SOCIOECONOMIC STATUS AS A CORRELATE OF PLASMA INFLAMMATORY MARKERS: AN ASSOCIATION THAT MAY BE MODULATED BY THE INTERLEUKIN 6 (-174) G/C PROMOTER POLYMORPHISM

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Growing evidence suggests that socioeconomic attributes of both individuals and communities of residence confer risk for cardiovascular morbidity and mortality. Despite decades of research demonstrating worse health behavior profiles with lower levels of SES, unexplained variance in the association between SES and cardiovascular disease persists. In this study, we examined the association between both individual and community SES and inflammatory markers relevant to cardiovascular pathophysiology (i.e. interleukin-6 and C-reactive protein) in a diverse sample of healthy community volunteers. We also examined whether a previously identified functional single nucleotide polymorphism on the IL-6 gene (-174 G>C) is associated with IL-6 and CRP in this sample, and whether it moderates any influence of SES on these inflammatory markers. Subjects were middle-aged men and women (m=424, f=427; Caucasian=76.7%, African-American=22.8%) from the Adult Health and Behavior Project. Individual SES was indexed by averaging educational attainment and family income, and community SES was a composite of five variables from the participants' US Census tract of residence. Regression analyses accounting for age, sex, and race showed individual SES to be associated with IL-6 ($B=-.126$, $p < .001$) and community SES to predict both IL-6 and CRP ($B= -.163$, $p <.0001$, $B= -.129$, $p = .002$, respectively). The inverse relationship between community SES and both inflammatory mediators persisted on multivariable adjustment for health behaviors (smoking, alcohol consumption, sleep, exercise, body mass index) and

individual SES (IL-6: B=-.091, p = .028 and CRP: B = -.086, p = .027). The relationship between individual SES and IL-6 did not withstand health behavior adjustment. Genetic analyses on the Caucasian subsample showed GG homozygotes to have lower CRP than participants with any C allele (i.e. GC & CC; t_{622}=2.00, p <.05), but genotype by SES interactions were not significant. In sum, our results show that regardless of individual income, educational attainment, or -174 G>C genotype, mid-life adults living in less advantaged neighborhoods have higher levels of circulating proinflammatory markers than residents of more affluent areas. This inflammation could mediate the association between lower community SES and atherosclerotic cardiovascular morbidity and mortality.

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PREFACE

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1.0 INTRODUCTION

It is well established that relative social and economic disadvantage increases risk for diverse health outcomes, including most major sources of population morbidity and mortality (e.g. Adler, Boyce, Chesney, Folkman & Syme, 1993; Adler et al., 1994; Adler & Ostrove, 1999). Despite an abundance of research, the mechanisms responsible for such associations are not well understood. Conceptually, socioeconomic position reflects one's ranking within a larger social structure, and this structure might conceivably affect health in a variety of ways. For example, variation in socioeconomic strata gives rise to differential access to preventative and health care resources and disparate levels of exposure to adverse (health-impairing) conditions of living (Lynch & Kaplan, 2000). Abundant speculation also exists surrounding stress and various neuroendocrine reactions to stressful life experiences, which may vary with socioeconomic position, contribute to disease etiology, or reflect a common biological process related to multiple health risks (e.g. McEwen, 2000). Whatever the mechanisms, however, any hypothesis to explain the relationship between socioeconomic position and health must be derived, at least in part, from a conceptualization of socioeconomic status itself. The purpose of this section of the paper, then, is to provide a context for the conceptualization of socioeconomic status, followed by a review of the research demonstrating an association between SES and cardiovascular disease.

1.1 CONCEPTUALIZATION OF SOCIOECONOMIC STATUS

Lynch & Kaplan (2000) summarize three sociological traditions that give rise to divergent ways of conceptualizing contemporary social stratification. Marxist theory is based on the notion that the class structure of a society arises out of a relationship between people and the production of commodities. This theory is essentially a structuralist view, positing that a person's social position (or social class) is dependent on his or her role in economic production. According to Marx, then, exploitation by owners generates distinct classes that are inherently in opposition to each other in a capitalist society. In contrast, Weber proposed an individualist perspective on social stratification that has also been highly influential in sociological thinking. Weber suggested that capitalism created different "life chances" for individuals. In Weberian sociology, the productive structure of society creates a systematically different distribution of resources, such as education, skills, assets, and opportunities that, in turn, contribute to an individual's likelihood of accruing wealth and attaining membership in any particular stratum of society. A third sociological tradition that has helped to shape current conceptualizations of socioeconomic status is the Functionalist perspective. Functionalists argue that because complex societies require diverse trades and skills (some trades requiring more skills than others), social stratification is, in a sense, inevitable. Although each employee class is important in such a society, compensation and esteem vary according to position type.

The conceptualization of socioeconomic status in most of the social sciences today relies heavily on these sociological traditions, particularly the Weberian school of thought. Thus, modern "measures" of socioeconomic position often attempt to quantify an individual's "life chances" by reference to income, educational attainment, occupation, or some combination of these factors. Education, in particular, may relate significantly to a person's "chances" of

succeeding in society, whereas income may better reflect the degree to which an individual has succeeded. Occupation functions as the connection between education and income. Presumably, all three of these factors correspond significantly with access to important resources and exposure to adverse conditions of life, with potential effects on health outcomes. In turn, these indices of "life chances" can be understood as an extension of political and economic structures. Recently, it has become apparent that analogous features of the social environment, such as the average income, educational attainment, and unemployment levels of an individual's community of residence, also contribute to disease risk (including that for cardiovascular disease) and may do so independently of individual level socioeconomic status (reviewed in Robert, 1999). Therefore, measures of socioeconomic status at the level of the community (neighborhood, census tract, postal code areas) provide additional information regarding the effects of variation in social and economic resources on health and will be considered as a unique measure of socioeconomic status in this paper**.**

1.2 SOCIOECONOMIC STATUS AND CARDIOVASCULAR DISEASE

A graded, inverse association exists between individual socioeconomic position and cardiovascular disease in industrialized, Western societies (Adler & Ostrove, 1999). This relationship exists irrespective of gender, race, or the index of individual socioeconomic status that is used (income, education, occupation, or a composite of these measures) (reviewed in Kaplan & Keil, 1993; Pickering, 1999). Variation in socioeconomic status is inversely related to both coronary heart disease (e.g. Cassel et al., 1971; Keil, Loadholt, Weinrich, Sandifer & Boyle, 1984; Liu et al., 1982; Lynch, Krause, Kaplan, Tuomilehto, & Salonen, 1997; Pocock,

Sharper, Cook, Phillips & Walker, 1987; Rose & Marmot, 1981; Siegel et al., 1987; Woodward, Shewry, Smith & Tunstall-Pedoe, 1992) and incidence of cardiovascular mortality (e.g. Keil, Sutherland, Knapp & Tyroler, 1992; Lynch et al., 1997; Rose & Marmot, 1981; Salonen, 1982; Virtanen & Notkola, 2002; Williams et al., 1992). Variation in socioeconomic indicators has also been associated inversely with both survival and ability to return to work following an acute cardiovascular event (e.g. Pell & D'Alonzo, 1964; Ruberman, Weinblatt, Goldberg, & Chaudary, 1984; Smith & O'Rourke, 1988; Weinblatt, Shapiro, Frank & Sager, 1966; Williams et al., 1992; Young, Waller, & Kahana, 1991). Finally, low childhood socioeconomic status predicts ischemic heart disease, including myocardial infarction and coronary death, in adulthood (Kaplan & Salonen, 1990; Notkola, Punsar, Karvonen & Haapakoski, 1985).

As mentioned previously, area level indices of socioeconomic status are also used in predicting health outcomes. Research indicates that inferior community socioeconomic characteristics predict greater cardiovascular morbidity (Eachus et al., 1996) and higher rates of coronary heart disease mortality (Eames, Ben-Shlomo, & Marmot, 1993). Importantly, recent work incorporating both individual and community SES reveals a unique contribution of area SES to cardiovascular outcomes (Waitzman & Smith, 1998; Smith et al., 1988; Diez-Roux et al., 1997; Diez-Roux et al., 2001; Hart et al., 1997). For example, cardiovascular mortality rates were inversely related to community SES, a relationship that remained significant after controlling for individuals' social class (Waitzman & Smith, 1998; LeClere, Rogers, & Peters, 1998). Prevalence of coronary heart disease (based on electrocardiographic indicators, history of coronary heart surgery or balloon angioplasty, or physician diagnosed myocardial infarction) and incidence of coronary events (probable myocardial infarction resulting in hospitalization, death due to coronary heart disease, or myocardial infarction documented as a change in ECG) were

also generally worse for those individuals living in lower SES communities, irrespective of individual indicators of SES (Diez-Roux et al., 1997; Diez Roux et al., 2001).

These literatures show that both individual and community measures of socioeconomic status are important predictors of the symptomatic expression of cardiovascular disease (i.e. angina pectoris, myocardial infarction, arrhythmogenesis, or sudden cardiac death). Recent work, however, indicates that SES is also important in the pathogenesis of cardiovascular disease. Individual SES predicts preclinical carotid and aortic atherosclerosis, as indicated by a greater thickening of the intimal and medial layers of the artery wall (Diez-Roux, Nieto, Tyroler, Crum & Szklo, 1995; Lynch, Kaplan, Salonen, Cohen & Salonen, 1995; Gallo, Matthews, Kuller, Sutton-Tyrrell & Edmundowicz, 2001; van Rossum, van de Mheen, Witteman, Mackenbach, & Grobbee, 1999). Variation in arterial elasticity is inversely associated with educational achievement in the four-site Atherosclerosis Risk in Communities (ARIC) study (Din-Dzietham et al, 2000), and, finally, individual educational attainment and income are associated with progression of carotid artery atherosclerosis in men with and without ischemic heart disease over a four-year period (Lynch, Kaplan, Salonen & Salonen, 1997). Community SES has also been shown to independently predict plaque occurrence in hypertensive men (Petersen et al, 2006.)

1.2.1 Mediators of the SES-cardiovascular disease relationship

Despite significant findings linking socioeconomic status to both preclinical cardiovascular disease and clinical events associated with more advanced stages of the disease, how socioeconomic status might be associated with these outcomes remains unclear. Variation in the socioeconomic attributes of both individuals and areas is associated with many adverse health

behaviors and cardiovascular risk factors. Lower community SES is associated with worse dietary habits of youth (Lee & Cubbin, 2002), increased smoking behavior (Smith et al., 1998; Diez-Roux, 1997; Reijneveld et al., 1998; Sundquist et al., 1998; Duncan et al., 1999), and decreased physical activity levels (Sundquist et al., 1998, Yen & Kaplan, 1998). Variation in physiological risk factors is also associated with socioeconomic characteristics. Left-ventricular mass and reactivity to stress are inversely related to community socioeconomic status in African-American children (Gump, et al., 1999). Also, higher systolic blood pressure is associated with both community and individual SES (Diez-Roux et al., 1997; reviewed in Pickering, 1999); greater airway resistance, higher BMI and obesity, and anginal symptoms are related to worse community quality (Diez-Roux et al., 1997; Smith et al., 1998; Sundquist et al., 1998). The association between cholesterol levels and socioeconomic status is less clear. One study shows some variation in cholesterol levels to be attributable to district of residence (Hart et al., 1997), another study suggests community SES is related inversely to cholesterol levels in 3 out of 4 ARIC test sites (Diez-Roux et al., 1997), and a final study does not report a significant, independent association between cholesterol levels and community SES (Smith et al., 1998). Results for individual SES and cholesterol are similarly mixed (reviewed in Pickering, 1999). A modest inverse association exists with individual SES and infection from bacteria and virus strains hypothesized to play a role in the pathogenesis of cardiovascular disease (e.g. Go, 2002; Romeo et al., 2001).

Adverse socioeconomic conditions are also associated with multiple psychosocial risk factors for cardiovascular disease. These risk factors include personality characteristics like hostility, hopelessness, and feelings of perceived control and mastery, and psychopathology, such as more prevalent depression in lower SES individuals (e.g. Rozanski et al., 1999). Lower

SES appears to be associated with significantly more chronic stress, as measured and conceptualized in multiple ways. For example, lower SES is associated with more environmental challenges (Baum et al., 1999)**,** fewer coping resources to deal with these challenges (Rosengren et al., 1998), greater job strain (including greater effort and lower reward as SES declines; e.g. Bosma et al., 1998), and greater measures of chronic life stress (i.e. health problems of significant others, problems with living conditions, financial stressors; Lynch et al., 1997). Related measures in community environments show that more stressful life events such as job loss and criminal victimization are reported in areas with more adverse socioeconomic conditions. Finally, social support, social integration, and social isolation are all worse in lower SES environments (e.g. Turner & Marino, 1994).

1.3 INFLAMMATION

1.3.1 Background

In most studies, multivariate adjustment for the aforementioned risk factors weakens, but does not eliminate, the graded relationship between socioeconomic status and cardiovascular morbidity and mortality (reviewed in Kaplan & Keil, 1993; Gallo et al., 2001; Lynch et al., 1995; Diez-Roux et al., 1995; Din-Dzietham et al., 2000; Gallo et al., 2001; Lynch et al., 1995; Diez-Roux et al., 1995, van Rossum et al., 1999). Thus, other factors must contribute to this relationship. One possible candidate is inflammation, which has recently been shown to be a risk factor for the development and exacerbation of atherosclerosis.

Prior to the 1990s, atherogenesis was thought to involve the passive accumulation of lipids in arterial walls, leading to progressive occlusion of the vessel and associated symptoms of cardiovascular disease. More recent models propose that inflammation plays a primary and essential role in atherogenesis and thus the incidence and course of CVD (e.g., Ross, 1999). In these models, the immune system is activated in response to vascular injury, i.e. from oxidized low density lipoproteins, free radicals (e.g. from smoking), hemodynamic perturbations**,** elevated plasma homocysteine**,** or infectious microorganisms. The inflammatory response should work to remove the offending agent and repair the tissue, but if the agent is not removed, the theory states that the inflammatory response will persist, activate portions of the adaptive immune response (i.e. T-cells) and become a chronic problem, rather than a solution. The result is a fibroproliferative process that leads to obstruction, and eventually to the development of vulnerable, complicated plaques with potential for rupture and thrombosis.

In a healthy artery leukocytes rarely attach to the endothelium. In response to injury , the endothelium expresses adhesion molecules that bind leukocytes and platelets. This leukocyte adherence is, presumably, the first stage of the response to injury. An increased permeability of the endothelium allows leukocytes to migrate into the intima through gaps between endothelial cells, initiating a localized inflammatory response. Here, monocytes mature into macrophages that express scavenger receptors for ingesting modified lipoproteins, and the foam cell is formed. In the continued presence of injury, multiple foam cells form and result in the earliest atherosclerotic lesion, the fatty streak. Activated macrophages proliferate within the lesion and release multiple pro-inflammatory cytokines, chemoattractants, growth factors, and hydrolytic enzymes, perpetuating this localized inflammatory response. If the response persists, it is thought that a fibrofatty plaque, with a necrotic, lipid-laden core, containing multiple layers

of smooth muscle cells, connective tissue, macrophages and T-lymphocytes develops. Processes of repair yield a fibrous cap that contains the lesion until sufficient macrophages accumulate. It is thought that this accumulation of macrophages erodes or thins the fibrous structure and leaves the plaque vulnerable to rupture (Ross, 1999; Libby, 2002; Libby, Ridker, & Maseri, 2002).

Research supporting this theory has proliferated in the last decade, and evidence now exists from several different lines of investigation to suggest a role for inflammation in the pathobiology of cardiovascular disease. Epidemiological evidence from diverse populations shows inflammatory markers, such as pro-inflammatory cytokines (e.g. IL-6, tumor necrosis factor-alpha, etc) and acute phase reactants (e.g. C-reactive protein, fibrinogen), to predict future cardiovascular events and mortality (e.g. Rattazzi, Puato, Faggin, Bertipaglia, Zambon, & Pauletto, 2003; Biasucci et al., 1996; Biasucci et al., 1999; Ridker, Rifai, Stampfer, & Hennekens, 2000; Pradhan et al., 2002, Ridker, Hennekens, Buring, & Rifai, 2000; Harris et al., 1999; Danesh et al., 2000; Danesh et al., 2004; Lagrand et al., 1999). Inflammatory markers, including cytokines, macrophages and T-cells, are present in all types of atherosclerotic lesions, from early foam cells to complicated lesions (Napoli et al., 1997; Stary et al., Schonbeck et al., 1997). Finally, data from the animal and in-vitro literatures provide the strongest evidence for a role of inflammation in cardiovascular disease. For example, mice genetically engineered to express human CRP show 34-48% greater lesion development compared to wild type mice (Paul et al., 2004), and transgenic mice that are deficient in monocyte chemoattractant protein-1 or its receptor show decreased lesion formation (Boring, Gosling, Cleary, & Charo, 1998; Gu et al., 1998). Mice engineered to produce significantly less vascular cell adhesion molecule-1 (i.e. 2-8% of wild type mice levels) show a reduction in atherosclerotic lesion development (Cybulsky et al., 2001). In later stages of the disease, activated macrophages have been shown to produce

enzymes that degrade the extracellular matrix leaving plaques more vulnerable to rupture (Nikkari et al., 1995; Galis, Sukhova, Kranzhofer, Clark, & Libby, 1995), and transgenic mice that express human CRP are at increased risk of thrombosis after mechanical vascular injury (Danenberg et al., 2003). The research from the animal and in-vitro literatures support the hypotheses generated from epidemiological research to suggest that inflammatory cells are not just markers of existing disease, but also function in a pathogenic role.

1.3.2 Interleukin-6.

Interleukin-6 (IL-6), a pro-inflammatory cytokine, is of particular interest because it is one of the best-documented indices of inflammation. IL-6 is induced primarily by another pro-inflammatory cytokine, interleukin-1. Multiple cell types are credited with producing IL-6 (i.e. vascular smooth muscle cells, adipose cells, monocytes, macrophages, lymphocytes, endothelial cells, fibroblasts, mast cells, astorcytes, microglia, osteoblasts, bone marrow stromal cells, keratinocytes, synoviocytes, chondrocytes, epithelial cells, folliculostellate cells of the pituitary, endometrial stromal cells, and trophoblasts). The multiple functions of IL-6 include, but are not limited to, induction of the acute phase response (i.e. stimulation of CRP and fibrinogen), differentiation and maturation of B cells (including inducing production of immunoglobulin), and proliferation and differentiation of T cells (Papanicolaou, Wilder, Manolagas, & Chrousos, 1998; Le & Vilcek, 1989.)

Prospective evidence shows higher levels of IL-6 in patients with unstable versus stable angina, and levels of IL-6 upon hospital admission predict coronary events (Biasucci et al., 1996; Biasucci et al., 1999). Importantly, large scale, prospective studies in presumably healthy men

(Ridker, Rifai, Stamnpfer, & Hennekens, 2000), post-menopausal women (Pradhan et al., 2002; Ridker, Hennekens, Buring, & Rifai, 2000), and older adults (Harris et al., 1999) show that elevated basal levels of IL-6 predict future cardiovascular events and mortality. Relative risk for mortality or incident CHD for those in the highest quartile of baseline IL-6 versus the lowest quartile ranged from 1.9 to 2.3 (Ridker et al., 2000; 2000b; Harris, 1999), and odds ratio for incident CHD in post-menopausal women was 3.3 (Pradhan et al., 1999) in these studies. After controlling for traditional risk factors (Harris et al., 1999, Pradhan et al., 2002, Ridker et al., 2000b) and other inflammatory markers (including C-reactive protein; Ridker et al., 2000b; Harris et al., 1999) the risk remained significant in most studies.

1.3.3 SES and Interleukin-6.

Currently five published studies examine the relationship between socioeconomic status and IL-6 (Woodward et al., 1999; Yudkin et al., 1999; Brydon et al, 2004; Steptoe et al., 2002; Mendall et al., 1997), albeit with inconsistent results. One study shows urban slum dwellers in India to have higher levels of IL-6 than residents of middle class neighborhoods, although it is unclear whether data were adjusted for concomitant infection, a likely correlate of living in congested areas of socioeconomic disadvantage (Yudkin et al., 1999). An analysis of a subsample of the Whitehall II cohort showed occupation levels in England (in women only) to be associated with IL-6. In this study, the lowest levels of IL-6 were found in the highest employment grade, but the findings were non-linear and there were no differences in men (Steptoe et al., 2002). Childhood SES, based on father's occupation type, was also inversely associated with IL-6, but the subjects' own occupation was not a significant predictor (Mendall et al., 1997). Two other investigations report no differences in IL-6 levels based on occupation type; although the sample size in one of

these studies was extremely small, limiting the study's power to reject null findings (n=38) (Woodward et al., 1999; Brydon et al., 2004). Finally, preliminary analyses involving 501 healthy Caucasian and African-American participants in the University of Pittsburgh Adult Health and Behavior Project (AHAB) showed a composite index of income and educational attainment to be inversely associated with IL-6 levels ($r = -.16$, $p < .01$) (Petersen et al., 2005). In sum, the few data regarding potential associations between SES and inflammatory markers are inconsistent and subject to a variety of methodological deficiencies (restricted sample sizes, dissimilar indices of social inequalities). Nonetheless, the AHAB findings, which are based on a reasonably large community sample and employ conventional indicators of socioeconomic position, along with the Whitehall II findings, offer promising evidence of at least a modest inverse association between SES and inflammation.

1.3.4 C-Reactive protein.

C-reactive protein (CRP) is perhaps the most frequently cited inflammatory marker in epidemiological studies showing an association between inflammation and cardiovascular outcomes. It is also the inflammatory marker that has been widely promoted as having clinical utility in establishing cardiovascular risk levels**,** suggesting that levels less than 1.0 mg/L represent low risk, 1.0-3.0 mg/L mid-level risk, and levels greater than 3.0 mg/L represent high risk for subsequent cardiovascular disease complications (Ridker, 2003; Pearson et al., 2003). As previously mentioned, CRP is an acute phase reactant that is induced by IL-6 and other pro-inflammatory cytokines (i.e. IL-1 and TNF-α). CRP is produced in cells of the liver, atherosclerotic lesions (by macrophages and smooth muscle cells), the kidney, neurons and macrophages in the alveoli (Jialal, Devaraj, & Venugopal, 2004). As part of the innate immune

response, the biological roles of CRP include activation of the classical complement immune pathway, which allows for elimination of cellular debris or infectious elements (Rattazi et al., 2003.)

Multiple recent review articles and meta-analyses of the literature investigating the role of CRP in cardiovascular disease show consistent evidence of the utility of CRP to predict future cardiovascular morbidity and mortality (Danesh et al., 2000; Danesh et al., 2004; Lagrand et al., 1999) differentially from total mortality (Tice et al., 2003). For example, in a 1999 review of 17 different studies, Lagrand and colleagues cite several studies showing that CRP predicts cardiovascular events in apparently healthy people and several studies showing the rise in CRP after myocardial infarction to predict future cardiovascular events in patients with unstable angina. Danesh and colleagues conducted a meta-analysis of 14 prospective studies of mostly middle-aged, healthy men. Those men with baseline CRP levels in the top third compared to men with levels in the bottom third produced an odds ratio of 1.9 (95% CI = 1.5, 2.3) for incident coronary heart disease (Danesh et al., 2000). A 2004 meta-analysis including the original studies (from the 2000 paper) and an additional 12 studies produced a slightly lower, but still significant, odds ratio of 1.5 (95% CI = 1.48, 1.68) for people in the top third versus the bottom third of baseline CRP levels (Danesh et al., 2004). The individual studies had taken into account standard risk factors for cardiovascular disease.

1.3.5 SES and C-Reactive Protein

Six studies examined the association between a measure of socioeconomic status and C-reactive protein in cross-sectional designs (Owen et al., 2003; Jousilahti et al., 2003; Panagiotakos et al., 2004; Mendall et al., 2000; Danesh et al., 1999; and Wu et al., 2002) and all studies provide

some evidence that socioeconomic status is inversely related to CRP levels. In a healthy, Caucasian subsample of the Whitehall II study of male and female British civil servants, subjects in low vs. high occupational groups were more likely to have a CRP reading greater than 1.0 mg/l (OR = 2.85), even after accounting for multiple confounding factors such as age, BMI and smoking status (Owen et al, 2003). This number is particularly significant because prospective cardiovascular studies have shown that CRP levels above 1.0 mg/L represent moderate to high risk for subsequent cardiovascular disease complications (Ridker et al, 2003; Pearson et al., 2003). These findings extend to a population based study of Finnish men (including men with chronic disease), where higher levels of CRP were found in groups with a lower composite SES score (based on income and education), even after accounting for presence of disease and other inflammatory risk factors (Jousilahti et al., 2003). Educational attainment was also inversely associated with CRP levels in a Greek sample (Panagiotakos et al., 2004) and in a multiracial sample (84% Caucasian, 11% African American and 5% Hispanic) (Wu et al, 2002). Finally, employment status (employed/unemployed; Danesh et al, 1999) and childhood SES based on father's occupation (manual/non-manual laborer; Mendall et al, 2000) were associated with CRP, such that lower SES predicted higher CRP levels. Several markers of SES used within these same studies did not predict CRP levels, however, including measures of car and home ownership, one measure of educational attainment (Danesh et al.), and manual employment of the individual (Danesh et al.; Mendall et al, 2000). In summary, however, the majority of evidence shows that SES is inversely related to CRP levels, even after controlling for known covariates.

1.3.6 Risk factors for heightened inflammation.

Levels of inflammatory cytokines and acute phase reactants can vary among individuals for a number of reasons. For example, age and obesity are positively associated with multiple measures of inflammation, including IL-6 and CRP (Bermudez, Rifai, Buring, Manson, & Ridker, 2002; Fernandez-Real et al., 2001; Mohamed-Ali et al., 1997; Ridker, Hennekens, Buring, & Rifai, 2000; Yudkin et al., 1999; reviewed in Kaplan & Fishmann, 2001). Adipose tissue produces pro-inflammatory cytokines, and weight reduction results in decreased levels of Il-6 (Tchernolf et al., 2002; Esposito et al., 2003). Other factors affecting inflammatory activity include high blood pressure, smoking, hyperlipidemia, insulin insensitivity and diabetes, physical inactivity, and chronic infection (Bermudez et al, 2002; Fernandez-Real et al., 2001; Mohamed-Ali et al., 1997; Ridker et al, 2000; Kaplan & Frishmann, 2001). Smoking and hyperlipidemia both cause endothelial dysfunction from oxidative stress (Altman, 2003). Hyperglycemia, a result of insulin insensitivity and/or diabetes, results in the production of advanced glycation end-products (AGE), which trigger endothelial dysfunction and the expression of proinflammatory molecules (Evan, Khan, & Rees, 1999). Acute physical activity can enhance the expression of inflammatory cells; however, routine, moderate physical activity is associated with lower levels of circulating pro-inflammatory cytokines (e.g. Taaffe, Harris, Ferrucci, Rowe & Seeman, 2000). Finally, chronic infection may function as an irritant stimulating a low level, chronic inflammatory response (as in the case of *C. pneumoniae* and cytomegalovirus) or by directly infecting the vessel wall (as in the case of *H. pylori*; Abgueguen, Delbos, Chennebault, Payan, & Pichard, 2003; Go, 2002; Griffiths & Emery, 1999; reviewed in Romeo, Clementi, Saldeen, & Mehta, 2001).

1.4 GENETIC INFLUENCES ON INFLAMMATORY MARKERS

1.4.1 Quantitative Genetic Studies.

While the risk factors for elevated inflammation mirror those of cardiovascular disease and are known to cluster in individuals of lower SES, the relationship between SES and inflammation is still quite modest (i.e. IL-6: $r = -.16$, $p < .01$ in AHAB). It is likely that heritable factors also contribute to inflammation, such that only individuals of lower SES harboring a certain genotype exhibit higher levels of IL-6 or CRP (i.e. gene by environment interaction). Recently, four quantitative genetic studies have examined relative genetic and environmental influences on IL-6 levels. Two such studies, one twin study and one non-twin family study, show the heritability of baseline plasma IL-6 levels to range from 17-24% (Pantsulaia, Trofimov, Kobyliansky, & Livshits, 2002; de Maat, Bladbjerg, Hjelmborg, Bathum, Jespersen, & Christensen, 2004). Two other quantitative studies examined the genetic influences of stimulated IL-6 levels, i.e. after ex-vivo stimulation by either lipolysaccharide (LPS) or amyloid-B. Both studies were of a twin design and showed high heritability for stimulated levels of IL-6 (57-67%) (de Craen, Posthuma, Remarque, van den Biggenlaar, Westendorp & DI Boomsma, 2005; Posthuma, Meulenbelt, De Craen, Geus, Slagboom, Boomsma, & Westendorp, 2005).

1.4.2 Molecular Genetic Studies

Several polymorphisms within the IL-6 gene have been examined to account for variation in IL-6 levels and disease susceptibility. The IL-6 gene is located on the short arm of chromosome 7 (p-15), and contains 5 exons and 4 introns. Functional variation in IL-6 levels has been attributed to

sites within the 5' flanking region of the IL-6 gene promoter. Here several single nucleotide polymorphisms (SNP) have been identified, including those at nucleotides -597 (G>A), -572 (G>C), -174 (G>C), and a variable AnTn tract at –392 to –373; all of which are in strong linkage disequilibrium (Fishman et al, 1998; Terry, Loukaci & Green, 2000**)**. Haplotype analyses reveal that although multiple SNPs function in the regulation of IL-6 expression, the -174 G>C SNP, located 1.2 kb upstream from the start of transcription, may be important since endothelial-like cells transfected with haplotypes including the -174 G-allele showed the highest transcriptional activity, irrespective of the other alleles in the haplotype (Terry et al., 2000). In this same study, however, hepatic cell lines showed a different pattern of results, suggesting that the regulation of IL-6 expression is a complex interaction of multiple SNPs and may vary in different types of tissue (Terry et al., 2000). Some evidence from an ex-vivo LPS stimulation of leukocytes supports the importance of the 174 G>C SNP in regulating ILG, however, as -174-GG individuals expressed higher IL-6 levels irrespective of alleles at -597 or –572 (Rivera-Chavez, Peters-Hybki, Barber, & O'Keefe, 2003).

Fishman (1998) reports the frequency of the less common C allele in Caucasians to be 0.40 (95% CI: 0.37-0.44), and studies since then report similar frequencies in Caucasians (e.g. Burzotta et al., 2001, Heesen et al., 2002). Allele frequencies vary by ethnicity, however, with one study showing only 1.2% of African-American women to carry the CC genotype, compared to 18.8% of Caucasian women, while 82.5% of African American women carried the GG genotype compared to 36.3% of Caucasian women (Ness, Haggerty, Harger,, & Ferrell, 2004). In reporter gene constructs, the G allele was associated with greater expression of IL-6 both in unstimulated cells and in response to interleukin-1 and LPS (Fishman et al, 1998). More specifically, in unstimulated cells, the C allele was associated with 0.62-fold lower luciferase

expression than the G allele. In cells stimulated with IL-1 and LPS, a 4.38-fold and 4.55-fold increased expression, respectively, was associated with the G allele relative to the C allele (Fishman et al., 1998). Higher IL-6 production associated with the G allele has also been demonstrated in adherent peripheral blood mononuclear cells (Olivieri et al., 2002), but one study of LPS stimulation of whole blood showed inconsistent results, with the highest levels of IL-6 produced in GG or CC genotypes, and the lowest levels produced in heterozygotes (Heesen, Bloemeke, Heussen, & Kunz, 2002.) In sum, the majority of evidence from in vitro models suggests that the -174 G allele enhances transcription and expression of IL-6.

In vivo studies examining plasma IL-6 levels by genotype report mixed findings. For example, plasma IL-6 levels were nearly twice as high in healthy Caucasians homozygous for the G allele vs. those with a CC genotype (Fishman et al, 1998). Serum IL-6 was significantly higher in men with a G allele (Olivieri et al., 2002) and in elderly men and women (Bonafe, 2001), but no differences in baseline IL-6 by genotype have also been reported (Endler, et al., 2004; Jerrard-Dunne et al., 2003; Margalionie, Bossone, Cappucci, Colaizzo, Grandone, Di Minno, 2001; [subset of women] Olivieri et al., 2002). Of note, sample size limitations and sensitivity of the IL-6 assays may have been a factor in the null findings.

Studies examining the association of the -174 G>C polymorphism and IL-6 levels in clinical populations and in regard to disease endpoints also show a complicated set of results. For example, no differences in IL-6 levels by genotype were reported in myocardial infarction (MI) patients (Bennermo et al., 2004; Lieb et al., 2004; Nauck, Winklemann, Hoffmann, Bohm, Wieland, & Marz, 2002), but the CC genotype was associated with higher IL-6 levels in patients with aortic aneurysm (Jones et al., 2001) and in heavy drinkers (Jerrard-Dunne et al., 2003). The presence of a G allele, however, was associated with higher plasma IL-6 levels in hemodialysis

patients (Balakrishnan et al., 2004). Further, the G allele or G homozygosity has also been shown to predict multiple adverse cardiovascular outcomes, including atrial fibrillation as a postoperative complication of coronary artery bypass grafting surgery (CABG) (Gaudino et al., 2003), peripheral artery occlusive disease (Flex et al., 2002), severe stroke (Greisenegger et al., 2003), mortality after coronary event (Antonicelli et al. 2005), adverse lipid profiles (Fernandez-Real, 2000**)** and greater intima-medial thickness (IMT) (Rundek et al., 2001). Not all studies are in agreement, however, as some report greater cardiovascular risk to be associated with the C allele [i.e. plaque and IMT in heavy drinkers (Jerrard-Dunne et al., 2003); MI (Georges et al., 2001); higher blood pressure and coronary heart disease (Humphries, Luong, Ogg, Hawe, & Miller, 2001)] or no association with the -174 G>C polymorphism [e.g. essential hypertension (Pola et al, 2002)].

Because IL-6 is crucial in orchestrating the acute inflammatory response, several studies have examined acute changes in IL-6 levels as a function of genotype. The -174-GG genotype was associated with higher IL-6 levels after surgery in several studies, (Burzotta et al., 2001; Travelyan, Brull, Needham, Montgomery, Morris & Mattu 2004; Gaudino et al. 2003), but one study reported no differences in peak IL-6 levels by genotype (Bown et al., 2003) and another reported differences in the timing of the peak IL-6 response by genotype (Brull et al., 2001). The GG genotype was also associated with higher IL-6 levels after vaccination with the *Salmonella typhi* vaccine (Bennermo et al., 2004) and ex-vivo LPS stimulation of leukocytes (Rivera-Chavez et al., 2003). However, young, healthy males injected with LPS showed no difference in IL-6 response by genotype (Endler et al., 2004). In sum, the majority of these studies suggest that the -174 G>C polymorphism modulates an individual's inflammatory (i.e. IL-6) response to an acute trigger, although the studies are not without contradiction.

Methodological differences between studies (i.e. timing of IL-6 measurement; sample characteristics) and limited statistical power (i.e. Endler et al., 2004; Bown et al., 2003) may account for some of the inconsistencies reported herein.

Finally, several studies have examined the association between -174 G>C and circulating CRP levels, given the role of IL-6 in the induction of CRP production. These studies produce largely null findings, showing no differences in circulating CRP levels by genotype in male elderly patients with acute coronary syndrome (Antonicelli et al., 2005), patients with coronary heart disease (Latkovskis, Licis, & Kalnins, 2004), Type II diabetic patients (Abrahamian et al., 2007), obese males (Eklund et al., 2006), or patients with an abdominal aortic aneurysm (Jones et al., 2001). However, another sample of Type II diabetes patients (Libra et al, 2006) and patients with severe periodontal disease (D'Aiuto, Parkar, Brett, Ready, Tonetti, 2004) having the GG genotype have also been shown to display higher levels of CRP. And while there was no difference in CRP levels related to the 174 G>C polymorphism in postmenopausal women, the -572/-174 promoter haplotype was predictive of circulating CRP levels in these women (Ferrari, Ahn-Luong, Garnero, Humphries, & Greenspan, 2003), with carriers of the 174 G allele showing higher levels of CRP. In sum, investigation of the 174 G>C polymorphism and circulating CRP levels has produced conflicting results. Many studies of patients with various illnesses (i.e. Type II diabetes, coronary heart disease, abdominal aortic aneurysm) have produced null findings, however, the majority of these studies had samples of less than 160 people. Two additional studies showed an association between 174 G>C and circulating CRP in periodontal patients and Type II diabetics, and one study showed that 174 G>C in combination with another site in the IL-6 promoter region (i.e. 572 G>C) was associated with variation in CRP levels.

1.4.3 Summary of genetic influences on IL-6 and CRP.

In sum, quantitative genetic studies suggest variation in IL-6 to be heritable, with estimates ranging from 17-24% for basal IL-6 and 57-67% for stimulated levels of IL-6. In-vitro studies suggest that presence of the G allele, and G homozygosity in particular, may be associated with increased transcription of IL-6, however all studies are not consistent. In-vivo studies of baseline IL-6 levels in healthy humans suggest that expression of IL-6 is also higher in individuals carrying the G allele, and while all studies are not in agreement, discrepant findings may be attributable to sample characteristics (e.g. age or health status), or limited statistical power. Results from clinical samples (i.e. patients with abdominal aortic aneurysms, myocardial infarction patients) are more difficult to interpret with some studies showing no difference in IL-6 levels by genotype, and some showing higher levels with the presence of the C allele. However, numerous studies show the -174 G>C polymorphism to be associated with distal cardiovascular phenotypes (e.g. IMT, plaque, atrial fibrillation, mortality). Studies also suggest that the -174 G>C polymorphism moderates the IL-6 response to an acute challenge (i.e. surgery, LPS stimulation, vaccination), and the majority of the studies suggest that the G allele is predictive of a greater IL-6 response. In sum, evidence from in-vitro and in-vivo studies suggest that the -174 G>C polymorphism alters function of the IL-6 gene promoter, with the majority of studies suggesting that the G allele is associated with higher IL-6 expression. Further, evidence from in vivo challenge studies suggests that the acute IL-6 response is also modulated by the -174 G>C polymorphism. Finally, studies examining the association between -174 G>C and CRP are conflicting, with several small studies showing no association, two studies showing G homozygosity to be associated with increased circulating CRP, and a final study showing the 174/572 promoter haplotype to be associated with CRP.

1.5 PURPOSE OF THE PROPOSED STUDY

Low socioeconomic status is associated with a myriad of adverse health conditions, including increased risk for cardiovascular disease. Despite numerous studies investigating potential mediating factors, the association between SES and cardiovascular disease remains largely unexplained. Inflammation is increasingly seen as a pathophysiological factor in the development and exacerbation of cardiovascular disease, and within this literature, two inflammatory markers, IL-6 and CRP, have emerged as prominent prognostic indicators. Accordingly, a few studies have begun to examine the potential for covariation between SES and these inflammatory markers. Results from these studies are inconsistent, with some support for an inverse association between SES and CRP, and two of the largest studies on SES and IL-6 suggesting that a modest association may exist. The primary aim of this study is to expand upon an early finding from our laboratory that showed an index of individual education and income to be associated with IL-6 levels. In a larger, community based sample, we will examine the association ~~of~~ both individual and community SES with IL-6 and CRP.

The preliminary individual SES-IL-6 finding in AHAB was modest ($r = -.16$, $p < .01$), and findings across studies are inconsistent. While multiple factors are known to influence circulating inflammatory markers (e.g. physical activity, alcohol consumption, smoking, etc.), quantitative genetic studies have also shown that a portion of interindividual variation in IL-6 is heritable. It is possible, therefore, that an association between SES and both IL-6 and CRP could be moderated not only by environmental exposure, but also by genetic influences. Thus, if the primary SES -IL-6 finding persists in the expanded sample and extends to CRP, a secondary aim of the paper will be to examine whether variation in a promoter polymorphism of the IL-6 gene (i.e. -174 G>C) modulates the influence of SES (both individual and community) on IL-6 and

CRP. Molecular genetic studies have identified a possibly functional single nucleotide polymorphism (-174 G>C) of the IL-6 promoter region. In vitro studies suggest the G allele may be associated with increased rates of IL-6 gene transcription, and results from studies in healthy individuals also suggest that the G allele is associated with higher levels of circulating IL-6. Finally, a gene by environment interaction has been demonstrated with the -174 G>C polymorphism, such that in vivo challenge by surgery and vaccination results preferentially in increased IL-6 levels among individuals possessing a G allele. It is possible that a more distal environmental stimulus (i.e. SES), then, might also be associated with higher IL-6 levels in individuals possessing the G allele, especially given the overall modest association between SES and IL-6 levels demonstrated in preliminary investigations. Since IL-6 stimulates the proinflammatory response and triggers the release of CRP, a few studies have evaluated the association between -174 G>C and CRP. These studies show a conflicting pattern of results with both null findings and some evidence of increased circulating CRP in individuals carrying a G allele. In this study our hypotheses are as follows:

1.5.1 Hypotheses:

Hypothesis 1 – Independent of demographic covariates (age, sex, and race), IL-6 will be associated inversely with individual SES (defined by composite index of income and education); we expect this relationship to extend to CRP, as well. Community SES has been shown to predict health outcomes (including those associated with cardiovascular disease). Accordingly, community SES (an index derived from sociodemographic variables characterizing U.S. Census tracts of residence) will be inversely associated with IL-6 and CRP in this study.

Hypothesis 2 –In accordance with evidence showing community SES is associated with health outcomes independently of individual SES, we expect community SES to be independently associated with IL-6 and CRP in this study, after also adjusting for age, sex, and race.

Hypothesis 3 –Assuming hypotheses 1 and 2 are supported, the extent to which any association between SES and IL-6/CRP may be independent of concomitant variability in other risk factors will also be determined (i.e. smoking, BMI, alcohol consumption, sleep, and physical inactivity).

Hypothesis 4 – In accordance with in-vitro studies showing the G allele of -174 G>C to be associated with greater transcription of IL-6 and CRP, the G allele is expected to be positively correlated with circulating IL-6 and CRP concentrations.

Hypothesis 5 – We expect a gene by environment interaction as follows:

- In low SES participants (both individual level and community SES), the G allele will be associated with higher concentrations of IL-6 and CRP, but in high SES individuals there should be no differences in IL-6 or CRP concentrations based on genotype.

2.0 METHODS

2.1 STUDY OVERVIEW

This investigation included 1379 individuals between the ages of 30 and 54 who participated in the Adult Health and Behavior project (AHAB) between 2001 and 2005. AHAB is a University of Pittsburgh registry of diverse behavioral and biological measurements collected on individuals recruited (by mail solicitation) from communities of Southwestern Pennsylvania (principally Allegheny County). Potential participants were excluded if they reported a history of any of the following conditions: myocardial infarction, stroke, or cancer treatment within the past year, chronic kidney or liver disease, major neurological disorders, schizophrenia or other psychotic illness. Pregnant women were also ineligible. Participants taking nitrates, digoxins, anti-arrhythmic medications, glucocorticoids, insulin, or weight-loss drugs were also excluded. Inflammatory assays were conducted on frozen plasma from 1081 subjects from the larger AHAB database. Given that AHAB eligibility did not include absence of common acute illnesses (e.g., recent colds or allergies), IL-6 levels greater than 10pg/ml or CRP levels greater than 10 mg/L were also exclusionary for the present analyses. Overall, 230 subjects were excluded due to elevated inflammatory markers or absence of an identifiable census tract (e.g., an address coded by PO number), resulting in a final sample of 851 individuals for all primary (i.e. non-genetic) analyses. This sample included 424 men and 427 women and

had a mean age of 44.9 years (SD = 6.5). European-Americans comprised 77 percent of study participants; the remaining 23 percent were African-American. When compared with AHAB subjects excluded from analysis due to elevated inflammatory measurements or an unidentifiable Census tract, study participants did not differ in age or distribution of sex and ethnicity, personal socioeconomic indicators (educational attainment, income) or community characteristics (described below). Subjects excluded from analyses due to elevated inflammatory markers also did not differ from those subjects included in the genetic analyses on distribution of genotypes. Informed consent was acquired in compliance with guidelines of the University of Pittsburgh Institutional Review Board.

2.1.1 Procedure.

Prior to arrival at the laboratory, participants were asked to fast and abstain from exercise for 12 hours, to abstain from alcohol for 24 hours and to abstain from nicotine for 1 hour. At the laboratory, a nurse then completed a medical history interview, height and weight measurements, and drew a 40 cc blood sample which was stored at -80^0C (see IL-6 measures).

2.2 MEASURES

2.2.1 Socioeconomic Status

2.2.1.1 Individual Socioeconomic Status

Participant income and education were assessed as individual-level indices of SES. Subjects were well-educated on average, with a mean of 15.3 (SD=2.8) years of schooling. Levels of educational attainment nonetheless varied appreciably among study participants, with 5% lacking a high school diploma, 19% having completed high school or technical training only, 24% with some college, 34% with a Bachelors degree, 15% with Masters degree, and 4% with an MD or PhD. Annual (pre-tax) family income also varied substantially across participants, averaging <$25,000 in 22.6% of subjects, $25,000-49,999 in 27.3%, $50,000-$79,999 in 28.4%, and >$80,000 in 21.6% of participants. An index of individual-level SES was calculated by standardizing the distributions of educational attainment and family income across subjects, averaging the resulting Z-scores for each individual, and re-standardizing the resulting distribution (M=0, SD = 1; range: -2.41-1.99). For four subjects lacking income data, individual SES was determined from educational attainment alone.

2.2.1.2 Community Socioeconomic Status

Participant's home addresses were used to identify their Census Tracts of residence, based on the 2000 US Census (**http://www.census.gov/main/www/cen2000.html**). Census tracts are geographical areas established to reflect a relatively homogeneous group of approximately 4000 people (range 2500-8000) and 1500 housing units (range 1,000-3,000). For the 285 identified tracts, we extracted five Census variables: (1) median household income, (2) percentage of

individuals in the work force who were unemployed, (3) proportion of households below the federally designated poverty level; (4) percentage of households that were headed by a single woman with one or more children under the age of 18; and (5) percentage of persons over 25 years of age who had completed a college education (Bachelor's degree). Distributions of all but the last variable were normalized by logarithmic transformation to reduce skew. Following Manuck et al (2005) and Petersen et al (2006), an index of community SES was calculated as the average of the standardized scores of the five Census-derived variables, with signs adjusted so that higher values of each variable reflected more advantaged communities. As with individual SES, this continuous index of community SES was then re-standardized (M=0, SD=1; range: –3.07 to 2.21). Illustrating the variability in community SES present in the sample, median values of the five extracted Census variables for upper and lower quartiles of the distribution of community SES were: $22,231 and $60,168 in median household income, 11.6% and 2.5% unemployment, 25.0% and 3.5% of households in poverty, 13.8% and 2.9% of households headed by a single mother, and 10.5% and 47.5% of adults over 25 having a college education.

Across subjects, community SES correlated significantly with individual SES ($r_{849} = 0.539$, $p < 0.00001$), as well as with its two components, educational attainment ($r_{849} = .408$, $p < 0.001$) and current annual income ($r_{845} = .485$, $p < 0.001$), indicating that participants with lower incomes and less education tended also to live in less advantaged communities. Nevertheless, variability in the economic and material circumstances of the Census tracts represented here could be accounted for only partially by overlapping variation in individual SES.

2.2.2 Inflammation

2.2.2.1 Interleukin-6

IL-6 levels were determined using a high sensitivity quantitative sandwich enzyme immunoassay kit (R & D Systems) according to manufacturer's directions. The assay standard range is from 0.156 to 10 pg/mL. IL-6 levels were extrapolated from a standard curve with linear regression from a log-linear curve. All samples were run in duplicate and the average coefficient of variation (CV) between samples was 5%. Reciprocal transformation was applied to normalize raw score distributions of the IL-6 values.

2.2.2.2 C-Reactive Protein

CRP was measured using the BNII nephelometer from Dade Behring utilizing a particle enhanced immunonephelometric assay. In this procedure, polystyrene particles are coated with monoclonal antibodies to CRP, which, in the presence of antigen agglutinate cause an increase in the intensity of scattered light. The increase in scattered light is proportional to the amount of CRP in the sample. The assay range is 0.175-1100 mg/L. Intra-assay CVs range from 2.3-4.4% and inter-assay CVs range from 2.1– 5.7%. Final CRP values were normalized by reciprocal transformation.

2.2.3 Genotyping

Genomic DNA will be isolated from EDTA anticoagulated whole blood using the Puregene kit (Gentra Systems,Minneapolis, MN). The IL6 (-174) polymorphism will be genotyped by the 5'-nuclease assay (TaqMan). Amplification will use the primers F5'

GACGACCTAAGCTGCATTTTC-3' and R5' GGGCTGATTGGAAACCTTATTAAGATTG-3', and detection oligonucleotides VIC5'-CCTTTAGCATCGCAAGAC-3' and FAM-5'-CTTTAGCATGGAAGAC-3' (Livak, 1999). The genetic analyses conducted in this paper were a subsample (n=811) of both the group used for the initial socioeconomic analyses (n=851) and the larger AHAB dataset (n=1379)**.** Use of this subsample was necessary because 40 of the 851 subjects for whom we had intact address of residence and inflammatory data did not have genotype data. With respect to Caucasians, genotyping in the larger AHAB sample consisted of 1167 participants, and genotyping was successful on 1045 people. Of note, distribution of genotypes in this larger sample (n=1045) conformed with Hardy-Weinberg equilibrium (X^2_1 = 4.01, ns), as did the subgroup we excluded based on lack of address or inflammatory data (X^2_1 =.15, ns). The distribution of genotypes in the Caucasian subsample mildly deviated from Hardy-Weinberg equilibrium (X_1^2 = 4.17, p = .04). Genotypes in the African-American subsample also conformed with Hardy-Weinberg equilibrium (X_1^2 = 0.73, p > .05). Please see Figure 1 for differences in frequency of -174 G>C genotypes by race (X_2^2 = 110.99, p < .001).

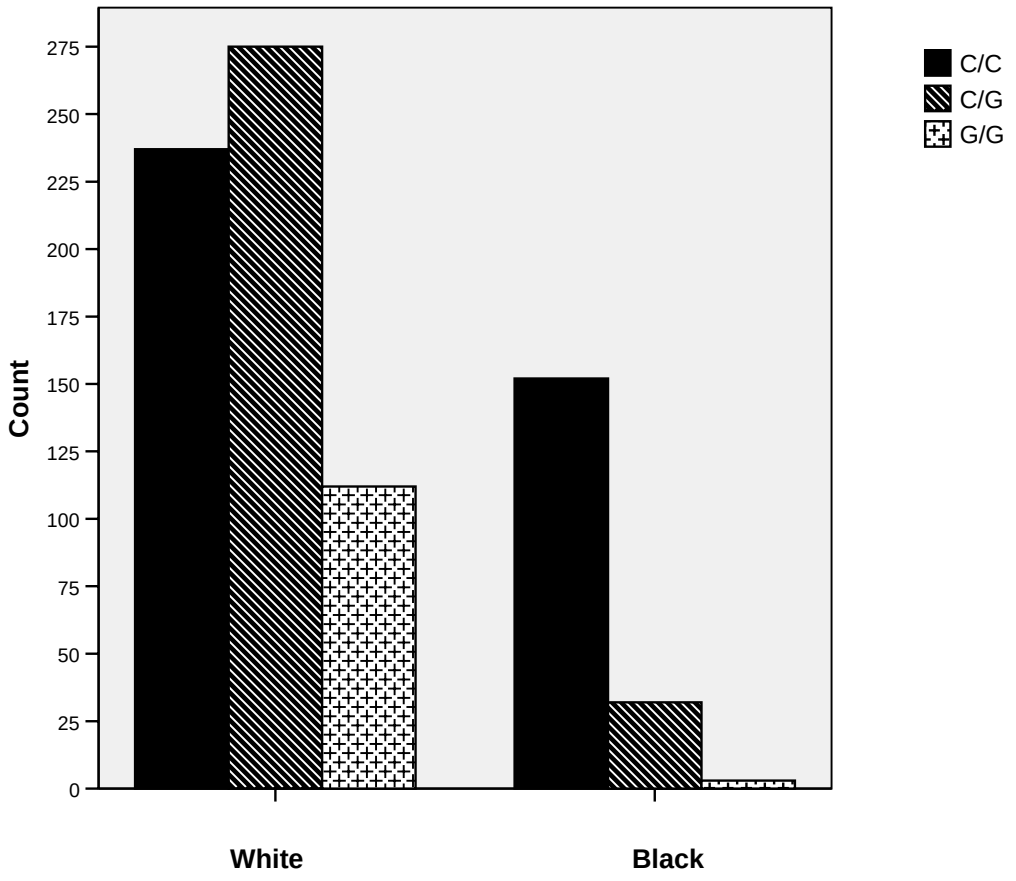


Figure 1: Frequency of Genotype by Race.

2.2.4 Additional Variables

A number of variables were assessed that might confound any association between SES and IL-6. These variables include age, sex, race, body mass index (kg/m^2), smoking status (current smoker versus ex/non-smoker), self- reported alcohol use (average number of alcoholic drinks/week), sleep volume (hours of sleep during last 7 nights = (average hours/week night x 5) + (average hours/weekend night X 2), and exercise. Exercise was measured using the Paffenbarger Physical Activity Questionnaire, which is a widely used questionnaire that yields

an estimated kilocalorie expenditure over a 7-day period based on self-report of leisure time activity, walking, and stair climbing (Paffenbarger et al., 1978). The questionnaire has adequate retest reliability (Washburn, Smith, Goldfield, & McKinlay, 1991; Washburn, Adams, & Haile, 1987; Ainsworth, Leon, Richardson, Jacobs & Paffenbarger, 1993), and is highly correlated with other measures of physical activity (summarized in Albanes et al, 1990). It is also highly correlated with maximal oxygen capacity (VO_2 max) ($r = .60$, $p < .05$; Ainsworth et al., 1993), and demonstrates good predictive validity for coronary heart disease (e.g. Paffenbarger et al., 1993).

2.3 ANALYTIC STRATEGY

Of note, due to the differences in the frequency distribution of -174 G>C genotypes in African-American and Caucasian subjects, all genetic analyses were performed separately in Caucasians and African-American groups ($X^2_2 = 110.99$, $p < .001$).

Hypothesis 1: Hierarchical linear regression was employed to examine the association between SES (i.e. individual and community), entered on step 2, and IL-6 and CRP levels, after adjusting for age, gender, and race on step 1.

Hypothesis 2: In order to determine the unique association of community and individual SES with IL-6 and CRP, hierarchical linear regression was used - with standard demographic characteristics (age, gender and race) entered on step 1 of the analyses, individual SES entered on step 2 of the analysis, and community SES entered on Step 3.

Hypothesis 3: To determine any mediating effect of health behaviors, the above analyses were repeated with the inclusion of standard demographic characteristics (age, gender and race) on step 1 of the analysis, health practices on step 2 (smoking, BMI, alcohol consumption, sleep and physical inactivity), individual SES on step 3, and community SES on step 4 of the analysis.

Hypotheses 4 and 5: Linear regression was again employed to determine whether there were mean differences in IL-6 or CRP levels based on genotype, and to determine whether there was an interaction between SES and genotype in predicting IL-6 and CRP. These analyses were run separately in Caucasians and African-Americans. In Caucasians, all variations of the -174 G>C polymorphism were coded separately (i.e. GG, GC, and CC). A secondary model was run with two dichotomous genotypes (i.e. C allele present vs. GG homozygotes; G allele present vs. CC homozygotes) in Caucasians. Since the frequency of the GG genotype was extremely rare in this African-American sample (i.e. less than 1%), analyses were only conducted with the genotype dichotomized (i.e. CC homozygotes vs GG+GC). Parallel analyses were employed using individual-level and community SES. SES and genotype variables were centered to reduce potential colinearity between the independent variables and their interaction product. Covariates were entered on step 1 of the model (age, sex), SES was entered on step 2, genotype on step 3, and the SES by genotype interaction term was entered on step 4. For any analyses where the genotype significantly predicted IL-6 or CRP, the regression was repeated with the inclusion of health behaviors on step 1 (smoking, BMI, alcohol consumption, sleep and physical inactivity).

2.4 POWER ANALYSES

Power calculations were conducted using the Sample Power program in SPSS. With an alpha-level of .05, the power to detect an SES effect accounting for 1% of the variance in IL-6 levels (after accounting for age, sex, and race) was 0.84 in the full sample (n=851). The power to detect a main effect for the -174 G>C polymorphism accounting for 1% of the variance, after adjusting for age, sex, and socioeconomic status, was 0.72 in whites (n=624) and 0.29 in blacks (n=197). The power for detecting an SES by genotype interaction effect accounting for 2% of the variance, after adjusting for age, sex, socioeconomic status, and genotype, was also 0.72 in whites, and 0.29 in blacks.

3.0 RESULTS

3.1 SOCIOECONOMIC STATUS AND INFLAMMATORY MARKERS

Hypotheses 1-3, investigating socioeconomic and behavioral correlates of inflammation, were tested using the 851 subjects for whom we had data on all measures of SES, covariates, and inflammatory markers. Bivariate correlations describing the associations of these subject characteristics with community SES and with IL-6 and CRP levels are presented in Table 1. For convenience, the signs of correlations involving reciprocally transformed measurements of IL-6 and CRP are reversed (as in all subsequent test statistics), so that positive (and negative) coefficients are interpreted as such. Consistent with prior literature, higher levels of IL-6 were associated with African American race, higher BMI, current smoking, consumption of 14 or more alcoholic drinks/week, fewer hours of sleep/week and less physical activity. Male gender also predicted higher levels of IL-6. Similarly, higher CRP was associated with African-American race, higher BMI, and less physical activity. Neither CRP nor IL-6 covaried with age, nor was CRP associated with sex, smoking status, alcohol use, or sleep duration. Community and individual SES were similarly associated with covariates, with higher SES (predictably) associated with white race, non-smoking status, older age, lower BMI, more physical activity, and more sleep in the previous week.

Table 1: Bivariate correlations with covariates, CRP, IL-6, Community and Individual SES.

	N=851 M (SD)	CRP	IL-6	Comm SES	Indiv SES
Age, yrs	44.9 (6.5)	-.035	.036	.113**	.132**
Gender, % ma	49.8%	.057	-.080*	-.004	.018
Race, % AA	23%	.094**	.162**	-.547**	-.434**
BMI, kg/m^2	27.5 (5.7)	.484**	.306**	-.146**	-.122**
Smoking,%current	18%	.055	.206**	-.278**	-.333**
Exercise	2407.1 (1836.1)	-.120**	-.149**	.151**	.186**
Alcohol, >14/wk	7%	-.025	.072*	-.031	-.058
Sleep, hrs/wk	47.7 (7.5)	-.046	-.093**	.094**	.068*
SES: Indiv	0 (1)	-.079*	-.170**	.539**	
Community	0 (1)	-.142**	-.201**		

**P<.01, *P<.05, $^{+}$P<.10

Individual and Community SES indices are expressed as z-scores (M = 0). Race coded 0 = White, 1 = Black. BMI = body mass index; Smoking Status (0=not current, 1=current); correlations were conducted on the transformed CRP and IL-6 variables, but signs were reversed in the table for ease of interpretation; $^{\dagger}r_{pb}$ = point biserial correlation.

3.1.1 Hypotheses 1: Does inflammation (i.e. IL-6 and CRP) covary with individual or community socioeconomic status?

As also shown in Table 1, circulating levels of IL-6 and CRP covaried inversely with both individual and community SES. Results of regression analyses examining the association between SES and inflammatory markers after adjusting for age, sex, and race can be found in Table 2. When entered on the first step of the model, age, sex and race accounted for 3.7% of the variance in IL-6 concentration ($F_{3, 847} = 10.91$, $p = .000$), and 1.2% of the variance in CRP ($F_{3, 847} = 3.55$, $p = .014$). Males had higher concentrations of IL-6, and African-Americans had higher concentrations of both IL-6 and CRP. Individual SES, entered on step 2 of the models, continued to predict IL-6 ($F_{1, 846} = 11.26$, B=-.126,$p < .001$), but not CRP ($F_{1, 846} = 1.55$, B= -.047, $p = .214$), whereas in similarly adjusted regression models, community SES predicted both IL-6 ($F_{1, 846} = 16.55$, B= -.163, $p <.001$) and CRP ($F_{1, 846}= 9.99$, B= -.129, $p = .002$). Regarding the latter associations, then, study participants living in less advantaged Census tracts showed higher plasma concentrations of the two inflammatory markers than did residents of more affluent neighborhoods. To examine the possibility that these community associations might exist only in analyses performed on data transformed to satisfy parametric assumptions of normality, box-plots of *untransformed* IL-6 and CRP measurements are displayed in Figure 2. The values depicted here are partitioned by quartiles of the distribution of participants' community 'scores', which are the standardized residuals created by regressing age, sex and race on participants' community SES values. For both inflammatory markers, median values among subjects comprising the lowest quartile of community SES were greater than those of the highest quartile, and non-parametric statistic (Mann-Whitney U) corroborated these end-quartile differences (Mann-Whitney U) (IL-6: $Z = 3.9$, $p < 0.0001$; CRP: $Z = 3.3$, $p < 0.0009$). The Spearman rank-

order correlation of community SES (adjusted for age, sex, and race) with untransformed inflammatory markers was likewise significant, across all subjects, for both IL-6 (rho = -0.139, $p < 0.0001$) and CRP (rho = -0.110, $p < 0.0015$) (For comparison, Pearson correlations between the adjusted community variable and reciprocally transformed IL-6 and CRP values (signs reversed) were virtually identical [IL-6: $r = -0.136$, $p < 0.0001$; CRP: $r = -0.107$, $p < 0.002$]).

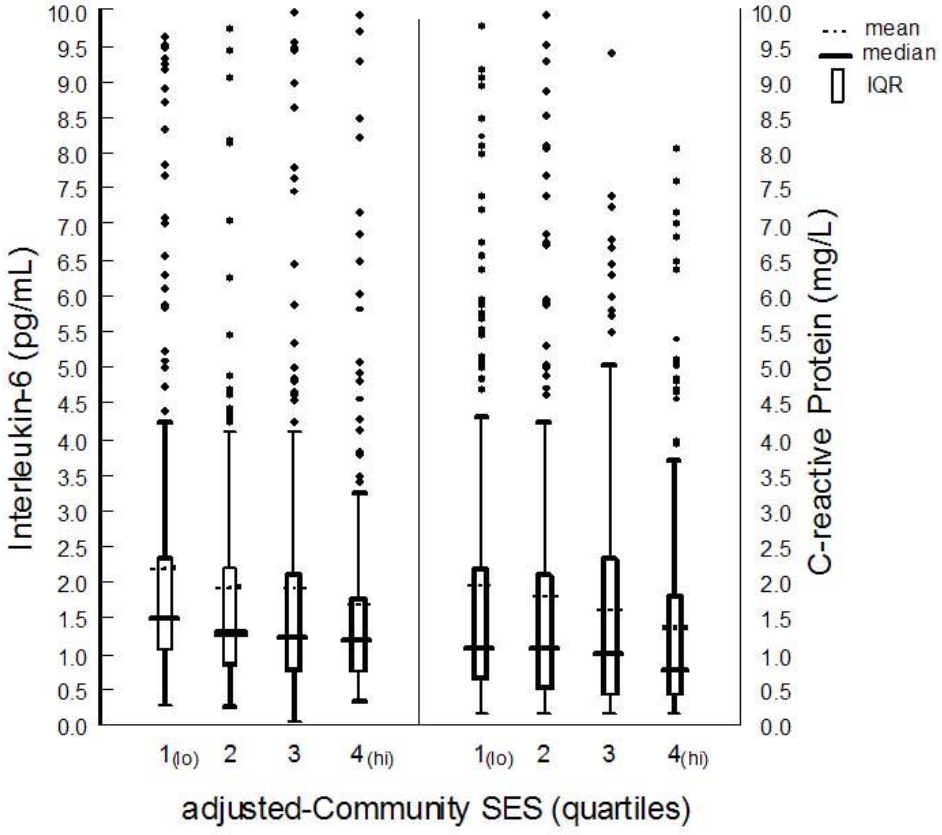
Table 2: Hierarchical regression analyses assessing the relationship between individual/community SES and inflammatory markers after adjusting for age, sex, and race (*Hypothesis 1)*.

Linear Regression	ΔR^2	B	P
DV: IL-6			
Step 1:	.037		.000
Age		.055	.107
Gender		-.091	.007
Race		.172	.000
Step 2:			
Individual SES	.013	-.126	.001
DV: CRP			
Step 1:	.012		
Age		-.029	.393
Gender		.054	.117
Race		.089	.010
Step 2:	.002		
Individual SES		-.047	.214
DV: IL-6			

Step 1:	.037		.000
Age		.055	.107
Gender		-.091	.007
Race		.172	.000
Step 2:			
Community SES	.018	-.163	.000
DV: CRP			
Step 1:	.012		.014
Age		-.029	.393
Gender		.054	.117
Race		.089	.010
Step 2:	.012		
Community SES		-.129	.002

*Dependent variables are the reciprocal transformations of CRP and IL-6, but signs have been reversed for ease of interpretation.

Figure 2: Distribution of the untransformed values of IL-6 and CRP depicted for quartiles of age, sex, and race-adjusted Community SES. IQR = inter-quartile range.



3.1.2 Hypothesis 2: Does community SES predict inflammatory markers independently of individual SES?

In additional regression analyses, community SES was entered as a predictor on step 3, after demographic covariates and individual SES, and again, the area-level socioeconomic index explained significant variability in both IL-6 (B=-.130, p =.003) and CRP (B=-.129, p=.004). Results are displayed in table 3. Thus, the association of community SES with these two inflammatory markers was not attributable to correlated variation in personal socioeconomic indicators.

Table 3: Hierarchical regression analyses assessing the relationship between community SES and inflammatory markers after adjusting for age, sex, race, and individual SES (*Hypothesis 2)*.

Linear Regression	ΔR^2	B	P
DV: IL-6			
Step 1:	.037		.000
Age		.055	.107
Gender		-.091	.007
Race		.172	.000
Step 2:	.013		
Individual SES		-.126	.001
Step 3:	.010		
Community SES		-.130	.003
DV: CRP			
Step 1:	.012		
Age		-.029	.393
Gender		.054	.117
Race		.089	.010
Step 2:	.002		
Individual SES		-.047	.214

Step 3:	.010		
Community SES		-.129	.004

3.1.3 Hypothesis 3: Is SES (both individual and community) associated with IL-6 and CRP independently of concomitant variation in health behaviors?

Finally, analyses were conducted to determine whether health practices could account for relationships of either individual or community SES with markers of inflammation, and results can be found in Table 4. As expected, after controlling for age, sex and race, higher BMI (B=.285, p = .000), current smoking (B=.172, p = .000), and less physical activity (B=-.074, p = .024) were associated with higher IL-6 levels. Similarly, higher BMI (B = .491, p = .000) and current smoking status (B = .073, p = .023) predicted higher CRP levels independently of demographic characteristics. Interestingly, individual SES was no longer associated with IL-6 when entered into regression after demographic covariates and both BMI and health behaviors (B=-.049, p = .186). Community SES, on the other hand, remained a significant, independent predictor of IL-6 (B = -.098, p = .011) and CRP (B = -.072, p=.049), with age, sex, race, BMI, smoking status, and physical activity in the model. And when entered after demographic covariates, BMI and health behaviors, and individual SES in a final regression analysis, community SES continued to predict both IL-6 and CRP (B=-.091, p = .028 and B = -.086, p = .027).

Table 4: Hierarchical regression analyses assessing the relationship between SES and inflammatory markers after adjusting for health behaviors.

Linear Regression	ΔR^2	B	P
DV: IL-6			
Step 1:	.037		
Age		.055	.107
Gender		-.091	.007
Race		.172	.000
Step 2:	.121		
Smoking		.172	.000
BMI		.285	.000
Sleep		-.049	.128
Physical Activity		-.074	.024
Alcohol		.061	.059
Step 3:	.002		
Individual SES		-.049	.186
Step 4:	.005		
Community SES		-.091	.028
DV: CRP			
Step 1:	.012		
Age		-.029	.393

Gender		.054	.117
Race		.089	.010
Step 2:	.238		
Smoking		.073	.023
BMI		.491	.000
Sleep		-.020	.505
Physical Activity		-.024	.436
Alcohol		.011	.708
Step 3:	.000		
Individual SES		.009	.802
Step 4:	.005		
Community SES		-.086	.027

*Dependent variables are the reciprocal transformations of CRP and IL-6, but signs have been reversed for ease of interpretation.

3.1.4 Stratified Analyses

Tests of potential interactions between community SES and race were not significant, suggesting that the foregoing associations were not qualified by participant race (IL-6: B = -.070, p = .199; CRP: B=.006, p = .910). Nonetheless, African American participants lived disproportionately in poorer Census tracts than whites, as reflected in our composite community index (African Americans: M = -1.00 [SD = 0.95]; Whites: M = 0.30 [SD = 0.80]; $t_{849} = 19.1$, p < 0.0001). Because inequalities among communities were thus partly confounded by race, we next asked whether community SES predicted inflammatory markers in analyses conducted separately on Whites (n = 654) and African Americans (n = 197). The bivariate correlation of community SES with IL-6 in Whites was -0.15 (p < 0.0001) and in Black participants, -0.08 (n.s.); corresponding correlations for CRP were -0.11 (p < 0.007) in Whites and -0.12 (p < 0.10) in Blacks. Linear regressions adjusting for age and sex further corroborated these associations (White, IL-6: B = -0.16, p < 0.001; CRP: B = -0.11, p < 0.01; African American, IL-6: B = -0.09, n.s.; CRP: B = -0.13, p < 0.08). Please see tables 5 & 6 for results from stratified regression analyses. When both demographic factors and individual SES were entered first, effects of community SES were weakened, but continued to predict IL-6 (B = -0.13, p < 0.002) and CRP (B = -0.09, p < 0.04) levels in Whites. Interestingly, among African Americans, community SES accounted for significant variance in CRP after entering age, sex, and individual SES, while the community association with IL-6 (B = 0.03) remained non-significant. Among White participants, community SES remained a significant predictor of IL-6 after age, sex, BMI and health behaviors were entered (B = - 0.11, p = 0.005), as well as in the fully adjusted model

including both these covariates and individual SES ($B = -0.10$, $p < 0.015$). However, the community variable did not account for significant variation in CRP in parallel analyses adjusting for other risk factors among Whites, and predicted neither IL-6 nor CRP levels in similarly adjusted models among Blacks.

Table 5: Hierarchical regression analyses assessing the relationship between community SES and inflammatory markers after adjusting for age, sex, race, health behaviors, and individual SES in whites alone. (*Hypothesis 3)*

Linear Regression	ΔR^2	B	P
DV: IL-6			
Step 1:	.016		
Age		.048	.222
Gender		-.118	.003
Step 2:	.109		
Smoking		.135	.000
BMI		.275	.000
Sleep		-.043	.255
Physical Activity		-.077	.042
Alcohol		.041	.283
Step 3:	.003		
Individual SES		-.057	.135
Step 4:	.008		
Community SES		-.098	.015
DV: CRP			
Step 1:	.001		

Age		-.028	.481
Gender		.027	.490
Step 2:	.292		
Smoking		.029	.402
BMI		.540	.000
Sleep		-.026	.440
Physical Activity		-.024	.489
Alcohol		-.011	.745
Step 3:	.000		
Individual SES		-.019	.572
Step 4:	.002		
Community SES		-.045	.211

Table 6: Hierarchical regression analyses assessing the relationship between community SES and inflammatory markers after adjusting for age, sex, race, and health behaviors, and individual SES in African-Americans alone. *(Hypothesis 3)*

Linear Regression	ΔR^2	B	P
DV: IL-6			
Step 1:	.007		
Age		.083	.246
Gender		-.006	.937
Step 2:	.184		
Smoking		.269	.000
BMI		.331	.000
Sleep		-.079	.240
Physical Activity		-.047	.501
Alcohol		.152	.024
Step 3:	.000		
Individual SES		-.011	.882
Step 4:	.000		
Community SES		-.012	.872
DV: CRP			
Step 1:	.019		
Age		-.032	.650

Gender		.135	.059
Step 2:	.160		
Smoking		.163	.019
BMI		.375	.000
Sleep		.006	.935
Physical Activity		-.004	.951
Alcohol		.102	.132
Step 3:	.009		
Individual SES		.108	.142
Step 4:	.014		
Community SES		-.133	.069

3.1.5 Summary Socioeconomic Status and Inflammatory Markers

In sum, hypotheses one, two, and three were largely supported, and our analyses show a significant relationship between socioeconomic variation at both the individual and community level in the prediction of inflammatory markers. IL-6 and CRP covaried with both individual and community SES in univariate models, and individual SES continued to predict IL-6 after adjustment for age, sex, and race. The association between community SES and both IL-6 and CRP persisted after adjustment for age, sex, race, health behaviors (i.e. alcohol, smoking, sleep, BMI, physical activity), and individual SES.

3.2 GENETIC INFLUENCES ON INFLAMMATORY MARKERS

3.2.1 Caucasian Subsample

As noted in the introduction, the multiple determinants of circulating IL-6 and CRP levels include cytokine-related genetic variation, and quantitative genetic studies estimate 17-24% of variation in IL-6 levels to be attributable to heritable factors. The objective of hypothesis four was to determine whether a previously identified functional single nucleotide polymorphism in the promoter region of the IL-6 gene (i.e. 174 G>C) was associated with both IL-6 and CRP in this middle-aged, relatively healthy sample. Hypothesis five examined whether community or individual SES interacted with the 174 G>C polymorphism in the prediction of IL-6 or CRP. Of the 851 participants used in the initial analyses (i.e. hypotheses 1-3), forty participants did not

have valid genotype data, leaving a final sample of 811 subjects for genetic analyses (n=624 Caucasian, n=187 African-American).

3.2.1.1 Preliminary Analyses

Genetic analyses in the Caucasian subsample used all three genotypes (i.e. GG, GC, CC) and were repeated with the genotype dichotomized two ways (i.e. GG vs GC+CC and GG+GC vs CC). To determine whether relevant participant characteristics (i.e. age, sex, BMI, smoking status, alcohol consumption, physical activity, individual and community SES) varied by genotype, we first subjected these variables to analysis of variance, Chi-square, and t-test. Results of these univariate analyses can be found in Table 7.

Table 7: Univariate participant characteristics by genotype in white subsample (n=624).

Participant Characteristics (n=624)	Genotype (CC,CG,GG)	Genotype (CC+CG,GG)	Genotype (CC,CG+GG)
Age	$F_{2, 624} = 0.72$	$t_{622} = 1.18$	$t_{622} = .64$
Sex	Cramer's V^2 = 0.04	$X_1^2 = .008$	$X_1^2 = .81$
BMI	$F_{2, 624} = 1.86$	$t_{622} = 0.73$	$t_{622} = -1.39$
Alcohol	X_2^2=3.95, eta = .06	**$X_1^2 = 3.94$***	$X_1^2 = .45$
Sleep	**$F_{2,624} = 3.47$***	$t_{622} = -2.42$	$t_{622} = .07$
Smoking	X_2^2 = 1.36, eta = .03	$X_1^2 = 0.03$	$X_1^2 = 1.29$
Exercise	$F_{2,624} = 0.170$	$t_{622} = -0.33$	$t_{622} = .33$
1/IL-6	$F_{2, 624} = 1.23$	$t_{622} = 0.11$	$t_{622} = -1.42$
1/CRP	**$F_{2,624} = 2.67$^**	**$t_{622} = 2.0$***	$t_{622} = -.34$
Ind SES	**$F_{2,624}$=4.71****	$t_{622} = -1.11$	**$t_{622} = 2.25$***
Com SES	$F_{2, 624} = 1.76$	**$t_{622} = 1.84$^**	$t_{622} = 1.01$

^ = $p < .10$; * = $p < .05$; **$p < .01$. Alcohol, 0-13.99 drinks/wk, > 14 drnks/wk; smoking, current vs non-smoker.

The 174 G>C genotype (i.e. coded CC, CG, GG) was associated with sleep and individual SES, but not with the other variables of interest (sleep: $F_{2, 624} = 3.47$, $p < .05$; CC M = 48.12, SD = 6.5; CG M = 47.48, SD = 7.6; GG M =49.56, SD = 6.7; individual SES: $F_{2, 624} = 4.71$, $p < .01$; CC M = .114, SD = 1.01; CG M = -.137, SD = 1.02; GG M =.095, SD = .875). Pairwise comparisons showed CG heterozygotes to sleep less than GG homozygotes (Mean difference: -2.08, $p < 01$; please see table 8). Individual SES was also significantly lower among CG heterozygotes, relative to both CC and GG homozygotes (M diff CC: -.25, $p < .01$, M diff GG: -.23, $p < .05$ – please see table 9).

Table 8: Pairwise Comparisons of Sleep by Genotype

(I) 1=C/C, 2=C/G, 3=G/G	(J) 1=C/C, 2=C/G, 3=G/G	Mean Difference (I-J)	Std. Error	Sig. (a)
C/C	C/G	.64	.62	.30
	G/G	-1.44	.81	.08
C/G	C/C	-.64	.62	.30
	G/G	-2.08(*)	.79	.01
G/G	C/C	1.44	.81	.08
	C/G	2.08(*)	.79	.01

Based on estimated marginal means

* The mean difference is significant at the .05 level.

Table 9: Pairwise Comparisons of Individual SES by Genotype

(I) 1=C/C, 2=C/G, 3=G/G	(J) 1=C/C, 2=C/G, 3=G/G	Mean Difference (I-J)	Std. Error	Sig. (a)
C/C	C/G	.252(*)	.088	.004
	G/G	.019	.114	.865
C/G	C/C	-.252(*)	.088	.004
	G/G	-.232(*)	.111	.037
G/G	C/C	-.019	.114	.865
	C/G	.232(*)	.111	.037

Based on estimated marginal means

* The mean difference is significant at the .05 level.

When the genotype was dichotomized (i.e. 1 = CG+CC, 2= GG), GG homozygotes consumed more alcohol ($X_1^2 = 3.94$, $p < .05$) and had higher community SES ($t_{622} = 1.84$, $p < .05$), than subjects carrying any C allele. This dichotomized genotype was not associated with age, sex, smoking status, BMI, or individual SES (ps >.10). Dichotomized another way (i.e. CC vs CG+GG), subjects carrying any G allele had higher individual SES than CC homozygotes ($t_{622} = 2.25$, $p < .05$).

These preliminary analyses also show mean circulating levels of reciprocally transformed IL-6 among Caucasian GG, GC and CC participants to be 0.44 (SD =.17 , untransformed M = 1.90 pg/mL), 0.43 (SD=.16, M = 1.93 pg/mL), and 0.45 pg/mL (SD = .15, M=1.64 pg/mL), and reciprocally transformed CRP values to be 0.55 (SD=.21, untransformed M = 1.39 mg/L), 0.50 (SD= .22, M = 1.69 mg/L), 0.52 mg/L (SD = 0.21, M = 1.56 mg/L). Subjects of the three IL-6 174 G>C genotypes did not differ in circulating IL-6 ($F_{2, 624} = 1.23$, $p = .29$), and showed a trend for significant differences in CRP (CRP: $F_{2, 624}$ 2.67, $p = .07$). Pairwise comparisons show this trend was attributable to a slightly higher CRP concentration in CG vs GG genotypes (please see table 10). Similar analyses on the dichotomized polymorphism (i.e. CC+CG vs GG) showed GG homozygotes to have lower mean CRP concentrations than subjects with any C allele ($t_{622} = 2.00$, $p < .05$; untransformed M CC+CG = 1.63 mg/L, M GG = 1.39 mg/L) but no differences in mean IL-6 levels were evident by genotype ($t_{622} = -0.11$, $p > .10$). Finally, when CC homozygotes were contrasted with subjects carrying any G allele, no differences were found in IL-6 or CRP concentrations ($t_{622} = -1.42$, $p > .10$; $t_{622} = -.34$, $p > .10$, respectively). In sum, examining IL-6 and CRP by the three possible genotypes of the polymorphism revealed no differences in circulating concentrations. Dichotomizing the polymorphism showed GG homozygotes to have lower CRP relative to subjects carrying any C

allele. The final dichotomization of genotype, contrasting CC homozygotes with subjects carrying any G allele, showed no differences in IL-6 or CRP concentrations.

Table 10: Pairwise Comparisons of Reciprocally Transformed CRP by Genotype

(I) 1=C/C, 2=C/G, 3=G/G	(J) 1=C/C, 2=C/G, 3=G/G	Mean Difference (I-J)	Std. Error	Sig. (a)
C/C	C/G	.022	.019	.247
	G/G	-.033	.025	.181
C/G	C/C	-.022	.019	.247
	G/G	-.055(*)	.024	.023
G/G	C/C	.033	.025	.181
	C/G	.055(*)	.024	.023

Based on estimated marginal means

* The mean difference is significant at the .05 level.

3.2.2 Hypotheses 4 and 5: Is the G allele associated with higher circulating levels of IL-6 and CRP? And, do SES (i.e. either individual or community) and genotype interact in the prediction of IL-6 or CRP?

Regression Analyses

Several parallel hierarchical regression models were executed to examine the association between inflammatory markers (i.e. IL-6 or CRP) and SES (i.e. either individual or community), genotype, and the genotype by SES interaction term. On step 1 of these analyses, age and sex were entered. Step 2 contained either individual or community SES, and genotype was entered on step 3 (either CC, CG and GG; CC+CG vs GG; or CC vs CG+GG), while the final step included the genotype by SES interaction term. Results of these models are displayed in tables 11-15. Because age, sex, and either individual or community SES associations with the dependent variable (i.e. IL-6 or CRP) do not vary among models, these results are presented only with the first regression model for each dependent variable.

3.2.2.1 Individual SES, Genotype, and IL-6

Results of the hierarchical regression for IL-6, where IL-6 174 G>C is entered by its three genotypes, are presented in Table 11**.** When entered on the first step of the analysis, female gender predicted higher IL-6 ($B = -.118$, $p < .01$), while age was not associated with IL-6. Higher individual SES, entered on step 2, predicted lower IL-6 ($B = -.11$, $p < .01$), as it had done in analyses of the entire sample presented earlier. Entered on the two subsequent steps, neither the main effect of genotype (i.e. CC, CG, GG) nor the interaction of genotype by individual SES accounted for significant variation in IL-6 (p's $> .10$). The pattern of results with the genotype

dichotomized both ways was identical, with no additional variance in IL-6 accounted for by either genotype or the genotype by individual SES interaction term (ps > .10).

Table 11: Hierarchical regression analyses assessing the relationship between genotype and IL-6 after adjusting for age, sex, individual SES and individual SES x genotype in whites alone. (*Hypotheses 4 and 5)*.

Linear Regression	ΔR^2	B	P
DV: IL-6			
Step 1:	.016		
Age		.052	.194
Gender		-.118	.003
Step 2:	.012		
Individual SES		-.111	.005
Step 3:	.001		
Genotype (GG, GC, CC)		.038	.341
Step 4:	.000		
Indiv SES x Genotype		-.006	.887
DV: IL-6			
Step 3:	.000		
Genotype (GC+CC, GG)		.004	.921
Step 4:	.000		

Individual SES x Genotype		.001	.980
Step 3:	.003		
Genotype (CC vs GG+GC)		.053	.181
Step 4:	.000		
Indiv SES x Genotype		-.006	.884

3.2.2.2 Individual SES, Genotype, and CRP

Results of parallel regression models with CRP can be found in Table 12. Step 1 of the analysis shows age and sex were not associated with CRP in this Caucasian subsample, but higher individual SES was marginally associated with less CRP (B=-.07, p = .08) when entered on step 2 of the model. Neither the main effect of genotype (i.e. CC, CG, GG), entered on step 3, nor the individual SES by genotype interaction terms, entered on the final step, were significant predictors (ps > .10). A parallel model with the dichotomized genotype shows that when GG homozygotes were contrasted with subjects carrying any C allele in step 3 of the model, GG homozygotes had lower CRP than subjects with CC+CG genotypes (B = -.08, p = .05), but the interaction of genotype by individual SES was not associated with CRP (p>.10). Finally, a third model showed no association between CRP and genotype when contrasting CC homozygotes with the combined GC and GG genotypes (ps > .10).

Table 12: Hierarchical regression analyses assessing the relationship between genotype and CRP after adjusting for age, sex, individual SES and individual SES x genotype in whites alone. (*Hypotheses 4 and 5)*.

Linear Regression	ΔR^2	B	P
DV:CRP			
Step 1:	.001		
Age		-.020	.626
Gender		.027	.497
Step 2:	.005		
Individual SES		-.070	.083
Step 3:	.001		
Genotype (GG, GC, CC)		-.037	.355
Step 4:	.000		
Indiv SES x Genotype		-.015	.712
DV: CRP			
Step 3:	.006		
Genotype (GC+CC, GG)		-.078	.052
Step 4:	.000		

Individual SES x Genotype		.014	.722
DV: CRP			
Step 3:	.000		
Genotype (CC vs GG+GC)		.006	.874
Step 4:	.001		
Indiv SES x Genotype		-.031	.435

3.2.2.3 Summary of Individual SES, Gene, and Inflammatory Markers

In sum, consistent with analyses presented earlier, subjects of lower individual SES had higher concentrations of IL-6, but neither main effect of genotype nor the interaction of genotype by individual SES were associated with IL-6. With regard to CRP, higher individual SES marginally predicted lower CRP, and GG homozygotes had lower circulating CRP relative to subjects carrying any C allele (i.e. CG + CC), but genotype did not interact with individual SES in predicting CRP.

3.2.2.4 Community SES, Genotype, and IL-6

In an additional set of regression analyses, we next considered a main effect of genotype after adjusting for age, sex, and community SES, and finally examined the possibility of a community SES by genotype interaction in predicting IL-6 (see table 13). After adjusting for age and sex, hierarchical regression analyses showed participants with higher community SES, entered on step 2, to have lower circulating IL-6 ($B=-.16$, $p < .01$), consistent with analyses presented earlier. There was neither a main effect of genotype (step 3) nor an interaction of genotype by community SES (step 4) in predicting IL-6, irrespective of the coding of the genotype (all $ps > .10$).

Table 13: Hierarchical regression analyses assessing the relationship between genotype and community SES x genotype and IL-6 after adjusting for age, sex, and community SES in whites alone. (*Hypotheses 4 and 5)*.

Linear Regression	ΔR^2	B	P
DV: IL-6			
Step 1:	.016		
Age		.052	.194
Gender		-.118	.003
Step 2:	.024		
Community SES		-.157	.000
Step 3:	.001		
Genotype (GG, GC, CC)		.031	.428
Step 4:	.001		
Comm SES x Genotype		-.032	.423
DV: IL-6			
Step 3:	.000		
Genotype (GC+CC, GG)		-.013	.747

Step 4:	.001		
Comm SES x Genotype		-.027	.499
DV: IL-6			
Step 3:	.003		
Genotype (CC vs GG+GC)		.056	.153
Step 4:	.001		
Comm SES x Genotype		-.031	.429

3.2.2.5 Community SES, Genotype, and CRP

In this Caucasian subsample, lower community SES predicted higher CRP (B=-.111, $p < .01$) after adjusting for age and sex, as it had done in analyses reported earlier. Three separate regressions were run with genotype for the 174 G>C polymorphism coded differently (see table 14), and only one main effect for genotype in the prediction of CRP was found. When the genotype was dichotomized (i.e. CC+CG vs GG), subjects carrying any C allele had higher CRP ($B = -.09$, $p < .05$) relative to GG homozygotes, but the genotype by community SES interaction was not significant ($p>.10$).

Table 14: Hierarchical regression analyses assessing the relationship between genotype and community SES x genotype with CRP after adjusting for age, sex, and community SES in whites alone. (*Hypotheses 4 and 5)*.

Linear Regression	ΔR^2	B	P
DV: CRP			
Step 1:	.001		
Age		-.020	.626
Gender		.027	.497
Step 2:	.012		
Community SES		-.111	.006
Step 3:	.002		
Genotype (GG, GC, CC)		-.042	.292
Step 4:	.000		
Comm SES x Genotype		-.022	.590
DV: CRP			
Step 3:	.008		
Genotype (GC+CC, GG)		-.089	.025

Step 4:	.000		
Comm SES x Genotype		-.010	.811
DV: CRP			
Step 3:	.000		
Genotype (CC vs GG+GC)		.008	.841
Step 4:	.002		
Comm SES x Genotype		-.046	.250

3.2.2.6 Health Behaviors

Additional analyses designed to explore whether health behaviors might affect the relationship between the dichotomized genotype (i.e. CC+CG vs GG) and CRP were conducted next. Results are displayed in table 15. On step 1 of the regressions, age, sex, sleep, BMI, smoking status (non vs. current), alcohol consumed per week (0-13.99 vs >14 drinks/week), and physical activity were entered. Step 2 included the relevant SES measure (either individual or community), with genotype entered on step 3, and the genotype by SES interaction term entered on step 4. Including these health behaviors eliminated the association between individual SES and CRP, and showed female gender ($B = .12$, $p < .01$) and higher BMI ($B = .54$, $p < .01$) to predict higher CRP. After including these health behaviors, the dichotomized genotype was marginally associated with CRP ($B = - .06$, $p = .07$). With regard to the community SES model, genotype remained a significant predictor of CRP ($B = -.07$, $p = .05$). To summarize, inclusion of health behaviors in parallel models with individual and community SES weakened, but did not eliminate, the association between genotype and CRP. Neither CRP nor IL-6 varied as a function of any genotype by SES interaction.

Table 15: Hierarchical linear regression analyses predicting CRP by genotype and SES (i.e. individual and community) after adjusting for age, sex, race, and health behaviors in whites alone.

Linear Regression	ΔR^2	B	P
DV: CRP			
Step 1:	.294		
Age		-.034	.320
Gender		.124	.001
Sleep		-.022	.536
BMI		.541	.000
Smoking Status		.033	.353
Alcohol		-.015	.662
Physical Activity		-.030	.390
Step 2:	.000		
Individual SES		-.018	.616
Step 3:	.004		
Genotype (GG vs GC+CC)		-.063	.065
Step 4:	.000		
Indiv x Genotype		.005	.889
DV: CRP			

Step 2:	.002		
Community SES		-.050	.151
Step 3:	.005		
Genotype (GG vs GC+CC)		-.068	.046
Step 4:	.001		
Comm SES x Genotype		.034	.320

3.2.3 African-American Subsample

Preliminary Subsample

In the African-American subsample (n=187), the distribution of genotypes conformed with Hardy-Weinberg equilibrium ($X_1^2 = 0.73$, $p > .05$). Due to the extremely low frequency of the GG genotype in African-Americans (n=3), GG homozygotes and GC heterozygotes are combined for analyses. Preliminary results showed baseline IL-6 and CRP levels did not vary by genotype (IL-6: $t_{185} = -.62$, $p > .05$; CRP: $t_{185} = -.92$, $p > .05$). Reciprocally transformed IL-6 means among subjects carrying any G allele (i.e. GG/GC) and CC homozygotes were 0.39 (SD = .13, untransformed M = 1.98 pg/mL) and 0.37 pg/mL (SD = .15, M = 2.44pg/mL), respectively, and reciprocally transformed CRP values were 0.43 (SD = .23, untransformed M = 2.37 mg/L) and 0.47 mg/L (SD = .24, M = 2.05mg/L). As summarized in table 16, age, sex, alcohol consumption, smoking status, body mass index, exercise, sleep, individual and community SES did not vary by genotype (p's > .05).

Table 16: Univariate participant characteristics by genotype in black subsample (n=187).

Participant Characteristics	Genotype (CC, CG+GG)
Age	$t_{185} = .01$
Sex	$X_1^2 = .06$
BMI	$t_{185} = .22$
Alcohol	$X_1^2 = .08$
Sleep	$t_{185} = -.21$
Smoking	$X_1^2 = 3.32$
Exercise	$t_{185} = .54$
1/IL-6	$t_{185} = -.50$
1/CRP	$t_{185} = .92$
Individual SES	$t_{185} = -.24$
Comm SES	$t_{185} = -.10$

^ = $p < .10$; * = $p < .05$; **$p < .01$. Alcohol, 0-13.99 drinks/wk, > 14 drnks/wk; smoking, current vs non-smoker.

3.2.3.1 Hypotheses 4&5: Is the G allele associated with greater circulating IL-6 and CRP, and is there an interaction between genotype and SES in predicting inflammation?

Parallel regression models examining the contribution of individual and community SES, alone and in interaction with 174G>C (1=CC, 2 = GG+CG), were also conducted. These models, adjusting for age and sex in step 1, are summarized in tables 17-18. They showed no association between individual SES (step 2), genotype (step 3), or the interaction of individual SES and genotype (step 4) in predicting levels of circulating IL-6 or CRP. Community SES, genotype, and the interaction of community SES and genotype also were not associated with IL-6 or CRP after adjusting for age and sex. In sum, analyses in the African-American subsample showed variation in inflammatory markers (i.e. IL-6 and CRP) was not accounted for by genotype in this study.

Table 17: Hierarchical regression analyses assessing the relationship between genotype and individual SES by genotype with inflammatory markers after adjusting for age and sex in blacks alone (*Hypotheses 4 and 5)*.

Linear Regression	ΔR^2	B	P
DV:IL-6			
Step 1:	.010		
Age		.100	.173
Gender		-.018	.810
Step 2:	.012		
Individual SES		-.111	.144
Step 3:	.001		
Genotype (CC, GG+CG)		-.035	.634
Step 4:	.000		
Indiv SES x Genotype		.006	.941
DV: CRP			
Step 1:	.017		
Age		-.021	.246
Gender		.128	.937
Step 2:	.000		

Individual SES		.006	.937
Step 3:	.005		
Genotype (CC, GG+CG)		.070	.341
Step 4:	.004		
Indiv SES x Genotype		-.067	.363

Table 18: Hierarchical regression analyses assessing the relationship between genotype and the community SES by genotype interaction withinflammatory markers after adjusting for age and sex in blacks alone. (*Hypotheses 4 and 5)*.

Linear Regression	ΔR^2	B	P
DV:IL-6			
Step 1:	.010		
Age		.100	.173
Gender		-.018	.810
Step 2:	.002		
Comm SES		-.048	.513
Step 3:	.001		
Genotype (CC, GG+CG)		-.037	.617
Step 4:	.000		
Comm SES x Genotype		.003	.970
DV: CRP			
Step 1:	.017		
Age		-.021	.246
Gender		.128	.937

Step 2:	.015		
Comm SES		-.122	.095
Step 3:	.005		
Genotype (CC, GG+CG)		.071	.330
Step 4:	.006		
Comm SES x Genotype		-.077	.290

4.0 DISCUSSION

Despite a well documented inverse association between individual level socioeconomic status and cardiovascular disease, and a more recent literature showing area-level measures of SES to be independently associated with cardiovascular morbidity and mortality, mechanisms explaining these associations are not fully understood. In the last decade, multiple lines of research have documented the pathogenic role of inflammation in the development of atherosclerotic cardiovascular disease. Subsequently, a limited literature has examined whether inflammation might covary with individual SES and mediate its association with atherosclerotic cardiovascular disease. Thus, hypotheses one to three of this study aimed to extend this literature and examine whether socioeconomic status, at the level of both the individual and the community, is associated with two markers of inflammation (i.e. IL-6 and CRP) that are frequently cited as predictive, and potentially pathogenic, in the development of atherosclerotic cardiovascular disease. Hypothesis four assessed whether the influence of a previously identified functional single nucleotide polymorphism (i.e. -174 G>C) in the promoter region of the IL-6 gene is associated with circulating levels of both IL-6 and CRP in this healthy, middle-aged sample. And hypothesis five examined whether this polymorphism modulates any influence of SES on IL-6 and CRP. This discussion examines the results of each aspect of the study in turn, with concluding comments at the end.

4.1 SOCIOECONOMIC CORRELATES OF INFLAMMATORY MARKERS

The current study contributes to an understanding of relationships between individual SES and inflammatory markers. Consistent with the extant literature (Rathmann et al., 2006; Mendall et al., 2000), we found an inverse association between individual SES and plasma IL-6 that was explained by behavioral factors, including BMI, smoking status and physical activity. A large literature supports an association between lower individual SES and poor health practices, with higher rates of smoking and obesity and lower levels of physical activity than among persons of higher SES (e.g. Marmot et al, 1991). Furthermore, these health practices have been associated with higher levels of peripheral proinflammatory mediators (e.g., Bruunsgaard, 2005; Frohlich et al., 2003). The current findings support a particularly strong relationship between BMI and plasma levels of IL-6 and CRP, which may be explained by the production and release of IL-6 by adipocytes (Mohamed-Ali et al., 1997). In sum, behavioral covariates of individual SES may provide a pathway linking lower SES to increased systemic inflammation.

In regard to CRP, we found no significant relationship between individual SES and levels of CRP after adjusting statistically for age, sex, and race. Although not all findings are consistent (Mendall et al, 1997), the current results contrast with increasing evidence of an inverse relationship between individual SES and plasma CRP (Owen et al., 2003; Jousilahti, et al., 2003; Panagiotakos et al., 2004; Danesh et al., 1999; Wu et al., 2002; Hemingway & Marmot, 1999; Rathmann et al., 2000). One possible explanation for our failure to replicate this effect is our recruitment of a younger and healthier sample than in many other studies (e.g., Jousilahti et al, 2003; Panagiotakos et al., 2004; Hemingway & Marmot, 1999). It has also been suggested that the presence of subclinical infections among individuals lower in SES may account for relationships between individual SES and inflammatory mediators (Mendall et al, 1997). In this

regard, we excluded individuals with levels of IL-6 or CRP in the range associated with acute infection, decreasing the probability that this confounder could have influenced findings. Finally, it is possible that the range of individual SES represented in this sample was limited in comparison with other studies. Indeed, 61% of our sample was college educated, with a mean of 15.3 years of education. It is possible that a clearer individual SES- inflammatory marker relationship would be seen with the inclusion of more individuals from lower socioeconomic strata.

The present study also provides initial evidence for an association between an area-based measure of socioeconomic inequalities and circulating markers of inflammation in a relatively healthy, mid-life community sample. Consistent with evidence that socioeconomic attributes of communities confer risk for preclinical atherosclerosis (Nordstrom, Diez Roux, Jackson & Gardin, 2004; Petersen et al, 2006) and predict cardiovascular morbidity and mortality (e.g., Lawlor et al., 2005; Steenland et al, 2004; Booth et al., 2001), our findings show that, when compared with residents of more advantaged communities, individuals living in census tracts of lower median income, higher rates of poverty and unemployment, and lower levels of education have higher levels of plasma CRP and IL-6. These associations were independent of individual level SES and of demographic and behavioral health risk factors, including age, sex, race, BMI, smoking, alcohol consumption, physical activity and sleep. There were no significant community SES by race interactions, and exploratory race stratified analyses yielded a similar pattern of results in whites, but results were not significant in blacks. These nonsignificant results need to be interpreted with caution, given the small African-American sample size (n=197) and consequent power limitations.

The current results are consistent with recently published evidence that levels of CRP covary inversely with unadjusted community SES (Pollitt et al., 2007), extending these findings to show a similar relationship between community SES and IL-6. Thus, our findings also are consistent with prior observations that IL-6 concentrations are elevated in 'slum dwellers' when compared with the urban middle class in India (Yudkin et al, 1999). Taken together, these findings raise the possibility that relationships between community SES and increased vulnerability to cardiovascular disease could be mediated, in part, through inflammatory pathways.

The mechanisms through which socioeconomic attributes of communities may affect systemic inflammation are unclear. Younger individuals in the sample were more likely to live in less advantaged communities than older individuals. This raises the possibility that community SES is a marker of accumulated wealth, which may be a more stable and global socioeconomic determinant than indicators of current income and educational attainment. While this interpretation is plausible, it suggests that community SES is actually a measure of an individual-level attribute functioning independently of the physical environment. In all likelihood, community SES affects the health of individuals by mechanisms associated with both the physical environment and the attributes of the individuals who live there.

Indeed, a growing literature suggests that community SES is a measure of exposure to environmental factors that increase risk for adverse health behaviors as well as a marker of access to health promoting resources (Booth et al., 2001). More specifically, physical aspects of the environment such as density of fast food restaurants, grocery stores with affordable fruits and vegetables, park and recreation facilities, access to medical care, and well lit and safe paths for walking are known to vary among neighborhoods, and these physical differences have been

shown to impact health behaviors (e.g. Brennan-Ramirez, et al., 2006; Glass, Rasmussen & Schwartz, 2006; Morland, Wing, & Diez Roux, 2002; Field et al., 2004; Pearce, Witten & Bartie, 2006; Roemmich et al, 2006). Further, targeted advertising for alcohol and tobacco use, combined with density of convenience stores and alcohol distributors in lower SES neighborhoods is associated with enhanced use of these substances (e.g. Alaniz, 1998; van Lenthe & Mackenbach, 2006; Chuang et al., 2005). In sum, physical differences in the environment of disadvantaged communities may be one area-level pathway leading to worse health behaviors and contributing to increases in inflammatory markers for individuals residing there.

In our study, a number of behavioral risk factors for inflammation were associated with lower community SES, including smoking, physical inactivity, less sleep, and higher BMI. These findings are consistent with a larger literature showing a number of risk factors for inflammation and atherosclerosis to cluster in areas of lower SES. For example, census tract variables reflective of social disorganization and urbanization have been shown to predict worse dietary habits of youth (Lee & Cubbin, 2002). Community socioeconomic status has been associated with smoking behavior in most (Smith et al., 1998; Diez-Roux et. al., 1997; Reijneveld, 1998 ; Sundquist et al., 1998; Duncan et al., 1999), but not all studies (Lee & Cubin, 2002; Hart, Ecob, & Smith, 1997). For example, the odds ratio for current smoking in individuals from lower SES communities varies from 1.10 to 1.73 across studies, depending upon which parameters are used to index community SES (Diez-Roux et al., 1997; Sundquist et al., 1998). Individuals in deprived areas are less likely to participate in any physical activity (Sundquist et al., 1998), with one longitudinal analysis showing residents of poverty areas to experience a decline in physical

activity over a 9-year period, irrespective of race or individual income level (Yen & Kaplan, 1998).

However, because the association between community SES and inflammatory markers in our study was independent of all the behavioral factors we analyzed, theorizing about other potential mediating factors is also warranted. There is a longer tradition of examining SES mediators at the individual-level, and several studies suggest that lower individual SES is associated with psychological and social sequelae, so this discussion will begin with a brief review of that literature. For example, individual SES is positively correlated with social support and social integration, such that low SES predicts low social support (Rosengren, Orth-Gomer, & Wilhemsen, 1998). Lower SES is also more commonly associated with social isolation than higher SES (Turner & Marino, 1994). People of lower SES report more job strain and effort-reward imbalance at work, relative to people of higher levels of SES (Bosma, Peter, Siegrist, & Marmot, 1998; Marmot, Bosma, Hemingway, et al, 1997). Perceived control and mastery are greater among high SES individuals (Bosma, Schrijvers, & Mackenbach, 1999; Lachman & Weaver, 1998), and hostility, depression and hopelessness are more commonly associated with relative socioeconomic deprivation (Fiscella & Franks, 1997; Gump et al., 1999; Turner & Lloyd, 1999). Finally, people of lower individual SES appear to experience chronic life stress (e.g. health problems of significant others, problems with living conditions, financial stressors) more frequently than individuals of higher SES (Lynch, Kaplan, & Shema, 1997; Stronks, van de Mheen, Looman, & Mackenbach, 1998; Rozanski et al., 1999). In sum, low individual SES is associated with adverse psychosocial profiles**,** although the direction of the relationship is unclear and may, in fact, be bi-directional.

Several studies also show that aspects of less advantaged neighborhoods, such as crowding, noise, unemployment, and crime contribute to chronic stress burden and other adverse psychological sequelae. For example, more stressful life events such as job loss, death of a loved one, and criminal victimization are reported in areas with more adverse socioeconomic conditions (Fang, Madhavan, Bosworth & Alderman, 1998; Massey & Shibuya, 1995; Krivo & Peterson, 1996; Sampson, Raudenbush, & Earls, 1997). In addition to greater exposure to stressors in lower SES environments, at least two studies have shown that stress has a greater impact on the health of persons living in lower SES neighborhoods (e.g. Boardman, 2004; Krieger, 1991), and factors that help to buffer the effects of chronic stress such as social support, social integration/cohesion, and informal social control are also worse in lower SES environments (e.g. Turner & Marino, 1994; Steptoe & Feldman, 2001). Depression is also more prevalent in communities with lower SES (Robert, 1998; Silver, Mulvey & Swanson, 2002; Matheson, Moineddin, Dunn, Creatore, Gozdyra, Glazier, 2006).

Interestingly, Steptoe & Feldman (2001) attempted to more directly address stress that is uniquely associated with neighborhoods. They measured both individual and neighborhood SES, and then created a "neighborhood problem questionnaire" reflecting variables such as threat from others, vandalism, access to amenities, noise, and safety for walking at night. Their results showed that these "neighborhood problems" were associated with psychological distress, poorer self-rated health, and reduced ability to carry out activities of daily living, arguing that physical attributes of the neighborhood environment have a direct correlation with psychological outcomes and health. Of note, these associations were independent of both individual and neighborhood SES. It is important to conceptualize the potential differences between stress related to low individual SES (e.g. unemployment), which might be more easily altered, and

stress attributable to neighborhood of residence. The chronic stress burden of living in a lower SES community is pervasive, likely longstanding, and wholly outside the individual's control. Upward mobility requires stability of employment and an ability to save and plan for the future; it is much more complicated than, for example, simply gaining employment that might help put food on the table. In this regard, it is easier to conceptualize potential reasons why community SES may have more pervasive effects on health than individual SES.

The pathways for psychological variables, including chronic stress, to affect inflammatory markers include both behavioral mediators and more direct psychophysiological mechanisms. Behavioral mediators would include, for example, changes in physical activity due to depression or stress, resulting in higher BMI and increased production of IL-6 through adipocytes. Direct physiological mechanisms include upregulation of innate inflammatory processes through endocrine pathways involving the hypothalamic-pituitary adrenal (HPA) axis or by activation of the sympathetic nervous system (e.g., Segerstrom & Miller, 2004; Maier & Watkins, 1998). More specifically, when the hypothalamus is activated, i.e. by stress, it secretes corticotrpoin releasing hormone, which stimulates the release of adrenocorticoptropin releasing hormone (ACTH) from the anterior pituitary, in turn triggering cortisol release into the bloodstream. White blood cells express receptors for cortisol, which functions to inhibit inflammatory processes acutely. With prolonged stress, however, white blood cells may downregulate cortisol receptor expression resulting in increased inflammation. Alternatively, cortisol secretion itself may diminish, resulting in the same enhanced inflammatory state (Miller, Cohen & Ritchey, 2002; Bower, Ganz, Aziz, Olmstead, Irwin, & Cole, 2007). The sympathetic nervous system, on the other hand, directly innervates immune organs. The cells of these organs express receptors for norephinephrine, a neurotransmitter that is released directly by the

sympathetic nervous system. Prolonged secretion of norepinephrine results in increased inflammation (Segerstrom & Miller, 2004; Maier & Watkins, 1998).

4.1.1 Summary

In sum, this paper provides evidence for an inverse association between individual and community SES with inflammatory markers relevant to the development of atherosclerotic cardiovascular disease. The association between individual SES and IL-6 was explained by adjustment for race, sex, and age, while the association between individual SES and CRP was explained by health practices (i.e. smoking, BMI, and physical activity.) Conversely, none of the variables we incorporated in these analyses, including individual SES and health practices, eliminated the association between community SES and IL-6 or CRP. Older individuals tended to live in better communities in our sample. It is therefore possible that community SES functions as a more global measure of wealth than individual SES, and is therefore operating as an extension of an individual-level attribute. However, evidence from other studies suggests that contextual variation in environments affects both the health practices of its individuals, as well as the degree of chronic stress to which they are exposed. Furthermore, people living in these environments face the added burden of social isolation and lack of community buffers such as informal social control and social cohesion, all of which are likely to mediate mental and physical health outcomes.

4.2 GENETIC INFLUENCES ON IL-6 AND CRP

Genetic analyses were conducted entering genotype of the polymorphism by three alternative groupings. In Caucasian subjects, there was no main effect of the polymorphism on IL-6 levels regardless of how 174 G>C was defined. When IL-6 174 G>C was defined by either its three genotypes (i.e. GG, GC, CC) or when CC homozygotes were contrasted with any G allele (i.e. CG+GG), there was also no main effect of genotype on CRP. However, GG homozygotes had lower CRP concentrations than subjects carrying any C allele (i.e. CC+CG). This association between dichotomized genotype and CRP seemed to be partially explained by gender and body mass index. There was also no evidence of an interaction between either individual or community SES and genotype in predicting IL-6 or CRP. Finally, results in the African-American subsample showed no association between genotype and IL-6 or CRP.

While the 174 G>C polymorphism is thought to be functionally related to the production of IL-6 both in-vitro (Fishman et al., 1998) and with circulating IL-6 and CRP in-vivo (e.g. Fishman et al., 1998; Olivieri et al., 2002; Bonafe, 2001; Jones et al., 2001; Jerrard-Dunne et al., 2003), not all studies show significant associations (e.g. Endler et al, 2004; Jerrard-Dunne et al, 2003; Margalionie, et al., 2001; Bennermo et al., 2004; Lieb et al., 2004; Nauck et al, 2002; Eklund et al., 2006; Antonicelli et al., 2005), and not all significant findings are in agreement. For example, some studies show higher IL-6 and/or CRP in GG homozygotes (Fishmann et al., 1998; Olivieri et al., 2002; Bonafe 2001; Nikolova et al, 2007; Libra et al., 2006), while others suggest either CC homozygotes (Jones et al, 2001) or carriers of any C allele are associated with higher IL-6 and/or CRP (D'Aiuto et al., 2004) .

These conflicting results may be at least partially explained by haplotype analyses showing multiple sites within the promoter region of the IL-6 gene to collectively regulate gene

expression (Terry, Loukaci & Green, 2000; Ferrari et al., 2003). For example, one study found an additive effect for risk alleles within two sites of the promoter region (i.e. -572G and -174C), with each additional risk allele present associated with an increase in production of circulating CRP (i.e. presence of 4 risk alleles showed a 79% increase in circulating CRP; Ferrari et al., 2003). In-vitro haplotype analyses show a much more complicated set of results, such that the type of cell line used (i.e. hepatic vs. endothelial) generates a different set of effects. These results suggest that regulation of IL-6 by four different sites within the promoter region of the gene is not simply additive, but is actually a complex interaction between the alleles of the haplotype (Terry et al., 2000).

Another potential reason for the largely null findings we have reported includes the deviation of our Caucasian subsample's genotype distribution from Hardy-Weinberg equilibrium (HWE). HWE states that a population's gene frequencies remain unchanged from one generation to the next. A departure from equilibrium could occur due to: genetic drift (i.e. a deviation in expected frequencies based on chance, rather than natural selection), migration or 'gene flow' (i.e. permanent movement of genes from one population to another), mutations, non-random mating, and natural selection operating on this locus. Additionally, study design or mechanical problems, such as sampling or genotyping errors, could affect the distribution's conformity with Hardy-Weinberg equilibrium (Hosking et al, 2004). The genetic analyses conducted in this paper were based on a subsample of participants (Caucasian n=624) for whom we had data on all relevant variables. Because the distribution of genotypes from both the larger AHAB database and from those subjects excluded from these analyses (data presented earlier) did not deviate from Hardy-Weinberg equilibrium, it is unlikely that the cause of the deviation in our subsample was due to genetic drift, mutation, non-random mating, or natural selection. One

would expect those effects to also be present in the larger dataset. It is possible that errors in genotyping were magnified in this smaller sample, and unfortunately there is no way to confirm or disconfirm this hypothesis. Nonetheless, the deviation from Hardy-Weinberg was quite small, and additional comparisons show that the Caucasian subsample used in this paper contrasted with those subjects from the larger dataset who were not included in these analyses did not differ on sex, current marital status, employment status (currently employed vs. not), family income, educational attainment, or median community income (ps = ns). They did differ on age, however, with a slightly older subsample in these analyses [M = 45.2 years (SD = 6.5) vs 44.0 years (SD = 7.2); t_{1043} = -2.93]. Given the lack of significant differences between the two samples, the mild deviation from Hardy-Weinberg equilibrium in our subsample is likely due to chance rather than forces of natural selection, genetic drift, mutation, assortative mating, or errors in genotyping.

4.2.1 Gene by Environment Interactions

Finally, several gene (-174 G>C) by environment (i.e. individual and community SES) interactions were tested in this study, and none of the results were significant. A recent article by Moffitt, Caspi & Rutter (2005), highlights the conditions under which these authors believe a gene by environment interaction is most likely to be discovered. Our study design was consistent with many of these conditions. For example, the literature shows heritability for circulating IL-6 to be estimated at 17-24% (Pantsulaia et al., 2002; de Maat et al., 2004), which is a necessary preliminary step before finding a specific gene to study. Secondly, preliminary analyses in the AHAB dataset showed a correlation between individual SES and IL-6 to be modest ($r = -.16$, $p < .01$), showing that individuals exposed to lower SES conditions produce variable IL-6 responses. This modest correlation suggests the possibility of a gene by environment interaction.

Additionally, Moffitt et al. point out that the environmental risk factor (i.e. SES) should have a plausible relationship with a neurobiological pathway for the disorder or trait. In this case, I have detailed plausible biological pathways through which SES could affect natural immunity, including arousal of the HPA and SAM systems.

Of particular relevance to this study, Moffitt et al. argue the importance of optimizing environmental risk measurement, based partly on proximity of the environmental influence to the disorder or trait. In the case of IL-6, previous results show two proximal environmental pathogens, i.e. surgery and vaccination, to affect the acute IL-6 response differentially such that GG homozygotes had a much higher reaction than those subjects possessing a C allele (Burzotta et al., 2001; Travelyan, et al., 2004; Gaudino et al., 2003; Bennermo et al., 2004). We sought to extend this research by examining whether a more distal environmental risk factor interacts with 174 G>C in predicting circulating levels of IL-6 and CRP. It is possible that these distal measures of environmental influence (i.e. SES) may not best capture environmental variation interacting with -174 G>C, and that a more proximal measure of SES might show different results. Such a measure might include an index of chronic stress burden associated with residential environment [i.e. neighborhood problem questionnaire, (Steptoe & Feldman, 2001], or other measures of chronic stress or depression. If these more proximal moderators vary by SES, as we have hypothesized, one might be more likely to discover an interaction with 174 G>C.

While it is a reasonable argument that more proximal measures would enhance the likelihood of revealing gene by environment interactions, evidence certainly exists that distal measures, such as SES, interact with genes in the prediction of phenotypes. For example, two studies show that a site on the promoter region of the serotonin transporter gene interacts with

individual (Manuck et al., 2004) and unadjusted community SES (i.e. measured by Census data; Manuck et al., 2005) in predicting central nervous system serotonergic responsivity. While Moffitt et al. argue against using a distal environmental pathogen, then, there is certainly precedence in the literature that both community and individual SES interact with genes in the prediction of biological traits.

A final point to consider regarding interpretation of the gene by environment interaction findings in this study includes the fact that the environmental pathogen we used, i.e. SES, is actually partly heritable. That is, Rowe, Vesterdal, & Rodgers report, for instance, that individual difference in IQ, income, and education are all significantly heritable and that a common genetic factor accounts for 59% of the covariation between IQ and income and 68% of the covariation between IQ and education in one large population study in the US (1999). It is likely, then, that SES is not solely environmental (i.e. determined by the political, social, and economic structures of a society). Rather, the attainment of a particular SES likely results from a complex interaction of personal characteristics, such as intelligence and certain personality variables, as well as societal-level variables. In this way, the interactions we tested here may actually be tests of gene by gene interactions as well as gene by environment interactions. Nonetheless, believing our null findings to be true is challenging given both the power limitations in the stratified analyses, and the possibility that our distal measures of SES may have dampened the likelihood of detecting a gene by environment interaction.

4.2.2 Summary of Genetic Influences on IL-6 and CRP

In sum, analyses examining the relationship between IL-6 174 G>C polymorphism and circulating IL-6 and CRP were conducted by three different genotype groupings. Only one

significant main effect was found, showing Caucasian subjects carrying any C allele to have higher levels of CRP than GG homozygotes. This effect was found in models adjusting for age, gender, and SES (i.e. individual or community). When parallel regressions including health behaviors, age and sex on step 1, SES (i.e. either individual or community) on step 2, genotype on step 3, and genotype by SES on step 4 were executed, the association between genotype and CRP was attenuated in both the individual and community SES models ($p = .07$ and $p = .05$, respectively).

These findings contribute to an existing literature of conflicting data regarding the prediction of circulating IL-6 and CRP based on the -174 G>C polymorphism. Of note, our power to detect a significant genetic main effect in this study was limited, particularly in African-Americans (i.e. 0.29 in blacks and 0.72 in whites.) Further complicating our interpretation of these largely null effects is the mild deviation of our genotype's distribution from HWE, which is likely due to chance, but could also be due to a number of other factors including errors in genotyping. A more likely explanation for both our null effects as well as the conflicting literature on this polymorphism is the reality that both IL-6 and CRP are determined by more than one site on the IL-6 gene, and indeed may be influenced by multiple genes. Finally, none of our gene by environment interactions were significant, which suggests that the measure we used to reflect SES did not interact with -174 G>C in predicting circulating IL-6 or CRP levels. However, more proximal measures of SES, such as the neighborhood problem index, or indices of chronic stress or depression may have elicited different results.

4.3 LIMITATIONS

There are a number of limitations to the current study. First, its cross-sectional design precludes causal interpretation. Alternative explanations for our results showing community SES to be associated with inflammatory markers even after adjustment for demographics and health behaviors include the possibility that inflammatory mediators and neighborhood of residence are independently related to a third factor we did not include in analyses such as personality, cognitive ability, or general health. Another limitation is the single assessment of IL-6 and CRP. Although evidence suggests that levels of these inflammatory mediators are relatively stable over extended periods (e.g. Rao et al., 1994), a more reliable indicator of chronic interindividual variability would be derived from multiple assessments over time. Further, because we did not screen individuals for acute illness when they presented for the study, we chose to eliminate anyone with an IL-6 level greater than 10 pg/mL or CRP level greater than 10 mg/L, which may indicate an acute infection. However, it is possible that when we eliminated these subjects we also eliminated some subjects with significant chronic inflammation which could have weakened our overall findings.

Regarding genetic analyses, the only significant finding showed GG homozygotes to have lower CRP concentrations than carriers of the C allele, which is not without support in the literature (D'Aiuto et al., 2004). Obviously, this site on the IL-6 gene is presumed to influence circulating CRP through induction of increased IL-6 production. We did not find an association between this site and IL-6, however, and given the number of analyses we conducted to obtain the significant association between -174 G>C and CRP, the possibility of Type I error must be considered. Also, our choice to examine one site in the promoter region of the IL-6 gene limits our understanding of gene by environment interactions. It is possible that SES interacts with

other functional polymorphisms on the IL-6 gene to influence circulating inflammatory markers. As discussed earlier, using more proximal measures of SES may have also helped detect a gene by environment interaction. Finally, race stratified analyses reduced our overall power to detect significant genetic effects. The low number of African-American participants (n=187), in particular, reduces our confidence in interpreting the null findings (i.e. both SES-inflammatory and genetic) from this study.

4.4 SUMMARY AND FUTURE DIRECTIONS

Despite these shortcomings, our findings provide initial evidence that socio-demographic characteristics of communities are associated with markers of inflammation that are widely thought to play a role in the pathogenesis of atherosclerotic cardiovascular diseases. Furthermore, these relationships are independent of demographic characteristics, measured health practices and socioeconomic attributes of individuals, raising the possibility that inflammatory mechanisms mediate relationships between community SES and increased vulnerability to cardiovascular disease. The -174 G>C polymorphism was also unrelated to circulating IL-6, while the C allele predicted higher levels of circulating CRP. This association was partially mediated by gender and BMI.

Future studies should prospectively investigate the role of both individual and community SES on inflammation, and attempt to address other possible mechanisms that might account for this relationship, including psychological activation of hypothalamic-pituitary-adrenal (HPA) and autonomic nervous system (ANS) pathways. Studies should begin in early adulthood, before significant atherosclerotic disease develops. A focus on more proximal neighborhood

characteristics such as access to health resources (i.e. parks, healthy food), density of fast food restaurants, violence/crime, and noise, and an examination of the psychological sequelae of living in these environments (i.e. chronic stress) would help inform the complex interaction of community effects on individual psychology and health. These proximal measures of neighborhood environment might also increase the likelihood of finding gene by environment interactions. These genetic studies should also have more subjects to optimize power. Investigation of resiliency factors such as social support, social integration, and exercise would also be informative. Since cardiovascular disease, and many of its risk factors, are more prevalent in African-Americans, additional investigation of the role of inflammation in racially diverse populations is warranted (Albert & Ridker, 2004).

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