The Dissertation Committee for John Michael Proffitt certifies that this is the approved version of the following dissertation:

Genetics of Plasma Cytokine Variation in Healthy Baboons and Humans

Committee:

Jeanne H. Freeland-Graves, Supervisor

Christopher Jolly

Michelle Lane

Anthony G. Comuzzie

Shelley A. Cole

Genetics of Plasma Cytokine Variation in Healthy Baboons and Humans

by

John Michael Proffitt, B.S.; B.A.

Dissertation

Presented to the Faculty of the Graduate School of

the University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Doctor of Philosophy

The University of Texas at Austin

August 2005

Acknowledgements

"A smooth sea never made a skilled mariner" – English Proverb

I would like to thank all of my dissertation committee members for their time, input, and support. My supervisor, Dr. Freeland-Graves, has been instrumental in guiding me on my intellectual and professional path. Her tireless patience and efforts have made this project possible. Dr. Jolly and Dr. Lane have provided valuable educational resources and personal encouragement. My mentors, Dr. Comuzzie and Dr. Cole, have far exceeded any reasonable expectations I could have asked for as a student. Their intellectual, scientific, and personal assistance have sustained me, and I remain indebted to them for all of their help.

I would also like to acknowledge all the assistance I have received from my fellow graduate students and the staffs of UT Austin and SFBR. Gouwen Cai and Liz Tejero have become good friends, and their academic experience has been invaluable in my educational process. Kyle Peebles and the members of the core lab have also been continually supportive. Catherine Jett, Vicki Mattern, Dev Rai, Ram Upadhyay, Wendy Vorndam, and Jennifer Darling have all been outstanding as well. Without the administrative assistance of Elsa Villaneuva, Selina Flores, and Tricia Curry, this work would not have been possible.

Finally, I must recognize the reason why I started this endeavor in the first place – my family. I am especially grateful to my beautiful wife Courtney and the ultimate motivator – my child.

Genetics of Plasma Cytokine Variation in Healthy Baboons and Humans

Publication No._____

John Michael Proffitt, Ph.D.

The University of Texas at Austin, 2005

Supervisor: Jeanne H. Freeland-Graves

The goal of this project was to investigate the genetic regulation of plasma cytokine variation in healthy baboons and humans. The first objective was to estimate the heritabilities of plasma levels of inflammatory cytokines in healthy baboons. In Study I, levels of the inflammatory cytokines TNF-α, GM-CSF, IL-1β, and IL-6, as well as sTNFR1, and sTNFR2 were measured in 370 related baboons. All of the traits exhibited significant evidence for genetic regulation. The heritabilities ranged from 61% for TNF-α to 14% for sTNFR1. The second objective was to identify chromosomal regions governing the production of resistin in healthy baboons. In Study II, resistin levels were measured in 416 related baboons. Variation in the plasma concentration of resistin was significantly linked to chromosome 18, between the markers *D18S475* and *D18S172* on the cytoband 18q12 (LOD = 4.0). The third objective of this study was to locate quantitative trait loci (QTLs) that affect circulating levels of plasma TNF-α and IL-1β levels in healthy humans. Study III examined plasma levels of TNF-α and IL-1β in a population of Caucasian Americans from the Midwestern United States. Quantitative trait loci were identified for TNF-α and IL-1β

(LOD = 3.0 and LOD = 4.0, respectively) on chromosome 18 between markers *ATA82B02* and *D18S1371*. The fourth objective was to detect QTLs affecting plasma levels of CRP, and to investigate its association with obesity phenotypes in healthy humans. Study IV revealed that chromosome 12 harbors a QTL regulating circulating CRP levels between markers *D12S375* and *D12S1052* (LOD = 4.1). CRP was genetically correlated with parameters of adiposity. The results from these baboon and human studies suggest that circulating cytokine levels in healthy animals are under significant genetic control. Chromosomes 12 and 18 appear to contain genetic differences that influence inflammation. Future work should aim to resolve the specific genetic elements through fine mapping and the positional candidate gene approach.

Table of Contents

List of Tables

List of Figures

Chapter 1

Review of Literature

1.1 Overview

In recent years, the endocrine function of adipose tissue has been shown to be a prominent component in obesity-related, metabolic abnormalities such as cardiovascular disease and type 2 diabetes (Frayn et al., 2005;Gimeno and Klaman, 2005). Adipose tissue-derived cytokines provide local and systemic metabolic feedback that directs the trafficking of nutrients throughout the body (Staiger and Haring, 2005). In obesity, this complex signaling network is disregulated, leading to the elevation of free fatty acids, cholesterol, triglycerides, and glucose in the circulation. The resultant change in metabolism increases the risk for hypertension, atherosclerosis, and insulin resistance. This cluster of diseases is known as the metabolic syndrome (Roman and Hancu, 2004;Reaven, 1987). The changes in blood chemistry profile that are associated with obesity are the same seen during the activation of the innate immune system (Pickup and Crook, 1998). This observation has led to the discovery that inflammatory cytokines are involved intimately in the endocrine signaling and nutrient trafficking of adipose tissue (You et al., 2005;Valyasevi et al., 2001;Trayhurn and Wood, 2004;Ruan et al., 2002).

The primary challenge for studying a complex disorder such as obesity is the assessment of the relative contributions of genetics and environment. In the hunt for genes that influence risk factors for disease, statistical methods have been developed to

allow for the partitioning of variance that is observed in any measurable trait into its genetic and environmental components (Blangero et al., 2001). These statistical methods rely on access to a large group of related subjects that have been genotyped for genetic differences. In this research, inflammatory markers will be monitored in the plasma of baboon and human populations. Initially, heritabilities will be estimated to evaluate the additive effects of genes. Genome scans will be conducted to identify specific chromosomal regions that influence these traits. Subsequently, bivariate quantitative genetic analyses will be performed to examine the extent of pleiotropy among the cytokines, and between cytokines and obesity phenotypes.

The overall goal of this project is to identify chromosomal regions that influence plasma cytokine variation in healthy baboons and humans. Future characterization of the differences in candidate genes will advance our understanding of the genetic contributions to the pathology of obesity-related disorders. Ultimately, these finding may have therapeutic implications for metabolic syndrome, as well as disorders that are influenced by cytokine phenotypes, such as rheumatoid arthritis, multiple sclerosis, and Crohn's disease.

1.2 Objectives

The objectives of this project are:

1) To estimate the heritabilities of plasma levels of inflammatory cytokines in healthy baboons

Hypothesis: Significant additive genetic effects contribute to the variation in plasma cytokine protein levels in healthy baboons.

2) To identify chromosomal regions governing the production of resistin in healthy baboons

   Hypothesis: Specific chromosomal regions regulate the expression of resistin.

3) To locate quantitative trait loci (QTLs) that affect circulating levels of plasma TNF-α and IL-1β levels in healthy humans

   Hypothesis: Genetic effects influence plasma TNF-α and IL-1β levels in humans.

4) To detect QTLs affecting plasma levels of CRP, and to investigate its association with obesity phenotypes in healthy humans

   Hypothesis: Significant genetic factors influence variation in plasma CRP production in humans.

1.3 Background

Obesity has emerged in the 20th century as one of the foremost health problems in the world. Sixty-five percent of adult Americans are classified as overweight or obese, and an excess of $90 billion is spent annually on healthcare related directly to obesity in America (Finkelstein et al., 2005). This chronic disease has a strong genetic component, and the metabolic disorders that frequently accompany it also demonstrate significant heritability (Bouchard et al., 1991). Insulin resistance, dyslipidemia, hypertension, and atherosclerosis are a constellation of co-morbidities associated with obesity that are

known as the metabolic syndrome (Reaven, 1987). Another component of obesity that could contribute to metabolic syndrome is the chronic activation of the innate immune system.

The initiation of the immune system by infection or injury elicits a myriad of metabolic cascades designed to thwart potential insults. The inflammatory cytokines, interleukin 1-beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α), are secreted by the innate immune system as a first step in conveying information about immunologic activity to other physiological systems. The resultant message is known as the "acute phase response." This reaction produces a systemic change in metabolism that favors increased nutrient availability for immune function, protection from free radical damage, and eventual tissue repair (Grimble, 1995). The acute phase response has survival value when activated as a potent, short-term change to restore homeostasis. In obesity, a chronic low-level activation of the innate immune system occurs, and instead of protection, produces metabolic complications.

Measures of adiposity are related directly to systemic inflammatory signals, but the mechanism underlying this relationship remains to be elucidated. There is growing evidence of a causal link between adipose tissue in obesity and the production of pro-inflammatory cytokines (You et al., 2005;Trayhurn and Wood, 2004;Gimeno and Klaman, 2005;Berg and Scherer, 2005). Because expansion of the size and number of adipocytes are the key characteristics of obesity, several research groups have investigated how these metabolic changes might induce inflammation.

One hypothesis proposed by Trayhurn and Wood (2005;2004) is that adipocyte cellular expansion by lipid accumulation in adipose tissue may restrict the nutrient access of adipocytes distant from the vasculature and induce local hypoxia. The transcription factor hypoxia-inducible factor-1 (HIF-1) is produced in adipocytes, and is known to regulate the expression of the potent adipokine leptin (Ambrosini et al., 2002;Hausman and Richardson, 2004). HIF-1 also has been shown to induce atherogenic factors in cultured mouse adipocytes (Lolmede et al., 2003). In addition, hypoxia may facilitate the endoplasmic reticulum (ER) stress observed in overweight animals. Obesity generates demand on the ER of all tissues, but particularly adipose tissue (Harding and Ron, 2002;Mori, 2000;Ozcan et al., 2004). The mechanical remodelling of adipocytes to accommodate lipid accumulation severely alters the tissue architecture and nutrient flux, and the ER strains to satisfy the cellular demands. The ER stress leads to activation of the JNK and NF-κB signalling pathways known to initiate cytokine expression (Hung et al., 2004;Srinivasan et al., 2005).

Oxidative stress may be another source of adipose tissue-derived inflammation (Furukawa et al., 2004). Increased glucose uptake by adipocytes and endothelial cells causes excess ROS production in mitochondria (Lin et al., 2005;Olivares-Corichi et al., 2005). This incursion inflicts oxidative damage inside these cells and mediates the inflammatory cascade.

Obesity-mediated adipocyte changes may initiate local inflammation in adipose tissue, but the processes that perpetrate systemic immune effects remain unresolved.

Adipose tissue is postulated to contribute to the production of plasma cytokines. Initially, the assumption was that adipocytes were the primary source of these proteins. Recent discoveries by Xu et al. (2003) and Weisberg et al. (2003) have challenged this idea by demonstrating that macrophages infiltrate adipose tissue. The accumulation of macrophages in adipose tissue was observed to be directly proportional to anthropometric measures of adiposity in both mice and humans. These potent immune cells are solicited to restore homeostasis to stressed adipocytes. It appears that macrophages recruited from sources outside the adipose tissue ingress and secrete the majority of the cytokines (Weisberg et al., 2003).

The progressive inflammatory processes instigated by obesity are likely the product of intricate interactions between adipocytes, their surrounding cell types in adipose tissue, and immune cells recuited from the periphery. This complexity presents a challenge for identifying appropriate traits that capture information about obesity-induced inflammation. Plasma cytokine levels in healthy organisms appear to effectively represent this phenomenon (Bullo et al., 2003;Ghanim et al., 2004;Hauner, 2005;Xydakis et al., 2004). Macrophages resident in adipose tissue produce cytokines that leak into the systemic circulation (Hauner, 2005). This spillover is proportional to the inflammatory state of adipose tissue, as the number of macrophages producing cytokines represent the cumulative stress in all fat depots (Fantuzzi, 2005). Circulating concentrations of these inflammatory signals effectively approximate the degree of inflammation in adipose

tissue of healthy animals because other tissues do not secrete these proteins in the absence of injury or infection.

The persistant elevation of cytokines that affects the pathogenesis and progression of chronic disease states differs from the acute inflammatory processes that serve protective functions in response to injury (Grimble, 1998;Trayhurn and Wood, 2004). Presumably, long-term levels of these markers in otherwise healthy individuals are the product of environmental factors acting on a genetic background. The question of whether genetic differences can predispose some individuals to obesity-mediated inflammation remains unanswered. In an attempt to evaluate how genes contribute to variation in plasma levels of these proteins, this research will examine these cytokines in extended families of baboons.

The baboon colony maintained at the Southwest Foundation for Biomedical Research in San Antonio, Texas has a pedigreed population of animals that have been genotyped for 330 polymorphic microsatellite markers used to establish IBD relationships. The baboon has been established previously as a primate model of inflammation (Kinasewitz et al., 2000;Kruithof et al., 1997;Munro et al., 1989). Gene sequence homology between baboons and humans is high, and long life spans of these animals can accurately reflect metabolic changes that accompany long-term disease states (Comuzzie et al., 2003). The baboons offer the additional benefit of minimal environmental effects, because diet, housing, and animal healthcare are strictly controlled.

A variance components approach will be employed to decompose the variation in these quantitative traits into portions attributable to the additive effects of genes and nongenetic causes. The proportion of the total variance influenced by genetic causes is referred to as the heritability (Falconer, 1989). Objective 1 in this study is to estimate the heritabilities of plasma levels of inflammatory cytokines in healthy baboons.

Resistin is a cytokine originally described in the rodent model as an adipocyte-derived protein linking obesity and insulin resistance. In humans, however, most of the circulating resistin protein is derived from peripheral mononuclear leukocytes and bone marrow cells (Patel et al., 2003). Circulating resistin levels are associated with the acute phase protein CRP and the pro-inflammatory cytokines TNF-α and IL-6, suggesting a role for resistin signaling in the inflammatory cascade (Bokarewa et al., 2005;Lehrke et al., 2004). Current research linking inflammation with obesity-related comorbidities makes resistin an attractive candidate for identifying disease risk. Objective 2 in this study is to identify chromosomal regions governing the production of resistin in healthy baboons.

To date, the genetic studies related to immune activity have been focused primarily on the transcriptional regulation of inflammatory genes. This candidate gene approach places emphasis on the specific regulatory elements in the promoter regions of cytokine genes, with the assumption that particular polymorphisms control the outcome of cytokine phenotypes. Association studies of the TNF-α gene are a classic example of this principle (Abraham and Kroeger, 1999;Heesen et al., 2003;Rodriguez-Perez et al.,

2005;Saarela et al., 2005). Several have identified promoter polymorphisms in the TNF gene associated with TNF-α expression in animal models of autoimmunity, or by artificial LPS stimulation (Abraham and Kroeger, 1999;Heesen et al., 2003;Saarela et al., 2005). Confusion often arises from these findings because the genetic effects identified are specific to the affliction or environment that created the variation, but these may be portrayed as variants with functional relevance for all of a cytokine's activities. However, it is inappropriate to extrapolate the relevance of polymorphisms from one circumstance to metabolic conditions unrelated to those findings. For example, the identification of a functional polymorphism relevant to endotoxin stimulation is not expected to serve any significant contribution to the inflammation associated with a progressive disease such as obesity. This distinction does not exclude the possibility that an inflammatory marker such TNF-α could play a role in both biological outcomes, but genetic differences controlling susceptibility would likely be unique for these two phenomena.

Plasma cytokine levels in healthy individuals represent a distinct set of traits largely unexamined by researchers in the field of immune genetics. As outlined above, this group of phenotypes represent inflammatory processes that are influenced by adipose tissue metabolism and confer risk for obesity-related metabolic abnormalities. This class of inflammatory markers reflect complex physiologic processes unlikely to be governed by single genes. The pro-inflammatory cytokines TNF-α and IL-1β are the two sentinels of this innate inflammatory process, and represent important risk factors indicative of

obesity-related inflammation. In this study, genome wide searches will be performed to identify chromosomal regions that influence these cytokine phenotypes.

The genome scan approach identifies genetic contributions in a manner far superior to the traditional candidate gene approach because no *a priori* assumptions are made about the relevance of particular genes. The basis of the genome scan is the concept of relatives sharing alleles identical by descent (IBD) (Amos, 1994). A linkage between gene alleles and a quantitative phenotype is established when IBD sharing segregates with variance in the trait among family members. This study will attempt to identify quantitative trait loci (QTLs) in a group of Caucasian American families recruited through Taking Off Pounds Sensibly (TOPS), a commericial weight loss program. A panel of 387 short tandem repeat polymorphisms were genotyped in this population, yielding an average map density of 10 centiMorgans (cM). This study will be the first to conduct genome scans for TNF-α and IL-1β. Therefore, Objective 3 in this project is to locate quantitiative trait loci (QTLs) that affect circulating levels of TNF-α and IL-1β in healthy humans.

In addition to the major pro-inflammatory cytokines such as TNF-α and Il-1β, acute phase proteins also are implicated in mitigating the deleterious effects associated with chronic inflammation. One such example is C-reactive protein (CRP), which has emerged as a consistent correlate of cardiovascular disease (Anand et al., 2004;Pannacciulli et al., 2001;Mazer and Rabbani, 2004).

C-reactive protein is a pentameric molecule that belongs to the pentraxin protein family. The CRP gene promoter region contains sequences that bind the cytokine-

responsive transcription factors STAT-3 and CCAAT-enhancer-binding-protein (C/EBP) beta. The pro-inflammatory cytokines interleukin-6 (IL-6) and interleukin -1 beta (IL-1 β) are the traditional activators of CRP production in the liver. As an acute phase reactant, the primary role for CRP is in recognition of bacterial membrane lipids and damaged host tissue, resulting in the subsequent complement of these cell types with macrophages for destruction.

Obesity is a common comorbidity of cardiovascular disease, and recent evidence suggests that it may mitigate its effects through a chronic inflammatory process (Hak et al., 1999). Central adipose tissue distribution is associated with increased CRP levels and cardiovascular complications (Saijo et al., 2004;Pannacciulli et al., 2001). CRP expression also is related to serum leptin levels, suggesting an overlap in the metabolic pathways that regulate these traits (Shamsuzzaman et al., 2004b). Adipose tissue in obese individuals also is a significant source of the pro-inflammatory cytokine interleukin 6 (IL-6) (Flower et al., 2003). IL-6 is the major signal regulating the release of CRP and other acute phase proteins from the liver and may link circulating CRP with adiposity.

Nutrient and pathogen-sensing systems have been highly conserved in organisms. As a result, metabolic and immune pathways also evolved to be closely linked and interdependent as a variety of cytokines, transcription factors and bioactive lipids function in both capacities. A compelling example of this overlapping biology is seen in the functions of macrophages and adipocytes. Their gene expression is highly similar. For example, macrophages express many traditional adipocyte gene products such as FABP aP2

and PPAR γ (Makowski et al., 2001;Tontonoz et al., 1998). Adipocytes in turn, produce immune proteins such as TNF-α, IL-6, and their respective receptors (Hotamisligil et al., 1993;You et al., 2005).

The functional capabilities of macrophages and adipocytes also overlap. Macrophages can phagocytosize and accumulate lipids in the process of becoming atherosclerotic foam cells (Gerrity, 1981). Preadipocytes can exhibit phagocytic properties under certain conditions and may even be able to differentiate into macrophages when given the proper stimuli (Charriere et al., 2003;Cousin et al., 2001). Furthermore, macrophages co-localize with adipocytes in the adipose tissue of obese animals.

The integration between macrophages and adipocytes makes sense in terms of immune activity, as both participate in the innate immune response. Both signal in paracrine fashion to relay information about local stress. Given this close physiologic coordination, it is likely that genes influencing adipose tissue physiology also may influence cytokine expression from immune cells such as macrophages.

This study will also examine the overlap in the genetic regulation between CRP and parameters of adiposity. The univariate quantitative genetic methods used to generate heritability estimates are readily extendable to multivariate traits (Lange and Boehnke, 1983). Bivariate variance-component analysis methods can be implemented to estimate the genetic, environmental, and phenotypic correlations between phenotypes collected. The phenotypic covariance is modeled so that the covariation between two individuals for two traits is given by a 2 × 2 covariance matrix. The resultant genetic correlation is an estimate

of the proportion of shared genes common to both traits (Lange and Boehnke, 1983). Objective 4 of this study is to detect QTLs affecting plasma levels of CRP, and to investigate its association with obesity phenotypes in healthy humans.

In summary, the overall goal of this project is to determine whether plasma cytokines in healthy baboons and humans are under genetic control. Quantitative trait linkage analysis will be employed to identify specific chromosomal regions influencing variation in these inflammatory protein levels. This study also will examine whether cytokines share genetic effects with other cytokines and parameters of adiposity.

Chapter 2

Genetics of Plasma Cytokine Variation in a Healthy Baboon Population

2.1 Abstract

Circulating levels of pro-inflammatory cytokines are the newest biomarkers used to assess and predict chronic disease outcomes. Previously, these levels in healthy organisms were assumed to be quiescent because expression of these phenotypes during host stress were orders of magnitude greater than in the unaffected state. High-sensitivity immunoassays are now available to detect variation in these cytokine measures in the absence of injury or infection, creating an avenue for studying these traits in novel ways. In this study, 370 healthy baboons from the pedigreed colony at the Southwest Foundation for Biomedical Research in San Antonio, TX, were assayed for circulating pro-inflammatory markers. Investigation of plasma cytokines in a population of related animals permits the evaluation of environmental and genetic contributions to their variation. A maximum likelihood-based, variance decomposition approach, implemented in the program SOLAR, was used to conduct quantitative genetic analyses on these immunity signals. All the cytokines tested demonstrated significant heritabilities. Bivariate genetic analyses revealed overlap in the genetic effects that control variation in these protein levels. This study revealed that inflammatory markers are under genetic control in the absence of injury or infection, and that a common set of genes influence these traits.

2.2 Introduction

Immunologists have long neglected components of the innate immune system as arcane, evolutionary remnants of a complex immune response (Pickup and Crook, 1998). These powerful cytokines were once viewed as non-specific, stopgap measures antithetical to the precision of activated immunity - a club versus scalpel approach to host defense. In recent years, pro-inflammatory signals have been implicated as a component of several chronic metabolic diseases (Binder et al., 2002;Festa et al., 2002;Libby, 2002;Pradhan et al., 2001). A sustained, low-grade inflammatory state is now firmly associated with obesity, insulin resistance, atherogenesis, and non-alcoholic fatty liver disease (Ghanim et al., 2004;Koruk et al., 2003;Lim et al., 2005).

The research establishing links between inflammation and metabolic dysfunction has employed the use of several biomarker assays, but the most predominant and consistent correlate of these conditions has been plasma cytokine levels (Binder et al., 2002;Festa et al., 2002). High-sensitivity immunoassays are now available to detect variation in these circulating proteins in the absence of injury or infection, creating a previously unavailable avenue for studying these traits. As a result, systemic, circulating levels of the cytokines in healthy individuals can be used as viable risk factors to predict chronic disease outcomes.

The question of whether genetic differences can predispose some individuals to chronic inflammation remains unanswered. The ability to assay plasma cytokines accurately now presents a novel set of quantitative phenotypes to estimate the effects of

genes on these traits. This study will establish the normal variation of plasma cytokines in a population of healthy baboons.

The baboon is a long-standing primate model of inflammation (Kinasewitz et al., 2000;Munro et al., 1989;Kruithof et al., 1997). The protein and gene sequence homology between baboons and humans is high, and the long life spans of these animals effectively reflect the age-related metabolic changes that accompany chronic disease states in humans (Comuzzie et al., 2003). Controlled diet, housing, and animal healthcare minimize random environmental effects and undiagnosed diseases common in human study groups. The pedigreed colony of baboons at the Southwest Foundation for Biomedical Research (SFBR) in San Antonio, TX are a unique resource for examining the genetic contributions to quantitative phenotypes. This population provides an excellent opportunity to examine hereditary components influencing variation in plasma cytokine levels. The goal of this paper is to establish the normal range of cytokine variation in a captive baboon population, and to estimate the environmental and genetic contributions to the variations in these phenotypes.

2.3 Methods

Subjects

The study group was comprised of 370 pedigreed baboons from two subspecies, *Papio hamadryas anubis* and *Papio hamadryas cynocephalus*. The distribution of relative pairs present in the study population is listed in Table 2.1. The baboons were fed ad

libitum on a standard diet (Harlan Tecklad 15% Monkey Diet, 8715, Indianapolis, IA), and housed in open-air group cages. Members of the SFBR veterinary staff performed all animal handling, and animals with injury or illness were excluded from the study.

Sampling

Blood samples were taken once from each animal after an overnight fast from the anitcubital vein under ketamine sedation. Weight was measured on a calibrated electronic scale (GSE, Chicago, IL). The 20 mL sample of blood was divided as follows: 8 mL in cell preparation tubes (BD vacutainer CPT mononuclear cell preparation, BD Biosciences, San Jose CA) containing a density gradient polymer gel and sodium citrate (Becton Dickinson, Franklin Lakes, NJ) for monocyte isolation; 4 mL in sodium fluoride tubes for glucose analysis; and 7 mL in an EDTA tube for plasma cytokine assays. All samples were centrifuged for 15 minutes at 2000 *g*. The resultant plasma from the tubes was aliquoted and stored at -80° C for subsequent analysis.

Cytokine Assays

Plasma measures of GM-CSF, IL-1β, IL-6, and TNF-α were assayed in a high throughput multiplex system using bioluminescence on the Luminex 100 platform (Linco Research, Inc., St. Charles, MO). Commercially available immunoassay kits developed from human antibodies by Boehringer Mannheim, Inc. (Ingelheim, Germany) were used to measure soluble TNFR1 and soluble TNFR2 in the collected plasma. All samples were

analyzed in duplicate and compared with standard curves. Replicates with variations greater than 5% were rerun.

Genotypes

Animals participating in this study were from a pedigreed population of baboons at SFBR with previously characterized genotyped markers. The genotyping protocol and details regarding the baboon linkage map have been described in detail elsewhere (Rogers et al., 2000). Briefly, human PCR primers amplified homologous, short tandem repeats in baboon DNA. A total of 330 markers were genotyped by gel electrophoresis on ABI platforms (Applied Biosystems, Foster City, CA), yielding a genome wide map density of 7.2 cM. The computer software PEDSYS managed the pedigree and genotype information.

Statistical Methods

A maximum likelihood-based, variance decomposition approach was utilized to conduct the genetic analyses (Almasy and Blangero, 1998). For a quantitative trait, the phenotypic variance is segregated into its additive genetic and environmental components. The proportion of the additive genetic effects that contribute to the total phenotypic variance is defined as the heritability (h^2) (Falconer, 1989). A likelihood ratio test is employed to compare a model in which the h^2 is estimated against a model where h^2 is constrained to zero. Twice the difference of these log likelihoods is asymptotically

distributed as a 1/2:1/2 mixture of a chi-squared distribution, with a single degree of freedom and a point mass at zero (Self and Liang, 1987).

The bivariate genetic analyses are an extension of the univariate method (Almasy et al., 1997). The bivariate phenotype is a function of the baboon's phenotypic values, population means, additive genetic values, and environmental effects (Lange and Boehnke, 1983). Genetic and environmental variance-covariance matrices are calculated and genetic (ρ_G) and environmental (ρ_E) correlations are estimated from this model. The phenotypic correlation (ρ_P) between two traits, accounting for the relatedness of the individuals, can be expressed as a function of their underlying genetic and environmental correlations using the equation:

$$\rho_P = \rho_G \sqrt{h_1^2} \sqrt{h_2^2} + \rho_E \sqrt{(1- h_1^2)} \sqrt{(1- h_2^2)},$$

where h^2_1 and h^2_2 correspond to the heritability values of the respective traits.

Both univariate and bivariate genetic analyses were conducted in the computer package called Sequential Oligogenic Linkage Analysis Routines (SOLAR) (Almasy and Blangero, 1998). Age, sex, age squared, and their interactions were included in all of the genetic analyses as covariates. A Student's independent *t* test was used to compare group means of male and female baboons.

2.4 Results

The descriptive statistics of the inflammatory cytokines, segregated by sex, are displayed in Table 2.2. Baboons exhibit strong sexual dimorphism, as reflected by

previously reported differences in weight and length (Comuzzie et al., 2003). Disparity in the inflammatory profile between the sexes was observed, with males having higher IL-1β, IL-6, and TNF-α levels than females.

Table 2.3 displays the heritabilities and genome-wide scan results of the plasma cytokines in baboons. TNF-α had the highest h^2 of 61%; TNFR1, the lowest at 14%. All of the cytokines demonstrated significant heritabilities, with p-values < 0.05. Genome wide scans for these traits revealed that the baboon chromosome regions equivalent to human chromosomes 15 and 22 may harbor genetic variations that influence inflammation. The maximum LOD scores for TNF-α and IL-1β co-localized to a region homologous to human chromosome 22. LOD scores for GM-CSF, IL-6 and sTNFR1 clustered on chromosome 15. The LOD score of 2.0 for IL-1β on chromosome 22 met the criteria for a signal suggestive of linkage (Morton, 1955).

The genetic (ρ_G) and phenotypic (ρ_P) correlations among the plasma cytokines in baboons are found in Table 2.4. A strong positive genetic correlation was observed between IL-1 β and TNF-α. The tests for complete pleiotropy were significant for IL-6 with both IL-1β and TNF-α. Plasma TNFR2 levels were negatively genetically related to both IL-1 β and TNF-α. Significant positive phenotypic correlations were observed between IL-1 β and TNF-α, and TNF-α and IL-6.

2.5 Discussion

This study has firmly established a genetic component to the variation in the inflammatory cytokines of healthy baboons, suggesting a genetic predisposition to elevated inflammation. To our knowledge, these data are the first to estimate heritabilities for IL-1β, GM-CSF, sTNFR1, and sTNFR2 in any species. The availability of accurate, high throughput immunoassay systems has created the opportunity to investigate a previously intangible category of quantitative inflammatory phenotypes. The use of plasma cytokines in healthy animals is ideal for monitoring inflammatory status associated with long-term, metabolic dysfunction.

The bivariate quantitative genetic analyses revealed that many innate immunity biomarkers are under common genetic control. IL-1β, TNF-α, and IL-6 are particular examples of this overlap. As part of the acute phase response, IL-1β and TNF-α function as the primary signaling molecules that initiate the cytokine signaling cascade (Grimble, 1998). The strong genetic and phenotypic correlations between these two proteins found in the present study substantiate this relationship and imply a close coevolution of their respective signaling pathways.

IL-1β and TNF-α initiate the expression of IL-6 in mononuclear cells through a p38 MAPK-dependent mechanism (Pickup and Crook, 1998). Since these cytokines signal upstream of IL-6, it is logical that genes influencing IL-1β and TNF-α also might affect the production of this inflammatory protein. The complete pleiotropy that IL-6 shares with IL-1β and TNF-α reflects the genetic hierarchy of these cytokines.

The soluble TNF-α receptors, sTNFR1 and sTNFR2, are shed from their membrane-bound state of expressing cells to serve as homeostatic regulators of TNF-α signaling (Holtmann and Neurath, 2004). The observed negative genetic correlations between sTNFR2 and TNF-α, and sTNFR2 and IL-1β reflect that shared genes are working in counter-regulatory ways to maintain the balance of inflammatory signals. The positive correlation between the two TNF-α receptors is likely due to genetic regulation governing the ectodomain shedding of these proteins (Gao et al., 2004).

Genome wide scans of these traits have revealed that chromosomes 14 and 20 may harbor genetic factors influencing inflammation. Although the current sample size lacked the statistical power to detect a major QTL, the clustering of maximum LOD scores on these two chromosomes suggest a common control as reflected in the genetic correlation calculations.

In conclusion, the baboon model successfully demonstrated that plasma cytokine levels in healthy animals are under genetic control. The genetic and phenotypic correlations that were significant in this study are consistent with the previously established mechanistic understanding of the innate immune system. For the first time, estimates of the extent of overlap in the genetic regulation of these interrelated characters have been elucidated. Future work should be conducted in humans to evaluate whether genetic effects of these traits can be estimated in populations where substantially larger environmental contributions could obfuscate these phenotypes.

Table 2.1 Distribution of Relative Pairs

Relationship	Number of Pairs
Parent-offspring	145
Siblings	219
Grandparent-grandchild	8
Avuncular	49
Half siblings	2870
Grand avuncular	769
Half avuncular	6
First cousins	55
First cousins, once removed	15
Half siblings and first cousins	129
Half siblings and half avuncular	12
Total	4277

Table 2.2 Descriptive Statistics in Baboons According to Sex

Trait	Male	N	Female	N	p-value*
Body weight (kg)	31.5 ± 4.5	130	19.5 ± 4.0	240	<0.0001
GM-CSF (pg/dL)	3.4 ± 1.2	81	2.6 ± 1.0	130	NS
IL-1β (pg/dL)	8.6 ± 2.7	86	5.2 ± 2.2	182	<0.01
TNF-α (pg/dL)	6.6 ± 3.0	100	3.9 ± 1.8	198	<0.01
IL-6 (pg/dL)	17.6 ± 7.3	114	12.6 ± 5.7	230	<0.05
sTNFR1 (pg/dL)	1.9 ± 0.5	128	2.1 ± 0.6	239	NS
sTNFR2 (pg/dL)	3.3 ± 1.0	128	3.4 ± 1.2	236	NS

*comparisons between males and females

Table 2.3 Heritabilities and Genome Wide Scans of Plasma Cytokines in Baboons

Trait	Heritability	p[†]	Max LOD[‡]	Chromosome*	Marker	Location[§]
TNF-α	0.61 ± 0.18	0.00001	1.5	20_22	D22S642	107
IL-1β	0.36 ± 0.21	0.01	2.0	20_22	D22S739	123
GM-CSF	0.52 ± 0.25	0.01	1.6	14_15	D15S114	127
IL-6	0.49 ± 0.16	0.00001	1.1	14_15	D15S79	144
sTNFR2	0.20 ± 0.17	0.05	1.4	14_15	D15S96	120
sTNFR1	0.14 ± 0.09	0.05	1.3	6	D6S17	11

[†] Significance of heritability estimate
[‡] Maximum LOD score produced by genome wide scan
* Human chromosome homolog(s) of baboon chromosome
[§] Distance (in cM) along chromosome

Table 2.4 Genetic (ρ_G) and Phenotypic (ρ_P) Correlations Among Plasma Cytokines in Baboons

Trait	ρ_G	p†	ρ_P	p‡
IL-1β correlated with:				
TNF-α	0.95	<0.01	0.78	<0.01
IL-6	1.0	<0.01	0.73	<0.01
sTNFR2	-0.83	<0.05	-0.45	<0.05
TNF-α correlated with:				
IL-6	1.0	<0.01	0.83	<0.01
sTNFR2	-0.74	<0.05	-0.51	<0.05
sTNFR1 correlated with:				
sTNFR2	0.81	<0.01	0.63	<0.05

† pleiotropy
‡ phenotypic correlation

Chapter 3

A Quantitative Trait Locus Influencing Plasma Resistin Levels in Healthy Baboons Resides on Chromosome 18

3.1 Abstract

Resistin is a cytokine originally described in the rodent model as an adipocyte-derived protein linking obesity and insulin resistance. In humans, however, most circulating resistin is from peripheral mononuclear leukocytes and bone marrow cells, suggesting a role for resistin signaling in the inflammatory cascade. Research linking inflammation with obesity-related comorbidities makes resistin an attractive candidate for identifying disease risk. The baboon was used as a non-human primate model for the study of the genetics of circulating resistin. A 10 mL blood sample drawn from 416 adult baboons from the colony at the Southwest Foundation for Biomedical Research in San Antonio, TX, was collected in fasting conditions under ketamine sedation. Serum resistin levels were assayed by bioluminescence. Maximum likelihood variance decomposition methods, implemented in the program SOLAR, were used to conduct univariate quantitative genetic analysis of this phenotype. The additive genetic heritability for resistin in this population was 71% ($p = 2.9 \times 10^{-10}$). A genome-wide scan for this trait revealed a quantitative trait locus (QTL) (LOD = 4.0) on the human homologue of chromosome 18, between the markers *D18S475* and *D18S72*. A secondary QTL (LOD = 1.5), suggestive of linkage, mapped to the homologue of human chromosome 6 near the

major histocompatability complex (MHC). These findings demonstrate that circulating resistin levels are under significant genetic regulation in the baboon, and identify specific chromosomal regions that affect the variation of this trait.

3.2 Introduction

Resistin belongs to a family of cysteine-rich proteins called "found in inflammatory zone" (FIZZ). The FIZZ family was first characterized by Holcomb in 2000 when the secreted protein product of the *FIZZ1* gene was associated with pulmonary inflammation. In 2001, Steppan et al. brought widespread attention to another FIZZ family member, resistin. This gene was identified in a screen of mRNA species differentially regulated by the transcription factor peroxisome poliferator activated receptor gamma (PPAR γ) in the 3T3-L1 murine adipocyte cell line. At the time of its discovery, resistin was purportedly an adipocyte-derived hormone linking obesity to diabetes. Its elevation in mice resulted in peripheral insulin resistance, hence, the name resistin.

Mice express resistin almost exclusively in white and brown adipose tissue (Steppan et al., 2001). In humans, however, the primary source for the homologue of this protein is found in peripheral mononuclear blood cells and bone marrow (Patel et al., 2003). When resistin was detected in human adipose tissue, the vascular-stromal fraction of cells was the source, rather than adipocytes (Janke et al., 2002). This distributional inconsistency between mice and humans has raised questions as to the relevance of this

protein in human insulin resistance and metabolism. Recent evidence has begun to suggest that resistin may, in fact, play an inflammatory role in humans (Bokarewa et al., 2005;Lehrke et al., 2004;Reilly et al., 2005).

Chronic inflammation is now linked with many metabolic dysfunctions commonly associated with obesity (Fantuzzi, 2005). A growing body of research has begun to implicate obesity-related adipocyte stress directly to the production of cytokines by immune cells recruited to adipose tissue. These pro-inflammatory markers serve protective functions when activated short-term. However, long-term exposure can result in damage (Grimble, 1996). CRP and PAI-1 are examples of two acute phase proteins whose long term elevation are known to potentiate atherosclerosis (Agirbasli, 2005;Tomiyama et al., 2005). New evidence has placed resistin in this category of cytokines. In human endothelial cells, Verma et al. demonstrated that resistin upregulates VCAM-1 and MCP-1 while downregulating the CD40 inhibitor TRAF-3 (2003). They also revealed that resistin promotes endothelial cell activation via promotion of ET-1 release. Kawanami et al. found ICAM-1 and pentraxin 3 increased in cultured human endothelial cells when resistin was added to the media (2004). The laboratory that performed the original resistin experiments in mice has now published results supporting resistin as a cardiovascular disease risk factor in humans (Reilly et al., 2005).

Significant strides have been made in the characterization of the role of resistin in the pathogenesis of metabolic diseases in mice and humans, but relatively little is known about the underlying genetic architecture that regulates this protein. Our lab group was

the first to identify a significant QTL for a resistin phenotype, mRNA levels in the omental adipose tissue of baboons (Tejero et al., 2005). This QTL localized to chromosome 19, near the structural *RSTN* gene. Circulating resistin has been associated with cardiovascular disease and it is possible that genes unique to this phenotype affect its variation and predispose individuals to increased atherosclerotic risk. In this study, the same baboon population in which the resistin mRNA QTL was identified will be used to search for novel chromosomal regions that regulate serum resistin levels.

3.3 Methods

Animals

The data for the present study were obtained from 416 randomly bred, pedigreed, adult baboons from the colony at the Southwest Foundation for Biomedical Research (SFBR) in San Antonio, TX. The baboons in this sample were comprised of 11 classes of relative pairs, and included 286 females and 130 males. Information regarding the distribution of relative pairs for this study is displayed in Table 3.1. The majority of the animals were olive baboons (*Papio anubis*), with a smaller proportion of yellow baboons (*Papio cynocephalus*), and crosses between those two subspecies. Animals were gang-housed and shared the same low-fat Chow diet (Harlan Teklad 15% Monkey Diet, 8715).

Sampling and phenotypic data

Prior to sample collection, baboons were subjected to an overnight fast and sedated with ketamine the morning of the blood draw. Body weight was measured on a calibrated electronic balance (GSC, Chicago, IL, USA) and body length was measured between head and feet, with the animals lying on their back. A 10 mL blood sample was collected from the antecubital vein. The serum was separated by centrifugation at 2000 g for 15 minutes, aliquoted, and stored at -80° C before analysis. Resistin, plasminogen activator inhibitor 1 (PAI-1), and C-reactive protein (CRP) were analyzed in multiplex by bioluminescence (Linco Research Inc., St. Louis, MO) in duplicate. Replicate measures with variance > 5% were repeated to ensure accuracy.

Multipoint linkage analysis

In this study, a variance components approach was employed to localize specific quantitative trait loci (QTLs) influencing plasma resistin levels. First, the total phenotypic variance (σ^2_p) was decomposed into genetic (σ^2_G) and environmental (σ^2_E) (nongenetic) parts. These components are additive, such that: $\sigma^2_p = \sigma^2_G + \sigma^2_E$ (Falconer, 1989). The σ^2_G can be separated further into additive genetic variance due to specific QTLs (σ^2_A) and residual genetic (non-QTL) variance. The heritability (h^2) is defined as the proportion of the total phenotypic variance explained by additive genetic effects.

A multipoint linkage method was employed to test for linkage between marker loci and plasma resistin. The concept of quantitative trait linkage is based on the genetic

covariances between family members expressed as a function of the identical by descent (IBD) relationships at a given locus. Two alleles are considered IBD when it is demonstrated that their commonality is a result of inheritance from the same ancestor. The expected IBD relationship between relative pairs is twice the kinship (Φ). Kinship is defined as the probability that two homologous genes drawn at random, one from each individual, will be IBD. IBD at a specific QTL locus is estimated using genotyped marker data.

The formal test for linkage uses a maximum likelihood approach for estimating QTL effects. The null hypothesis is that the additive genetic variance of the specific QTL (σ^2_q) for the trait equals zero. The likelihood of the null hypothesis (H_0), where σ^2_q is constrained to zero, is compared with the likelihood of the alternative hypothesis (H_a), where σ^2_q is estimated. Twice the difference between the log likelihood of the two models yields a test statistic. This test statistic is asymptotically distributed as a 1/2:1/2 mixture of a chi-square distribution with one degree of freedom (Self and Liang, 1987). The logarithm of odds (LOD) score demonstrates the significance of this test. It is calculated as follows: LOD = $\log_{10}$ (likelihood of H_a) – $\log_{10}$ (likelihood of H_0)

Locus-specific IBDs were calculated and the multipoint linkage analyses were conducted using SOLAR (Almasy and Blangero, 1998). Data greater or less than four standard deviations from the mean resistin value were blanked.

3.4 Results

The descriptive statistics of the baboons are displayed according to sex in Table 3.2. Females were older and had higher PAI-1 and CRP values than males. The additive genetic heritability for circulating resistin levels was 0.71 ± 0.14 ($P = 2 \times 10^{-10}$), which was substantially higher than the estimate for PAI-1 ($h^2 = 0.47 \pm 0.14$, $P = 3 \times 10^{-6}$). A significant genetic correlation was detected between resistin and PAI-1 levels ($\rho_G = 0.44 \pm 0.18$, $P = 0.03$). Positive phenotypic correlations, adjusted for the relatedness of individuals, were identified between resistin and PAI-1 ($\rho_P = 0.64$, $P = 3 \times 10^{-12}$), resistin and CRP ($\rho_P = 0.53$, $P = 0.02$), and PAI-1 and CRP ($\rho_P = 0.34$, $P = 0.001$).

Multipoint linkage analysis was conducted for resistin and is displayed by chromosome in Figure 3.1. The strongest signal for resistin resides on chromosome 18, with a maximum LOD score of 4.0. Figure 3.2 displays the peak located 36 cM pter, between markers *D18S475* and *D18S172*, on the cytoband 18q12. A secondary QTL (LOD = 1.5), suggestive of linkage, mapped to the homologue of human chromosome 6 near the major histocompatability complex (MHC).

3.5 Discussion

This study has identified a quantitative trait locus on chromosome 18 influencing plasma resistin levels in healthy baboons. Our finding represents the first QTL for this particular phenotype in any species. In addition, this data set is the first to yield a

significant heritability estimate for plasminogen activator inhibitor 1 (PAI-1) in baboons and establish a genetic correlation between resistin and an acute phase reactant (PAI-1).

Despite the recent explosion of research dedicated to understanding resistin physiology, relatively little is known about this protein. The identity of the resistin receptor remains a mystery, leaving questions about its signaling mechanism. As a consequence, much of the recent resistin work has focused on identifying correlates of resistin mRNA and protein production. Resistin expression in human monocytes is increased markedly by treatment with endotoxin (Lu et al., 2002), as well as pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) (Lehrke et al., 2004). In turn, recombinant resistin also upregulates cytokine and adhesion molecule production in human endothelial cells. These findings substantiate an inflammatory role for resistin (Kougias et al., 2005;Lehrke et al., 2004).

Previously, our research group was the first to identify a major quantitative trait locus influencing resistin mRNA expression in omental adipose tissue of baboons. The QTL on chromosome 19 suggests that transcriptional activation of resistin mRNA may be mediated by genetic differences in the promoter of the resistin gene. However, resistin protein levels reflect a fundamentally different phenotype than mRNA. Other processes such as translation, post-translational modification, and extracellular release also may contribute to the variation of plasma resistin levels. The QTL identified on chromosome 18 for plasma levels of resistin in this study demonstrates that distinct genetic effects control the various stages of a protein's production.

The 1-LOD support interval for this QTL is narrow (12 cM), and the maximum peak resides in a region with relatively few identifiable candidate genes. However, two potential candidates are the STAT 3 interacting protein (STATIP3) and the protein inhibitor of activated STAT 1 (PIAS2). The STAT family of transcription factors regulate the signaling of many important cytokines, such as IL-6 in the liver, leptin in adipose tissue, and insulin in skeletal muscle (Bjorbaek et al., 1997;Chen et al., 1997;Horvath, 2004). It is possible that polymorphisms in these candidate genes influence resistin levels by mediating the action of the STAT transcription factors. A secondary QTL on chromosome 6 resides near the MHC, a chromosomal region containing many genes related to host : pathogen interactions in immunity, including TNF-α. While this signal is only suggestive of linkage (LOD = 1.5), it may represent evidence for resistin regulation by genes involved in inflammatory processes.

In conclusion, this study demonstrates that substantial genetic contributions affect the expression of circulating resistin and PAI-1 levels in the plasma of healthy baboons. These two proteins are genetically correlated, suggesting overlap in the regulation and function of these inflammatory markers. The quantitative genetic analyses conducted have identified a previously unknown QTL on chromosome 18 for resistin. Future work should aim to identify positional candidate genes and polymorphisms in this region that might mediate plasma resistin production.

Table 3.1 Distribution of Relative Pairs

Relationship	Number of Pairs
Parent-offspring	125
Siblings	274
Grandparent-grandchild	3
Avuncular	64
Half siblings	2995
Half avuncular	660
First cousins	1
Half first cousins	17
Half siblings and first cousins	2
Half siblings and half first cousins	64
Half siblings and half avuncular	8
Total	4213

Table 3.2 Descriptive Statistics in Baboons According to Sex

Trait	Male	N	Female	N	p-value*
Body weight (kg)	31.5 ± 4.5	130	19.5 ± 4.0	240	<0.0001
Resistin (pg/dL)	3.4 ± 1.2	81	3.3 ± 1.0	130	NS
PAI-1 (pg/dL)	81.6 ± 29.8	86	104 ± 32.6	182	<0.01
CRP (pg/dL)	450 ± 81	100	464 ± 73	198	<0.01

*comparisons between males and females

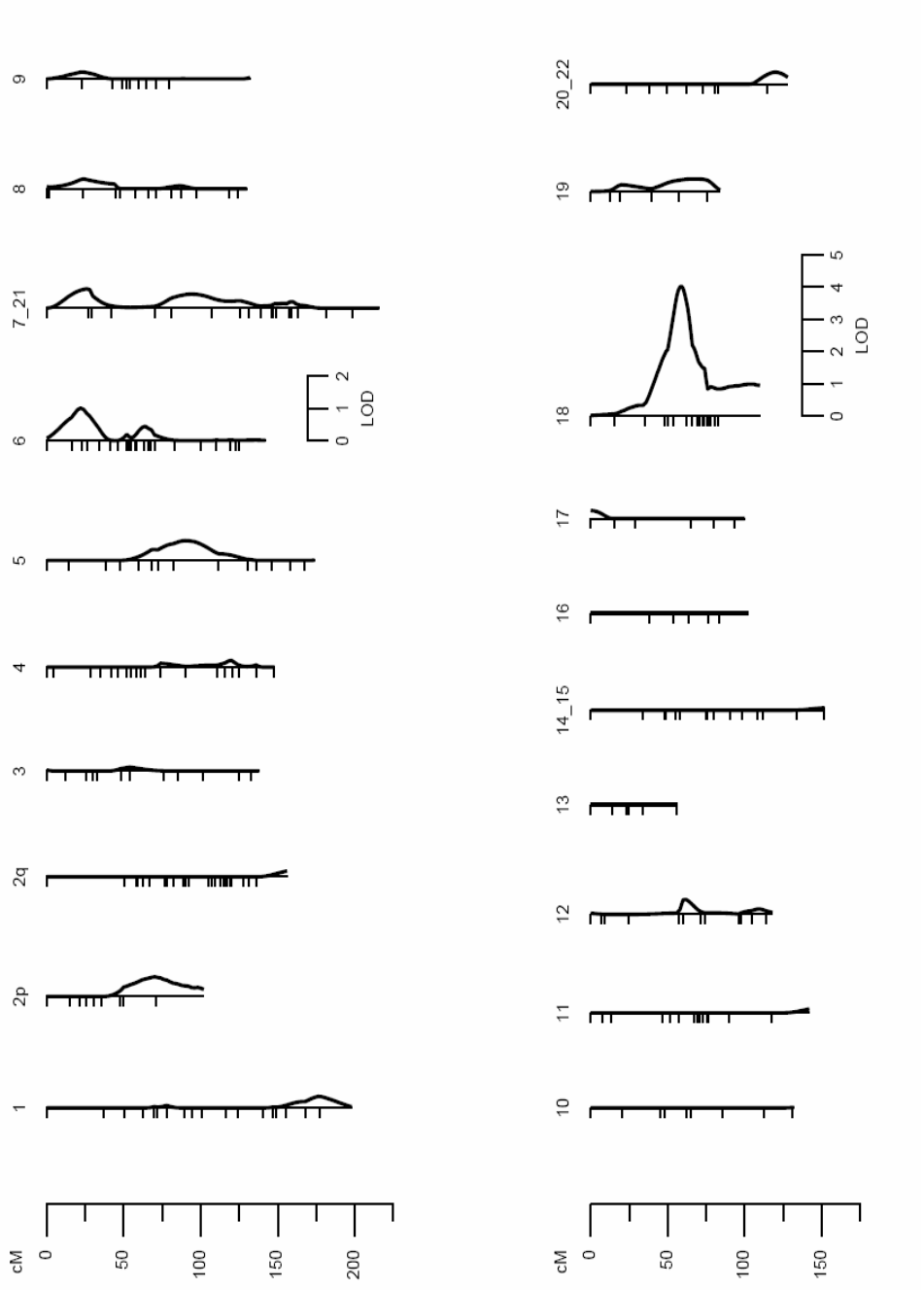

Figure 3.1: Genome-wide scan for plasma resistin, displayed by chromosome. Numeric values reflect the human homologs of the baboon chromosomes. Maximum log of odds ratio (LOD) scores are represented on the y axis and chromosomal location on the x axis. Hash marks reflect marker spacing and density.

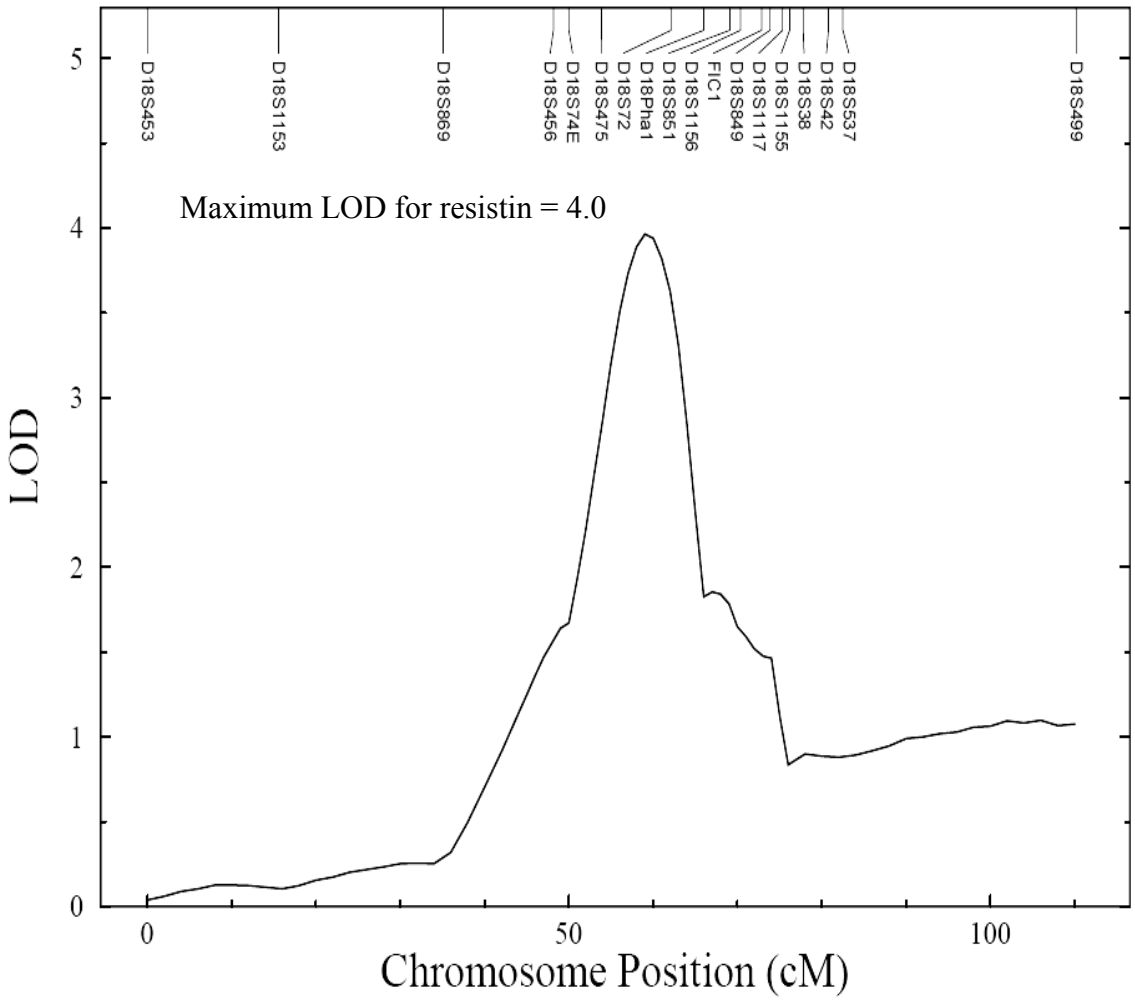

Figure 3.2: Multipoint linkage map and estimated LOD score on Chromosome 18 for plasma resistin. Maximum log of odds ratio (LOD) scores are represented on the y axis and chromosomal location on the x axis.

Chapter 4

Quantitative Trait Loci Influencing Plasma TNF-α and IL-1β in Humans Co-localize to Chromosome 18

4.1 Abstract

Pro-inflammatory cytokines are now widely recognized as a central component in the pathogenesis of obesity-related insulin resistance and atherosclerotic disease. The specific regulatory pathways governing subclinical inflammation remain a mystery. The purpose of this study was to examine whether circulating cytokines in healthy individuals are under genetic control, and to identify quantitative trait loci (QTLs) that might influence these phenotypes. Plasma levels of tumor necrosis factor - alpha (TNF-α) and interleukin -1 beta (IL-1β) were assayed in 500 healthy Caucasian adults recruited through TOPS, Inc. (Taking Off Pounds Sensibly), a commercial weight control program. Maximum likelihood variance decomposition methods, implemented in the program SOLAR, were used to conduct quantitative genetic analyses of these phenotypes. TNF-α and IL-1β levels demonstrated additive genetic heritabilities of 44% ($p = 1.0 \times 10^{-10}$) and 41% ($p = 1.4 \times 10^{-8}$), respectively. Genome-wide scans for these traits revealed significant QTLs for both phenotypes on chromosome 18. Maximum LOD scores of 3.0 for TNF-α and 4.0 for IL-β appeared between markers *ATA82B02* and *D18S1371*, approximately 28 cM pter. The concomitant location of these QTLs suggest that a common genetic component exerts an effect on these two cytokines. This study is the

first to identify significant QTLs for TNF-α and IL-1 β in healthy individuals, and demonstrates that inflammatory markers are under substantial genetic control even in a healthy state.

4.2 Introduction

The cytokines tumor necrosis factor - alpha (TNF-α) and interleukin -1 beta (IL-1β) are two powerful effector molecules of the innate immune response. TNF-α and IL-1β are produced primarily by monocytes, macrophages, and dendritic cells, and operate synergistically to enhance inflammatory responses (Staros, 2005). Pattern recognition receptors on immune cells identify common molecular features of pathogens and initiate the transcription of TNF-α and IL-1β through activation of the NF-κB transcription factor (Cao et al., 1999). These multifunctional proteins elicit numerous systemic functions, which include the hypothalamic induction of fever and sleep, activation of neutrophils, extravasation of leukocytes to damaged tissues, and an IL-6 - mediated release of acute phase proteins from the liver (Faggioni et al., 1995;Lampinen et al., 2004;Streetz et al., 2001). TNF-α and IL-1β also potentiate significant regulatory effects on nutrient trafficking, by increasing blood glucose, free fatty acids, and LDL cholesterol in the circulation (Chu et al., 2001;Grimble, 1998;Sethi and Hotamisligil, 1999).

Traditionally, the functional capabilities of TNF-α and IL-1β were thought to be limited to the brief, temporal periods when defense cells secreted high concentrations of these cytokines in response to foreign insults. Recent findings have begun to identify

novel signaling roles for these proteins in the absence of acute stress events. Chronic inflammation is now receiving attention as a separate category of immune activity, where long-term exposure of host tissues to lower levels of cytokines and chemokines exerts deleterious effects on metabolism (Grimble, 1996;Pickup and Crook, 1998;Sethi and Hotamisligil, 1999). Persistant elevation of these proteins now constitutes a novel set of risk factors that predict and exacerbate metabolic diseases such as type-2 diabetes, cardiovascular disease, and nonalcoholic steatohepatitis (Binder et al., 2002;Crespo et al., 2001;Hansson, 2005;Koruk et al., 2003).

Obesity is associated with these metabolic abnormalities that are affected by inflammatory pathways. Evidence now suggests that obesity-related changes in adipose tissue may initiate systemic inflammatory effects that exacerbate chronic diseases. Markers such as TNF-α and IL-1β track with anthropometric estimates of fatness (Maachi et al., 2004;Lim et al., 2005). Pro-inflammatory cytokines increase with prolonged weight gain and decrease with diet and exercise-mediated weight loss (Clement et al., 2004). Studies characterizing transcriptional regulation of genes in adipose tissue of obese animals have demonstrated that pro-inflammatory and stress-related genes are among the most differentially controlled mRNA species (Xu et al., 2003). It is possible that the metabolism accommodating the deposition of lipids in adipocytes creates stress on these cells and the surrounding tissue. This process could recruit immune cells to mitigate damage and, in turn, release cytokines that contribute to the systemic circulation.

Subclinical inflammation defines a group of cytokine phenotypes that confer risk for chronic metabolic abnormalities. We are interested in investigating the genetic regulation of two instrumental inflammatory molecules, TNF-α and IL-1β, in a healthy human population. Genome-wide scans were performed to identify chromosomal regions affecting the variation in these quantitative traits. This study also estimated the shared genetic effects of these cytokines with parameters of adiposity to determine the extent to which genes regulating obesity might influence low-grade inflammation.

4.3 Methods

Subjects

The study population was a subset of Caucasian individuals participating in the Medical Risks and Complications of Obesity Genes Project (Kissebah et al., 2000). The 700 individuals (384 women, 316 men) from 167 families in these genetic analyses were among individuals recruited through Taking Off Pounds Sensibly (TOPS, Inc.), a commercial weight control program. Study ascertaiment was based on recruiting families with at least two obese sibs (body mass index (BMI) > 30), availability of one (preferably, both) parent, and at least one never-obese sib and/or parent (BMI < 27). The list of relative pairs represented in the current analyses is displayed in Table 4.1. The Medical College of Wisconsin Institutional Review Board approved all protocols, and all subjects provided informed, written consent to participate in this study.

Phenotypes

Plasma samples drawn after a 12-hour overnight fast were used to measure circulating levels of TNF-α and IL-1β. These cytokines were assayed by bioluminescence on the Luminex 100 platform (Linco Research, Inc., St. Louis, MO). All assays were performed in duplicate, and the intra-and inter-assay coefficients of variation were less than 5%. Due to kurtotic distribution of these traits, outliers (± 4 SD) were removed, and the values were log transformed prior to genetic analyses.

Genotypes

DNA was obtained from whole blood of consenting family members using a nonphenol-based, commercially available kit (Puregene, Gentra Systems, Minneapolis, MN). The DNA samples were stored at 4°C, and reserve aliquots were kept in ethanol at -80°C. More than 200 ug genomic DNA (with a 260/280 ratio of 1.8–2.1) was available from each family member.

Marshfield Medical Research Foundation (Marshfield, WI) conducted the genotyping of 387 short tandem repeat polymorphic markers using the Weber Screening Set 9 (Research Genetics, Inc., Huntsville, AL). The genotyping protocol has been described in detail elsewhere (Kissebah et al., 2000). Briefly, this panel included 366 autosomal, 17 X-linked, and 4 Y-linked markers, yielding an average map density of 10 centiMorgans (cM). The genotypic data were examined for Mendelian inconsistencies, and those genotypes proven to be inconsistent were removed. The mean (+/-SD)

heterozygosity of these markers was 0.79 +/- 0.06, and the sex-averaged genetic spacing was 9.1 +/- 3.8 cM.

Genetic Analyses

Variance Components Linkage Analysis

A variance components model was employed to search for quantitative trait loci (QTLs) influencing the plasma cytokine levels of TNF-α and IL-1β. This method was implemented in the program package SOLAR (Almasy and Blangero, 1998), and assesses whether genetic variation at a specific chromosomal location explains the variation in the phenotype (Blangero et al., 2001). This approach is predicated on prescribing the expected genetic covariances between arbitrary relatives as a function of identical by descent (IBD) relationships at a given marker (Amos, 1994). Variance components linkage analysis also includes a QTL-specific component, which is used to test for linkage. The null hypothesis, that the additive genetic variance because of a QTL equals zero (no linkage), was tested by comparing the likelihood of this restricted model with that of a model in which a QTL is estimated. The difference between these log likelihoods produces a logarithm of odds (LOD) score. Twice the difference in log likelihoods of these models produces a test statistic that is asymptotically distributed as a1/2:1/2 mixture of a chi-squared variable with a point mass at zero (Self and Liang, 1987).

In these analyses, an extension of the technique developed by Fulker and colleagues (1995) was employed to permit multipoint analysis for QTL mapping. This multipoint procedure yields substantially greater power to localize QTLs than a two-point method. It enables direct localization of the QTL and construction of support intervals. The one LOD-unit support intervals is obtained by identifying the peak of the maximum LOD score on the plot in the multipoint results and descending one LOD-unit to identify the chromosomal region defining the shoulders of the curve.

Bivariate Quantitative Genetic Analyses

Bivariate analyses were conducted to explore genetic relationships between the cytokines as well as among the cytokines and phenotypes related to adiposity. Bivariate variance-components methods were implemented to estimate the genetic, environmental, and phenotypic correlations among the phenotypes (Lange and Boehnke, 1983). A detailed explanation of this method has been reported elsewhere (Hopper and Mathews, 1982). Briefly, the phenotypic covariance is modeled where covariation between two related individuals for two traits is given by a 2 × 2 covariance matrix. Its elements are defined by:

$$\Omega_{ab} = 2\Phi\rho_G\sigma_{ga}\sigma_{gb} + I\rho_E\sigma_{ea}\sigma_{eb},$$

where a and b assume the values of 1 or 2, and ρ_G and ρ_E are the additive genetic and environmental correlations of the phenotypes, respectively. The genetic correlation

estimates the extent of pleiotropy, or proportion of genes shared by two traits. The phenotypic correlation (ρ_P), is given by:

$$\rho_P = \rho_G \sqrt{h_1^2} \sqrt{h_2^2} + \rho_E \sqrt{(1- h_1^2)} \sqrt{(1- h_2^2)},$$

where $h_1^2$and h_2^2 represent the respective heritabilities of the traits. This estimate accounts for the relatedness of individuals in the calculation of the correlation between measures.

4.4 Results

Mean levels (±SD) of plasma TNF-α and IL-1β were 3.1 (± 2.2) ng/dL and 4.5 (± 3.0) ng/dL, respectively. Both of these phenotypes demonstrated significant genetic contributions due to the additive effects of genes. The heritabilities for TNF-α and IL-1β were 0.44 ± 0.08 ($P = 1 \times 10^{-10}$) and 0.41 ± 0.08 ($P = 1 \times 10^{-8}$), respectively. These findings are consistent with a study of cytokine heritabilities by Pantsulaia et al. (2002). TNF-α and IL-1β demonstrated a strong phenotypic correlation ($\rho_P = 0.86$, $P = 1 \times 10^{-12}$). A significant genetic correlation between TNF-α and IL-1β also was observed ($\rho_G = 0.94 \pm 0.02$, $P = 1 \times 10^{-9}$).

Phenotypic and genetic correlations between these cytokines and parameters of adiposity were also investigated. Table 4.2 lists the genetic correlations (ρ_G) of TNF-α and IL-1β with body mass index (BMI), waist circumference (WC), and serum leptin. TNF-α exhibited significant positive genetic correlations with all three adiposity-related phenotypes, while IL-1β was only genetically correlated with leptin. Circulating TNF-α

levels were positively associated with BMI, WC, and leptin. IL-1β levels in plasma were associated with serum leptin levels.

Multipoint linkage analysis was conducted for TNF-α and IL-1β, as displayed by chromosome in Figure 4.1. The strongest signal for TNF-α was detected on chromosome 18, with a maximum LOD score of 3.0. The peak is located 29 cM pter, between markers *ATA82B02* and *D18S1371*, on the cytoband 18q21. Chromosome 18 also produced the largest signal for circulating IL-1β. This QTL had a LOD score of 4.0 at 28 cM pter, between the same two markers as the signal for TNF-α. These findings are represented graphically in Figure 4.2.

4.5 Discussion

This is the first study to identify significant QTLs for a TNF-α and IL-1β in a human population. Genome-wide scans revealed that chromosome 18 plays a significant role in the genetic regulation of circulating TNF-α and IL-1β, the two primary inflammatory signals of the innate immune response. The plasma assays of the basal TNF-α and IL-1β expression in healthy individuals represent a novel set of traits separate from the traditional phenotypes previously used to characterize the genetic regulation of inflammation. Prior work has focused on the associations between cytokine polymorphisms and susceptibility to infections or autoimmune diseases. The panoply of information regarding a single promoter polymorphism such as the -308 G→A substitution in the *TNF* gene is a classic example of this emphasis (Abraham and

Kroeger, 1999;Dedoussis et al., 2005;Pawlik et al., 2005). However, it is important to recognize that potent molecules such as TNF-α and IL-1β operate under strict transcriptional regulation. Stimulation of the immune cascade by antigens (such as LPS) to elicit cytokine production represents a fundamentally different phenotype, as this trait characterizes the amplitude of the host response to a particular stressor. In contrast, plasma cytokine levels in healthy individuals are intended to represent the progressive inflammatory processes that accompany the pathogenesis of chronic diseases.

Several intriguing candidate genes reside in this region for the QTLs on the long arm of chromosome 18. These genes include the TNF superfamily member 11A (the RANK receptor), the suppressor of cytokine signaling six (*SOCS6*), the CD226 antigen, and the cytosolic nuclear factor of activated T-cells one (*NFATC1*). RANK and CD226 exhibit putative roles in inflammatory progression through NF-κB activation and T cell differentiation and proliferation, respectively (Anderson et al., 1997;Shibuya et al., 2003). In contrast, SOCS6 and NFATC1 facilitate suppression of inflammatory responses by blocking the transcription of inducible genes during an immune response (Fasshauer et al., 2004;Peng et al., 2001).

There is now a wealth of evidence indicating close ties between metabolic and immune systems. The identification of genes that contribute to this overlap could have importance in preventing and treating chronic disease conditions. Growing interest has mounted for the role of adipose tissue in the pathogenesis in chronic inflammation.

It seems likely that the inflammatory response is initiated in adipose tissue, as adipocytes are the first cells affected by the development of obesity. Physiologic changes in adipose tissue that accompany the accumulation of lipids induce metabolic stress on adipocyte endoplasmic reticulum and mitochondria (Harding and Ron, 2002;Olivares-Corichi et al., 2005;Ozcan et al., 2004). In response to this insult, adipocytes initiate a cascade of events to recruit immune cells, specifically monocytes and macrophages (Weisberg et al., 2003;Xu et al., 2003). The specific circumstances that potentiate this ingress remain unclear, but several inflammatory processes specific to adipose tissue may develop in response to obesity. Local inflammatory signals from stressed adipose tissue might contribute to chronic systemic inflammation.

To test this inter-relationship between adiposity and systemic inflammation, we investigated the degree of pleiotropy (i.e., shared additive genetic effects) between inflammatory markers and metabolic characteristics related to adiposity. Analysis of the data revealed that TNF-α, but not IL-1β, demonstrated evidence of shared genetic effects with waist circumference and BMI. However, the genetic correlations of the cytokines with leptin exhibited a greater degree of overlap than with BMI or WC. Leptin secretion is positively related to weight gain, and facilitates numerous endocrine signaling functions related to appetite, nutrient trafficking, fertility, and even immune function. The stress sensing transcription factor HIF-1α is now known to affect the production of leptin (Ambrosini et al., 2002). This finding suggests that the phenotype most directly related to the endocrine

signaling of adipocytes shares more genes with inflammatory markers than the anthropometric approximations of adiposity.

In conclusion, there is substantial evidence for genetic contribution to the expression of circulating inflammatory cytokines TNF-α and IL-1β. Our analyses have provided evidence of linkage for a previously unidentified QTL on chromosome 18 with TNF-α and IL-1β. These inflammatory signals are phenotypically and genetically correlated with parameters of adiposity, further reinforcing the role of adipose tissue in the development of chronic inflammation. These linkage analyses demonstrate that chromosomal regions other than the structural gene sites contribute to the differential regulation of cytokine phenotypes. Future work will aim to clarify the role for this region on chromosome 18 in the signaling cascade mediating the control of inflammation.

Table 4.1 Distribution of Relative Pairs

Relationship	Number of Pairs
Parent-offspring	458
Siblings	689
Grandparent-grandchild	40
Avuncular	289
Half siblings	13
Half avuncular	1
First cousins	194
Half first cousins	2
Identical sib pair	2
Total	1688

Table 4.2 Genetic Correlations (ρ_G) Between Cytokines and Parameters of Adiposity

Trait	IL-1β		TNF α	
	ρ_G	p	ρ_G	p
Body Mass Index (BMI)	0.15 ± 0.14	0.43	0.37 ± 0.18	0.05
Waist Circumference	0.19 ± 0.16	0.41	0.38 ± 0.19	0.05
Leptin	0.46 ± 0.21	0.04	0.54 ± 0.20	0.01

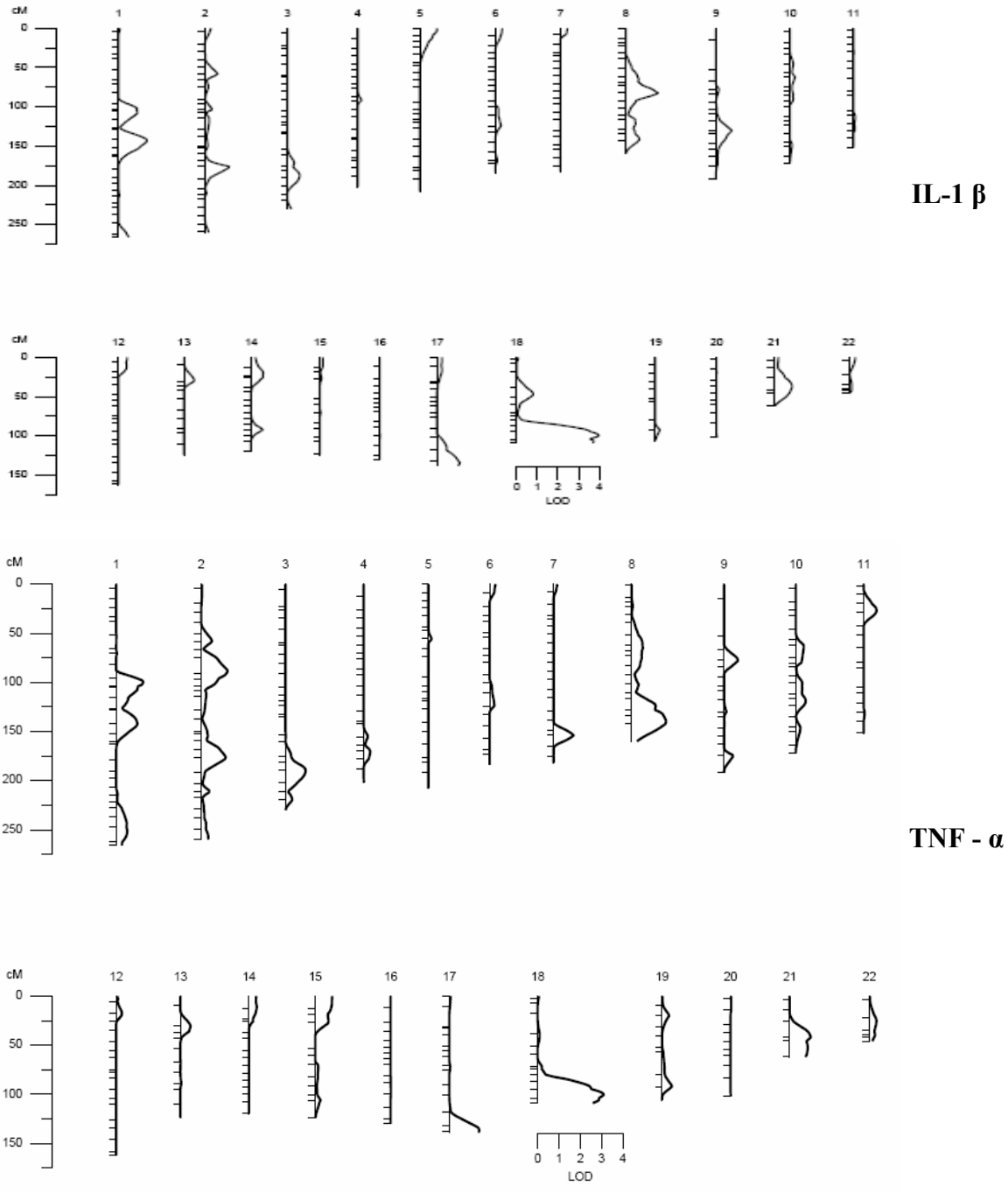

Figure 4.1: Results of the genome-wide scans, displayed by chromosome, for plasma TNF-α and IL-1β. Maximum log of odds ratio (LOD) scores are represented on the x axis and chromosomal location on the y axis. Hash marks reflect marker spacing and density.

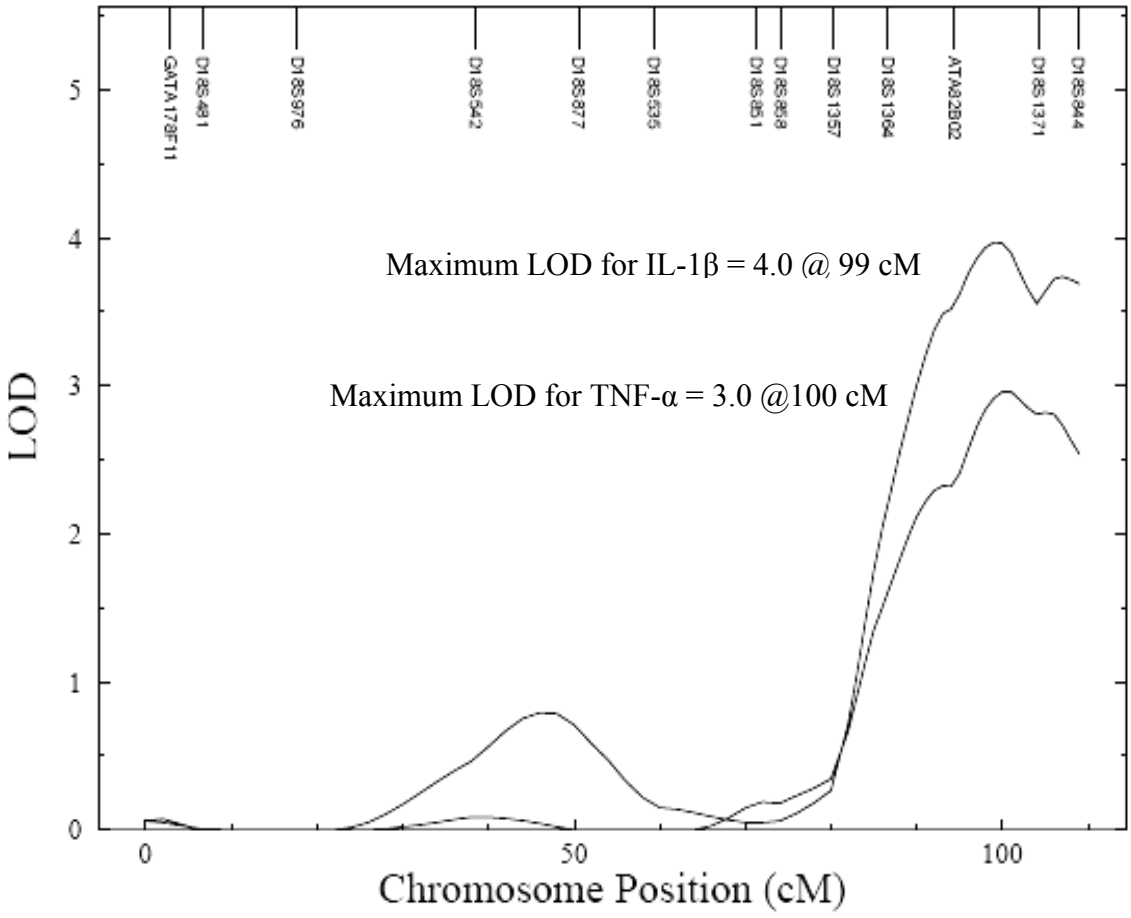

Figure 4.2: Multipoint linkage map of estimated LOD scores on Chromosome 18 for plasma TNF-α and IL-1 β. Maximum log of odds ratio (LOD) scores are represented on the y axis and chromosomal location on the x axis.

Chapter 5

A Quantitative Trait Locus Influencing Plasma CRP Levels Maps to Chromosome 12

5.1 Abstract

Inflammation is now widely recognized as a central feature in the pathogenesis and progression of many chronic diseases. The acute phase marker C-reactive protein (CRP) is a widely accepted cardiovascular disease (CVD) risk factor. In this study, circulating CRP levels were assayed in 500 healthy Caucasian adults recruited through TOPS, Inc. (Taking Off Pounds Sensibly), a commercial weight control program. Maximum likelihood variance decomposition methods, implemented in the program SOLAR, were used to conduct quantitative genetic analysis of this phenotype. Plasma CRP levels demonstrated an additive genetic heritability of 37% ($P = 6.3 \times 10^{-10}$). A genome-wide scan for CRP revealed a significant quantitative trait locus (QTL) on chromosome 12, with a maximum LOD score of 4.1 between markers *D12S398* and *D12S1294*. Bivariate quantitative genetic analyses were conducted to estimate the shared genetic effects between CRP and adiposity-related parameters. CRP was correlated genetically with serum leptin, waist circumference, waist-to-hip ratio, body mass index, and total body weight. This study is the first to identify a significant QTL for CRP in healthy individuals, and demonstrates that this important inflammatory phenotype shares genes with anthropometric indexes of fatness.

5.2 Introduction

Inflammatory pathways exacerbate many chronic disease conditions, but the specific mechanisms underlying their etiology remain poorly understood. Significant progress has been made in identifying particular immune components that track with insulin resistance, dyslipidemia, and atherosclerosis (Hansson, 2005;Sampietro et al., 2005;Vanderlaan and Reardon, 2005;Wellen and Hotamisligil, 2005). One such example is C-reactive protein (CRP), which has emerged as a consistent correlate of other cardiovascular disease (CVD) risk factors (Vikram et al., 2003;Anand et al., 2004;Rutter et al., 2004;Pannacciulli et al., 2001;Mazer and Rabbani, 2004). Recently, clinical and physiological evidence has strengthened the link between CRP and CVD (Pasceri et al., 2001;Pannacciulli et al., 2001).

The prognostic value of CRP measurements in determining CVD risk was once thought to be epiphenomenal to the pathophysiology underlying atherosclerosis. CRP is now implicated directly in this process through a variety of mechanisms. The vasodilatory capacity of blood vessels is impaired by the capacity of CRP to inhibit endothelial eNOS expression and, by extension, NO production (Venugopal et al., 2002). CRP also increases the production of monocyte chemoattractant protein-1 (MCP-1) in human endothelial cells, contributing to macrophage recruitment at the blood vessel walls (Pasceri et al., 2001). Additionally, CRP can mediate the uptake of LDL particles at atherosclerotic lesions by binding these particles and inducing their complement with

macrophages (Bhakdi et al., 1999). The net effect of these interactions is an increased capacity for foam cell formation.

The effects of CRP extend into vascular smooth muscle cells (VSMCs) as well. In 2001, Yasojima et al. demonstrated that VSMCs and macrophages from human atherosclerotic plaques were a significant source of CRP mRNA, and that the gene transcription was more than seven times higher in these plaques than the liver. *In vitro* experiments demonstrated that CRP exposure induced VMSC proliferation and migration (Wang et al., 2003).

Obesity is a common comorbidity of CVD, and recent evidence suggests that it may mitigate its effects through a chronic inflammatory process (Hak et al., 1999). Visceral adiposity is associated with increased CRP levels and cardiovascular complications (Saijo et al., 2004;Pannacciulli et al., 2001). CRP expression also is related to serum leptin levels, suggesting an overlap in the metabolic pathways that regulate these traits (Shamsuzzaman et al., 2004a). Adipose tissue in obese individuals is also a significant source of the pro-inflammatory cytokine interleukin 6 (IL-6) (Flower et al., 2003). IL-6 is the major signal regulating the release of CRP and other acute phase proteins from the liver and may link circulating CRP with adiposity.

Although the mechanistic actions of CRP in the pathogenesis of CVD continue to be elucidated, less is known about the underlying genetic regulation of CRP production. Studies from several ethnic populations have demonstrated that baseline CRP levels are heritable (Venugopal et al., 2002;De Maat et al., 2004;Austin et al., 2004). The current

challenge lies in the identification of specific genetic differences that affect CRP production. Within the last year, Brull et al. (2003) and Carlson et al. (2005) have identified CRP gene polymorphisms with putative effects on variation in circulating CRP. In this study, we have employed a genome-wide scan approach to search for novel quantitative trait loci (QTLs) that influence plasma CRP in a healthy, Caucasian population. Bivariate quantitative genetic techniques were used to explore the extent to which CRP expression shares genetic effects with parameters of adiposity.

5.3 Methods

Subjects

Plasma CRP levels were assayed, as part of the Metabolic Risk Complications of Obesity Genes project, from 505 participants (234 men, 271 women) from 154 families. Subject ascertainment and exclusion criteria are outlined in detail elsewhere (Kissebah et al., 2000). The distribution of relative pairs in the sample is displayed in Table 5.1. These subjects participated in a medical examination and blood draw in the morning after a 12 hour fast. Blood collected by venipuncture provided samples of whole blood, plasma, and serum, which were stored in aliquots at -80° C for future biochemical analyses. The Institutional Review Board of the Medical College of Wisconsin approved all procedures and informed, written consent was obtained from all subjects.

CRP Phenotype

Serum CRP levels were assayed by a high-sensitivity, immunonephelometry method using the BNII Systems (Dade Behring Diagnostics, Inc., Newark, DE). Briefly, polystyrene particles coated with monoclonal antibodies specific to human CRP, aggregate with CRP from the serum and scatter a wavelength of light passed through the sample. The intensity of the refracted light is proportional to the concentration of CRP in the serum. The results are determined by comparison with standards of known concentrations. The coefficient of variation for this assay was 8%, and the limit of detection for CRP was 0.175 mg/L. The trait was examined for normality, and measurements that exceeded four standard deviations were blanked from the genetic analyses. Non-normal distribution of the CRP measurements resulted in this trait being natural log transformed.

Genetic Analyses

Univariate Quantitative Genetic Analyses

Quantitative genetic analyses were performed using a maximum likelihood variance decomposition method implemented in the computer program package SOLAR (Almasy and Blangero, 1998). This approach partitions the phenotypic variance (σ^2_P) in a quantitative trait into components corresponding to genetic (σ^2_G) and environmental (σ^2_E) effects. These constituents are additive, such that $\sigma^2_P = \sigma^2_G + \sigma^2_E$. The heritability of a phenotype, defined as the proportion of the variance attributable to the additive effects of genes, is estimated as $h^2 = \sigma^2_G / \sigma^2_P$ (Falconer, 1989). The variance component system

models variance and covariance between relatives as a function of their relatedness. The covariance matrix (Ω) for a nuclear family is given by:

$$\Omega = 2\Phi\sigma^2_g + I\sigma^2_e,$$

where Φ defines the kinship matrix and I is an identity matrix.

Simultaneously, the effects of sex and age can be estimated as covariates. If a covariate effect is significant at the $P \leq 0.10$ level, it will be retained and its effect reestimated simultaneously with the genetic effects. Likelihood ratio tests are used to obtain P-values for the heritability estimates. The likelihood of the model which includes a heritability component is estimated and compared with the likelihood of the model in which the heritability is constrained to zero. Twice the difference in the natural logarithmic likelihoods is asymptotically distributed as a 1/2:1/2 mixture of a χ^2 variable, with a single degree of freedom and a zero point mass (Self and Liang, 1987).

Bivariate Quantitative Genetic Analyses

The quantitative genetic methods employed in the univariate genetic analyses are readily extendable to multivariate traits. Bivariate analyses were conducted to explore genetic relationships between CRP and anthropometric phenotypes. Large genetic correlations between these components imply overlap in the genes that influence these traits.

Bivariate variance-component analysis methods were implemented to estimate the genetic, environmental, and phenotypic correlations between CRP and the

anthropometric phenotypes collected. The bivariate method has been reported in detail elsewhere (Hopper and Mathews, 1982). Briefly, the phenotypic covariance is modeled so that the covariation between two individuals for two traits is given by a 2 × 2 covariance matrix. Its elements are defined by:

$$\Omega_{ab} = 2\Phi\rho_G\sigma_{ga}\sigma_{gb} + I\rho_E\sigma_{ea}\sigma_{eb},$$

where *a* and *b* assume the values of 1 or 2, and ρ_G and ρ_E are the additive genetic and environmental correlations between the traits, respectively. The genetic correlation is an estimate of the proportion of shared genes common to both traits. The phenotypic correlation (ρ_P) is given by:

$$\rho_P = \rho_G \sqrt{h_1^2}\sqrt{h_2^2} + \rho_E \sqrt{(1- h_1^2)}\sqrt{(1- h_2^2)},$$

where h_1^2 and h_2^2 represent the respective heritabilities of the traits.

Linkage Analysis

Multipoint linkage analysis was used to detect evidence of linkage with plasma CRP. Genetic loci influencing a quantitative trait covary between relatives as a function of their identical by descent (IBD) relationships (Amos, 1994). The variance component approach is employed in this method to partition the total genetic covariance into locus-specific, additive genetic, covariate, and environmental effects using relative pair relationship information. The maximum likelihood ratio test is performed to test for the significance of a linkage signal. The technique of Fulker et al. (1995) was extended to generate estimates of the IBDs at any point on a chromosome to allow for multipoint

analysis. The multipoint analysis for CRP was corrected for age, sex, age squared, and their interactions. The criteria for significant linkage between a marker and a trait is a LOD score ≥ 3 (Fulker et al., 1995;Morton, 1955).

5.4 Results

Mean levels of CRP were 3.6 (± 1.6) mg/l for males and 2.7 (± 1.4) mg/l for females. The additive genetic heritability for CRP was 0.37 ± 0.06 ($P = 6.3 \times 10^{-10}$), which is consistent with estimates from other family studies (De Maat et al., 2004;Austin et al., 2004). Table 5.2 displays the genetic and phenotypic correlations between plasma CRP and parameters of adiposity. Serum leptin, body mass index (BMI), waist circumference (WC), waist-to-hip ratio (WHR), and weight all were related genetically and phenotypically to circulating CRP. Circulating leptin yielded the highest genetic correlation with CRP ($\rho_G = 0.53$), body weight the lowest, ($\rho_G = 0.31$). Positive phenotypic correlations ranged from 0.24 for BMI to 0.41 for leptin.

Multipoint linkage analysis was conducted for CRP, and the results are displayed by chromosome in Figure 5.1. Serum LDL cholesterol levels was a significant covariate ($p < .01$), and the linkage analysis accounted for these effects. The strongest QTL resides on chromosome 12, with a maximum LOD score of 4.1. The peak resides approximately 82 cM pter, between the markers *D12S375* and *D12S1052,* and corresponds to the cytoband 12q14. The 1-LOD unit support interval for this signal spans approximately 40 cM, from 57 cM to 97 cM.

5.5 Discussion

To date, this study is the first to identify a significant QTL for plasma CRP levels in a healthy human population. A previously unknown region on chromosome 12 is responsible for contributing to the variation in CRP levels in this study population. Several intriguing candidate genes reside in the region identified on chromosome 12. These include interferon gamma (IFN γ), interleukin 22 (IL-22), interleukin 26 (IL-26), and the interleukin 1 receptor associated kinase three (IRAK3).

These candidates potentially mediate effects on CRP expression through regulation of CRP transcription factor availability. The CRP gene promoter region contains sequences that bind the cytokine-responsive transcription factors STAT-3 and CCAAT-enhancer-binding-protein (C/EBP) beta (Agrawal et al., 2003). The pro-inflammatory cytokines IL-6 and IL-1β are the traditional activators of CRP production in the liver. IL-6 utilizes a JAK2 - STAT-3 intracellular signaling cascade to initiate CRP gene expression while IL-1β relies on C/EBP beta to induce CRP transcription (Ochrietor et al., 2000;Zhang et al., 1996;Ramji et al., 1993). Therefore, IL-6 and IL-1β can act synergistically to enhance CRP availability.

STAT-3 activation can be mediated by IFN γ, IL-22, and IL-26 as well. IFN γ ligand binding results in STAT-3 dimerization in activated lymphocytes (Hibbert et al., 2003). Recombinant human IL-22 was found to activate STAT-3 in several hepatoma cell lines, and upregulate the production of acute phase reactants such as serum amyloid A in HepG2 cells (Nagalakshmi et al., 2004;Radaeva et al., 2004). The JAK - STAT pathway

is the main mechanism through which IL-26 conducts its signaling. In contrast, IRAK3 can mediate IL-1 β production of C/EBP beta and control innate immune responsiveness by negative regulation of toll receptors (Kobayashi et al., 2002).

It is now clear that adipose tissue performs substantial immunomodulatory functions. Nutrient regulation and host protection are two essential components of organism success, and these systems have coevolved. This relationship is seen most clearly with starvation-mediated immunosuppression and obesity-mediated inflammation. Components of the innate immune system, in particular, participate in the cross-talk between adipocytes and immune cells (Makowski et al., 2001;Tontonoz et al., 1998;Weisberg et al., 2003). TNF-α and IL-6 are two examples of cytokines produced in subcutaneous and visceral fat depots that track with the amount of lipid accumulation in these tissues (Weisberg et al., 2003;Xu et al., 2003).

Genes affecting adiposity phenotypes might also influence a number of inflammatory phenotypes, including CRP measures. To test this hypothesis, bivariate quantitative genetic analyses were performed between CRP and adiposity phenotypes. All the anthropometric measures of obesity were positively correlated with CRP, both genetically and phenotypically. Not surprisingly, the adipokine leptin produced the highest correlation, as this phenotype most accurately reflects the endocrine signaling of adipocytes. Body weight, BMI, WC, and WHR only approximate fatness without reference to adipose tissue deposition.

This study is a first step in elucidating the complex network of genetic and environmental interactions among risk factors that ultimately confer CVD susceptibility. We have identified a genetic link between obesity-related anthropometric measures and CRP, a marker of chronic, low-grade inflammation. Ample evidence of molecular and physiological signaling overlap exists between these two classes of risk factors, reinforcing the potential for shared genetic connections. The QTL discovered on chromosome 12 identifies a novel genetic region that contributes to plasma CRP levels in healthy individuals. Future work will be aimed at identifying specific genes responsible for the variation in this trait.

Table 5.1 Distribution of Relative Pairs

Relationship	Number of Pairs
Parent-offspring	355
Siblings	646
Grandparent-grandchild	53
Avuncular	47
Half siblings	19
Half avuncular	4
First cousins	11
Half first cousins	5
Identical sib pair	4
Total	1153

Table 5.2 Genetic (ρ_G) and Phenotypic (ρ_P) Correlations Between CRP and Parameters of Adiposity

Trait	ρ_G	p†	ρ_P	p‡
Leptin	0.53	0.02	0.41	1.9e-02
Waist Circumference	0.51	0.04	0.40	3.3e-03
Waist-to-Hip Ratio	0.49	0.004	0.35	7.8e-04
Body Mass index (BMI)	0.40	0.007	0.24	2.1e-03
Weight	0.31	0.07	0.39	4.8e-04

† *p* value for pleiotropy
‡ *p* value for phenotypic correlation corrected for the relatedness of individuals

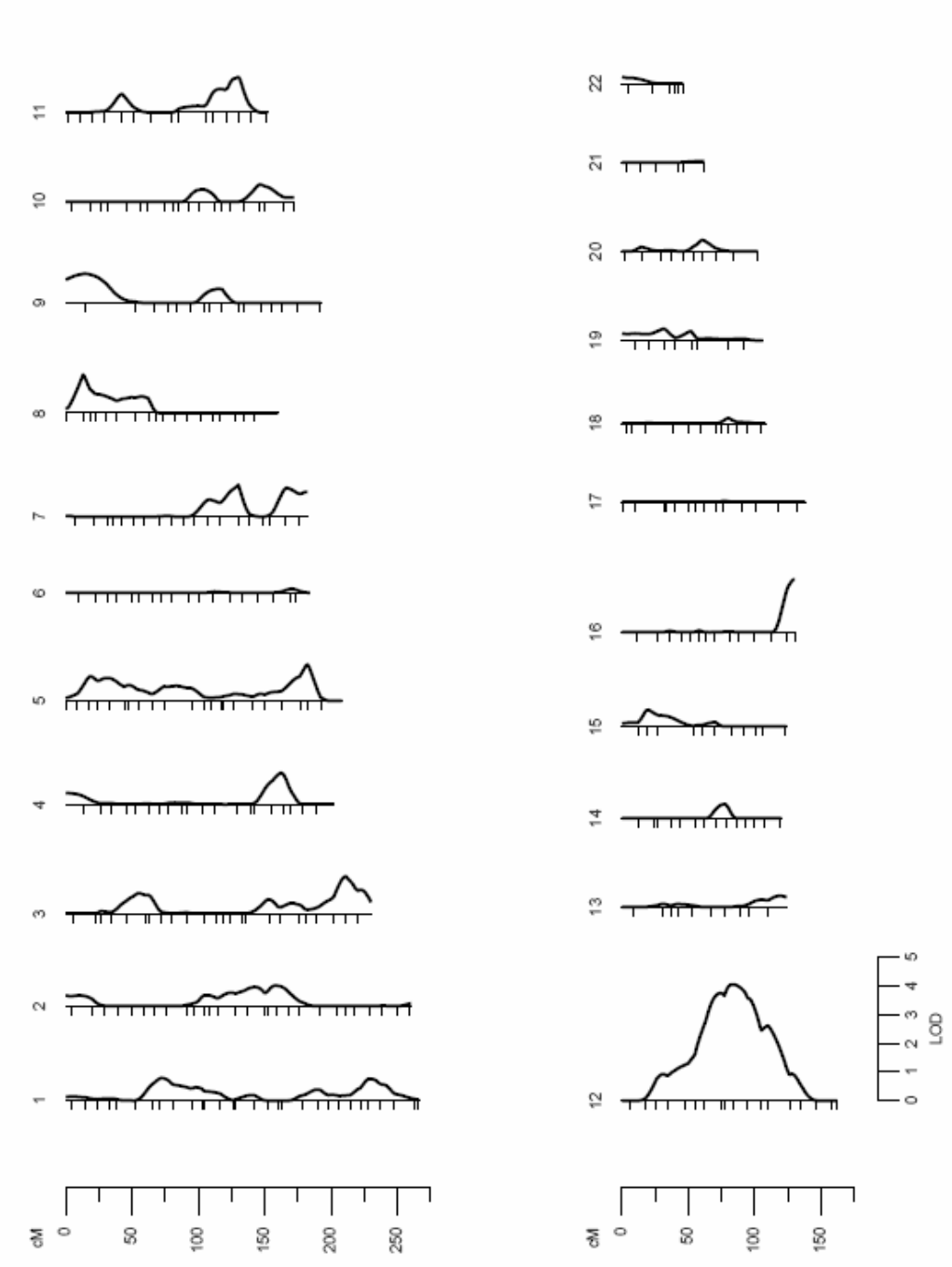

Figure 5.1: Results of the genome-wide scan, displayed by chromosome, for plasma CRP. The maximum log of odds ratio (LOD) scores are represented on the y axis and chromosomal location on the x axis. Hash marks reflect marker spacing and density.

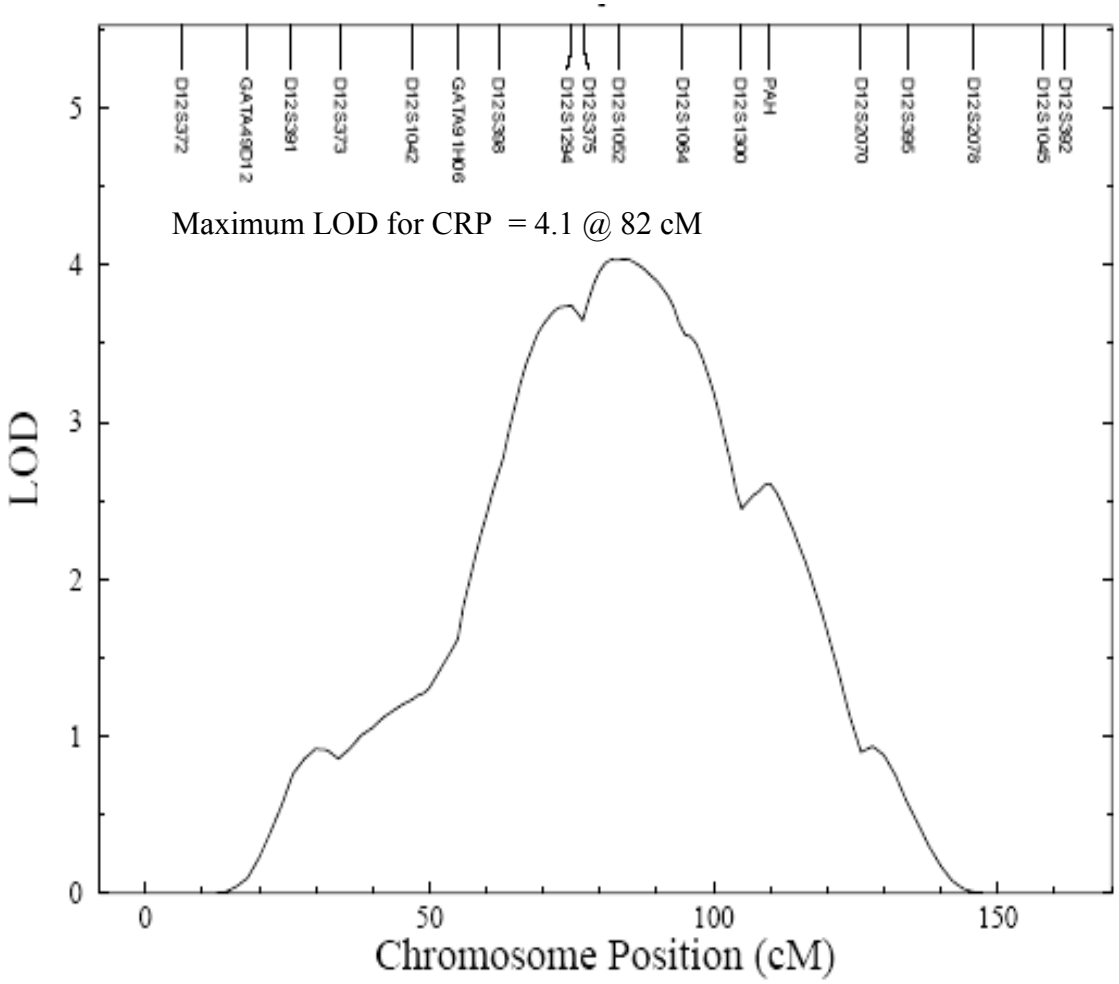

Figure 5.2: Multipoint linkage map of the estimated LOD score on Chromosome 12 for plasma CRP. Maximum log of odds ratio (LOD) scores are represented on the y axis and chromosomal location on the x axis.

Chapter 6

Discussion

Obesity is a common progenitor of many chronic metabolic conditions, such as insulin resistance, dyslipidemia, atherosclerosis, and lipotoxicity. The disregulation seen in these complications is presumably a product of changes in adipose tissue metabolism related to increased adiposity. Once considered an inert energy depot, adipose tissue is now widely known to be one of the more intriguing endocrine tissues of the body. This tissue is capable of relaying information about nutritional status, stress, and inflammation.

An important recent development is the emergence of the concept that obesity is characterized by chronic, low-grade inflammation. Adipose tissue itself is recognized as an important site for the production of inflammatory proteins. Evidence suggests that these signals from adipose tissue potentiate the metabolic dysfunctions associated with obesity.

The goal of this project was to investigate the genetic regulation of plasma cytokine variation in healthy baboons and humans. The first objective was to determine whether circulating levels of inflammatory markers were influenced by genetic factors in baboons. In Study I, levels of the pro-inflammatory cytokines TNF-α, GM-CSF, IL-1β, and IL-6, as well as sTNFR1 and sTNFR2, were measured in 370 related baboons. All of the traits exhibited significant evidence for genetic regulation. The heritabilities ranged from 61% for TNF-α to 14% for sTNFR1.

Linkage analyses were performed on the traits and revealed that the baboon homologs of human chromosomes 14 and 20 may contribute to variation in cytokine production. Genetic (ρ_G) correlations were observed among the plasma cytokines in baboons as well. A strong positive genetic correlation was observed between IL-1 β and TNF-α. The tests for complete pleiotropy were significant for IL-6 with both IL-1β and TNF-α. Plasma TNFR2 levels were negatively genetically related to both IL-1 β and TNF-α.

The second objective was to identify chromosomal regions governing the production of resistin in baboons. In Study II, resistin levels were measured in 416 related baboons. Variation in the plasma concentration of resistin was found to be significantly linked to chromosome 18, between the markers *D18S475* and *D18S172*, on the cytoband 18q12 (LOD = 4.0). This linkage signal is located on a different chromosome from a previously identified signal for mRNA levels of resistin in baboon adipose tissue. This finding suggests that separate genetic mechanisms regulate the expression and release of resistin.

The acute phase proteins plasminogen activator inhibitor 1 (PAI-1) and C-reactive protein (CRP) also were measured in the baboon plasma collected in Study II. These inflammatory markers, like resistin, are related to atherosclerotic risk. PAI-1 was found to be a heritable trait, and demonstrated a genetic correlation with resistin. While plasma CRP levels failed to yield a significant heritability, it was phenotypically correlated with both resistin and PAI-1. Several reasons could explain why CRP did not produce

evidence of a genetic effect. The sensitivity of the CRP measure was higher than other cytokines, hence, the range and standard error was larger for this trait. Transformations of the data failed to correct completely the skewness and kurtosis of the distribution. However, CRP also interacts with cholesterol. Since the baboon is a model of dyslipidemia, genetic factors affecting lipid metabolism might change the phenotype in a manner that obfuscates the genetic effects.

The third objective of this study was to locate quantitative trait loci (QTLs) that affect circulating levels of plasma TNF-α and IL-1β levels in humans. Study III examined plasma levels of TNF-α and IL-1β in a population of Caucasian Americans. Quantitative trait loci were identified for TNF-α and IL-1β (LOD = 3.0 and LOD = 4.0, respectively) on chromosome 18 between markers *ATA82B02* and *D18S1371*. Bivariate genetic analyses in this study demonstrated that TNF-α was genetically correlated with body mass index (BMI), waist circumference, and leptin. This finding reinforces the relationship between adipose tissue physiology and systemic inflammation, and suggests that a common set of genes regulate adiposity and TNF-α levels in blood.

The present marker density available on chromosome 18 in this study limits the ability to effectively establish support intervals for TNF-α and IL-1β. Future work should genotype additional markers in this region to clarify the specific region that contributes to these triats. The identification of QTLs on chromosome 18 substantiates the hypothesis that genetic loci distant from the structural site of the gene can influence variance on cytokine phenotypes.

The fourth objective was to detect QTLs affecting plasma levels of the acute phase protein CRP, and investigate its association with obesity phenotypes in humans. Study IV revealed that chromosome 12 harbors a QTL regulating circulating CRP levels between markers *D12S375* and *D12S1052* (LOD = 4.1). CRP was genetically correlated with parameters of adiposity. While the maximum LOD score on chromosome 12 was highly significant, this phenotype would also benefit from the typing of additional markers to clarify this interval.

The first two studies of this project were restricted by several shortcomings. The small sample sizes of the two baboon populations reduced the power to detect linkage between the plasma cytokine phenotypes and the genetic markers. This study also was not able to test the relationship between adiposity characteristics and cytokines because anthropometric measures were not available. Weight is not a reliable reflection of adiposity in babooons, and other estimates such as bioimpedance or skinfold were not taken on these animals at time of the blood collection. These studies also could not account for autoimmune conditions. Undiagnosed autoimmune diseases in baboons might contribute to plasma cytokine elevation that is regulated differently than the majority of the animals in the population. Finally, stress on the animals associated with being sequestered for the blood draw process might influence pathways that could affect cytokine expression.

Experimental limitations also existed for Studies III and IV. As in the baboon populations, the human group sample size was relatively small. In addition, the potential

for variation of cytokine levels due to environmental effects was greater. Unreported infections or injuries would elicit an inflammatory response. Other variables such as anti-inflammatory medications or food consumption conceivably could confound the cytokine measures.

In summary, the results from these baboon and human studies suggest that circulating cytokine levels in healthy animals are under significant genetic control. Chromosomes 12 and 18 contain genetic differences that influence inflammation. Genetic effects regulating IL-1β and TNF-α appear to share significant overlap in both the baboon and human studies. This relationship is evident from the strong genetic correlation observed and the clustering of the max LOD scores in baboons on chromosome 20, and in humans on chromosome 18. Future work should aim to resolve the specific genetic elements governing these cytokine phenotypes through fine mapping and the positional candidate gene approach.

Appendix A. Institutional Review Board Approval

IRB APPROVAL – IRB PROTOCOL # 2005-06-0006

TITLE: Genetics of the Innate Immune System and its Association with Obesity

Literature Cited

Abraham LJ and Kroeger KM. 1999. Impact of the -308 TNF promoter polymorphism on the transcriptional regulation of the TNF gene: relevance to disease. J Leukoc Biol 66:562-566.

Agirbasli M. 2005. Pivotal role of plasminogen-activator inhibitor 1 in vascular disease. Int J Clin Pract 59:102-106.

Agrawal A, Cha-Molstad H, Samols D, and Kushner I. 2003. Overexpressed nuclear factor-kappaB can participate in endogenous C-reactive protein induction, and enhances the effects of C/EBPbeta and signal transducer and activator of transcription-3. Immunology 108:539-547.

Almasy L and Blangero J. 1998. Multipoint quantitative-trait linkage analysis in general pedigrees. Am J Hum Genet 62:1198-1211.

Almasy L, Dyer TD, and Blangero J. 1997. Bivariate quantitative trait linkage analysis: pleiotropy versus co-incident linkages. Genet Epidemiol 14:953-958.

Ambrosini G, Nath AK, Sierra-Honigmann MR, and Flores-Riveros J. 2002. Transcriptional activation of the human leptin gene in response to hypoxia. Involvement of hypoxia-inducible factor 1. J Biol Chem 277:34601-34609.

Amos CI. 1994. Robust variance-components approach for assessing genetic linkage in pedigrees. Am J Hum Genet 54:535-543.

Anand SS, Razak F, Yi Q, Davis B, Jacobs R, Vuksan V, Lonn E, Teo K, McQueen M, and Yusuf S. 2004. C-reactive protein as a screening test for cardiovascular risk in a multiethnic population. Arterioscler Thromb Vasc Biol 24:1509-15.

Anderson DM, Maraskovsky E, Billingsley WL, Dougall WC, Tometsko ME, Roux ER, Teepe MC, DuBose RF, Cosman D, and Galibert L. 1997. A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. Nature 390:175-179.

Austin MA, Zhang C, Humphries SE, Chandler WL, Talmud PJ, Edwards KL, Leonetti DL, McNeely MJ, and Fujimoto WY. 2004. Heritability of C-reactive protein and association with apolipoprotein E genotypes in Japanese Americans. Ann Hum Genet 68:179-188.

Berg AH and Scherer PE. 2005. Adipose tissue, inflammation, and cardiovascular disease. Circ Res 96:939-949.

Bhakdi S, Torzewski M, Klouche M, and Hemmes M. 1999. Complement and atherogenesis: binding of CRP to degraded, nonoxidized LDL enhances complement activation. Arterioscler Thromb Vasc Biol 19:2348-54.

Binder CJ, Chang MK, Shaw PX, Miller YI, Hartvigsen K, Dewan A, and Witztum JL. 2002. Innate and acquired immunity in atherogenesis. Nat Med 8:1218-1226.

Bjorbaek C, Uotani S, da Silva B, and Flier JS. 1997. Divergent signaling capacities of the long and short isoforms of the leptin receptor. J Biol Chem 272:32686-32695.

Blangero J, Williams JT, and Almasy L. 2001. Variance component methods for detecting complex trait loci. Adv Genet 42:151-181.

Bokarewa M, Nagaev I, Dahlberg L, Smith U, and Tarkowski A. 2005. Resistin, an adipokine with potent proinflammatory properties. J Immunol 174:5789-5795.

Bouchard C, Despres JP, and Tremblay A. 1991. Genetics of obesity and human energy metabolism. Proc Nutr Soc 50:139-147.

Brull DJ, Serrano N, Zito F, Jones L, Montgomery HE, Rumley A, Sharma P, Lowe GD, World MJ, Humphries SE, and Hingorani AD. 2003. Human CRP gene polymorphism influences CRP levels: implications for the prediction and pathogenesis of coronary heart disease. Arterioscler Thromb Vasc Biol 23:2063-2069.

Bullo M, Garcia-Lorda P, Megias I, and Salas-Salvado J. 2003. Systemic inflammation, adipose tissue tumor necrosis factor, and leptin expression. Obes Res 11:525-531.

Cao Z, Tanaka M, Regnier C, Rothe M, Yamit-hezi A, Woronicz JD, Fuentes ME, Durnin MH, Dalrymple SA, and Goeddel DV. 1999. NF-kappa B activation by tumor necrosis factor and interleukin-1. Cold Spring Harb Symp Quant Biol 64:473-483.

Carlson CS, Aldred SF, Lee PK, Tracy RP, Schwartz SM, Rieder M, Liu K, Williams OD, Iribarren C, Lewis EC, Fornage M, Boerwinkle E, Gross M, Jaquish C, Nickerson DA, Myers RM, Siscovick DS, and Reiner AP. 2005. Polymorphisms within the C-Reactive Protein (CRP) Promoter Region Are Associated with Plasma CRP Levels. Am J Hum Genet 77:64-77.

Charriere G, Cousin B, Arnaud E, Andre M, Bacou F, Penicaud L, and Casteilla L. 2003. Preadipocyte conversion to macrophage. Evidence of plasticity. J Biol Chem 278:9850-9855.

Chen J, Sadowski HB, Kohanski RA, and Wang LH. 1997. Stat5 is a physiological substrate of the insulin receptor. Proc Natl Acad Sci U S A 94:2295-2300.

Chu NF, Spiegelman D, Hotamisligil GS, Rifai N, Stampfer M, and Rimm EB. 2001. Plasma insulin, leptin, and soluble TNF receptors levels in relation to obesity-related atherogenic and thrombogenic cardiovascular disease risk factors among men. Atherosclerosis 157:495-503.

Clement K, Viguerie N, Poitou C, Carette C, Pelloux V, Curat CA, Sicard A, Rome S, Benis A, Zucker JD, Vidal H, Laville M, Barsh GS, Basdevant A, Stich V, Cancello R, and Langin D. 2004. Weight loss regulates inflammation-related genes in white adipose tissue of obese subjects. FASEB J 18:1657-1669.

Comuzzie AG, Cole SA, Martin L, Carey KD, Mahaney MC, Blangero J, and VandeBerg JL. 2003. The baboon as a nonhuman primate model for the study of the genetics of obesity. Obes Res 11:75-80.

Cousin B, Andre M, Casteilla L, and Penicaud L. 2001. Altered macrophage-like functions of preadipocytes in inflammation and genetic obesity. J Cell Physiol 186:380-386.

Crespo J, Cayon A, Fernandez-Gil P, Hernandez-Guerra M, Mayorga M, Dominguez-Diez A, Fernandez-Escalante JC, and Pons-Romero F. 2001. Gene expression of tumor necrosis factor alpha and TNF-receptors, p55 and p75, in nonalcoholic steatohepatitis patients. Hepatology 34:1158-1163.

De Maat MP, Marie BE, Von Bornemann HJ, Bathum L, Jespersen J, and Christensen K. 2004. Genetic Influence on Inflammation Variables in the Elderly. Arterioscler Thromb Vasc Biol.

Dedoussis GV, Panagiotakos DB, Vidra NV, Louizou E, Chrysohoou C, Germanos A, Mantas Y, Tokmakidis S, Pitsavos C, and Stefanadis C. 2005. Association between TNF-alpha -308G>A polymorphism and the development of acute coronary syndromes in Greek subjects: The CARDIO2000-GENE Study. Genet Med 7:411-416.

Faggioni R, Benigni F, and Ghezzi P. 1995. Proinflammatory cytokines as pathogenetic mediators in the central nervous system: brain-periphery connections. Neuroimmunomodulation 2:2-15.

Falconer DS. 1989. *Introduction to Quantitative Genetics.* London, UK: Longman Scientific & Technical.

Fantuzzi G. 2005. Adipose tissue, adipokines, and inflammation. J Allergy Clin Immunol 115:911-919.

Fasshauer M, Kralisch S, Klier M, Lossner U, Bluher M, Klein J, and Paschke R. 2004. Insulin resistance-inducing cytokines differentially regulate SOCS mRNA expression via

growth factor- and Jak/Stat-signaling pathways in 3T3-L1 adipocytes. J Endocrinol 181:129-138.

Festa A, D'Agostino R, Jr., Tracy RP, and Haffner SM. 2002. Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. Diabetes 51:1131-1137.

Finkelstein EA, Ruhm CJ, and Kosa KM. 2005. Economic causes and consequences of obesity. Annu Rev Public Health 26:239-257.

Flower L, Gray R, Pinkney J, and Mohamed-Ali V. 2003. Stimulation of interleukin-6 release by interleukin-1beta from isolated human adipocytes. Cytokine 21:32-37.

Frayn KN, Fielding BA, and Karpe F. 2005. Adipose tissue fatty acid metabolism and cardiovascular disease. Curr Opin Lipidol 16:409-415.

Fulker DW, Cherny SS, and Cardon LR. 1995. Multipoint interval mapping of quantitative trait loci, using sib pairs. Am J Hum Genet 56:1224-1233.

Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, and Shimomura I. 2004. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 114:1752-1761.

Gao Y, Hansson M, Calafat J, Tapper H, and Olsson I. 2004. Sorting soluble tumor necrosis factor (TNF) receptor for storage and regulated secretion in hematopoietic cells. J Leukoc Biol 76:876-885.

Gerrity RG. 1981. The role of the monocyte in atherogenesis: I. Transition of blood-borne monocytes into foam cells in fatty lesions. Am J Pathol 103:181-190.

Ghanim H, Aljada A, Hofmeyer D, Syed T, Mohanty P, and Dandona P. 2004. Circulating mononuclear cells in the obese are in a proinflammatory state. Circulation 110:1564-1571.

Gimeno RE and Klaman LD. 2005. Adipose tissue as an active endocrine organ: recent advances. Curr Opin Pharmacol 5:122-128.

Grimble RF. 1995. Interactions between nutrients and the immune system. Nutr Health 10:191-200.

Grimble RF. 1996. Interaction between nutrients, pro-inflammatory cytokines and inflammation. Clin Sci (Lond) 91:121-130.

Grimble RF. 1998. Nutritional modulation of cytokine biology. Nutrition 14:634-640.

Hak AE, Stehouwer CD, Bots ML, Polderman KH, Schalkwijk CG, Westendorp IC, Hofman A, and Witteman JC. 1999. Associations of C-reactive protein with measures of obesity, insulin resistance, and subclinical atherosclerosis in healthy, middle-aged women. Arterioscler Thromb Vasc Biol 19:1986-91.

Hansson GK. 2005. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 352:1685-1695.

Harding HP and Ron D. 2002. Endoplasmic reticulum stress and the development of diabetes: a review. Diabetes 51 Suppl 3:S455-S461.

Hauner H. 2005. Secretory factors from human adipose tissue and their functional role. Proc Nutr Soc 64:163-169.

Hausman GJ and Richardson RL. 2004. Adipose tissue angiogenesis. J Anim Sci 82:925-934.

Heesen M, Kunz D, Bachmann-Mennenga B, Merk HF, and Bloemeke B. 2003. Linkage disequilibrium between tumor necrosis factor (TNF)-alpha-308 G/A promoter and TNF-beta NcoI polymorphisms: Association with TNF-alpha response of granulocytes to endotoxin stimulation. Crit Care Med 31:211-214.

Hibbert L, Pflanz S, de Waal MR, and Kastelein RA. 2003. IL-27 and IFN-alpha signal via Stat1 and Stat3 and induce T-Bet and IL-12Rbeta2 in naive T cells. J Interferon Cytokine Res 23:513-522.

Holtmann MH and Neurath MF. 2004. Differential TNF-signaling in chronic inflammatory disorders. Curr Mol Med 4:439-444.

Hopper JL and Mathews JD. 1982. Extensions to multivariate normal models for pedigree analysis. Ann Hum Genet 46 (4):373-383.

Horvath CM. 2004. The Jak-STAT pathway stimulated by interleukin 6. Sci STKE 2004:tr9.

Hotamisligil GS, Shargill NS, and Spiegelman BM. 1993. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 259:87-91.

Hung JH, Su IJ, Lei HY, Wang HC, Lin WC, Chang WT, Huang W, Chang WC, Chang YS, Chen CC, and Lai MD. 2004. Endoplasmic reticulum stress stimulates the expression of cyclooxygenase-2 through activation of NF-kappaB and pp38 mitogen-activated protein kinase. J Biol Chem 279:46384-46392.

Janke J, Engeli S, Gorzelniak K, Luft FC, and Sharma AM. 2002. Resistin gene expression in human adipocytes is not related to insulin resistance. Obes Res 10:1-5.

Kawanami D, Maemura K, Takeda N, Harada T, Nojiri T, Imai Y, Manabe I, Utsunomiya K, and Nagai R. 2004. Direct reciprocal effects of resistin and adiponectin on vascular endothelial cells: a new insight into adipocytokine-endothelial cell interactions. Biochem Biophys Res Commun 314:415-419.

Kinasewitz GT, Chang AC, Peer GT, Hinshaw LB, and Taylor FB, Jr. 2000. Peritonitis in the baboon: a primate model which stimulates human sepsis. Shock 13:100-109.

Kissebah AH, Sonnenberg GE, Myklebust J, Goldstein M, Broman K, James RG, Marks JA, Krakower GR, Jacob HJ, Weber J, Martin L, Blangero J, and Comuzzie AG. 2000. Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome. Proc Natl Acad Sci U S A 97:14478-14483.

Kobayashi K, Hernandez LD, Galan JE, Janeway CA, Jr., Medzhitov R, and Flavell RA. 2002. IRAK-M is a negative regulator of Toll-like receptor signaling. Cell 110:191-202.

Koruk M, Taysi S, Savas MC, Yilmaz O, Akcay F, and Karakok M. 2003. Serum levels of acute phase proteins in patients with nonalcoholic steatohepatitis. Turk J Gastroenterol 14:12-17.

Kougias P, Chai H, Lin PH, Yao Q, Lumsden AB, and Chen C. 2005. Effects of adipocyte-derived cytokines on endothelial functions: implication of vascular disease. J Surg Res 126:121-129.

Kruithof EK, Mestries JC, Gascon MP, and Ythier A. 1997. The coagulation and fibrinolytic responses of baboons after in vivo thrombin generation--effect of interleukin 6. Thromb Haemost 77:905-910.

Lampinen M, Carlson M, Hakansson LD, and Venge P. 2004. Cytokine-regulated accumulation of eosinophils in inflammatory disease. Allergy 59:793-805.

Lange K and Boehnke M. 1983. Extensions to pedigree analysis. IV. Covariance components models for multivariate traits. Am J Med Genet 14:513-524.

Lehrke M, Reilly MP, Millington SC, Iqbal N, Rader DJ, and Lazar MA. 2004. An inflammatory cascade leading to hyperresistinemia in humans. PLoS Med 1:e45.

Libby P. 2002. Inflammation in atherosclerosis. Nature 420:868-874.

Lim W, Hong S, Nelesen R, and Dimsdale JE. 2005. The association of obesity, cytokine levels, and depressive symptoms with diverse measures of fatigue in healthy subjects. Arch Intern Med 165:910-915.

Lin Y, Berg AH, Iyengar P, Lam TK, Giacca A, Combs TP, Rajala MW, Du X, Rollman B, Li W, Hawkins M, Barzilai N, Rhodes CJ, Fantus IG, Brownlee M, and Scherer PE. 2005. The hyperglycemia-induced inflammatory response in adipocytes: the role of reactive oxygen species. J Biol Chem 280:4617-4626.

Lolmede K, Durand dSF, V, Galitzky J, Lafontan M, and Bouloumie A. 2003. Effects of hypoxia on the expression of proangiogenic factors in differentiated 3T3-F442A adipocytes. Int J Obes Relat Metab Disord 27:1187-1195.

Lu SC, Shieh WY, Chen CY, Hsu SC, and Chen HL. 2002. Lipopolysaccharide increases resistin gene expression in vivo and in vitro. FEBS Lett 530:158-162.

Maachi M, Pieroni L, Bruckert E, Jardel C, Fellahi S, Hainque B, Capeau J, and Bastard JP. 2004. Systemic low-grade inflammation is related to both circulating and adipose tissue TNFalpha, leptin and IL-6 levels in obese women. Int J Obes Relat Metab Disord 28:993-997.

Makowski L, Boord JB, Maeda K, Babaev VR, Uysal KT, Morgan MA, Parker RA, Suttles J, Fazio S, Hotamisligil GS, and Linton MF. 2001. Lack of macrophage fatty-acid-binding protein aP2 protects mice deficient in apolipoprotein E against atherosclerosis. Nat Med 7:699-705.

Mazer SP and Rabbani LE. 2004. Evidence for C-Reactive Protein's Role in (CRP) Vascular Disease: Atherothrombosis, Immuno-Regulation and CRP. J Thromb Thrombolysis 17:95-105.

Mori K. 2000. Tripartite management of unfolded proteins in the endoplasmic reticulum. Cell 101:451-454.

Morton NE. 1955. Sequential tests for the detection of linkage. Am J Hum Genet 7:277-318.

Munro JM, Pober JS, and Cotran RS. 1989. Tumor necrosis factor and interferon-gamma induce distinct patterns of endothelial activation and associated leukocyte accumulation in skin of Papio anubis. Am J Pathol 135:121-133.

Nagalakshmi ML, Rascle A, Zurawski S, Menon S, and de Waal MR. 2004. Interleukin-22 activates STAT3 and induces IL-10 by colon epithelial cells. Int Immunopharmacol 4:679-691.

Ochrietor JD, Harrison KA, Zahedi K, and Mortensen RF. 2000. Role of STAT3 and C/EBP in cytokine-dependent expression of the mouse serum amyloid P-component (SAP) and C-reactive protein (CRP) genes. Cytokine 12:888-899.

Olivares-Corichi IM, Ceballos G, Medina-Santillan R, Medina-Navarro R, Guzman-Grenfell AM, and Hicks JJ. 2005. Oxidation by reactive oxygen species (ROS) alters the structure of human insulin and decreases the insulin-dependent D-glucose-C14 utilization by human adipose tissue. Front Biosci 10:3127-3131.

Ozcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E, Tuncman G, Gorgun C, Glimcher LH, and Hotamisligil GS. 2004. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science 306:457-461.

Pannacciulli N, Cantatore FP, Minenna A, Bellacicco M, Giorgino R, and De Pergola G. 2001. C-reactive protein is independently associated with total body fat, central fat, and insulin resistance in adult women. Int J Obes Relat Metab Disord 25:1416-20.

Pantsulaia I, Trofimov S, Kobyliansky E, and Livshits G. 2002. Genetic and environmental influences on IL-6 and TNF-alpha plasma levels in apparently healthy general population. Cytokine 19:138-146.

Pasceri V, Cheng JS, Willerson JT, Yeh ET, and Chang J. 2001. Modulation of C-reactive protein-mediated monocyte chemoattractant protein-1 induction in human endothelial cells by anti-atherosclerosis drugs. Circulation 103:2531-2534.

Patel L, Buckels AC, Kinghorn IJ, Murdock PR, Holbrook JD, Plumpton C, Macphee CH, and Smith SA. 2003. Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. Biochem Biophys Res Commun 300:472-476.

Pawlik A, Florczak M, Ostanek L, Brzosko M, Brzosko I, and Szklarz BG. 2005. TNF-alpha -308 promoter polymorphism in patients with rheumatoid arthritis. Scand J Rheumatol 34:22-26.

Peng SL, Gerth AJ, Ranger AM, and Glimcher LH. 2001. NFATc1 and NFATc2 together control both T and B cell activation and differentiation. Immunity 14:13-20.

Pickup JC and Crook MA. 1998. Is type II diabetes mellitus a disease of the innate immune system? Diabetologia 41:1241-1248.

Pradhan AD, Manson JE, Rifai N, Buring JE, and Ridker PM. 2001. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA 286:327-334.

Radaeva S, Sun R, Pan HN, Hong F, and Gao B. 2004. Interleukin 22 (IL-22) plays a protective role in T cell-mediated murine hepatitis: IL-22 is a survival factor for hepatocytes via STAT3 activation. Hepatology 39:1332-1342.

Ramji DP, Vitelli A, Tronche F, Cortese R, and Ciliberto G. 1993. The two C/EBP isoforms, IL-6DBP/NF-IL6 and C/EBP delta/NF-IL6 beta, are induced by IL-6 to promote acute phase gene transcription via different mechanisms. Nucleic Acids Res 21:289-294.

Reaven GM. 1987. Non-insulin-dependent diabetes mellitus, abnormal lipoprotein metabolism, and atherosclerosis. Metabolism 36:1-8.

Reilly MP, Lehrke M, Wolfe ML, Rohatgi A, Lazar MA, and Rader DJ. 2005. Resistin is an inflammatory marker of atherosclerosis in humans. Circulation 111:932-939.

Rodriguez-Perez JM, Cruz-Robles D, Hernandez-Pacheco G, Perez-Hernandez N, Murguia LE, Granados J, Reyes PA, and Vargas-Alarcon G. 2005. Tumor necrosis factor-alpha promoter polymorphism in Mexican patients with Chagas' disease. Immunol Lett 98:97-102.

Rogers J, Mahaney MC, Witte SM, Nair S, Newman D, Wedel S, Rodriguez LA, Rice KS, Slifer SH, Perelygin A, Slifer M, Palladino-Negro P, Newman T, Chambers K, Joslyn G, Parry P, and Morin PA. 2000. A genetic linkage map of the baboon (Papio hamadryas) genome based on human microsatellite polymorphisms. Genomics 67:237-247.

Roman G and Hancu N. 2004. Metabolic syndrome--new insights into a growing entity. Rom J Intern Med 42:257-266.

Ruan H, Miles PD, Ladd CM, Ross K, Golub TR, Olefsky JM, and Lodish HF. 2002. Profiling gene transcription in vivo reveals adipose tissue as an immediate target of tumor necrosis factor-alpha: implications for insulin resistance. Diabetes 51:3176-3188.

Rutter MK, Meigs JB, Sullivan LM, D'Agostino RB, Sr., and Wilson PW. 2004. C-reactive protein, the metabolic syndrome, and prediction of cardiovascular events in the Framingham Offspring Study. Circulation 110:380-5.

Saarela T, Hiltunen M, Helisalmi S, Heinonen S, and Laakso M. 2005. Tumour necrosis factor-{alpha} gene haplotype is associated with pre-eclampsia. Mol Hum Reprod 11:437-440.

Saijo Y, Kiyota N, Kawasaki Y, Miyazaki Y, Kashimura J, Fukuda M, and Kishi R. 2004. Relationship between C-reactive protein and visceral adipose tissue in healthy Japanese subjects. Diabetes Obes Metab 6:249-58.

Sampietro T, Bigazzi F, Rossi G, Dal Pino B, Puntoni MR, Sbrana F, Chella E, and Bionda A. 2005. Upregulation of the immune system in primary hypercholesterolaemia: effect of atorvastatin therapy. J Intern Med 257:523-530.

Self SG and Liang KY. 1987. Asymptotic properties of maximum likelihood ratio tests under nonstandard conditions. J Am Stat Assoc 82:605-610.

Sethi JK and Hotamisligil GS. 1999. The role of TNF alpha in adipocyte metabolism. Semin Cell Dev Biol 10:19-29.

Shamsuzzaman AS, Winnicki M, Wolk R, Svatikova A, Phillips BG, Davison DE, Berger PB, and Somers VK. 2004a. Independent association between plasma leptin and C-reactive protein in healthy humans. Circulation 109:2181-2185.

Shamsuzzaman AS, Winnicki M, Wolk R, Svatikova A, Phillips BG, Davison DE, Berger PB, and Somers VK. 2004b. Independent association between plasma leptin and C-reactive protein in healthy humans. Circulation 109:2181-2185.

Shibuya K, Shirakawa J, Kameyama T, Honda S, Tahara-Hanaoka S, Miyamoto A, Onodera M, Sumida T, Nakauchi H, Miyoshi H, and Shibuya A. 2003. CD226 (DNAM-1) is involved in lymphocyte function-associated antigen 1 costimulatory signal for naive T cell differentiation and proliferation. J Exp Med 198:1829-1839.

Srinivasan S, Ohsugi M, Liu Z, Fatrai S, Bernal-Mizrachi E, and Permutt MA. 2005. Endoplasmic reticulum stress-induced apoptosis is partly mediated by reduced insulin signaling through phosphatidylinositol 3-kinase/Akt and increased glycogen synthase kinase-3beta in mouse insulinoma cells. Diabetes 54:968-975.

Staiger H and Haring HU. 2005. Adipocytokines: fat-derived humoral mediators of metabolic homeostasis. Exp Clin Endocrinol Diabetes 113:67-79.

Staros EB. 2005. Innate immunity: New approaches to understanding its clinical significance. Am J Clin Pathol 123:305-312.

Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, and Lazar MA. 2001. The hormone resistin links obesity to diabetes. Nature 409:307-312.

Streetz KL, Wustefeld T, Klein C, Manns MP, and Trautwein C. 2001. Mediators of inflammation and acute phase response in the liver. Cell Mol Biol (Noisy -le-grand) 47:661-673.

Tejero ME, Cole SA, Cai G, Peebles KW, Freeland-Graves JH, Cox LA, Mahaney MC, Rogers J, VandeBerg JL, Blangero J, and Comuzzie AG. 2005. Genome-wide scan of

resistin mRNA expression in omental adipose tissue of baboons. Int J Obes Relat Metab Disord 29:406-412.

Tomiyama H, Okazaki R, Koji Y, Usui Y, Hayashi T, Hori S, and Yamashina A. 2005. Elevated C-reactive protein: a common marker for atherosclerotic cardiovascular risk and subclinical stages of pulmonary dysfunction and osteopenia in a healthy population. Atherosclerosis 178:187-192.

Tontonoz P, Nagy L, Alvarez JG, Thomazy VA, and Evans RM. 1998. PPARgamma promotes monocyte/macrophage differentiation and uptake of oxidized LDL. Cell 93:241-252.

Trayhurn P. 2005. Adipose tissue in obesity--an inflammatory issue. Endocrinology 146:1003-1005.

Trayhurn P and Wood IS. 2004. Adipokines: inflammation and the pleiotropic role of white adipose tissue. Br J Nutr 92:347-355.

Valyasevi RW, Jyonouchi SC, Dutton CM, Munsakul N, and Bahn RS. 2001. Effect of tumor necrosis factor-alpha, interferon-gamma, and transforming growth factor-beta on adipogenesis and expression of thyrotropin receptor in human orbital preadipocyte fibroblasts. J Clin Endocrinol Metab 86:903-908.

Vanderlaan PA and Reardon CA. 2005. Thematic review series: the immune system and atherogenesis. The unusual suspects:an overview of the minor leukocyte populations in atherosclerosis. J Lipid Res 46:829-838.

Venugopal SK, Devaraj S, Yuhanna I, Shaul P, and Jialal I. 2002. Demonstration that C-reactive protein decreases eNOS expression and bioactivity in human aortic endothelial cells. Circulation 106:1439-1441.

Verma S, Li SH, Wang CH, Fedak PW, Li RK, Weisel RD, and Mickle DA. 2003. Resistin promotes endothelial cell activation: further evidence of adipokine-endothelial interaction. Circulation 108:736-740.

Vikram NK, Misra A, Dwivedi M, Sharma R, Pandey RM, Luthra K, Chatterjee A, Dhingra V, Jailkhani BL, Talwar KK, and Guleria R. 2003. Correlations of C-reactive protein levels with anthropometric profile, percentage of body fat and lipids in healthy adolescents and young adults in urban North India. Atherosclerosis 168:305-13.

Wang CH, Li SH, Weisel RD, Fedak PW, Dumont AS, Szmitko P, Li RK, Mickle DA, and Verma S. 2003. C-reactive protein upregulates angiotensin type 1 receptors in vascular smooth muscle. Circulation 107:1783-1790.

Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, and Ferrante AW, Jr. 2003. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 112:1796-1808.

Wellen KE and Hotamisligil GS. 2005. Inflammation, stress, and diabetes. J Clin Invest 115:1111-1119.

Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, and Chen H. 2003. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest 112:1821-1830.

Xydakis AM, Case CC, Jones PH, Hoogeveen RC, Liu MY, Smith EO, Nelson KW, and Ballantyne CM. 2004. Adiponectin, inflammation, and the expression of the metabolic syndrome in obese individuals: the impact of rapid weight loss through caloric restriction. J Clin Endocrinol Metab 89:2697-2703.

Yasojima K, Schwab C, McGeer EG, and McGeer PL. 2001. Generation of C-reactive protein and complement components in atherosclerotic plaques. Am J Pathol 158:1039-1051.

You T, Yang R, Lyles MF, Gong D, and Nicklas BJ. 2005. Abdominal adipose tissue cytokine gene expression: relationship to obesity and metabolic risk factors. Am J Physiol Endocrinol Metab 288:E741-E747.

Zhang D, Sun M, Samols D, and Kushner I. 1996. STAT3 participates in transcriptional activation of the C-reactive protein gene by interleukin-6. J Biol Chem 271:9503-9509.

VITA

John Michael Proffitt was born on July 23rd, 1978, the son of Larry and Peggy Proffitt in San Antonio, TX. In 1996, Michael graduated 6th in his class from John Marshall High School in San Antonio, and entered Southern Methodist University in Dallas, Texas as a Hunt Leadership Scholar. He graduated from Southern Methodist University with a Bachelor of Science in Biology and a Bachelor of Arts in Anthropology in 2000. Michael entered the Graduate School of The University of Texas in the fall of 2000.

Permanent Address: 6307 Rue Sophie, San Antonio, TX 78238

This dissertation was typed by the author.