acted as a warning of a physical altercation, or loss of protection from the community. Those who were able to mount an early immune response to distressing social situations would have had an advantage in preparing for resultant infections and wounds. Indeed, the biological changes that result from early psychosocial stress appear to ready the body for injuries (Cole et al, 2007; Cole et al., 2011). Cole and colleagues have completed elegant molecular genetic studies showing that psychosocial stress, particularly feelings of loneliness, changes the expression of immunological genes. Older adults who feel lonely much of the time show increased expression in a large profile of over 200 pro-inflammatory genes and decreased expression of anti-inflammatory factors in leukocytes (white blood cells), compared to adults

who report low levels of loneliness (Cole et al., 2007). Further analysis of the type of leukocytes in which gene expression differs revealed that two cell types (monocytes and plasmacytoid dendritic cells) from the ancient, non-specific immune response to tissue damage were responsible for the differences, as opposed to the more recently evolved adaptive immune response (Cole, Hawkley, Arevalo, & Cacioppo, 2011). These observations lend support to the theory that the immune system has evolved to adapt to psychosocial stress by shifting toward a more general, fast-acting inflammatory response that lends itself to wound healing.

According to allostatic load theory, these changes are maladaptive when the child is no longer exposed to danger, and/or when the psychosocial stressors do not result in wounding. Therefore, cross-talk among the immune system, SNS and HPA axis that once promoted optimal functioning in environments that contained both pathogens and predators has become a liability for psychopathology and chronic disease.

Stress, Inflammation and Depression

Research on the relationship between early interpersonal stress and inflammatory outcomes has recently been joined with evidence that childhood stressors are associated in a dose-dependent manner with later depression symptoms (Bifulco, Moran, Baines, Bunn, & Stanford, 2002; Caspi et al., 2003; Nanni, Uher, & Danese, 2012). Given that depression and childhood stress have each been associated with inflammation, the experience of both may be particularly detrimental in terms of disease risk (Danese et al., 2009; Miller & Blackwell, 2006). Thus, the kinds of childhood stressors that evoke biological dysregulation and elevate risk for depression are worth identifying. Two prospective studies have examined the hypothesis that experiencing acute stress in childhood predicts the co-occurrence of depression and inflammation. In the first, adolescent women at risk for depression (defined as either family

history or cognitive vulnerability measured by the Dysfunctional Attitudes Scale), but with no psychiatric history, reported on acute stressors prior to age 15, including: birth to a teenage mother, parental divorce or death, low parental educational attainment, and limited economic resources, as measured by the family's leasing rather than owning the home (Miller & Cole, 2012). Exposure to stressors in childhood was marginally associated with the onset of depression during the two and a half year study. Additionally, the onset of a depressive episode was accompanied by increases in CRP and IL-6, which were more pronounced in young women with one or more early stressors (Miller & Cole, 2012). Young women with early stress were not only more prone to inflammatory elevations at the onset of a depressive episode, but were more likely to maintain the elevations after their depression had resolved. Lingering inflammation, in turn, predicted a recurrence of depression during the study. The authors controlled for a number of confounds affecting inflammatory levels, including waist-to-hip ratio, contraceptive use, and race. Authors did not examine outcomes by stressor content (i.e., interpersonal stress vs. socioeconomic), preventing the characterization of stressors that are particularly predictive of concomitant depression and inflammation.

In a second study, men and women were assessed at regular intervals from age three to age thirty, and were compared based on their experience of one or more acute or chronic stressors in childhood, which included low socioeconomic status; "maltreatment", defined as one or more of the following: maternal rejection, harsh discipline, a change in primary caregiver, sexual or physical abuse; and chronic social isolation between ages 5 and 11 (Danese et al., 2009). Participants were additionally compared by the presence or absence of a depressive episode in the past year. This created four comparison groups representing the co-occurrence of depression and childhood stress, one of these risks, or neither. Elevated CRP levels were

observed in adults who had both childhood stress and a past year depression, compared to those with either depression or childhood stress alone (Danese et al., 2009). The majority of research linking stress to inflammatory markers measures acute stressors, or stressful events that have a discrete time frame, while depression is known to be predicted both by acute ordeals and by stressful situations that persist chronically (Breslau & Davis, 1986; Hammen, Kim, Eberhart, & Brennan, 2009; Hammen, Marks, Mayol, & DeMayo, 1985). Although not specifically discussed by Danese and colleagues, the design of this study affords the opportunity to examine the separate contributions of chronic and acute stress in childhood to the clustering of inflammation and depression. Children exposed to acute maltreatment or chronic social isolation were all more likely to become depressed, and together these experiences accounted for 32% of the variance in depression. Interestingly, socioeconomic status was not related to depression outcomes. A similar pattern was described for CRP levels, where high levels of chronic or acute stress were each associated with a one and a half fold increase in CRP levels. Again, socioeconomic status was not a significant predictor of CRP. Thus, acute and chronic social stressors made depression more likely and predicted CRP levels. Although the authors do not address the lack of results for socioeconomic status, the absence of any socioeconomic effect on inflammation and depression may indicate that interpersonal stressors were more relevant than socioeconomic disadvantage to increases in inflammation and depression.

      The review of the research to this point indicates that interpersonal stress in childhood is a potent predictor of depression and inflammatory dysregulation. Therefore, taken together, the literature regarding exposure to childhood stress appears to demonstrate the dissertation's proposed relationships among stress, depression and inflammation. Early childhood stress activates biological responses, including inflammation, that may have been adaptive in response

to stress in our evolutionary past. This activation puts wear and tear on the stress response, leading to further biological dysregulation and heightened risk of depression. Depression, in turn, is known to promote further interpersonal difficulties, inflammation and glucocorticoid resistance. Thus, biological responses to stress that may have been adaptive in our ancestry may now promote a continuation of vulnerability to disease.

Proposed G×E Between Stress and Genes of the Innate Immune System

The research discussed thus far outlines a pathway from experiences of childhood stressors to depression via the inflammatory response to stress. Despite a large literature connecting the biological pathways that are altered by stress to the pathophysiology of depression, most children are in fact resilient to stress, and do not develop biological or emotional problems in adulthood (Dumont & Provost, 1999).

Candidate gene studies have been crucial in understanding why some people develop biological changes and depression after emotionally painful experiences. The substitution of one nucleotide can lead to drastic differences in the biological response to stress. For example, substitution of a guanine for a cytosine at *CRHR1* (rs. 110402), changes the configuration of the receptor for corticotrophin releasing hormone (CRH), leading to greater cortisol release by the HPA axis in individuals exposed to early maltreatment. Similarly, the 'short' variant of the serotonin transporter gene (*5-HTTLPR*) enhances cortisol reactivity to the TSST, and makes depression onset more likely in persons exposed to early abuse and other stressors (Taylor, Way, et al., 2006; Taylor, Way, & Seeman, 2011). The immune system is newly implicated in depression etiology, and the inconsistent relationship between elevated pro-inflammatory cytokine production and depression across research samples encourages exploration of potential genetic moderators of cytokine production in response to stress. It may be that only persons with

stress-sensitive inflammatory genotypes produce excessive inflammation in response to stress, which impacts the functioning of the SNS and HPA axis, and over time promotes depression.

This section of the review will describe how genes of the innate immune response are likely to act as diatheses for depression in response to stress. A description of how polymorphisms in the promoter regions of three pro-inflammatory cytokine genes meet the necessary criteria for investigating interactions between genes and the environment will be outlined, and emerging evidence that these genes promote depression in response to interpersonal stress will be reviewed (Moffitt, Caspi & Rutter, 2005).

Genes of interest must meet three criteria to be considered plausible as a diathesis for depression: they must have functional effects on a biological system that is implicated in depression, they must alter stress reactivity, and the identified variation must occur with enough frequency to account for measurable differences in depression susceptibility. The present dissertation focuses on a single nucleotide polymorphism (SNP) located in the promoter region of *IL6,* that meets each of these criteria, and additionally investigates SNPS in *TNF* and *IL1β*, which have received less attention in the psychiatry literature, but appear promising as moderators of the stress-depression relationship.

Variation at *IL6* (-174 G > C, rs.1800795; Smith & Humphries, 2009) increases the production of IL-6 mRNA (Cole et al., 2010; Cole et al., 2011) and has been associated with higher levels of CRP, which is released by the liver in response to IL-6. A SNP in the promoter region of *IL1β* (-511C > T, rs.16944) is associated with increased IL-1β expression in T-allele carriers, although the mechanism of increased expression is unknown. A nearby polymorphism (-31, T > C) may be the functional SNP responsible for increased expression, as this locus has the strongest association with heritable differences in *IL1β* mRNA. The two are in high linkage

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disequilibrium, however, making them indistinguishable for the purposes of the dissertation (Dixon et al., 2007). Finally, A SNP of *TNF* (-308 G >A, rs1800629; not to be confused with rs76917, mentioned earlier, which is associated with depression by GWAS and has no known functionality) is hypothesized to increase production of TNF-α based on its association with infectious and autoimmune disease (Abraham & Kroeger, 1999; McGuire, Hill, Allsopp, Greenwood, & Kwiatkowski, 1994; Nadel, Newport, Booy, & Levin, 1996). In vivo studies have produced mixed results regarding the impact of this SNP on actual plasma levels of TNF-α (Bayley, Ottenhoff, & Verweij, 2004 in Smith & Humphries, 2009).

IL6 (-174G>C) has received attention in the literature for its effects on interpersonal stress-related *IL6* expression (Cole et al., 2010; Schultze-Florey et al., 2012). In vitro, the stress hormone norepinephrine increases transcription of IL-6 mRNA, which is in agreement with the findings presented thus far, indicating a shift toward pro-inflammatory mechanisms in response to interpersonal stress (Cole et al., 2010). Further, an interactive effect was detected such that a G allele at *IL6* led to increased transcription following application of norepinephrine, compared to the CC genotype, which showed negligible changes in *IL6* transcription in response to norepinephrine. In a second in vitro study, carcinoma cells extracted from women with ovarian cancer were examined for effects of social support and depressive symptoms on gene expression. In agreement with their first findings, Cole et al. (2011) identified a norepinephrine-dependent transcription factor that was elevated in patients with low social support and high levels of depressive symptoms, compared to those with high social support and few depressive symptoms. Further, in the presence of the "high-transcription" G allele at *IL6*, the norepinephrine-dependent factor was able to bind and increase transcription of *IL6* mRNA, compared to the CC genotype. A naturalistic study of women who were recently widowed supports the in vitro finding that the

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stress of social disconnection interacts with *IL6* genotype to increase the production of IL-6. Scores on an inventory of complicated grief symptoms were found to interact with *IL6* genotype to predict higher circulating levels of IL-6 in women with the GG genotype, compared to GC or CC genotypes (Schultze-Florey et al., 2012).

Although not previously investigated as moderators, *TNF* -308G>A has been directly linked to depressive symptoms in women with breast cancer, a context likely to include stress exposure, and to major depressive disorder, and a higher rate of attempted suicide among patients with MDD (Bower et al., 2013; Jun et al., 2003; Kim et al., 2013, but see Lotrich, Ferrell, Rabinovitz, & Pollock, 2010, for non-significant relationship to depression in interferon-alpha patients), and *IL1β* -511C>T has been found to predict failure to remit after antidepressant treatment (Baune et al., 2010). Stress exposure was not measured in these investigations, and to our knowledge no study has undertaken a gene-environment approach to understanding the impact of these loci on risk for depression.

Although the interaction between interpersonal stress and *IL6* genotype as a predictor of depression has not been examined, two studies have shown effects of *IL6* on depression in the context of IFN-α therapy and cancer treatment. The results of these studies suggest that the effects of medically increased inflammation on depression are moderated by *IL6, TNF* and *IL1β* (Bower et al., 2013; Bull et al., 2008). Among women with early stage breast cancer who recently completed surgery, radiation or chemotherapy, a count of alleles associated with greater gene expression in the promoter regions of *IL6, TNF* and *IL1β,* was predictive of depression and fatigue symptoms (Bower et al., 2013). It is possible that the psychosocial stress and elevated inflammation inherent in cancer diagnosis and treatment contributed to an underlying interaction between stress/inflammation and genetic risk that resulted in differing levels of depression by

genotype. Thus, what was reported as a main effect of genotype on depressive symptoms might actually be the result of an underlying interactive effect, in which the high expression genotypes are more depressogenic than the low expression genotypes under conditions of stress.

Previous work in IFN-α treatment also supports an increased liability for depression in patients with the GG or GC genotype at *IL6* (Bull et al., 2008). Patients with the low-expressing *IL6* genotype (CC) demonstrated the established sensitizing effect of *5-HTTLPR* on depression outcomes, such that S allele carriers were more likely to become depressed compared to LL homozygotes. However, participants with the high expressing *IL6* genotype (GC/GG) were susceptible to depression during IFN-α treatment regardless of *5-HTTLPR* genotype. As described earlier, possession of the minor S allele at the *5-HTTLPR* results in decreased transcription of the serotonin transporter, which may enhance serotonin changes caused by inflammation (Fredericks et al., 2010).

Antagonistic Pleiotropy

A single study has found evidence that *IL6* genotype may have opposite effects on stress-induced inflammation in the young and old. In adolescents, the high transcription genotype (GG) of *IL6* was associated with *lower* circulating CRP under conditions of social and economic stress compared to C allele carriers (Cole et al., 2011). Allelic variation that has contrasting effects on fitness is known as antagonistic pleiotropy. This study did not examine depression or illness outcomes, but provides evidence that the relationship between pro-inflammatory cytokine genotype and systemic inflammation may differ by age. Cole et al. (2011) hypothesize that the G allele has undergone natural selection for its protection against stress-induced inflammation during the reproductive years, and additionally posit that G allele carriers' inflammatory response becomes sensitized to stress after child-bearing age (Cole et al., 2011). Therefore,

young people may benefit from having the GG genotype, as it protects against stress-induced inflammation, but have been shown to be worse off than their C allele carrier peers once past reproductive age, based on the their increased production of IL-6 in response to stress (Schultz-Florey et al., 2012), higher incidence of inflammatory illness in the context of depression (Cole et al., 2010), and propensity for depression in response to medically induced inflammation (Bower et al., 2013; Bull et al., 2008).

Summary

The role of inflammation in depression etiology has received increasing attention in the past decade, and research into the neurobiological mechanisms linking inflammatory processes to depressive symptoms has developed substantially. What is currently known is that high levels of inflammation, as in interferon alpha treatment, can be causal in depression, and the neurobiological mechanisms of this reaction have been partially elucidated. Inflammation crosses the blood brain barrier, disrupting serotonin neurotransmission and neuron growth processes, leading to psychological and behavioral changes identical to those seen in depression. Further, abnormal inflammatory activity occurs in about one-third of depressed patients, even in the absence of other inflammatory illness. This is likely a symptom of deterioration of the biological stress response through overuse. While these developments help to explain possible mechanisms of depression-induced inflammation and inflammation-induced depression, they fail to explain why only a subset of depressed patients develop abnormal immune function. The specific vulnerabilities that make the confluence of depression and inflammation more likely remain unknown. Depression and chronic inflammation are each associated with increased risk for inflammatory diseases, and their co-occurrence is more likely in the context of stress (Danese et al., 2008, 2009; Miller & Cole, 2012).The present dissertation will explore the relationships

among interpersonal stress, depressive symptoms and C-reactive protein, and a possible moderation of these relationships by genetic variation of the immune system. The following section summarizes key unresolved issues and presents a general plan for addressing several of these issues in the two studies that comprise the dissertation.

Project Overview

Identifying factors that increase liability for the co-occurrence of depression and inflammation is an important and logical next step in the study of depression etiology (Raison & Miller, 2011). Although the idea that the immune system could be involved in the pathophysiology of depression was once novel and even controversial, our understanding has now moved beyond simply observing innate immune activity in depressed patients, and empirical investigation must progress from broad association studies to the investigation of individual differences that lead to the co-occurrence of depression and inflammation. A greater understanding of the mechanisms by which each contributes to the other is not only at the edge of our knowledge of depression etiology, it has the potential to shed light on the increased prevalence of inflammatory illnesses in depressed patients, which is of growing concern in our aging population (Lutz, Sanderson, & Scherbov, 2008).

The present dissertation seeks to address several unanswered questions regarding genetic and psychosocial factors that characterize persons at greatest risk of developing both chronic inflammation and depression. In the first study, data drawn from a community sample of 444 Australian youth with a high prevalence of familial risk for depression will be used to examine allelic variation at *IL6*, *IL1β* and *TNF* as vulnerabilities for interpersonal stress-related depression. A gene-environment interaction between genetic predisposition toward the

production of pro-inflammatory cytokines, and the amount of exposure to interpersonal stress in the 12 months prior to age 20 will be examined as a predictor of symptom counts.

In study 2 of the dissertation, the relationship across time between depressive symptoms and chronic inflammatory activity will be modeled using data drawn from 4,348 participants in the Coronary Artery Risk Development in Young Adults (CARDIA) study. CARDIA collected longitudinal epidemiologic data and was funded by the National Heart, Lung, and Blood Institute (NHLBI). Measurements of depressive symptoms, operationalized by the Center of Epidemiological Studies Depression Scale (CES-D), and plasma concentrations of C-reactive protein were taken at 5 and 4 assessment time points, respectively, between mean ages 30 and 50. The dissertation will address the following five questions in these two datasets:

1) What is the nature of the relationship between depressive symptoms and CRP across late adolescence and into midlife?

Study 2 examines the relationship between self-reported depressive symptoms and blood plasma concentrations of C-reactive protein between mean ages 30 and 50, using a longitudinal dataset.

2) Does early life stress contribute to depressive symptoms across late adolescence and into midlife? To CRP?

Study 2 examines a retrospective account of adverse family environment prior to age 18, as a predictor of: 1) the initial status and subsequent growth in self-reported depressive symptoms across time; 2) the initial status and subsequent growth in blood plasma C-reactive protein concentration, and 3) the relationship between the two growth curves.

3) Does early life stress strengthen the relationship between depressive symptoms and CRP concentration?

Study 2 examines the contribution of early life stress to self-reported depressive symptoms and levels of plasma C-reactive protein in adulthood. Further, study 2 examines early life stress as a moderator of the relationship between self-reported depressive symptoms and growth in CRP concentration.

4) Does allelic variation at *IL6, IL1β* and *TNF* affect the risk of depression in response to stress?

Study 1 examines the moderation of the stress-depression relationship by each of the three SNPs, while study 2 examines moderation by *IL6* only. Together, study 1 and study 2 offer the opportunity to compare the effects of *IL6* on the stress-depression relationship in youth at mean age 20, to participants followed from mean age 30 to mean age 50, allowing for a preliminary examination of the antagonistic pleiotropy hypothesis. Study 1 additionally examines whether genetic moderation is specific to chronic interpersonal stress, or generalizes across interpersonal and non-interpersonal stressors.

5) Does *IL6* predict plasma CRP concentrations? Does *IL6* strengthen the predictive relationship from an early adverse environment to adult plasma CRP concentrations? Or the relationship between depressive symptoms and plasma CRP concentrations?

Study 2 examines genetic variation at *IL6* as a direct predictor of plasma CRP concentrations, and also as a moderator of plasma CRP concentrations as a function of exposure to early life stress. Finally, *IL6* is examined as a moderator of the relationship between depressive symptoms and CRP.

Chapter 2: Effects of chronic interpersonal stress exposure on depressive symptoms are moderated by genetic variation at *IL6* and *IL1β* in youth

Abstract

The aim of the present study was to investigate a trio of functional SNPs in the promoter regions of *IL6* (-174G>C, rs1800795), *IL1β* (-511C>T, rs16944), and *TNF* (-308G>A, rs1800629) as moderators of the relationship between chronic stress exposure and elevations in depressive symptoms. Participants were 444 Australian youth (mean age = 20.12) whose exposure to chronic stress in the past 6 months was assessed using the semi-structured UCLA Life Stress Interview, and who completed the Beck Depression Inventory II at ages 15 and 20. Between ages 22 and 25, all participants in the selected sample provided blood samples for genotyping. In line with a hypothesized moderation effect, -174G allele carriers at *IL6* had *fewer* depressive symptoms following interpersonal stress, relative to C/C homozygotes with equal interpersonal stress exposure. However, *IL6* genotype did not moderate the effects of non-interpersonal stress exposure (i.e., financial, work and health-related difficulties) on depression. Also in line with hypotheses, the -511C allele in *IL1β*, previously associated with higher IL-1β expression, was associated with more severe depression following chronic interpersonal stress exposure, relative to T/T homozygotes. Again, the moderating effect did not generalize to non-interpersonal stress. *TNF* was not a moderator of the effects of either type of stress on depression outcomes. Findings were consistent with the hypothesis that pro-inflammatory genetic variation increases the risk of stress-induced depression.

Introduction

Depression is moderately heritable and often manifests following exposure to psychosocial stress (Kendler et al., 1995; Kendler, Thornton, & Gardner, 2000, 2001). Activation of the inflammatory response, measured by increased plasma levels of pro-inflammatory cytokines, including interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor-necrosis factor alpha (TNF-α), and C-reactive protein (CRP), has been shown to follow exposure to psychosocial stress, and is associated with rises in depressive symptoms and with major depressive disorder (Matthews et al., 2007, 2010; Miller & Cole, 2012; Pasco et al., 2010; van den Biggelaar et al., 2007). Genetic variants that enhance immune reactivity may enhance depressive reactions to stress, creating vulnerability to depressive disorders. To our knowledge, the present study is the first to examine the interaction between stress exposure and polymorphisms of the inflammatory response as a predictor of depressive symptoms.

Interpersonal stress strongly elicits depressive symptoms, and also increases pro-inflammatory cytokine production (Dickerson et al., 2009; Monroe et al., 1999; Slavich et al., 2010). Chronic exposure to interpersonal stress is associated with persistent and unhealthy inflammatory activity (Dhabhar, 2009, 2014; Miller, Maletic, & Raison, 2009). For instance, older adults who endorse high levels of loneliness show increased expression of pro-inflammatory genes and decreased expression of anti-inflammatory factors in leukocytes, compared to those who feel socially integrated (Cole et al., 2007).

A functional SNP in *IL6* (-174G>C) has received attention in the literature for its effects on interpersonal stress-related *IL6* expression (Cole et al., 2010; Schultze-Florey et al., 2012). Possession of a G allele at -174 increases expression of IL-6 mRNA and plasma levels of IL-6,

following exposure to the stress hormone norepinephrine, and also in response to grief in midlife and older adults (Cole et al., 2010; Schultz-Florey et al., 2012).

Of developmental relevance to our study, this locus may have opposite effects on cytokine expression in response to interpersonal stressors in youth. In a study of 17-19 year olds exposed to varying levels of psychosocial stress, those with CC genotypes had greater plasma CRP, a marker of chronic IL-6 production, compared to GG or GC genotypes (Cole et al., 2011). One interpretation of the changing allele of risk by age is that the G allele provides protection against inflammation prior to reproductive age, but becomes sensitized to stress during the transition to adulthood, likely reflecting evolutionary adaptations to promote survival early on in life (Cole et al., 2010; Williams, 1957).

The primary aim of the present study was to examine *IL6* (-174G>C, rs1800795; Smith & Humphries, 2009) as a moderator of depressive reactions to chronic stress exposure in a large cohort of adolescents, given the relevance of *IL6* to stress-induced inflammation and mood. We were also interested in examining *IL1β*, (–511C>T, rs16944; Dixon et al., 2007) and *TNF* (-308G>A, rs1800629, Abraham & Kroeger, 1999; McGuire et al., 1994; Nadel et al., 1996, but see Bayley et al., 2004) as moderators of the stress-depression relationship, based on their association with changes in pro-inflammatory cytokine expression, and preliminary evidence of their relevance to mood symptoms (Baune et al., 2010; Jun et al., 2003; Kim et al., 2013).

Given the young age of the present sample, and the findings of Cole et al. (2010), we hypothesized that the C allele in *IL6* would confer risk for stress-induced changes in depression. We hypothesized that the high expression variants of *IL1β* and *TNF* would potentiate increases in depressive symptoms following exposure to chronic stress. We were particularly interested in

whether genetic moderation would be specific to chronic interpersonal stress, or would

generalize to non-interpersonal stressors as well.

Method

Participants

Participants were 444 young adults drawn from the much larger University of

Queensland Study of Pregnancy (MUSP) birth cohort, based on their participation in a genetic

substudy. The parent study enrolled 7,223 publicly supported maternity patients and their

children, who were part of a birth cohort at Mater Misericordiae Mothers' Hospital, in Brisbane,

Australia between 1981 and 1984 (Keeping et al., 1989). Mothers in the original MUSP cohort

completed self-report questionnaires that measured symptoms of depression using the Delusions-

Symptoms States Inventory (DSSI; Bedford, Foulds, & Sheffield, 1976; Bedford & Foulds,

1977) during pregnancy, at birth, and at child ages 6 months and 5 years (see Najman et al., 2005

for full details of the sample selection). When the children turned 15 years old, a sample of 815

families was selected, oversampling for maternal depression, for a substudy of outcomes in

offspring of depressed and never-depressed mothers. Further details concerning the selection of

this sample are provided in Hammen & Brennan, 2001.

Participants in the selected sample were re-contacted when youth turned 20 for additional

psychological assessment, and 706 mother-child pairs completed the age 20 data collection.

Between the ages of 22 and 25, the 815 youth who participated in the age 15 assessment were

invited to provide blood samples for genetic analyses. Failure to respond to requests for

participation, medical problems, death and invalid DNA samples resulted in a total of 444

participants who contributed blood samples for the genetic data collection, and the current study

sample is limited to these individuals, focusing on psychosocial measures taken at ages 15 and

20, supplemented by health survey data collected in early life that assessed the presence or absence of asthma. This final sample was evenly split by gender (49.4% female), predominantly Caucasian (92.1%), and came from lower and lower-middle income homes. Non-Caucasian participants were Asian (4.1%), Maori/Pacific Islander (1.1%), Australian Aborigine (2%) and other (0.2%). Those who completed the genetic study were more likely to be female ($\chi^2(1) =$ 33.66, $p < .001$), but were no more likely to have mothers with a history of depression ($\chi^2(1) =$ .013, $n.s.$) compared to youth that did not complete the genetic study. There were no differences in ethnicity ($\chi^2(5) = 6.40$, $n.s.$), depressive symptoms at ages 15 or 20 ($t(631) = -1.44$, $n.s$; $t(803) = -.83$, $n.s.$), or in chronic stress exposure in the 12 months prior to age 20 (interpersonal: $t(813) = -1.97$, $n.s$; non-interpersonal: $t(813) = 1.26$, $n.s$), between the overall sample and the genetic subsample.

Procedure

Mothers and children in the sample completed the Structured Clinical Interview for DSM-IV (SCID-IV) and self-report measures of symptoms, social functioning, and family environment when offspring turned 15, and again when they turned 20. Information was gathered in the family home or a location convenient for the participants and interviewer. Postgraduate psychology students were trained to appropriately conduct and reliably score these interviews. Participants all gave informed consent, or assent in the case of minors. The institutional review or ethics panels of the University of Queensland, University of California, Los Angeles, and Emory University approved the research protocol.

Youth were contacted in 2006, between ages 22 and 25, regarding participation in a blood draw for genotyping purposes. Those who wished to participate in the genotyping study were mailed consent forms, a blood collection kit, and questionnaires. They were instructed to have a

blood draw completed by a local phlebotomist, and the samples were then picked up by courier

and transported to the Genetic Epidemiological Laboratory of the Queensland Institute of

Medical Research (QIMR). DNA extraction from leukocytes took place via the salting out

method (Miller, Dykes, & Polesky, 1988). The resultant "stock" DNA was eluted in 400μl of 1 x

Tris-EDTA buffer (10mM Tris pH 8.0, 1mM EDTA pH 8.0) and ranged in concentration from

100ng/μl - 100μg/μl. DNA samples were stored at the QIMR laboratory until 2011, when

aliquots of DNA were sent to the UCLA Social Genomics Core Laboratory for genotyping.

Allelic variation at *IL6* (G > C; rs1800795), *IL1β* (-511 C > T; rs16944) and *TNF* (-308 G >A;

rs1800629) was assayed by a commercial TaqMan Genotyping Assay (Applied Biosystems,

Foster City, CA) performed on an iCycler real-time PCR instrument (BioRad, Hercules, CA)

following the manufacturer's specified protocol (Cole et al., 2010).

Test-retest reliability of duplicated specimens yielded a total genotyping error rate below

1%. All three polymorphisms were in Hardy-Weinberg equilibrium (χ^2 (1, 444) values: *IL1β* -

511 = 0.26, *IL6* -174 = 0.13, *TNF* -308 = 0.08, all *p*-values > .05). Population-level minor allele

frequencies of the three SNPs were 18.5% at *IL6*, 46.5% at *IL1β*, and 9.6% at *TNF*, indicating

that allelic variation at these loci is common, and could reasonably account for differences in

depressive symptoms.

Measures

Depression symptoms. Youth self-report of depression symptoms was collected at ages

15 and 20 using the Beck Depression Inventory-II (BDI-II; (Beck, Steer, & Brown, 1996), a

continuous measure that has shown excellent internal consistency, and has been validated against

clinical ratings of depression (Beck, Steer, & Carbin, 1988). Internal consistency was very good

in the present sample (Crohnbach's α =.90). Scores on the BDI-II at age 15 ranged from 0 to 32

(M = 6.20, SD = 6.19). At age 20, scores ranged from 0-52 (M = 7.44, SD = 8.40; see Table 1). Use of a continuous measure of depressive symptoms provides a sensitive test of the present study's gene-environment interaction hypotheses, while the use of diagnostic categories of depressive disorders would greatly reduce the ability to detect effects. Further, diagnostic categories of depressive disorders have shown only slight advantage over subsyndromal depressive symptoms as indicators of functional impairment (Judd, Paulus, Wells, & Rapaport, 1996).

Chronic Interpersonal Stress. Youth were interviewed at age 20 using the semi-structured UCLA Life Stress Interview (LSI; Hammen & Brennan, 2001), which was developed to assess chronic, ongoing stressful conditions in major life domains, as well as acutely stressful life events, and has been validated for use in young adult samples (Adrian & Hammen, 1993; Rao, Hammen, & Daley, 1999). Using a set of standardized questions and follow up prompts, interviewers asked participants to describe their functioning in eight different life domains, half of them related to social functioning, over the past six months. Functioning in each domain was then scored on a scale of 1 to 5, with half-points permitted, where 1 indicates superior functioning and 5 indicates significantly impaired functioning. For example, in the Romantic Relationship domain, a score of 1 would be given to an individual who has a stable romantic relationship that is low in conflict and high in self-disclosure, and who engages in frequent positive interactions with their partner. An individual who reports a close relationship that is lacking in disclosure, or who experiences regular conflict in their relationship would score a 3. An individual who reports isolation from their partner or severe or frequent conflict with their partner would receive a score of 5. The four domains with social content (romantic relationships, relationship with a best friend, family relationships, and social life) were then summed to create

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a composite score reflecting chronic interpersonal stress, where higher scores indicate greater distress. The remaining four domains (work, finances, personal health, family health) were summed to create a composite measure of non-interpersonal chronic stress. Composite scores across interpersonal domains ranged from 4 to 18.5 with a mean of 10.04 (SD = 2.59). Composite scores across non-interpersonal domains ranged from 3 to 15 with a mean of 7.59 and a standard deviation of 2.02.

Maternal Depression. Mothers were interviewed when their child turned 15, using the Structured Clinical Interview for DSM-IV (SCID-IV; (First, 1995; First, Spitzer, Gibbon, & Williams, 2012). Nearly half (43.5%) of the mothers in this high-risk sample met criteria for a current or past episode of major depression or dysthymic disorder by youth age 15.

Statistical Analysis

All statistical tests were two-sided and conducted using SPSS statistical package, version 18.0 for Macintosh. The simple effects of significant interactions were examined in Stata 12.0 for Macintosh. All stress variables were mean-centered to reduce multicollinearity, and missing data (3 cases at age 15, and 6 cases at age 20) were replaced with the variable mean. Maternal depression was entered as a covariate in all analyses, as depression in the mother has been linked to increased exposure to psychosocial stress and also contributes heritable risk for depression. Additionally, gender and scores on the BDI-II at age 15 were entered as covariates to insure that earlier depressive symptoms did not better account for current symptoms. Childhood asthma was the most common inflammatory illness based on health diagnoses recorded at age 5, and was entered as a covariate to control for possible confounding effects of heightened inflammation on depression.

Linear regression was used to test stress exposure in the past 6 months, inflammatory genotypes, and the interactions of stress and genotype, as predictors of depressive symptoms at age 20. All genotypes were coded as three-level variables (0 = minor allele homozygotes, 1 = heterozygotes, 2 = major allele homozygotes). Post-hoc contrasts were planned to evaluate the simple effects of significant interactions between genotype and stress. At *IL1β* and *TNF*, contrasts evaluated the hypothesis that dominant homozygotes would have greater levels of depression compared to heterozygotes and to recessive homozygotes. In adolescents, the allele of risk at *IL6* has been identified as -174C, and post-hoc contrasts compared CC homozygotes to CG and GG genotypes. In light of the conflicting findings regarding the allele of risk at *IL6*, we also compared CG and GG genotypes. Given the a priori hypotheses and exploratory nature of the analyses, correction for multiple testing was not planned. Following the primary analyses, the models were re-run restricting the sample to Caucasians, the largest ethnic group, to examine the impact of ethnicity on genetic effects.

Results

We first tested the specificity of chronic interpersonal stress as a predictor of depressive symptoms. Both interpersonal and non-interpersonal stress remained significant when entered in a single model predicting age 20 BDI-II score (b = 0.92, SE = 0.17, p < .001 for interpersonal stress; b = 0.91, SE = 0.21, p < .001 for non-interpersonal stress).

In line with previous work in young, physically healthy samples (Misener et al., 2007), the individual genetic polymorphisms did not predict depressive symptoms at age 20 (all p values > .10). Inflammatory genotypes were then examined as predictors of the magnitude of chronic stress exposure. *IL1β* genotype was found to predict chronic non-interpersonal stress exposure (b = -.36, SE = .14, p = .01), such that exposure increased with the number of minor

alleles. There were no significant associations of *IL6* or *TNF* with either interpersonal or non-interpersonal stress exposure (*p* values between .54 and .89).

Moderation of the depression and stress exposure relationship by genotype

In all models, maternal depression, age 15 BDI-II score, and exposure to chronic interpersonal stress remained significant as predictors of age 20 depressive symptoms following the addition of the main and interactive effects of genotype and stress (all *p*'s < .01, see Table 2 for interpersonal stress results).

Moderation by *IL6* genotype. Linear regression was used to determine whether allelic variation at *IL6* might moderate the impact of stress exposure on age 20 depression severity, over and above the effects of the covariates. An interaction of *IL6* genotype and chronic interpersonal stress exposure was detected and deconstructed into its simple effects (*b* = -.44 [95% CI: -.803 to -.078], ΔR^2 = 0.01, ΔF = 5.71, *p* = .017, see Table 2). Post-hoc contrasts revealed significantly higher depressive symptoms in CC homozygotes compared with GG homozygotes (*b* = -0.98, SE = 0.42, *p* = .02), and marginally higher depressive symptoms in CC homozygotes compared with heterozygotes, given equal exposure to chronic interpersonal stress (*b* = -0.58, SE = 0.31, *p* = .07; see Figure 1). The contrast between GG and GC genotypes was not significant (*b* = 0.40, SE = 0.41, *p* = .34).

IL6 genotype did not moderate the effects of chronic non-interpersonal stress on depressive symptoms at age 20 (*b* = -.36 [95% CI: -0.82 to 0.10], ΔR^2 = 0.004, ΔF = 2.40, *p* = .12).

Moderation by *IL1β* and *TNF* genotypes. The interaction of *IL1β* genotype and chronic interpersonal stress exposure predicted later depressive symptoms, even accounting for the effects of the covariates (*b* = 0.44, [95% CI: 0.06 to 0.82], ΔR^2 = .009, ΔF = 5.29, *p* = .022, see

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Table 2 and Figure 2). Contrasts between genotypes at *IL1β* revealed marginally significant

simple effects, such that CC homozygotes developed more depressive symptoms relative to TT

homozygotes ($b = 0.88$, SE $= 0.45$, $p = .05$), and relative to heterozygotes ($b = 0.59$, SE $= 0.31$, p

$= .06$).

Allelic variation at *IL1β* was not a significant moderator of the effects of non-

interpersonal stress exposure on depressive symptoms ($b = .43$, SE $= .26$, $p = .10$).

Variation at *TNF* was not associated with differences in depressive symptoms as a

function of chronic interpersonal stress ($b = -.41$, SE $= 0.26$, $p = .11$, see Table 2), but had a

marginally significant effect on depressive symptoms in response to non-interpersonal stress ($b =$

-0.60 [95% CI: -1.27 to 0.06], $\Delta R^2 = 0.005$, $\Delta F = 3.18$, $p = .08$). The simple effects of the *TNF*

$\times$ non-interpersonal stress interaction were not significant (AA vs. GA: $b = -0.69$, SE $= 2.01$, $p =$

0.73; AA vs GG: $b = -1.12$, SE $= 2.00$, $p = .57$)[1].

Following the primary analyses, we reran the models restricting the sample to

Caucasians, the predominant ethnicity. The interaction effect of chronic interpersonal stress and

IL6 genotype on depression severity held under these conditions: $b = -.48$ [95% CI: -0.86 to $-$

0.11], $\Delta R^2 = 0.01$, $\Delta F = 6.36$, $p = .01$. Consistent with the results in the full sample, *IL6*

genotype was not a moderator of the relationship between chronic non-interpersonal stress and

depression ($b = -.39$, [95% CI: -0.87 to -0.08], $\Delta R^2 = 0.01$, $\Delta F = 2.63$, $p = .11$). The interaction

of chronic interpersonal stress and *IL1β* continued to predict depressive symptomatology: $b =$

0.49, [95% CI: 0.10 to 0.88], $\Delta R^2 = .01$, $\Delta F = 6.01$, $p = .02$, when restricted to Caucasians.

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[1] Based on the results obtained with the individual genotypes, an exploratory analysis of additive risk at *IL6* and *IL1β* (-174C in *IL6* and -155T in *IL1β*) was conducted. A risk index was created with a range of 0-4 risk alleles, and tested as a moderator of chronic interpersonal stress at age 20, predicting BDI-II score, controlling for maternal depression, asthma, gender and age 15 BDI-II. The interaction of the genetic index and stress was significant (B = 0.45, SE = 0.14, $p = .001$), with each additional risk allele predicting greater mean depressive symptoms, *except* for participants who had zero risk alleles. Participants with zero risk alleles had the second lowest mean depression scores, between participants with one and participants with two risk alleles. When repeated in the sample's largest ethnic group (Caucasians), the interaction term held (B = .51, SE = .14, $p < .001$). Although this analysis had increased power to detect effects, it was not based on our original hypotheses, and for this reason, we do not include it as a primary analysis.

Further, *IL1β* remained non-significant as a moderator of non-interpersonal stress, although the *p*-value was reduced to .08: $b = 0.49$, [95% CI: -0.05 to 1.03], $\Delta R^2 = .01$, $\Delta F = 3.17$, $p = .08$. The interaction effects of *TNF* and chronic stress remained non-significant (*TNF* × interpersonal stress: $b = -.45$, $SE = 0.27$, $p = .10$, *TNF* × non-interpersonal stress: $b = -0.58$, $SE = 0.35$, $p = .10$). Analyses were repeated using similar stress and depression variables at the age 15 measurement time point, when the range in BDI-II symptoms (0-32) reflected lower severity of depression, compared with 0-52 at age 20, and no pattern of gene-environment interaction was identified [details available on request].

Discussion

The aim of the present study was to evaluate the interaction of chronic stress with three pro-inflammatory polymorphisms as precipitants of depressive symptoms in young adulthood. We hypothesized that variation at *IL6, IL1β* and *TNF* would potentiate the effects of interpersonal stress on the development of depressive symptoms. As hypothesized, interactive effects were revealed at loci in *IL6* and *IL1β* that accounted for differences in depressive symptoms, over and above the contributions of maternal depression history, earlier depressive symptoms, gender, and asthma diagnosis. These interactive effects were specific to interpersonal stress, as findings for non-interpersonal stress exposure were not significant. Findings were also specific to the age 20 time point. Of note, the range of BDI-II scores at age 15 (0-32) indicated lower severity of depression compared to age 20, when scores ranged from 0-52. A possibility is that the identified genetic effects emerge in late adolescence, which is in line with the antagonistic pleiotropy hypothesis generated by Cole et al. (2011) for the effects of *IL6*. Previous longitudinal work with twins has also found changes in genetic and environmental influences on depression (Kendler, Gardner, & Lichtenstein, 2008; Lau & Eley, 2008).

There were no main effects of genotype on depressive symptoms. Interestingly, the present study also found evidence of a gene-environment correlation at *IL1β,* so that exposure to non-interpersonal stress increased with each -511C allele. Further explication of this effect was not an aim of the present study and awaits future replication.

The pattern of results for *IL1β* is consistent with previous work that identified the high expression -511C allele as a risk for poor response to pharmacotherapies for depression, and for early onset of depression and higher depressive symptom severity (Dixon et al., 2007; Hwang et al., 2009; Tadić et al., 2008; Yu, Chen, Hong, Chen, & Tsai, 2003). No previous empirical work has examined a gene-environment interaction at this locus, but the finding is in line with the pathogen host defense theory (PATHOS-D), which posits that pro-inflammatory genotypes enhance depression risk (Raison & Miller, 2013).

Results of analyses at *IL6* revealed that participants homozygous for -174C developed higher levels of depressive symptoms following exposure to chronic interpersonal stress when compared to those with one or more -174G alleles. This finding is consistent with a previous investigation that found elevations in plasma CRP following stress exposure in adolescents homozygous for -174C compared with -174G carriers (Cole et al., 2011). In contrast, -174G is associated with increased IL-6 expression following stress exposure in older adults (Cole et al., 2010; Schultz-Florey et al., 2012). To explain these conflicting results, Cole et al. (2011) hypothesize that -174G has undergone selection for its protection against stress-induced inflammation in adolescence, and becomes sensitized to stress after child-bearing age is reached. This is consistent with age-dependent antagonistic pleiotropy, as any detrimental effects of -174G on survival after reproductive age would not affect its retention in the population (Williams, 1957). No study has directly evaluated the hypothesis that aging leads to a change in

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the allele of risk at *IL6*, despite opposing findings in older adults and adolescents. Replication of the present results is needed, and further study should include measures of stress exposure, inflammatory markers and depressive outcomes in a single investigation.

Taken together, the present results suggest that possession of high expression alleles at *IL6* and *IL1β* increase depression risk following interpersonal stress exposure in the present sample. Further, these effects were specific to interpersonal stress and did not generalize across other types of adverse experience, potentially suggesting a specific biological vulnerability to interpersonal stress. Nesse and Ellsworth (2009) hypothesized that in our ancestry, interpersonal stress may have acted as a warning signal for physical confrontation or social abandonment. Individuals who were able to mount an early immune response to distressing social situations would have had an advantage in healing infections and wounds resulting from physical conflict or attack after the loss of community protection. In support of this theory, the biological changes induced by interpersonal stress mitigate injury and infection (Cole, et al., 2007, 2011; Dhabhar, 2009). Although brief activation of the inflammatory response promotes healing, chronic activation leads to dysregulation of immunological processes and elicits depressive symptoms (Dhabhar, 2014; Dowlati et al., 2010; Howren et al., 2009; Raison & Miller, 2011). Theoretically, individuals genetically predisposed to inflammation would be at increased risk for developing depressive illness when exposed to chronic interpersonal stress.

The present study was not designed to assess possible mechanisms linking stress exposure and inflammatory genotypes with depression. It may be that risk genotypes increase the production of pro-inflammatory cytokines in response to stress, which increases the risk of depression onset via action on neurotransmitter systems involved in depression (Dantzer et al., 2011; Myint et al., 2007). Consistent with this theory, findings from longitudinal studies in older

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populations indicate that rises in inflammation precipitate later depression (Matthews et al., 2007, 2010; Pasco et al., 2010; van den Biggelaar et al., 2007). In never depressed youth, however, inflammatory levels were normative before depression onset, and a first depressive episode was found to increase inflammatory factors. Copeland et al. (2012) found that increases in pro-inflammatory cytokines arise as a consequence of depression onset in youth. Further, Miller and Cole (2012) found that in young women, the first onset of a major depressive episode was accompanied by increases in plasma CRP and IL-6 that were more pronounced in women with higher levels of childhood stress. In women with a history of early stress, heightened inflammation persisted beyond the resolution of the MDE and predicted a second depressive episode. The role of age in determining the complex relationship between inflammation and depression is an important question for future research. In addition, future studies should explore the role of psychological resiliency and risk factors, including cognitive appraisal of stressful events and coping skills that may affect inflammatory changes and depression risk following stress exposure.

The present study had a number of strengths, including hypotheses based in theory, longitudinal assessment of the relationship between stress and depression, and validated, independently-rated measurement of chronic interpersonal and non-interpersonal stress using the semi-structured UCLA Life Stress Interview, which reduced the sample size needed to detect a significant interactive effect, and increased the validity of the findings (Caspi et al., 2010). Several alternative explanations for the present results were ruled out, including effects of maternal depression, a factor that influences genetic makeup and psychosocial stress exposure, as well as prior elevations in depressive symptoms, gender and inflammatory illness (asthma).

Limitations of the present study include the retrospective assessment of chronic stress, and narrow age range of participants, which prevents a direct evaluation of the hypothesis that the allele of risk at *IL6* differs by age. The sample size was relatively small for a candidate gene study, which reduced our statistical power. The present study is an analysis of gene-environment interaction with specific a priori hypotheses dictated by theory, and we present the findings without correction for multiple analyses. Because of the limited sample size and restricted power, we present the analyses as an extension of previous work that identifies these SNPs as depression or stress-relevant. We readily acknowledge that the present results are not a conclusive description of the effects of the identified SNPs on the stress-depression relationship, as epistasis and other effects could also be relevant. Therefore, replication in a larger sample is necessary before any firm conclusions can be drawn from the present analyses.

When analyses were repeated within the largest ethnic group (Caucasians), the primary results held. Of interest, the interaction of *IL1β* with non-interpersonal stress remained non-significant, although a slightly larger difference between depression symptom means of allele groups (*p* reduced from .10 to .08) suggested that sample size may play a role in the magnitude of effects for different ethnicities. In fact, each of the gene × chronic stress effects moved closer to significance (*p*-values between .01-.11) following the restriction to Caucasians, indicating that moderation by each genotype examined in the present study may be worth investigating further in larger samples. The small number of non-Caucasian subjects prevented an ethnically stratified analysis. Even considering these limitations, the present data are novel in finding that inflammatory genotypes moderate the effect of chronic interpersonal stress on depressive symptoms.

Chapter 3: Contribution of *IL6* genotype to depressive symptoms and CRP in the CARDIA study

Abstract

The aim of the present study was to shed light on the complex relationship between depressive symptoms and circulating inflammation beginning in early adulthood and continuing into middle age. Early childhood stress and genetic variation in the gene coding for pro-inflammatory cytokine IL-6 were examined as potential moderators of these relationships. In line with previous investigations, early childhood stress was a potent predictor of depressive symptoms at age 30, which were in turn weakly predictive of growth in plasma C-reactive protein from mean age 32 to 50. The prediction of CRP from depression was more pronounced among Caucasian subjects, and was rendered non-significant when health and lifestyle covariates were accounted for. Genetic moderation of the relationship between early stress exposure and trajectories of change in CRP was identified among African Americans, so that -174G carriers showed stress-dependent change in CRP, while CC homozygotes, and Caucasians, regardless of genotype, showed no effect of stress on CRP levels. Future investigations would benefit from inclusion of health and lifestyle factors as mediators, rather than covariates, to assess the role of BMI, smoking and exercise on the relationships among early stress, depressive symptoms and inflammation. Further, the emergence of ethnically stratified effects suggests that risks for inflammation differ between Caucasians and African Americans. These findings imply that race and risks potentially associated with race, including exposure to discrimination stress and heightened health risks, should be investigated further as potential mediators of the relationships among stress, depression and inflammation.

Introduction

A burgeoning literature implicates pro-inflammatory activity as a mechanism of stress-related illnesses, including major depression. However, many people with depressive symptoms, and/or severe stress exposure have normal inflammatory activity. Thus, environmental and innate biological differences likely determine when and for whom inflammation accompanies depression. The main purpose of the present study is to test the hypothesis that elevated depressive symptoms in young adulthood are associated with increased plasma levels of C-reactive protein (CRP), a marker of chronic inflammatory activity, across adolescence and into middle age, and that early stress exposure strengthens this longitudinal relationship. Further, the hypothesis that genetic predisposition to inflammatory activity also strengthens the relationship between depressive symptoms and later inflammation will be examined.

To date, the relationship between depressive symptoms and inflammation has been examined in age-restricted samples. Results in youth conflict with findings in older adults, indicating a complex and shifting relationship between depression and inflammation over the lifespan. In a large, multi-wave study of youth free of depression and medical diseases at study entry (N = 1,490; ages 9-21), new depressive symptoms, a first major depressive episode, and recurrent depressive episodes all predicted subsequent circulating levels of C-reactive protein (CRP), a marker of chronic inflammatory activity found in the blood plasma (Copeland et al., 2012; Danese et al., 2009; Raison & Miller, 2013). Recurrent episodes were particularly potent in predicting high CRP concentrations. Reverse associations from CRP to later depressive symptoms, episodes and recurrent episodes, however, were all non-significant, suggesting that the relationship is directional in youth, moving from depression to inflammation. Both the rates of depression in the population and plasma CRP naturally increase with age (Chung et al., 2009),

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and as these parameters change over the life course, their predictive relationship appears to shift as well. This suggests a dynamic, changing relationship due to age and likely additional variables. A number of investigations have found a prospective relationship from inflammation to depressive symptoms in older adults (Gimeno et al., 2009; Matthews et al., 2010; Pasco et al., 2010; Stewart, Rand, Muldoon, & Kamarck, 2009; van den Biggelaar et al., 2007), but the findings have been heterogeneous. The inconsistent results may be attributable to gender differences as well as discrepancies in the measurement of depression among studies.

Gimeno et al. (2009) found that plasma CRP in men and women between 35 and 55 years old predicted cognitive symptoms of depression 11.8 years later, even after controlling for a number of behavioral and biological risk factors. The reverse relationship, from depressive symptoms to CRP concentrations, was not predictive. In contrast, Stewart et al. (2009) found a weak, bidirectional relationship between depression and CRP among men and women aged 50-70; however, this study enrolled only 263 people. A number of studies have looked at the relationship between depressive symptoms and CRP concentrations exclusively in women. Matthews et al. (2010) found a bidirectional relationship between depressive symptoms and CRP levels, starting when women were between 42 and 52 years old, and measured again 7 years later. Covarying for body mass index, physical activity and health conditions reduced the prediction of CRP from earlier depressive symptoms to marginal significance, while the prospective relationship from CRP to later depressive symptoms held with the covariates in the model. Pasco et al. (2010) looked at baseline CRP as a predictor of de novo cases of depression during 10 years of follow up in women aged 20-84. Each additional standard deviation in baseline CRP was associated with a 44% increase in risk for new onsets of depression. Finally, a Dutch birth cohort study followed nearly all residents of the city of Leiden who turned 85

between 1997 and 1999, for 5 years. A plasma CRP concentration in the highest tertile at age 85 predicted a more rapid increase in depressive symptoms between ages 85 and 90 (van den Biggelaar et al., 2007). Plasma CRP was measured only once, at baseline, in both the Leiden study and by Pasco et al. (2010), and thus, the prediction of CRP by depressive symptoms could not be assessed in either study. By and large, the findings in older adults indicate that elevations in CRP predict later increases in depressive symptoms and also onset of depressive episodes, while only limited evidence supports an effect of earlier depression on later plasma concentrations of CRP.

In addition to depressive illness itself, many of the risks for depressive illness, namely stressful life experiences, are associated with increases in pro-inflammatory activity (Dickerson et al., 2009; Dickerson et al., 2004; Shelton & Miller, 2011). Stressors that disrupt important relationships are some of the most potent and well-studied precipitants of depression, and are also known to trigger an inflammatory response (Aschbacher et al., 2012; Chiang et al., 2012; Dickerson et al., 2009; Murphy et al., 2012). Although many different types of stress have been associated with depression onset, disruption of important relationships in childhood via maternal rejection, neglect, or the death of a parent are some of the most consistent predictors of later major depressive disorder (Burge & Hammen, 1991; Heim et al., 2008; Kendler et al., 1992; Kessler & Magee, 1993; Monroe et al., 1999). Scores on the childhood trauma questionnaire, which measures parental abuse and neglect, have also been associated with profound long-term changes in stress-activated biological pathways, including diminished cortisol response and increased plasma levels of interleukin-6, an acute marker of the inflammatory response (Carpenter et al., 2007; Pace et al., 2006). In a previous, cross-sectional analysis of the CARDIA cohort, low levels of affection paired with high conflict in the childhood home predicted higher

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concentrations of plasma CRP in adulthood via their influence on a measure of psychological health that included depressive symptoms (Taylor, Lehman, et al., 2006). The present study will extend the findings of Taylor, Lehman, et al. (2006) by taking a longitudinal approach to describing the impact of the family environment on the development of depressive symptomatology and plasma CRP, and will additionally explore how genetic vulnerability to stress-induced inflammation affects these relationships.

Several investigations of inflammation and depression in youth and young adults have found that early stress exposure enhances the inflammation/depression relationship. In a study of adolescent, never depressed women (ages 15-19) with varying exposure to economic and psychological stressors in childhood, the onset of a first depressive episode was found to be accompanied by increases in plasma CRP and IL-6 (Miller & Cole, 2012). Compared to no exposure, one or more stressors in childhood increased vulnerability to depression, to depression-induced inflammation and to more prolonged elevations in plasma CRP that persisted after the depressive episode resolved. Further, lingering elevations in CRP and IL-6 after the resolution of a depressive episode increased vulnerability to a second major depressive episode at 6 month follow up in the context of two or more childhood stressors, but not in women with fewer than two childhood stressors.

Danese et al. (2009) found a far-reaching impact of childhood stress on depression, inflammation, and their co-occurrence. The experience of early childhood stress, operationalized as the experience of caregiver abuse, economic disadvantage and social isolation, was predictive of elevated plasma CRP into the third decade of life (Danese et al., 2009). Further, when the presence vs. absence of a past year depressive episode was examined as an additional risk factor,

childhood stress and a recent depressive episode interacted to predict significantly higher plasma CRP compared to one or none of these risks (Danese et al., 2009).

Biological variability likely plays a role in the relationships among stress exposure, circulating inflammatory factors and depressive symptoms. Variation in the gene coding for the pro-inflammatory signaling molecule interleukin-6 (IL-6), which stimulates CRP production in the liver, has been found to sensitize the production of IL-6 to stress. A single nucleotide variation in the promoter region of *IL6* (rs.1800795, G > C, Smith & Humphries, 2009) increases IL-6 mRNA transcription in response to the stress hormone norepinephrine (Cole et al., 2010). Further, possession of the norepinephrine-responsive G allele at *IL6* has been associated with greater plasma levels of IL-6 following stressful life events, such as bereavement (Schultze-Florey et al., 2012). In older adults, chronic social isolation is also associated with greater CRP in individuals with the G allele at *IL6* (Cole et al., 2011). Thus, *IL6* influences the sensitivity of IL-6 production to stress, both when stress is operationalized as the hormone norepinephrine, and when stress is operationalized as an acute or chronically painful life event. The present study aims to explore whether *IL6* genotype might create vulnerability to elevated plasma CRP as a function of early childhood stress exposure, and/or depressive symptoms.

Study covariates were carefully chosen based on literature describing the impact of biobehavioral factors on circulating CRP (O'Connor et al., 2009). As discussed, aging is associated with increases in CRP regardless of gender. Although CARDIA is a cohort study, the wide range of ages at first assessment (17 to 31) merits controlling for initial age. Female gender is also associated with greater circulating CRP, and changes in hormonal status affected by phases of the menstrual cycle and use of hormonal birth control may contribute additional variation. Further, women experience more depressogenic stressors and have greater overall

genetic risk for depression (Kendler, Gardner, & Prescott, 2002; Nolen-Hoeksema, 2001). In the present study, gender was included as a time-invariant covariate, and women who were pregnant at the time of CRP measurement were excluded from that time point. Racial differences in the strength of the relationship between depression and plasma CRP have previously been reported in the CARDIA cohort, and the effects of race will be examined in all analyses. Adipose tissue can account for up to 30% of circulating IL-6, a stimulant of CRP (Mohamed-Ali, Pinkney, & Coppack, 1998). Cigarette use is also linked to greater CRP, although this effect may be specific to men (Bo et al., 2005; Nazmi, Oliveira, & Victora, 2008). Finally, cardiovascular fitness levels are inversely associated with circulating levels of CRP (Plaisance & Grandjean, 2006). Therefore, physical activity, BMI and cigarette use were included as covariates at all time points.

The Coronary Artery Risk Development in Young Adults (CARDIA) Study is a longitudinal investigation designed to assess cardiovascular disease risk, funded by the National Heart, Lung, and Blood Institute (NHLBI). CARDIA began in 1985-6 with over 5,000 African American and Caucasian men and women, selected to create an even number of participants in subgroups of race, gender, education and age. The 25-year time span of CARDIA affords the opportunity to explore change in plasma CRP and in depressive symptoms over the course of young adulthood and into middle age, potentially addressing unanswered questions about their relationship over time. The present sample includes 4,276 adult men and women who were followed from mean age 30.05 (3.63), to mean age 50.17 (SD = 3.64, range of 42-59). Data were collected at six intervals, and depression symptoms were assessed at five of these visits, and plasma C-reactive protein at four. Latent growth curve (LGCM) and autoregressive models will be used to explore the relationship between CRP and depressive symptoms across time.

Hypotheses

1) We hypothesize that exposure to early life stress, as measured by the Risky Families Questionnaire, will predict the latent intercept and slope of the growth curve models of depressive symptoms (measured by the CES-D), and of plasma CRP. In order to test these hypotheses, two separate growth curves will be constructed, one for depressive symptoms and one for CRP.

2) We hypothesize that higher initial levels (the latent intercept) of depressive symptoms, as measured by the CES-D, will predict the latent slope of CRP, reflecting the direction of effect reported in previous studies of young, healthy samples (Miller & Cole, 2012; Copeland et al., 2012). This hypothesis will be tested using a parallel process model that combines the individual growth curves established in hypothesis 1. Any effect of CES-D intercept on the slope of CRP identified in the parallel process model will be explored in greater detail using an autoregressive model.

3) We hypothesize that exposure to early life stress will interact with the latent intercept of depression to predict the latent slope of CRP. This hypothesis will be tested in the parallel process model established for hypothesis 2.

4) Finally, it is hypothesized that the minor 'G' allele at the -174 locus of *IL6* will be associated with greater initial status and growth in CRP, and additionally will interact with early life stress to predict higher plasma CRP concentrations and CES-D symptoms. This hypothesis will be tested using the individual growth curves of CRP and CES-D described in hypothesis 1.

5) In an exploratory analysis, it is hypothesized that -174G will moderate the predictive relationship from depressive symptoms to plasma CRP concentrations, such that G-allele

carriers will have a stronger relationship from CES-D intercept to CRP slope. This hypothesis will be tested using the parallel process model established for hypothesis 2.

Method

Participants

Participants were enrolled in the Coronary Artery Risk Development in Young Adults (CARDIA) study, a longitudinal, epidemiologic assessment of coronary risk factors and their determinants from young adulthood until early middle age funded by the National Heart, Lung, and Blood Institute (NHLBI). An initial cohort of 5,115 participants (mean age of 24.76, SD = 3.61, with a range of 17-31) was recruited mainly by telephone, and in small part by door-to-door recruiters, between 1985 and 1986, with the goal of representing the populations of African American and Caucasian adults in four US cities: Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA. The cohort was designed to have equal groups of African Americans and Caucasians, women and men, and further, to be equally divided by age (18-25 and 25-30 at study entry), and education (high school or less and more than high school). This initial cohort was comprised of 53.9% women and 49.5% African Americans. Seven follow up time points were conducted at 2 (1987-1988), 5 (1990-1991), 7 (1992-1993), 10 (1995-1996), 15 (2000-2001), 20 (2005-2006), and 25 (2010-2011) years after enrollment.

The present study explores change in repeated measurements of the CES-D and plasma C-reactive protein during follow-up. Depressive symptoms were measured beginning at year 5, when participants had a mean age of 30.05 (SD = 3.63), and reassessed at years 10, 15, 20 and 25. Plasma C-reactive protein (CRP) was measured beginning in year 7, when participants had a mean age of 32.05 (SD = 3.62) and again at years 15, 20 and 25. Participants with missing data at all time points on one or both of the primary outcome variables were not included in the present analyses (n = 679). Women pregnant at the time of CRP measurement at any time point

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were excluded based on significantly higher plasma CRP in pregnant women at year 7, compared with women who were not pregnant (t (60.20) = -4.42, p < .001). However, there were no differences in plasma CRP for pregnant women at years 15 (t (1577) = -0.64, p = .53), or 20 (t (1502) = -1.25, p = .21), and no pregnancies were reported at year 25. Plasma concentrations of CRP greater than 10 mg/L are indicative of current, acute illness and were excluded from analyses (169 participants at year 7; 45 at year 15; 136 at year 20 and 207 at year 25), as were concentrations lower than 0.16 mg/L, which indicate measurement error (196 participants at year 7; 362 at year 15; 209 at year 20 and 67 at year 25). These exclusions left a total of 4,276 participants, 39.3% of whom had complete depression and CRP data, and 84.4% of whom completed at least 5 of the 9 assessment time points.

The sample had a fairly even distribution of men and women (52.4% women), and African-Americans and Caucasians (48.2% African American, see Table 3 for demographic information). In year 7, when plasma CRP was first measured, the majority of participants were overweight (30.5% overweight; 22.7% obese; see Table 4 for study variable descriptive statistics). Close to one quarter currently used tobacco, and those who used tobacco smoked an average of 12.98 (SD = 9.00) cigarettes per day. Participants, on average, had completed a high school education, and this was stratified by race (African American mean = 13.13, Caucasian mean = 14.61; t (4032.35) = 23.42, p < .001).

Genetic Sample. *IL6* genotype was available for 3,128 participants within the chosen sample. Of these, 2,895 completed year 15; 2,027 completed year 20; and 1,832 completed year 25, meaning that they had data available for the CES-D, plasma CRP, or both at those years. Participants with available *IL6* data were no different from those without *IL6* data in gender composition (χ^2 (1, 4276) = 1.30, p = .25), plasma CRP concentration (t (3181) = 0.13, p = .90),

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exposure to early adverse experience (t (3490) = 1.06, p = .29), frequency of cigarette smoking (t (1106) = -0.12, p = .90), participation in physical activity (t(3913) = -0.57, p = .57) or BMI (t (3933) = -0.03, p = .98). Participants with *IL6* data were less likely to be African American (χ^2 (1, 4276) = 65.71, p < .001; 45.6% of the genetic sample vs. 59.7% of the non-genetic sample), were older (mean age of 30.12 at first CES-D measurement vs. 29.81 for the non-genetic sample; t(1756.40) = -2.42, p < .05), more educated (13.99 years in the genetic sample vs. 13.56; t (4265) = -5.69, p < .001), less depressed (t (3901) = 2.65, p < .01 (mean CES-D score of 10.88 vs. 11.65), and less likely to smoke (25.9% vs. 34.6%; χ^2 (1, 3940) = 29.26 p < .001).

Procedure

Depressive Symptomatology. Depressive symptoms were measured using the Center of Epidemiological Studies Depression Scale (CES-D; (Hertzog, Van Alstine, Usala, Hultsch, & Dixon, 1990; Radloff, 1977). The CES-D is comprised of 20 items that participants self-rate on a 4-point Likert scale, indicating how often the symptom was experienced in the past week, from "rarely or almost never (less than 1 time)", to "most or all of the time (5-7 days)". Items assess feelings of guilt and worthlessness, helplessness and hopelessness, psychomotor retardation, loss of appetite, and sleep disturbance. The CES-D is a reliable measure of the severity and duration of depressive symptoms across race, gender and age (Knight, Williams, McGee, & Olaman, 1997; Foley, Reed, Mutran, & DeVellis, 2002). An overall score of 16 or more is indicative of clinically significant depressive symptoms (Haringsma, Engels, Beekman, & Spinhoven, 2004).

Inflammation. Information concerning the collection and analysis of blood plasma samples for CRP measurement has been detailed elsewhere (Cho, Bower, Kiefe, Seeman, & Irwin, 2012; Lakoski, Herrington, Siscovick, & Hulley, 2006). Briefly, plasma CRP was measured at years 7, 15, 20 and 25 using a BNII nephelometer (Siemens Dade Behring,

Deerfield, Ill). The assay range was 0.175-1100 mg/L (Deverts et al., 2010). All measurement time points of plasma CRP had negatively skewed values, and were transformed with the natural log function (Ln). Following the transformation, skewness and kurtosis indicated a normal distribution of CRP data at all time points.

Early Life Stress. Family environment prior to age 18 was measured using the Risky Families Questionnaire (RFQ; Felitti et al., 1998; Taylor, Lerner, Sage, Lehman, & Seeman, 2004). The RFQ assesses for the frequency of seven different indicators of a harsh early environment, including whether participants felt loved, supported and cared for; were verbally abused; experienced physical warmth and affection; were physically abused; whether family members knew what they were up to; whether they lived with a parent who abused substances; and whether the home was organized and well-managed. Items were rated from 1 "rarely or never happened", to 4, "occurred most or all of the time". Based on previous publications examining the RFQ in CARDIA, each item was scored 0-3, before taking an average across the seven items of the RFQ for each participant, creating a mean score with a range of 0-3, and mean of 0.67 (SD = 0.59) (Loucks, Almeida, Taylor, & Matthews, 2011). The RFQ has been validated against an interview assessment of early family environment (Taylor et al., 2004) and has been found to predict depressive symptoms in multiple study samples (Taylor, Lehman, et al., 2006; Taylor et al., 2004), including a previous analysis of CARDIA data. In the present subsample of CARDIA, the RFQ demonstrated adequate internal consistency ($\alpha = 0.76$). Participants endorsed an average of 4.69 items on the Risky Families Questionnaire, indicating that the sample was exposed to relatively low levels of childhood adversity. Items most commonly endorsed were poor household organization/management, low levels of physical affection, and families' negligence about participants' whereabouts.

Time-varying covariates. Tobacco use was assessed at each time point by asking whether participants smoked cigarettes regularly. Regular smokers were then asked to report the number of cigarettes smoked per day. Body mass index was calculated according to the National Heart, Lung, and Blood Institute guidelines as weight (kg) / height (m)^2 . BMI ranged from 10.52–64.16 (M = 26.04) in the first year of CES-D measurement when participants had a mean age of 30.04 (SD = 3.64). Participation in a range of heavy and moderate physical activities in the past 12 months was assessed using the CARDIA Physical Activity History Questionnaire (Sidney et al., 1991) at each time point. Based on previous publications (Cho et al., 2012), a summary score was computed in physical activity units (EU). The American College of Sports Medicine recommendations for the amount of physical activity needed to support weight loss is equivalent to 300 EU (Parker, Schmitz, Jacobs, Dengel, & Schreiner, 2007). In order to provide a more understandable unit of measurement, physical activity levels were divided by 300, so that a one unit increase in physical activity represents 300 EU.

Genotyping. Of the 4,276 participants chosen for the present study, 3,128 had rs.1800795 genotype available. Genotyping was completed as part of the Inflammation Genomics and Atherosclerosis Prevention (IGAP) ancillary study to CARDIA, using TaqMan Assays By Design (ABI). Of these, 292 were -174G homozygotes, 1,018 were heterozygotes, and 1,818 were -174C homozygotes. Hardy-Weinberg equilibrium testing revealed significant genetic heterogeneity in the full sample (χ^2 (2) = 66.50, p < .001), which is likely due to ethnic stratification. Analyzed separately, each ethnic group was genetically homogeneous: Caucasians (GG = 279, CG = 790, CC = 630) χ^2 (1, 1699) = 1.39, p = .24; African Americans (GG = 13, CG = 228, CC = 1,188) χ^2 (1, 1429) = 0.31, p = .57).

Statistical Analysis

Hypotheses were examined using latent growth curve and autoregressive models in MPlus version 7 (Muthén & Muthén, 2012). Latent growth curve modeling (LGCM) is an application of structural equation modeling, meaning that some variables are measured, while others are latent (estimated based on the observed variables). In LGCM, repeated measurements are used to estimate the sample mean and variability in the initial status (i.e., intercept), and subsequent growth (i.e., slope) of some outcome.

Missing data were accommodated by full information maximum likelihood estimation (FIML; Enders, 2001), which allows for the use of all available data, and is appropriate even when data are not missing at random.

Participants eliminated from the present sample due to missing data at all time points on one or both of the outcome variables did not differ from included subjects by gender (χ^2 (1, 5027) = 0.07, p = .79), exposure to early life stress (t (3577) = -0.93, p = .35), likelihood of being a cigarette smoker (χ^2 (1, 1826) = 3.50, p = .06), the number of cigarettes smoked per day (t(1225) = 0.39, p = .69), or the amount of physical activity they engaged in (t (4217) = -1.03, p = .30). Those with missing data were twice as likely to be African American (χ^2 (1, 5011) = 64.17, p < .001), were slightly younger (t (4267) = -2.64, p < .01), had greater BMI scores (t (331.41) = 4.45, p < .001, 28.20 vs. 26.04) and fewer years of education (t (5025) = -2.61, 13.57 vs. 14.06, p < .01).

Initially, separate growth models were estimated for depression and CRP in order to establish a best fit for each curve, and to test hypothesis 1, that exposure to childhood stress would predict the latent factors of these models. These individual models used the repeated measures of CES-D and plasma CRP to estimate mean initial status (intercept), mean linear

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growth, variance in the initial status and growth, and also covariance between initial status and growth for each outcome.

Once statistically significant linear growth was established for both depression and plasma CRP, a final model was fitted by adding time-invariant covariates (age at enrollment, gender, race and RFQ) as predictors of the latent intercept and growth factors, and, time-varying covariates (cigarette use, physical activity, and body mass index) were added by regressing each observation of depression or CRP on the corresponding measurement of the covariate (e.g., year 5 CES-D regressed on year 5 BMI, cigarette use and physical activity). Time-varying covariates and participant age were centered at the year 5 mean for ease of interpretation. Effects of time-varying covariates that were not statistically significant were omitted from the final models to improve fit.

A "parallel process" model combining the latent growth curves of depression and CRP just described was used to examine study hypotheses 2, 3 and 5, regarding the relationship between depressive symptoms and CRP over time, and potential moderators of this relationship. Within a parallel process model, the latent factors of one curve can be regressed on the latent factors of the other curve. Thus, the parallel process model allows a test of hypothesis 2, that depression intercept predicts CRP slope, by regressing the slope of CRP on the initial status of depression. The parallel process model was initially estimated as an unconditional model, and time-invariant covariates were then added, followed by the addition of the time-varying covariates (i.e., cigarette use, physical activity, BMI) from the best fitting individual growth curves.

To gain more detailed information about the timing of effects as proposed in hypothesis 2, an autoregressive path model was estimated using cross-lagged regressions between

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depression and plasma CRP, so that each measurement of depression was examined as a predictor of the following measurement of CRP and vice versa.

Hypothesis 3 was tested by adding an interaction term to the parallel process model, so that CRP slope was regressed on the intercept of CES-D, RFQ score, and the interaction of RFQ score with CES-D slope, to determine whether the relationship between depression and CRP differed by childhood stress exposure.

Hypothesis 4, which proposed direct effects of *IL6* on CRP, as well as moderating effects on the stress-CRP and stress-depression relationships, was tested using the initial, separate growth curve models for CRP and CES-D. Models testing the effects of *IL6* were stratified by race in order to account for ethnic differences in the distribution of alleles at *IL6*. In these models, race was included both as a stand-alone predictor of the latent intercept and growth, and also in interaction with genotype as a predictor of the latent factors.

Hypothesis 5, which is exploratory in nature, proposes moderation of the relationship between CES-D and CRP by *IL6* genotype. Hypothesis 5, like hypothesis 3, was tested by creating interaction terms within the parallel process model. CRP slope was regressed on CES-D intercept, *IL6* genotype, race, and the two- and three-way interactions of these predictors.

Results

Hypothesis 1: Effects of Early Stress Exposure on C-Reactive Protein and CES-D.

Initial Latent Growth Curve Model of CRP. The initial status and change in CRP was modeled in a step by step process to obtain the best fit to the data, and to assess the effects of early childhood stress (hypothesis 1) and also of the covariates of race, gender, age, cigarette use, body mass index and physical activity on the latent factors. Modeling of plasma CRP began with the estimation of a random intercept and no latent growth factor, to serve as a comparison of fit

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for subsequent models. This initial, null model of CRP had a poor fit (Table 5), and a mean intercept significantly different from zero, with significant variance in intercepts between participants (see Table 6 for parameters). A random slope was added, allowing the model to accommodate individual variation in the trajectory of CRP change. The random slope was positive and significantly different from zero, indicating that growth in CRP was occurring over time, and that the slope of change in CRP also varied significantly among individuals (Table 6). Overall, participants' plasma CRP concentrations gradually increased between years 7 and 25 of the study, with the exception of year 15, when CRP concentrations were higher than at any other time point. Close to one quarter of the sample exceeded clinical guidelines for heart disease risk ($\geq$ 3mg/L) at each time point. The intercept and linear growth terms were negatively correlated, indicating that higher initial CRP concentrations were associated with more shallow increases in plasma CRP over the next 18 years.

Time invariant covariates were added to the model by regressing the latent factors on gender, race, age and early life stress (RFQ). The model was a good fit to the data with the covariates included (Table 5). Female gender was associated with higher initial CRP measurement, and a more shallow increase in CRP across time. African Americans had higher initial concentrations of CRP and steeper growth over time, compared to Caucasians. Neither age nor exposure to early life stress were significant predictors of initial status or growth in CRP (Table 6). This model was also run substituting the individual items of the RFQ for the average RFQ score, which resulted in a good fit to the data (X^2 (3289, 25) = 160.01, p < .001, RMSEA = 0.04 [90% CI = 0.04-0.05], SRMR = 0.02, CFI = 0.97), but RFQ items were not significant predictors of the intercept or slope of CRP. Thus, hypothesis 1, that RFQ would predict the latent factors of the CRP model, was not supported.

A final model was estimated adding the effects of time varying covariates, by regressing each measurement of CRP on its corresponding BMI, cigarette use and physical activity scores (Table 6). This model was run using Monte Carlo integration, which prevents the calculation of indicators of absolute fit, and can be compared with other models using the Bayesian Information Criterion (BIC), a comparative fit index (see Table 5 for a comparison of BIC among models). In the final model, the effects of the time-invariant covariates held. Additionally, BMI was a significant, positive predictor of plasma CRP at all four measurements (main effect: $b = 0.08$, SE < 0.01, $p < .001$); physical activity was associated with lower plasma CRP at years 15, 20 and 25 (main effect: $b = -0.04$, SE $= 0.01$, $p < .01$), and cigarette use was positively associated with CRP values at all years (marginal significance at year 25; main effect $b = 0.02$, SE < 0.01, $p < .05$).

These descriptive analyses suggest that women and African Americans began the study with greater plasma CRP concentrations compared to men, and Caucasians, respectively. Interestingly, women went on to have more shallow growth in CRP across time compared to men, while African Americans showed steeper growth in CRP compared to Caucasians. As expected, cigarette use and BMI were robust predictors of greater plasma CRP, while exercise had a protective effect. Contrary to hypothesis 1, there was no effect of early childhood stress exposure on CRP, even when the 7 items of the RFQ were examined separately.

Initial Latent Growth Curve Model of Depressive Symptoms[2]. Model estimation of the intercept and growth in CES-D across time was completed using the same steps described for

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[2] CES-D scores were negatively skewed and analyses were compared between a square root transformation that corrected for non-normality, and non-transformed data using maximum likelihood estimation to account for non-normality. There were no meaningful differences between the two sets of analyses (results not reported), and all models were run using non-transformed CES-D data using maximum likelihood estimation to account for non-normality, for ease of interpretation.

the CRP model. First, a null model was estimated for depression using a latent intercept and no linear growth factor. This model was a poor fit, and indicated that initial depression status was significantly different from zero, and varied significantly among participants (Table 5). Allowing the slope to account for individual variation in depressive symptom trajectories improved model fit and revealed that average change in depressive symptoms was negative and varied significantly among participants (see Table 6 for model parameters). Initial depression status was negatively correlated with growth, indicating that participants with high levels of depressive symptoms at study entry had a more shallow decrease in symptoms over time.

Following the establishment of the base model, the latent components were regressed on age, gender, race and early childhood stress. The addition of the covariates improved model fit and indicated that women started the study with an average of 1.14 more depressive symptoms, and had a more shallow decline in their symptoms over time, compared to men (see Table 6 for covariate effects). There was an inverse relationship between depressive symptoms and age, indicating that each year older than 30 (the mean) at first depression measurement, was associated with a 0.07 symptom decrease in CES-D score. The relationship between age and slope was marginal and positive, indicating that participants older than 30 at first measurement had a more rapid decline in their depressive symptoms, compared to younger participants.

African American participants entered the study with an additional 2.81 depressive symptoms, and had a more shallow decline in depressive symptoms over time, compared with Caucasians. Each item endorsed on the Risky Families Questionnaire was associated with an additional 3.07 depressive symptoms at study entry, but did not impact the change in depressive symptoms across time. This model was also run substituting the individual items of the RFQ for the average RFQ score. The fit of this model was good: $X^2 (3289, 40) = 182.68$, $p < .001$,

RMSEA = 0.03 [90% CI = 0.03-0.04], SRMR = 0.02, CFI = 0.98, and RFQ items "How often did you feel loved?" (reverse coded; b = 0.87, SE = 0.21, $p < .001$), "Was your house well organized/managed?" (reverse coded; b = 0.44, SE = 0.15, $p < .01$), "Did your family know what you were up to?" (reverse coded; b = 0.63, SE = 0.15, $p < .001$), and "How often were you sworn at or insulted?" (b = 0.82, SE = 0.17, $p < .001$) were all positive, significant predictors of depression intercept. No RFQ items predicted the slope of depressive symptoms.

Finally, the time-varying covariates (BMI, cigarette use, physical activity) were added to the depression model to complete the fitting process (see Table 6 for model parameters). This final model provided adequate fit, and continued to have mean initial status and linear growth significantly different from zero. BMI was not a significant predictor of depressive symptoms at any time point (main effect b = -0.01, SE = 0.01, $p = .92$), while cigarette use became a significant, positive predictor at years 15 and 20 (main effect b = 0.04, SE = 0.02, $p < .05$), and physical activity was inversely associated with depressive symptoms at all years (main effect b = -0.48, SE = 0.07, $p < .001$). In this final model, initial depression status continued to correlate negatively with the slope, indicating that a high number of depressive symptoms at baseline predicted a smaller decrease in symptoms over time. There were no meaningful changes in the effects of age, gender, race or RFQ on depression slope or intercept from those reported in the previous model.

In summary, results of the initial model of change in depressive symptoms measured by the CES-D indicate that on average, participants endorsed between 10 and 11 symptoms at study entry, and that overall, participants' depressive symptoms declined over time. Women and African Americans began the study with higher CES-D scores and showed less of a decrease in symptoms over time, compared with men and Caucasians, respectively. Childhood stress

exposure was a strong predictor of initial CES-D measurement and did not affect the change over time in depressive symptoms. Older participants began the study with fewer depressive symptoms and showed a more rapid decline in depressive symptoms. Interestingly, BMI was not associated with depressive symptoms, while exercise protected against depressive symptoms, and cigarette use became a significant predictor of depressive symptoms later in life.

Hypothesis 2: Prediction of Growth in CRP from initial Depression Measurement

A parallel process model was constructed to test hypothesis 2, that initial depression severity would predict growth in plasma CRP. The LGCM models described above for CRP and depressive symptoms were combined into a single, parallel process model, and the latent slope of CRP was regressed on the latent intercept of the CES-D model. To test the hypothesized direction of effect, the latent slope of depression was also regressed on the intercept of CRP.

An unconditional model was a good fit to the data (see Table 5 for fit statistics). Consistent with hypothesis 2, the slope of CRP was predicted by initial depression status, so that each additional symptom endorsed on the CES-D at age 30 was associated with an increase in the slope of CRP between ages 32 and 50 (b < 0.01, SE < 0.01, β = 2.57, p < .05; see Table 7 for full model parameters). Also consistent with hypothesis 2, the regression of CES-D slope on CRP intercept was not significant (b < 0.01, SE = 0.01, p = .97). When the covariates of age, gender, race and early life stress were added to the model, the regression of CRP slope on CES-D intercept became marginal (see parameter values for time-invariant covariates, as well as regressions in Table 7). Taken together, the results of the two regressions indicate that initial depression status at age 30 is predictive of growth in plasma CRP levels over an 18 year period, spanning from early adulthood to middle age, as predicted by hypothesis 2. Further, in line with

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hypothesis 2, initial plasma CRP levels were not predictive of change in depressive symptoms

across the same time span.

Finally, the repeated measures of BMI, cigarette use and physical activity retained from

the best-fitting individual growth models were added to the parallel process model, using Monte

Carlo integration. Slopes and intercepts continued to differ significantly from zero and to vary

among participants (see Table 7 for parameters). The prediction of change in CRP from CES-D

intercept remained marginally significant: $b < .01$, $SE < .01$, $\beta = 1.76$, $p = .08$, indicating that the

demographic, health behavior and lifestyle covariates of gender, race, body mass index, cigarette

smoking and exercise account for much of the individual variation in trajectories of plasma CRP,

although an attenuated effect of depressive symptoms could still be detected following the

addition of all covariates. The effects of gender, race, age, RFQ, BMI, cigarette use and physical

activity from the individual LGCMs held in this final parallel process model (statistics for time-

varying covariates not reported) indicating significant contributions to increases in CRP across

time, over and above the contribution of depressive symptoms measured at age 30.

In order to gain a more detailed understanding of the timing of relationships between

depressive symptoms and CRP, we constructed an autoregressive path model of cross-lagged

associations between CRP measurements and depression measurements. Autoregressive models

provide a stringent test of the relationship across time between two repeated measures, by

controlling for the previous measurement of each construct (e.g., regression of year 20 CRP on

year 15 depression, while controlling for both year 15 CRP and year 10 depression), as well as

the health and lifestyle covariates retained from the best-fitting individual growth models. To

create an even number of measurements for the two outcomes, year 5 CES-D was not included.

Model fit was initially poor, but the addition of seven residual correlations led to an adequate fit (X^2 (72, 4276) = 41,738.12 , $p < .001$, RMSEA = 0.07 [90% CI = 0.07-0.08], SRMR = 0.07 CFI = 0.85; see Figure 3). The need for residual correlations indicates that depression and CRP share additional variance at years 15, 20 and 25 that is not accounted for by the model. Of the six primary regressions evaluated, only the regression of year 15 CRP on year 10 depression was significant, as shown in Figure 3. Thus, the autoregressive model supports the findings of the parallel process model, in that depression is a predictor of CRP, while CRP is not a predictor of depression. When the autoregressive model was run using a grouping function to produce separate model parameters for African Americans and Caucasians, Caucasians had a marginal, positive effect of year 10 CES-D on year 15 CRP (b < 0.01, SE < 0.01, β = 1.78, $p = .075$), and African Americans had no significant predictive relationships. Taken together, the findings of the parallel process model and the cross-lagged model indicate that depression in young adulthood has a lasting but tenuous effect on the cumulative growth in CRP, and further, that when relationships between depression and CRP are examined on a smaller time scale, there are few predictive relationships, at least when using a rigorous statistical approach.

Hypothesis 3: Moderation of the Changes in Depression and CRP by Early Life Stress

Hypothesis 3, that early life stress would moderate the relationship between depression intercept and growth in plasma CRP, was tested by regressing the slope of CRP on CES-D intercept, RFQ score, and their interaction, within the parallel process framework. The use of an interaction prevented the calculation of indicators of absolute fit, and the model was assessed using the Bayesian Information Criterion (BIC). Model fit compared favorably with the unconditional parallel process model (N = 3,492, df = 25, BIC = 134,083.67; see Table 5 for fit statistics). Without covariates, the interaction and main effects were each non-significant (CES-

D int.× RFQ: b < 0.01, SE < 0.01, p = .17). Controlling for gender, age, and race improved

model fit (BIC =129,943.82), and brought the interaction term to marginal significance (b < 0.01,

SE < 0.01, β = 1.69, p = .09), while the main effects of RFQ (b < 0.01, SE < 0.01) and CES-D

intercept (b < 0.01, SE < 0.01) remained non-significant. The addition of time-varying covariates

(BMI, cigarette use and physical activity) led to problems with the covariance structure of the

model. Thus, the hypothesis that exposure to early life stress would moderate the depression-

inflammation relationship was not supported.

Hypothesis 4: Genetic Contributions to Trajectories of CRP and Depression

As previously discussed, Hardy-Weinberg analyses indicated genetic heterogeneity in the

overall sample due to ethnic stratification, and therefore, genetic models were run including the

main effects of race and the interaction of race with genotype, as predictors of the latent factors.

Because of the low number of African American recessive homozygotes, *IL6* was coded as a

binary variable grouping recessive homozygotes (GG) with heterozygotes (GC).

Genetic prediction of CRP intercept and slope. Hypothesis 4, that *IL6* would directly

predict the intercept and growth of the plasma CRP model, was tested using the individual

growth curve model of CRP established for hypothesis 1, with *IL6* genotype, race and their

interaction as predictors of intercept and slope. This model had acceptable fit: X^2 (3,128, 11) =

122.95, p < .001, RMSEA = 0.06 [90% CI: 0.05-0.07], SRMR = 0.03, CFI = 0.97, and only race

was revealed to be a significant predictor of CRP intercept (b = 0.41, SE = 0.08, p < .001), so

that African Americans began the study with higher CRP concentrations than Caucasians. There

were no direct or interactive effects of *IL6* on intercept or slope. These effects were no different

when age, sex and the time varying effects of BMI, physical activity and cigarette use were

added to the model (results not reported). Therefore, in contrast to hypothesis 4, *IL6* genotype was not a direct predictor of CRP concentrations, nor of the change in CRP across time.

Genetic moderation of the stress-CRP relationship. Hypothesis 4 also predicted that *IL6* would moderate the relationship between early life stress and the latent factors of the CRP model. Moderation by early life stress was tested using the single, latent growth curve model of CRP established for hypothesis 1, with *IL6*, race, RFQ and their 2- and 3-way interactions entered as predictors of intercept and slope. This model was a good fit to the data: X^2 (2,848, 22) = 136.52, $p < .001$; RMSEA = 0.05 [90% CI = 0.04-0.04], SRMR = 0.02, CFI = 0.97, and the three-way interaction of *IL6* × race × RFQ was a significant predictor of CRP slope (b = 0.02, SE = 0.01, $p < .05$), but not intercept (b = -0.27, SE = 0.18, $p = .13$). This effect held controlling for age and gender (statistics not shown), and model fit was improved by the addition of these covariates: X^2 (2,695, 23) = 132.12, $p < .001$; RMSEA = 0.04 [90% CI = 0.04-0.05], SRMR = 0.02, CFI = 0.97). Finally, the repeated measures of BMI and physical activity were added to the model. Cigarette use was omitted as the large amount of missing data on this measure (due to most participants being non-smokers) led to difficulties with the covariance matrix of the model. This final model had adequate fit: X^2 (2,695, 119) = 868.34, $p < .001$; RMSEA = 0.05 [90% CI = 0.05-0.05], SRMR = 0.07, CFI = 0.85, and reduced the 3-way interaction to marginal significance ($p = .09$; statistics not shown). Although the relationship between stress and CRP differed by race, there were no differences in stress exposure by race (t (3481.11) = -0.01, $p = .48$).

The three-way interaction was examined further to determine how the relationship between stress and CRP differed by race and *IL6* genotype. Examination of the two-way interactions revealed that among -174G carriers, the effect of RFQ on CRP slope was dependent

on race (b = -0.02, SE = 0.01, β = -2.54, $p < .05$), while among CC homozygotes, the effect of

RFQ on CRP slope did not differ by race (b < .01, SE = .01, β = 0.13 $p = .89$). Further, among

African Americans, the relationship between RFQ and CRP slope depended on genotype (b = -

0.02, SE =0.01, β = -1.87, $p < .05$), while Caucasians' effect of RFQ on CRP slope did not

depend on genotype (b < 0.01, SE < 0.01, β = -0.38, $p = .71$). Finally, the two-way interactions

were broken into their simple effects. Among African Americans, RFQ had a significant effect

on CRP intercept and slope only among -174G carriers, so that a higher RFQ score was

positively associated with RFQ intercept (b = 0.28, SE = 0.13, $p < .05$), and had a dampening

effect on the slightly positive slope of CRP (b = -0.02, SE = 0.01, $p < .05$; see Figure 4). The

effect of RFQ on CRP intercept and slope was non-significant for African Americans with CC

genotype (see Figure 5), and for Caucasians, regardless of genotype. Thus, the hypothesized

effect of -174G allele on the stress-inflammation relationship was supported only among African

Americans in the present sample. Visual representation of the interaction revealed that stress

exposure had little impact on CRP concentrations in African Americans with CC genotype (see

Figure 5). However, African Americans carrying -174G showed stress-dependent changes in

plasma CRP across the 18 years of the study, so that high stress exposure predicted increasing

CRP across the 18 years of measurement, while -174G carriers exposed to low levels of stress in

the family home had negligible change in their CRP across time (see Figure 4).

Genetic moderation of the stress-depression relationship. To test the hypothesis that

IL6 moderates the relationship between exposure to early life stress and later depressive

symptoms, *IL6*, RFQ, race and their 2- and 3-way interactions were examined as predictors of

the latent intercept and growth terms of the depression model. Without other covariates, the

model was a good fit to the data: X^2 (2,848, 31) = 176.23, $p < .001$; RMSEA = 0.04 [90% CI =

0.04-0.05], SRMR = 0.02, CFI = 0.97. The three-way interaction was not a predictor of CES-D intercept or slope. In line with the results of previous models, African Americans began the study with two more depressive symptoms than Caucasians (b = 1.99, SE = 0.81, $p < .05$), and for each one unit increase past the mean in RFQ, a 3-symptom increase in CES-D score was predicted (b = 2.79, SE = 0.36, $p < .001$). The slope of change in depressive symptoms was negative and was not predicted by race, *IL6*, or RFQ.

When age and gender were added to the three-way interaction model, fit remained good: X^2 (2,695, 37) = 172.62, $p < .001$; RMSEA = 0.04 [90% CI = 0.03-0.04], SRMR = 0.02, CFI = 0.97, and there were no meaningful differences from the effects reported for the unconditional model. Thus, the hypothesis that *IL6* would moderate the stress-depression relationship was not supported.

Hypothesis 5: Exploratory Analysis of Genetic Moderation of the Depression-CRP Relationship

Finally, hypothesis 5, that *IL6* would moderate the regression of CRP slope on depression intercept, was explored using a the parallel process model. The main and interactive effects of depression intercept, *IL6* and race were examined as predictors of the latent slope of CRP. The use of an interaction prevented the calculation of indicators of absolute fit, and the model was assessed using the Bayesian Information Criterion (N = 3,128, df = 29, BIC = 117,060), which compared favorably with the BIC of the unconditional parallel process model (BIC = 150,235.83). The three-way interaction was non-significant (b < .01, SE < .01, β = 1.40, p = .16), and of the two-way interactions, only depression intercept × race was a significant predictor of CRP slope (b < -0.01, SE < 0.01, β = -2.60, $p < .01$). Thus, results did not support hypothesis 5: *IL6* genotype did not moderate the relationship from CES-D intercept to CRP slope.

The significant interaction effect of CES-D intercept with race to predict CRP slope was further explored in a new, simplified parallel process model that included only CES-D intercept, race and their interaction as predictors of CRP slope. The simplified parallel process model increased the available sample size, and decreased model fit (N = 4,276, df = 25, BIC = 150,110.13). The intercept and slope of CES-D, and the intercept of CRP were significantly different from zero and varied among participants, while the slope of CRP was not statistically different from zero (no mean change across time) but had significant variance among participants. The effect of depression intercept on CRP slope was conditional on race at marginal significance (b < -0.01, SE < 0.01, β = -1.90, p = .06), so that Caucasians had a stronger relationship between CES-D intercept and CRP slope. Adding age and gender to the model increased the goodness of fit (N = 3950, df = 27, BIC = 142,511.84), and the significance of the interaction term (b < -0.01, SE < 0.01, β = -2.04, p = .04). Adding the effects of the time varying covariates to the model rendered the interaction non-significant.

Simple effects of the depression intercept × race interaction were explored by running the regression of CRP slope on depression intercept within a grouped parallel process model that provided separate model parameters for Caucasians and for African Americans. The grouped model was a good fit to the data: X^2 (4,276, 46) = 335.59, p < .001; RMSEA = 0.05 [90% CI = 0.04-0.05], SRMR = 0.03, CFI = 0.97. Caucasians' CES-D intercept was a marginally significant predictor of CRP slope (b < 0.01, SE < 0.01, β = 1.94, p = .05), while African Americans' CES-D intercept did not predict the slope of change in CRP (b < 0.01, SE < 0.01, β = 0.73, p = .47). Controlling for gender and age decreased the effect of CES-D intercept on CRP slope in each ethnic group (Caucasians: p = .07; African Americans' p = .94). Controlling for health and lifestyle factors, the model was a good fit, and effects were non-significant regardless of race

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(statistics not reported). Thus, it appears that the effects of CES-D intercept on CRP slope identified in the full sample can be attributed primarily to Caucasians.

Discussion

The aim of the present study was to shed light on the complex relationship between depressive symptoms and circulating inflammation beginning in early adulthood and continuing into middle age. Childhood stress exposure and allelic variation at *IL6* were hypothesized to moderate the influence of depressive symptoms on inflammation. Trajectories of change in depression and CRP were first modeled separately. Overall, participants began the study with a mean score of 10.6 on the CES-D, with women starting at 11.7, and African Americans at 13.4 (a score of 16 or greater on the CES-D approximates clinically significant depression), and on average, participants' depressive symptoms declined over time, with women and African Americans' depressive symptoms declining more slowly. Mean initial plasma CRP was 1.98 mg/L, which gradually increased from mean age 32 to mean age 50, with the exception of the age 40 measurement time point, when CRP concentrations were higher than at any other time point. Women and African Americans began the study with greater CRP concentrations, and African Americans' CRP increased more steeply across time. Based on previous work, we hypothesized that exposure to early life stress would predict the severity of depression, the concentration of C-reactive protein in the blood plasma, and growth in these outcomes across time. Exposure to early life stress was a strong predictor of the intercept of depression (predicted CES-D scores at age 30), an effect that remained after controlling for a number of demographic, health, and lifestyle factors. Early stress exposure was not associated with change in depressive symptoms across time. Contrary to hypotheses, exposure to early life stress was not a predictor of the intercept or growth in CRP.

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In addition to hypothesizing that early life stress would lead to increases in depressive symptoms and plasma CRP, we expected the intercept of the depression growth curve to predict the slope of the CRP growth curve, and this hypothesis was supported: depression intercept at age 30 was a direct predictor of increasing plasma CRP across the next 18 years. Further, no effect was found in the opposite direction, from CRP measurement at age 32 to growth in depressive symptoms over the next 16 years. Thus, the direction of effect hypothesized in the current study was confirmed. This predictive relationship remained after controlling for age, gender, and ethnicity, and is consistent with a previous, cross-sectional examination of RFQ, psychological wellbeing and CRP in CARDIA, which found an indirect effect of childhood stress exposure on CRP, via its contribution to overall psychological wellbeing (Taylor, Lehman, et al., 2006). When health and lifestyle covariates known to affect depression and CRP were added to the model, the regression of CRP slope on depression intercept was reduced to marginal significance. The reduction in significance suggests that the repeated measures of body mass index, daily cigarette use and physical activity accounted for much of the increase in CRP across the length of the study, and contributed far more to the variance in CRP than did depressive symptoms measured at age 30. Further, despite an overall significant relationship while controlling for ethnicity, the effect of depressive symptoms on CRP was found to differ by race, so that Caucasians' depressive symptoms led to increases in CRP, while African Americans' symptoms were not predictive. African Americans in the present sample were at much greater risk for inflammation based on higher BMI, more frequent cigarette use and less frequent/strenuous physical activity compared to Caucasians. Given that in the overall sample, these health and lifestyle factors accounted for much of the variance in CRP slope, and reduced the effect of depression to marginal significance, it is likely that the heightened risks from these

factors were far more impactful in African Americans, eclipsing the minor contribution of depressive symptoms to CRP levels.

Little information was gleaned from a more fine-grained examination of the relationships between CRP and CES-D measurements using an autoregressive model. Results of this rigorous statistical approach found that only year 10 depression uniquely predicted year 15 CRP over and above the contributions of the previous depression and CRP measurements and concurrent BMI, cigarette use and exercise. We had also hypothesized that early life stress exposure would lead to a stronger relationship between initial levels of depressive symptoms and growth in CRP, as found in previous work looking at these constructs in youth (Danese et al., 2009; Miller & Cole, 2012), but found no moderating effect, even when examined separately by ethnicity. Instead, results indicate that early life stress is a strong contributor to the development of depressive symptoms, which are then a weak contributor to CRP growth.

We addressed a number of hypotheses regarding G×E effects on depression and inflammation at a polymorphic locus in the promoter region of *IL6*. A G allele at this locus has been associated with greater production of interleukin-6, for which C-reactive protein is a stable, long term biomarker. However, findings linking this locus with greater inflammation have been inconsistent, suggesting the possibility of interactive effects between the genetic locus and the environment on inflammatory outcomes (Fishman et al., 1998; Smith & Humphries, 2009). We found no direct relationship between this locus and the intercept or slope of CRP in the present study, regardless of race. Hypotheses that stress exposure might moderate the relationship between *IL6* genotype and depression or CRP were then tested using interactions stratified by race. *IL6* had no moderating effect on the stress-depression relationship. It did, however, moderate the stress-CRP relationship in African Americans, so that exposure to childhood stress

was predictive of the intercept and change in CRP among G allele carriers at -174, but not CC homozygotes. Among Caucasians, the relationship between early childhood stress and CRP was null regardless of genotype. Stress exposure did not differ by ethnicity, and a visual inspection of the interaction showed that stress exposure was related to increased plasma CRP levels for G-allele carriers, while CC homozygotes showed no effect of stress on CRP concentrations.

Finally, variation at *IL6* was examined as a moderator of the depression-inflammation relationship in the present sample, based on previous work demonstrating increased mortality from inflammatory diseases among male and female G allele carriers with high levels of depression (Cole et al., 2010). No moderating effect of *IL6* was identified, even when stratifying results by ethnicity.

In sum, the results of the present study imply that the relationships among stress, depression and C-reactive protein differ for African Americans and Caucasians. Across ethnicities, childhood stress was a strong predictor of depressive symptoms at age 30, and a weaker relationship from depression to later growth in CRP was detected in the full sample, controlling for ethnicity. Neither ethnic group showed a direct effect of childhood stress on plasma concentrations of CRP, and instead, moderating effects specific to each ethnicity were found. Caucasians showed a stronger relationship from depressive symptoms to growth in CRP across time, implying an overall pattern of early stress exposure leading to depressive symptoms, which then predict growth in inflammation over time. African Americans had a much weaker relationship between initial depressive symptoms and growth in CRP. Instead, African Americans' stress exposure was linked directly to depressive symptoms, and was indirectly related to CRP concentrations via genetic moderation at *IL6*. These results suggest that among Caucasians, any effect of childhood stress on inflammation is fully accounted for by its influence

on depression, while among African Americans, the effects of childhood stress on CRP were moderated by *IL6* genotype.

Deverts et al. (2010) found an opposite effect of ethnicity on the relationship between CES-D and CRP at years 15 and 20 of the CARDIA study, such that African Americans showed a significant prediction of year 20 CRP from year 15 CES-D, and Caucasians did not, after controlling for a number of health and demographic covariates. Although the effect of ethnicity found by Deverts et al. (2010) conflicts with the present results, the present study asked a slightly different question, of whether depression predicted the *change* in plasma CRP across 18 years of follow up, rather than whether depression predicted a single measurement of CRP. Further, the present study was able to include an additional 3 measurements of CRP and 4 measurements of CES-D from CARDIA, and followed up on these findings using a stringent, cross-lagged regression model that did not find the effect that Deverts et al. (2010) identified from year 15 CES-D to year 20 CRP.

In the present study, the effects of depression in early adulthood on the growth in CRP through middle age were most easily detected in the absence of other health and lifestyle risks for inflammation. In the full sample, the effect of depression on CRP became marginal accounting for these health and lifestyle factors, and among African Americans, whose health risks were significantly greater than Caucasians' at all time points, there was no significant effect of depression. Thus, the contribution of depression to CRP was detectable only when other risk factors were absent or attenuated in the present study, and this may be true in the general population as well, based on previous work (Matthews et al., 2010; Copeland et al., 2012), but would require further study among different age groups. The specificity of the genetic moderation of childhood stress exposure predicting adult CRP concentrations to African

Americans is puzzling. There were no race differences in childhood stress exposure measured by the RFQ, however, minority status may be associated with greater exposure to discrimination and other sources of stress in childhood and adulthood that are not accounted for by the RFQ. Perceived discrimination has previously been shown to impact cardiovascular health in African Americans (Guyll, Matthews, & Bromberger, 2001), and more generally, interpersonal stress in adulthood has been shown to moderate the relationship between *IL6* and inflammation (Schultze-Florey et al., 2012; Cole et al., 2010).

Childhood stress and inflammatory genetics were hypothesized to lead to the confluence of depression and inflammation based on prior work (e.g, Cole et al., 2010), and the results of the present study suggest that while stress and inflammatory genetics play a role in the relationship between depression and inflammation, the impact of health on these outcomes, and of race, and possibly its associated exposure to stress, is equally, if not more relevant.

Further, the self-report measurement of depression in the present study may have affected the strength of the relationship between depression and inflammation. Previous work has found that diagnosed depressive episodes, particularly cumulative depressive episodes, predict later CRP, and it is possible that using a self-report symptom measure diluted an effect specific to those with more severe depressive symptomatology (Copeland et al., 2012; Miller & Cole, 2012; Pasco et al., 2010). Further, mean BMI in the present sample was unusually high compared to the population average. U.S. adults between 20 and 70 years of age have an average BMI of 26.6 (Centers for Disease Control, 2010), while the present sample had BMI > 27 by age 35, which increased to an average BMI of 30 by age 50. Body mass index may have been particularly influential on the present results, given that obesity has been linked to both depressive symptoms and inflammation (Benson et al., 2008; Dong, Sanchez, & Price, 2004).

The present study also had a number of limitations. While the RFQ has been validated as a measure of exposure to childhood stress, its use in the present study was problematic. The RFQ was administered when participants were age 40, which required recall and self-report of the family environment prior to age 18. Thus, stress was measured 22 years after it occurred, and then used as a predictor of outcomes measured 12-14 years after it occurred. Experienced stress in the present sample was also quite mild, with participants endorsing a severity of less than 5 out of a possible 21 points on the RFQ, meaning that on average, participants endorsed each item as either "rarely or never happened" or "sometimes happened". Further, the only question assessing abuse ("How often were you marked from getting hit?"), showed low frequency of this experience: only 55 participants said that this happened to them very often, and only 258 participants said that this happened more than "sometimes". The stress measured in the present study may have been too distal and mild to directly affect resting inflammation levels in adulthood. While severe stress in childhood, such as high social isolation, abuse or loss of a caregiver is associated with changes in resting CRP at age 32 (Danese et al., 2009), and with changes in the acute inflammatory response to stress (Carpenter et al., 2010), more mild stressors have been associated only with short-term changes in inflammation (Slavich et al., 2010; Murphy et al., 2012). A previous investigation of the CARDIA cohort examined the cross-sectional relationships among RFQ, psychological functioning (which combined several measures, including the CES-D), and CRP, at the age 40 measurement. Results of this investigation were in agreement with the present, longitudinal results, in finding an indirect relationship from childhood stress exposure to plasma CRP in CARDIA, mediated by depressive symptoms.

The temporal separation of the first two measurements of depression at years 5 and 10 from the first measurement of CRP at year 7, may also have weakened associations among childhood stress, depression, and CRP. Depressive symptoms and plasma CRP were not measured concurrently until year 15 of the CARDIA study. The CARDIA study has previously received criticism for measuring CRP only once at each time point, when multiple measurements over a two-week period might have led to more stable readings, and thus increased power to detect relationships among stress, depression and CRP (Deverts et al., 2010).

In the present study, the elimination of subjects with data missing at all time points resulted in a healthier subset of the CARDIA sample, and is therefore limited in its applicability to the full population of African American and Caucasian American adults that CARDIA was designed to represent. The present sample had a higher proportion of Caucasians, was older, had lower CES-D scores, lower body mass index and more years of education compared to excluded participants. Despite the loss of African American subjects due to missing data, significant results were obtained stratifying models by race, indicating that the sample remained powered to detect genetic effects specific to African Americans.

Strengths of the present study include the large sample of over 4,000 participants, over 3,000 of whom had genetic data. The longitudinal dataset with more than three repeated measures for each of the primary outcome variables also contributed to the power of the models, and inclusion of time-varying health and lifestyle variables ensured that a number of alternative explanations of the findings were explored.

In sum, the present study found evidence that exposure to stress in the childhood family environment influences the development of depressive symptoms in early adulthood (age 30), and in turn, depressive symptoms in early adulthood account for change in blood plasma

concentrations of CRP into middle age (age 50). While childhood stress was not found to directly influence plasma C-reactive protein, depression represented a weak moderating pathway, regardless of ethnicity, and a G×E effect of *IL6* was identified specifically in African American participants, so that G allele carriers showed stress-related increases in CRP. Future investigations of G×E effects at *IL6* would benefit from an ethnically diverse sample to further understand ethnic differences in effects. Effects for African Americans need to be replicated to determine whether the effect is due to race, or to a risk factor correlated with race. Possible correlated risk factors that could be more fully explored in future studies are chronic stress, health, behavioral determinants of health, and acute stressful life events. Given that exercise was a strong predictor of depression in the present study, and further, that chronic stress, particularly of an interpersonal nature has been found to interact with *IL6* genotype to predict inflammation previously, these factors would be important for future investigations to address. An interview-based assessment of interpersonal stress in adulthood would align better with the measurements used by previous studies identifying G×E effects at *IL6*, and would increase the power to detect effects.

Chapter 4: General Discussion

The current dissertation tested exposure to early life stress and genetic variation in pro-inflammatory genes as potential risks for the co-occurrence of elevated depressive symptoms and pro-inflammatory cytokines. Previous work has found that close to one-third of persons with depression show abnormally elevated cytokines, including IL-6 and CRP, and have even suggested a subtype of depression characterized by inflammatory changes that is best treated with anti-inflammatory drugs (O'Brien et al., 2007; Raison et al., 2013). Few investigations have addressed the question of how inflammation and depression co-occur, and what factors may predispose to having both of these outcomes, rather than one. Neurobiological mechanisms that allow depressive processes to elicit change in immune function have been identified, and yet, knowledge of these mechanisms has not increased our understanding of why some persons with depression are susceptible to inflammation, while others are not.

Based on a handful of previous studies identifying interpersonal stress as a risk for both depression and inflammation, the present pair of studies examined interpersonal stress exposure as a risk for the co-occurrence of inflammation and depression. A new line of translational work by Cole and colleagues has also identified a single-nucleotide polymorphism in *IL6* that leads to stress-dependent changes in IL-6 transcription (Cole et al., 2010; Cole et al., 2011). The two studies presented here examined variation at this locus as a potential moderator of the relationships between stress exposure and later development of depression and/or inflammation.

The dissertation sought to confirm that in young, healthy adults, depressive symptoms tend to develop before, and contribute to elevations in pro-inflammatory cytokines, and, to determine whether functional variation in immunological genes would create susceptibility to depression and inflammation in response to stress, and/or create susceptibility to inflammation

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following an elevation in depressive symptoms. The dissertation explored these questions in a pair of longitudinal cohort studies: the first followed Australian youth from ages 15 to 20, and included assessments of depressive symptoms, a detailed interview to characterize stress exposure, and genotypic information for a trio of immunological genes (Study 1). The second was a large, multi-site study of American adults (CARDIA) who self-reported depressive symptoms and completed blood draws for measurement of C-reactive protein at regular intervals over a 25 year period, and for whom *IL6* genotype was available (Study 2). While the first study offered an opportunity to examine the interaction of exposure to chronic stress with allelic variation in genes coding for three different pro-inflammatory cytokines as a predictor of depressive symptoms, the second study allowed for the characterization of the relationship between inflammation and depressive symptoms across time, and to test exposure to stress and allelic variation at *IL6* as potential contributors to depression, inflammation and their co-occurrence.

The results of both studies confirmed the strong influence of chronic interpersonal stress exposure on the development of depressive symptoms. In Study 1, exposure to interpersonal or non-interpersonal chronic stress in the year prior to age 20 was strongly associated with depressive symptoms at age 20, while Study 2 found that retrospective ratings of chronic interpersonal stress in childhood, operationalized as levels of neglect, emotional warmth and harsh discipline in the family environment prior to age 18, were associated with depressive symptoms at age 30. These results are consistent with the well-established temporal and presumably contributory effect of stress on depression (Breslau & Davis, 1986; Hammen et al., 2009; Hammen et al., 1985).

Regarding the potential influence of depression on inflammation, Study 2 found that elevations in depressive symptoms at age 30 predicted growth in resting plasma CRP over time, but found no relationship from resting CRP at age 32 to later depression. This pattern of results aligns with previous studies of healthy young adults showing that changes in depressive symptoms precede and predict changes in inflammation (Copeland et al., 2012; Miller & Cole, 2012). It is possible that the depression-inflammation link may be specific to certain types of populations, or may be most easily detected when other risk factors are absent. The present study found that health and lifestyle risks for inflammation were robust predictors of C-reactive protein, and overshadowed the contribution of depression when added to models. Further, the effect of depressive symptoms on growth in CRP was more pronounced in Caucasians in Study 2 (not tested in Study 1). This finding may be explained by the significantly greater health risks for inflammation among African Americans in this cohort, which may obscure any contribution of depression in this population.

In contrast to Study 2 hypotheses, and previous investigations, no direct relationship was observed between childhood stress exposure and age 32 levels of plasma CRP in the CARDIA sample, even controlling for age, race, gender and health risks for heightened inflammation. Stress may have been too mild, as evidenced by a mean severity of 4.69 out of a possible 21 on the Risky Families Questionnaire and low frequency of endorsed physical abuse, or too distal (12 years) to the inflammatory measures to have a direct influence on plasma C-reactive protein. In support of this explanation, previous studies of mild to moderate stressors, including social rejection and the TSST, have identified increases in circulating pro-inflammatory factors immediately following the stressor (Slavich et al., 2010) and 6 months after (Murphy et al., 2012). Thus, long-term differences in resting inflammatory markers as a consequence of

relatively mild stress exposure may be unlikely. Health and lifestyle risks of smoking, body mass index and low levels of physical activity were each associated with increases in CRP in the present sample, and childhood stress has previously been found to predict these risks through its effects on lifestyle choices (Raposa, Bower, Hammen, Najman, & Brennan, 2014). Thus, although not a hypothesis of the present study, it is possible that childhood stress might increase CRP concentrations via its effects on unhealthy lifestyle choices. Future examinations may benefit from including health and lifestyle risks as mediators of stress/depression/CRP relationships, rather than controlling for their effects.

A significant G×E effect on depression was found in Study 1, so that exposure to chronic interpersonal stress in the year prior to depressive symptom measurement interacted with variation at *IL6* and, separately with variation at *IL1β*, to predict depressive symptoms. Greater exposure to chronic interpersonal stress was associated with elevations in depressive symptoms in the presence of -174C at *IL6*, and also -511C at *IL1β*. Study 2 failed to replicate an interactive effect of *IL6* × chronic stress exposure on later elevations in depressive symptoms. A number of factors specific to Study 2, including inferior measurement of stress, may have prevented detection of an interaction. Stress measurement in Study 1 was accomplished with a detailed and objective interview that sorted stressful experiences over the past 12 months into validated categories of interpersonal vs. non-interpersonal experiences. Study 2 used a self-report measure of stress, which likely led to a reduction in power to detect an interaction (Karg, Burmeister, Shedden, & Sen, 2011; Moffitt et al., 2005). The assessment of stress in Study 2 also focused on childhood experience, which lost the advantage Study 1 had of assessing stress that was recent and therefore might have greater effects on current depressive symptoms. In past analyses of G×E effects on depression, interview assessments of stress exposure have fared better than self-

report measures, for example, in showing effects of the serotonin transporter polymorphism (*HTTLPR*) on depression outcomes, (Karg et al., 2011; Moffitt et al., 2005). Thus, the methodological differences in stress measurement between Studies 1 and 2 may have contributed to the discrepancy in results regarding a potential G×E at *IL6*.

Surprisingly, although Study 2 failed to replicate moderation of the stress-depression relationship by *IL6*, an ethnically stratified moderation of the stress-CRP relationship was found, so that exposure to childhood stress was predictive of the intercept and change in CRP among African American participants with the ancestral G allele at -174. This relationship remained null among African Americans with the CC genotype, and among Caucasians, regardless of genotype. Stress exposure did not differ by ethnicity, and a visual inspection of the data indicated that under conditions of high stress exposure (RFQ median split), G-allele carriers' CRP increased, while CC homozygotes' CRP levels began at a moderate level and remained relatively flat through middle age. Taken together, the findings of Studies 1 and 2 show that -174C was associated with greater depressive symptoms in response to stress among primarily Caucasian, Australian, 20-year-olds, while -174G was associated with greater CRP concentrations in response to stress among African Americans from age 32 to age 50. These findings are not directly comparable, due to the many differences in the samples, beginning with the methodological differences in stress measurement.

However, when placed in the context of Cole et al.'s hypothesis that antagonistic pleiotropy is occurring at *IL6*, we may speculate that the pair of findings is consistent with this theory. Cole et al. (2011) posit that the G allele, which is the dominant, ancestral allele, provides protection from inflammation prior to reproductive maturity. Cole et al. (2011) support their hypothesis with the finding that 64 Hispanic and Caucasian adolescents carrying -174G had

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significantly lower plasma CRP in response to life stress, compared to CC homozygotes (Cole et al., 2011). Beyond reproductive maturity, any emergent gene functions would not affect selection at this locus. Cole et al. (2011) therefore hypothesize that the role of -174G reverses following reproductive maturity, becoming a liability for inflammation, rather than a protection. This hypothesis has been supported in a handful of studies showing a stress-dependent increase in IL-6 and CRP among older adults with -174G (Cole et al., 2010; Schultze-Florey et al., 2012). The present pair of studies finds a similar pattern in comparing results for adolescents (age 20), for whom -174C was a risk for depression, with young adults (age 32), for whom -174G is a risk for CRP. Whether these findings constitute evidence of antagonistic pleiotropy at *IL6* awaits replication in a cohort that spans from adolescence into middle adulthood. Previous work examining racial differences in inflammation, for example the higher prevalence of breast cancer in African American women, compared to Caucasian women, has suggested that the higher allelic ratio of G > C at -174 in African Americans might be responsible for the greater prevalence of inflammatory disease in African Americans. In contrast with population level allelic distribution at *IL6*, African Americans in the present sample were primarily CC homozygotes, and thus a higher prevalence of the G allele does not account for the present findings.

The results of Study 2 found evidence that exposure to stress in the childhood family environment (prior to age 18) influences the development of depressive symptoms in early adulthood (age 30), which in turn, account for change in blood plasma concentrations of CRP into middle age (age 50). While childhood stress was not found to directly influence plasma C-reactive protein, depression represented a weak transitional pathway from stress to CRP,

regardless of ethnicity, and a G×E effect of *IL6* was identified specifically in African American participants, so that G allele carriers showed stress-related increases in CRP.

Each of the dissertation studies had several limitations. Although the findings of each study indicated that G×E effects were occurring at *IL6*, neither study was equipped to evaluate the hypothesis that antagonistic pleiotropy is occurring at this locus, and neither study corrected for multiple analyses given the exploratory nature of the gene-environment hypotheses. The sample size of Study 1 was relatively small for a candidate gene study, which reduced statistical power. The selection of the final subsample of the CARDIA cohort selected for analysis in Study 2 was based on completion of at least one measurement of depressive symptoms or CRP, and was healthier than the overall CARDIA sample as evidenced by less cigarette use, lower BMI and more frequent physical activity among those included in the dissertation, as compared to CARDIA participants excluded from the present analyses. These health and lifestyle factors have been linked with the outcomes of interest, and therefore the results of the study may not be applicable to the full CARDIA sample. The retrospective measurement of stress in the family environment in Study 2 was also problematic. At mean age 40, participants were asked to recall stressful experiences that occurred prior to age 18, and this self-report measure was then used as a predictor of depressive symptoms and CRP concentrations beginning at age 30. The convoluted and distal assessment of stress in this study, along with the use of a self-report rather than an interview measure of stress exposure, may have limited the ability to detect effects, particularly when examining G×E hypotheses. Finally, the G×E effects reported in each study are in need of replication in an ethnically diverse sample that combines a validated, interview assessment of chronic interpersonal stress exposure, along with multiple measurements of CRP and of depressive symptoms, as well as diagnosis of depressive episodes.

In summary, findings from the dissertation suggest that depression plays a detrimental and lasting role in the development of inflammation. Based on the results of the current pair of studies, the effects of stress experienced in childhood and late adolescence have consequences into middle age that may decrease overall health and longevity via contributions to chronic, low grade inflammation. Future study of the long-term impact of stress on physical health would benefit from including measures of both depression and lifestyle choices (cigarette use, physical activity, body mass index) as mediators. Further, the nature of these relationships potentially differs by ethnicity, and risks associated with minority status, including stress resulting from discrimination, merit further clarification.

Future studies would benefit from continuing to test the antagonistic pleiotropy hypothesis using exacting measurements of multiple types of stress exposure, and by combining multiple measurements of resting immune activity with acute immunological responses to stress. A more thorough understanding of the relationships among depression, inflammation and stress, and the role of *IL6* and other relevant immunological genes may lead to targeted interventions for depression. For example, early experimental tests of anti-inflammatory treatments for depression have shown benefit in patients whose depression is marked by inflammatory activity. Early psychological interventions to prevent the development of depressive symptoms following exposure to early life stress may mitigate the potentially life-long disadvantage of early stress exposure, and prevent some of the effects of stress on poor health and lifestyle choices, and subsequently, depression and inflammation. The roots of life-limiting disease appear to begin in childhood, and the physical and psychological consequences of stress exposure are tightly related and mutually increasing. As our knowledge of the interdependence of the psychological and

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biological contributions to inflammatory disease become better known, interventions that

combine medical and psychological interventions will likely be more effective than either alone.

Tables

Table 1.
Socio-Demographic and Genetic Characteristics of Study Participants (Study 1)

	Males (*n* = 170)	Females (*n* = 250)
BDI-II Age 15 (SD)	5.20 (6.76)	6.30 (7.43)
BDI-II Age 20 (SD)	4.84 (6.08)	7.86 (9.71)
MDE at age 20 (yes/no)	12/158	31/219
Maternal Depression History (yes/no)	76/94	111/139
IL6 -174G>C		
CC	61	95
GC	85	110
GG	24	45
TNF -308G>A		
AA	2	8
GA	43	73
GG	125	169
IL1β -511C>T		
TT	76	113
TC	72	115
CC	22	22
Asthma (yes/no)	12/155	23/222

Table 2.

Hierarchical linear regression analyses of inflammatory genotypes, chronic interpersonal stress severity in the past 6 months, and their interactions, predicting depressive symptoms at age 20 (Study 1)

| | Age 20 BDI-II Score | | | |
Predictor	*b*	*SE*	*β*	*F change for interaction term*
IL6 Model				
Maternal Depression	2.01	0.65	3.09**	
Gender	0.51	0.65	0.78	
Asthma	-0.14	0.88	-0.01	
Age 15 BDI-II Score	0.41	0.05	0.32***	
Chronic Interpersonal Stress Age 20	1.39	0.19	0.43***	
IL6⁺	-0.17	0.45	-0.02	
Chronic Interpersonal Stress Age 20*IL6	-0.44	0.18	-0.14*	5.71*
IL1β Model				
Maternal Depression	1.96	0.65	0.12**	
Gender	0.53	0.65	0.03	
Asthma	-0.00	0.89	0.00	
Age 15 BDI-II Score	0.41	0.05	0.32***	
Chronic Interpersonal Stress Age 20	0.76	0.19	0.24***	
IL1β ⁺	0.38	0.49	0.03	
Chronic Interpersonal Stress Age 20*IL1β	0.44	0.22	0.13*	5.29*
TNF Model				
Maternal Depression	2.07	0.65	0.13**	
Gender	0.46	0.65	0.03	
Asthma	-0.21	0.88	-0.01	
Age 15 BDI-II Score	0.41	0.05	0.32***	
Chronic Interpersonal Stress Age 20	1.74	0.46	0.54***	
TNF⁺	0.56	0.60	0.04	
Chronic Interpersonal Stress Age 20*TNF	-0.41	0.26	-0.22	2.53

$*p < .05$, $**p < .01$, $***p < .001$; ⁺Coded as 0/1/2 minor alleles

Table 3.
CARDIA Selected Sample Demographics (Study 2)

Study Time Point	N	Age, Mean (SD)	% Women	% African Am
Year 5	3950	30.04 (3.63)	53.8	47.9
Year 7	3826	32.06 (3.62)	53.6	48.1
Year 10	3678	35.05 (3.63)	54.1	48.5
Year 15	3511	40.20 (3.63)	54.5	47.3
Year 20	3397	45.24 (3.62)	55.5	46.7
Year 25	3355	50.17 (3.64)	55.4	47.0

Table 4.
CARDIA Variable Means by Ethnicity (Study 2)

	All Participants			African Americans			Caucasians		
Study Variable	**M (SD)**	**N**	**% in clinical range**	**M (SD)**	**N**	**% in clinical range**	**M (SD)**	**N**	**% in clinical range**
Risky Families Questionnaire, Year 15[†]	0.67 (0.59)	3496		1.68 (0.57)	1642		1.66 (0.60)	1854	
Physical Activity, year 5	1.27 (0.98)	3915		1.20 (1.03)	1862		1.34 (0.93)	2053	
Physical Activity, year 7	1.14 (0.91)	3762		1.08 (0.98)	1794		1.19 (0.84)	1968	
Physical Activity, year 10	1.12 (0.93)	3651		1.04 (0.97)	1751		1.19 (0.89)	1900	
Physical Activity, year 15	1.17 (0.95)	3498		1.08 (0.99)	1643		1.24 (0.91)	1855	
Physical Activity, year 20	1.13 (0.92)	3376		1.00 (0.94)	1561		1.24 (0.88)	1815	
Physical Activity, year 25	1.13 (0.92)	3323		0.96 (0.89)	1543		1.28 (0.92)	1780	
Body mass index, year 5	26.04 (5.65)	3935		27.21 (6.32)	1876		24.98 (4.72)	2059	
Body mass index, year 7	26.65 (5.91)	3783		27.86 (6.49)	1801		25.54 (5.08)	1982	
Body mass index, year 10	27.38 (6.24)	3628		28.78 (6.77)	1741		26.08 (5.40)	1887	
Body mass index, year 15	28.71 (6.68)	3494		30.32 (7.14)	1641		27.28 (5.90)	1853	
Body mass index, year 20	29.40 (7.05)	3379		31.13 (7.38)	1561		27.92 (6.40)	1818	
Body mass index, year 25	30.05 (6.96)	3347		31.89 (7.36)	1561		28.45 (6.16)	1786	
Cigs smoked/day, year 5	12.98 (9.00)	1108		10.66 (7.09)	642		16.17 (10.30)	466	
Cigs smoked/day, year 7	12.43 (9.33)	784		10.64 (7.95)	611		16.42 (10.45)	432	
Cigs smoked/day, year 10	11.95 (9.13)	667		10.91 (7.55)	558		15.91 (10.03)	371	
Cigs smoked/day, year 15	11.12 (7.82)	583		10.89 (8.08)	482		14.90 (10.60)	302	
Cigs smoked/day, year 20	13.04 (9.50)	1043		9.81 (7.20)	404		15.23 (10.70)	263	
Cigs smoked/day, year 25	12.91 (8.96)	929		9.81 (7.11)	364		13.30 (8.47)	219	
CES-D, year 5	11.08 (8.05)	3903	23.70%	12.47 (8.44)	1852	29.8%	9.82 (7.47)	2051	18.2%
CES-D, year 10	10.62 (8.17)	3619	21.60%	12.09 (8.88)	1732	28.1%	9.27 (7.21)	1887	15.7%
CES-D, year 15	9.10 (7.81)	3467	16.60%	10.44 (8.33)	1623	22.4%	7.91 (7.11)	1844	11.6%
CES-D, year 20	9.27 (7.83)	3303	17.60%	10.41 (8.38)	1518	22.0%	8.29 (7.20)	1785	13.9%
CES-D, year 25	9.40 (7.69)	3314	16.90%	10.28 (8.23)	1540	20.8%	8.64 (7.09)	1774	13.6%
C-reactive protein (mg/L), year 7	1.98 (2.11)	3401	22.90%	2.30 (2.31)	1618	27.8%	1.70 (1.87)	1783	18.5%
C-reactive protein (mg/L), year 15	2.25 (2.26)	3119	26.40%	2.71 (2.46)	1439	34.0%	1.85 (1.98)	1680	19.9%
C-reactive protein (mg/L), year 20	2.11 (2.26)	3054	23.90%	2.56 (2.49)	1415	30.8%	1.73 (1.97)	1639	17.9%
C-reactive protein (mg/L), year 25	2.11 (2.11)	3095	24.00%	2.58 (2.33)	1395	31.8%	1.73 (1.83)	1700	17.6%

[†] All study variables in table differed by race at significance <.001, *except for the Risky Families Questionnaire, which was no different for African Americans vs. Caucasians.* High-risk range for CRP is >/= 3 mg/L, CESD Scores >/= 16 considered equivalent to clinical depression, Physical Activity given in units of 1 = 300 Exercise Units.

Table 5.

Fit Indices of Latent Growth Curve Models (Study 2)

Model	N	(df) χ2 fit	RMSEA (90% CI)	CFI	SRMR	AIC	BIC
C-reactive protein							
Null (random intercept)	4276	(8) 283.61***	0.09 (0.08-0.10)	0.93	0.06	33,099.84	33,138.01
Unconditional	4276	(5) 143.81***	0.08 (0.07-0.09)	0.97	0.04	32,966.04	33,023.29
Conditional: time invariant	3289	(13) 144.51***	0.06 (0.05-0.06)	0.97	0.02	27,650.05	27,753.72
Conditional: time variant	3289	Monte Carlo Integration				204,204.23	205,003.12
CESD							
Null (random intercept)	4276	(13) 432.06***	0.09 (0.08-0.09)	0.93	0.07	117,463.06	117,507.59
Unconditional	4276	(10) 145.55***	0.06 (0.05-0.07)	0.98	0.04	117,182.56	117,246.17
Conditional: time invariant	3289	(22) 152.69***	0.04 (0.04-0.05)	0.98	0.02	99,728.47	99,838.24
Conditional: time variant	3289	(217) 12,887.88***	0.13 (0.13-0.14)	0.27	0.16	269,613.65	270,674.76
Parallel Process	**N**	**(df) χ2 fit**	**RMSEA (90% CI)**	**CFI**	**SRMR**	**AIC**	**BIC**
Unconditional	4276	(31) 302.20***	0.05 (0.04-0.05)	0.97	0.03	150,089.53	150,235.83
Conditional: time invariant	3289	(51) 311.43***	0.04 (0.04-0.04)	0.97	0.02	127,373.39	127,611.23
Conditional: time variant	3289	Monte Carlo Integration				301,925.47	302,949.99

***$p < .001$

Table 6.

Latent Growth Curve Model Parameters for CRP and Depression Models (Study 2)

C-reactive Protein Model	Mean Intercept	Mean Slope	Intercept Variance	Slope Variance	Intercept, Slope Covariance
Null (random intercept)	0.267***	not estimated	0.653***	Not estimated	Not estimated
Unconditional	0.192***	0.007***	0.703***	0.001***	-0.008***
Time invariant	0.186***	0.007*	0.631***	0.001***	-0.006***
Age effect	0.005	0.000			
Gender effect	0.313***	-0.005*			
RFQ effect	0.001	0.002			
Race effect	0.369***	0.007**			
Time variant[b]	0.121*	-0.005*	0.427***	0.001***	-0.004*
Age Effect	-0.006	0.000			
Gender effect	0.294***	-0.007**			
RFQ effect	-0.038	0.002			
Race effect	0.145***	0.005*			

CES-D Model	Mean Intercept	Mean Slope	Intercept Variance	Slope Variance	Intercept, Slope Covariance
Null (random intercept)	10.008***	No slope.	32.800***	No slope.	Not estimated.
Unconditional	10.849***	-0.079***	35.403***	0.047***	-0.358
Time invariant	10.600***	-0.079***	27.339***	0.042***	-0.239
Age effect	-0.073*	0.004[a]			
Gender effect	1.139***	-0.027[a]			
RFQ effect	3.074***	-0.002			
Race effect	2.814***	-0.058***			
Time variant[b]	10.684***	-0.086*	27.257***	0.043***	-0.260***
Age effect	-0.082*	0.004*			
Gender effect	0.996***	-0.032*			
RFQ effect	3.056***	-0.003			
Race effect	2.847***	-0.067***			

*$p <.05$, **$p <.01$, ***$p <.001$, [a]$p <.10$, [b]Results for covariates of BMI, frequency of cigarette use and physical activity reported in text.

Table 7.
Parallel Process Model Parameters (Study 2)

	Mean Intercept	Mean Slope	Intercept Variance	Slope Variance	Intercept, Slope Covariance	Intercept covariance	Slope covariance	CRP slope regressed on CESD intercept	CESD slope regressed on CRP intercept
Unconditional Model									
C-reactive protein	0.176***	0.008	0.739***	0.001***	-0.010***	0.539***	-0.001ᵃ	0.001**	< 0.001
Depressive symptoms	10.853***	-0.079***	35.366***	0.047***					
Time Invariant Covariates									
C-reactive protein	0.172***	0.007	0.661***	0.001***	-0.009***	0.120	<.001	0.001ᵃ	0.008
Age effect	0.005	<.001							
Gender effect	0.322***	-0.005*							
RFQ effect	-0.004	0.001							
Race effect	0.355***	0.005*							
Depressive symptoms	10.599***	-0.079*	27.335***	0.042***	-0.237***				
Age effect	-0.073*	0.004ᵃ							
Gender effect	1.139***	-0.030*							
RFQ effect	3.074***	-0.002							
Race effect	2.813***	-0.061***							
Time Variant Covariatesᵇ									
C-reactive protein	0.130*	-0.005**	0.455***	0.001***	-0.006**	-0.068	<.001	<..001ᵃ	0.017
Age effect	-0.007	<.001							
Gender effect	0.319***	-0.008***							
RFQ effect	-0.043	0.001							
Race effect	0.127**	0.004ᵃ							
Depressive symptoms	10.616***	-0.081*	27.287***	0.043***	-0.259**				
Age Effect	-0.080*	0.004*							
Gender Effect	0.967***	-0.036*							
RFQ effect	3.068***	-0.003							
Race effect	2.790***	-0.067***							

*p <.05, **p <.01, ***p <.001, ᵃ p <.10, ᵇ Results for covariates of BMI, frequency of cigarette use and physical activity reported in text

Figures

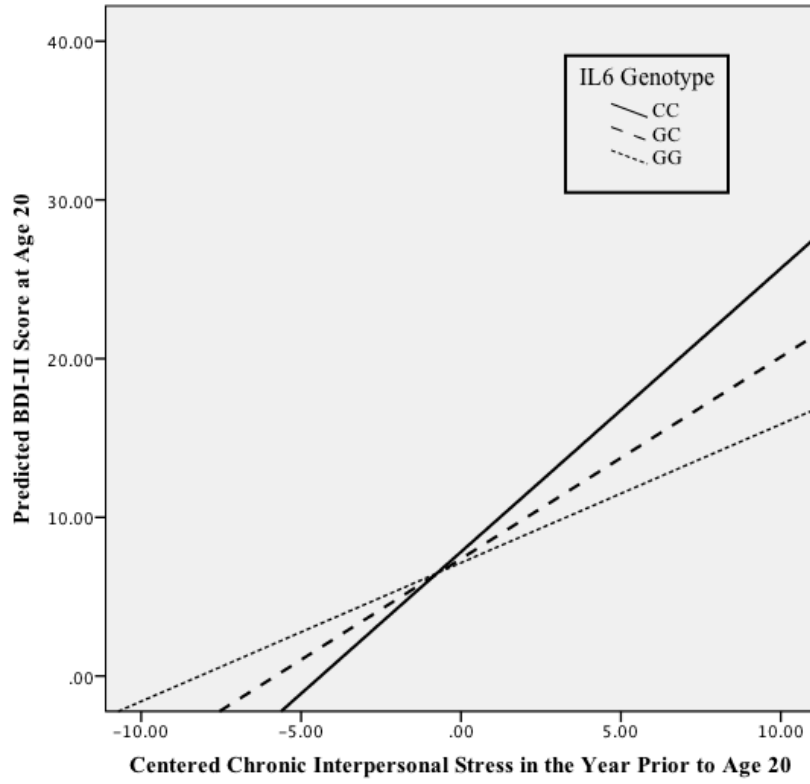*Figure 1.* Interaction of *IL6* with chronic interpersonal stress predicts BDI-II (Study 1).

Figure 2. Interaction of *IL1β* with chronic interpersonal stress predicts BDI-II (Study 1).

Figure 3. Cross-lagged model of regressions among CES-D and CRP measurements in CARDIA (Study 2).
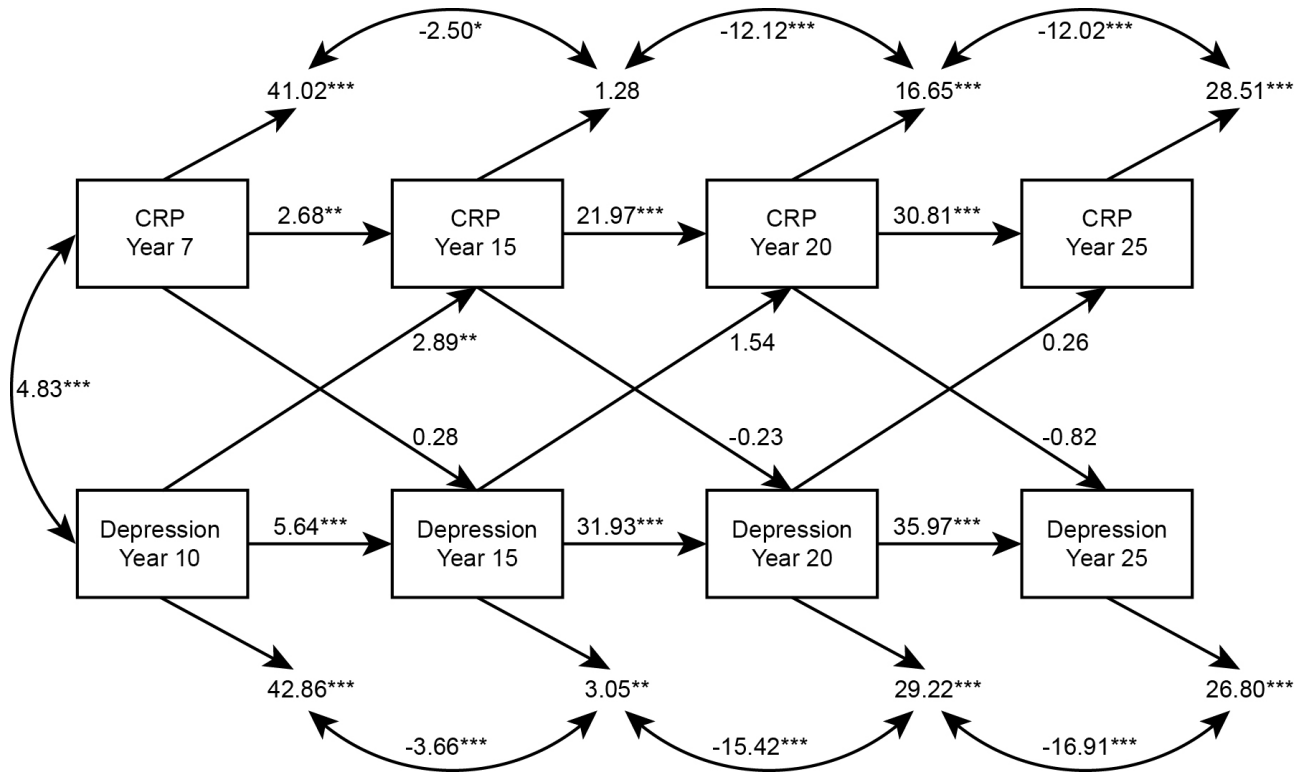
Figure 4. Interaction of *IL6* genotype, race, and stress exposure, predicts C-reactive protein in the CARDIA sample: Effects of differing stress exposure among African Americans carrying the -174G allele (Study 2).
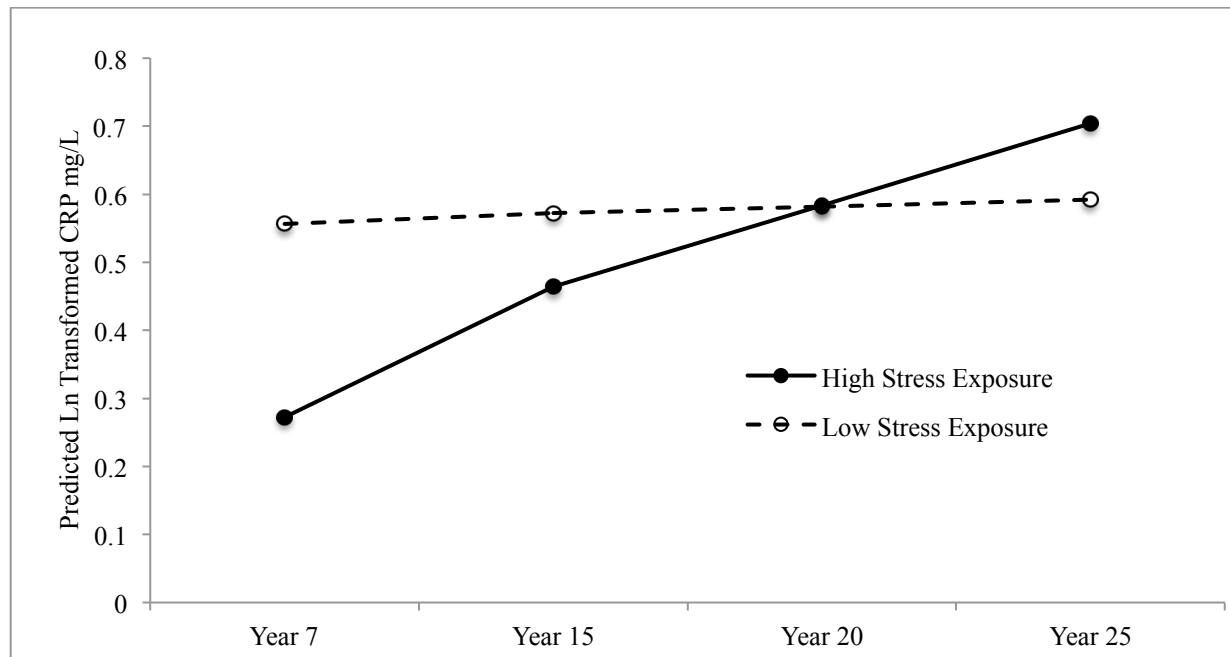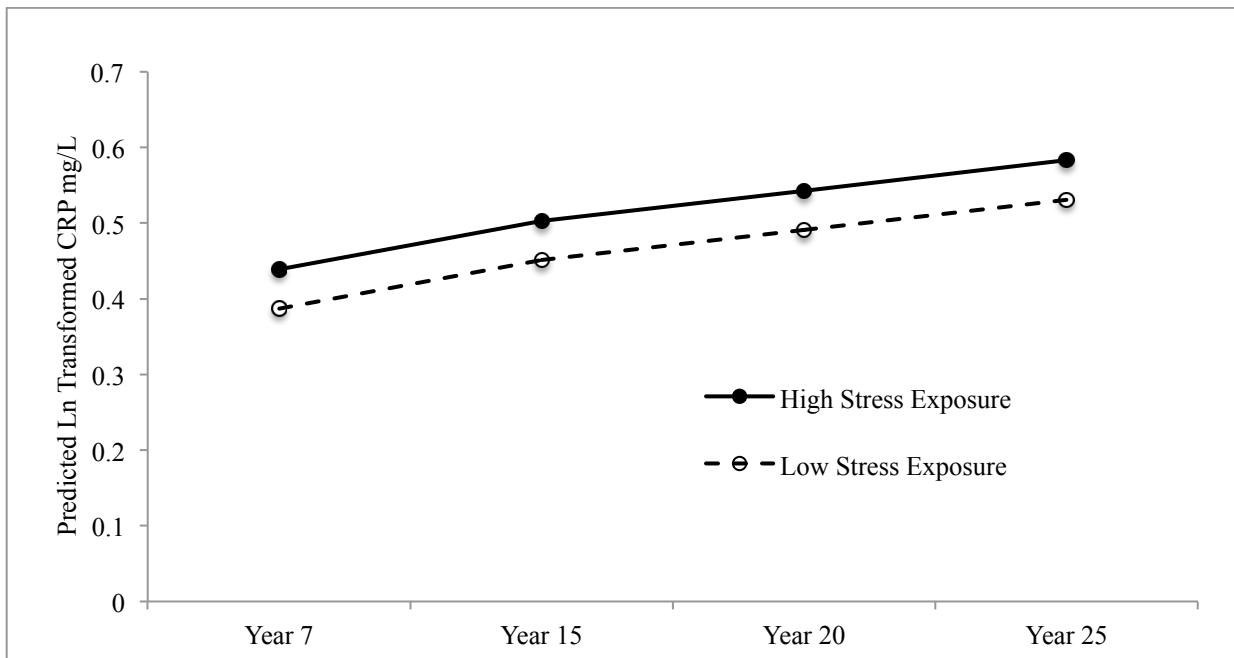
Figure 5. Interaction of *IL6* genotype, race, and stress exposure, predicts C-reactive protein in the CARDIA sample: Effects of differing stress exposure among African Americans homozygous for the -174C allele (Study 2).

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