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ORIGINAL INVESTIGATION

Genetic admixture, adipocytokines, and adiposity in Black Americans: the Health, Aging, and Body Composition study

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Abstract Adipocytokines are a subset of cytokines produced by adipose tissue and are associated with risk of type II diabetes and atherosclerosis. Levels of adipocytokines differ between Black and White Americans, even after adjustment for differences in adiposity, diseases associated with adipocytokines including type 2 diabetes and cardiovascular disease, and general socioeconomic status indicators such as income. We used a series of ancestry informative markers to estimate genetic ancestry in a population-based study of older Black Americans, and examined the association between genetic ancestry and adipocytokines and soluble receptors to help determine which of these may be most amenable to admixture mapping. We typed 35 ancestry informative markers in 1,241 self-reported Black Americans with available DNA from the Health, Aging, and Body Composition (Health ABC) study with available DNA and used a maximum likelihood approach to estimate percent European ancestry. We used linear regression models to determine the association between these adipocytokines and percent ancestry, and staged models to examine whether adiposity or other measures affected the associations of genetic ancestry and adipocytokines. Mean European ancestry was $22.3 \pm 15.9\%$. In multivariate adjusted models, the strongest associations observed were between higher European ancestry and interleukin-6 soluble receptor (IL-6 SR), C-reactive protein (CRP), and adiponectin levels, with interleukin-2 soluble receptor (IL-2 SR) and soluble tumor necrosis factor receptor II (TNF-α SR II) also showing more modest but significant associations. The association with adiponectin became stronger after adjustment for adiposity. These novel findings suggest that admixture mapping may identify genetic factors influencing the levels of IL-6 SR, CRP, IL-2 SR, and adiponectin.

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Introduction

Adipocytokines are a subset of cytokines that are partially produced or regulated by adipose tissue and are known to be associated with several complex age-associated chronic diseases. Higher levels of interleukin (IL)-6 (Pickup et al.

2000; Pradhan et al. 2001), leptin (Haffner et al. 1999), plasminogen activator inhibitor (PAI)-1 (Festa et al. 2002; Kanaya et al. 2006a), tumor necrosis factor (TNF)-α (Pickup et al. 2000; Winkler et al. 1998), C-reactive protein (CRP) (Pradhan et al. 2001; Yuan et al. 2006) and lower levels of adiponectin (Weyer et al. 2001) are associated with increasing insulin resistance and type 2 diabetes. Higher levels of CRP (Cesari et al. 2003; Cushman et al. 2005; Tuomisto et al. 2006), TNF-α (Cesari et al. 2003; Tuomisto et al. 2006), PAI-1 (Kohler and Grant 2000; Agirbalsi 2005), and IL-6 (Cesari et al. 2003; Tuomisto et al. 2006), and lower adiponectin (Pischon et al. 2004) are associated with increased risk of cardiovascular events.

Levels of several adipocytokines are known to differ by ethnicity. Mean levels of CRP (Khera et al. 2005), leptin (Ruhl and Everhart 2002), TNF-α (Visser et al. 2002), IL-6 (Visser et al. 2002), PAI-1 (Festa et al. 2003), and adiponectin (Hulver et al. 2004; Kanaya et al. 2006b) have all been found to differ between Black and White Americans. Distribution and amount of adipose tissue, particularly abdominal visceral fat, have also been found to differ by ethnicity (Ruhl et al. 2004; Ryan et al. 2002; Araneta and Barrett-Connor 2005). Since adipose tissue is known to secrete adipocytokines, and distribution of adipose tissue and levels of adipocytokines often differ by ethnicity, adiposity may mediate any relationships between adipocytokines and ethnicity, as might other factors that differ systematically by race, including certain chronic diseases and socioeconomic factors.

African Americans are known to be an admixed population with ancestry from Africans, and to a smaller degree Europeans (Parra et al. 1998). Genetic ancestry may be estimated among African American individuals using a set of ancestry informative markers (Chakraborty et al. 1986; Shriver et al. 1997) and individual ancestry estimates can be used to assess the association between ancestry and disease within an admixed population. In turn, these studies of ancestry and phenotypic traits can help to identify those traits most likely to benefit from admixture mapping, a method for efficiently mapping complex traits (McKeigue 2005). This approach takes advantage of the long-range linkage disequilibrium (LD) between ancestry informative markers in recently admixed populations (McKeigue et al. 2000). If a trait is associated with individual ancestry in an admixed population, admixture mapping may be a useful method of identifying loci that underlie it (Hoggart et al. 2004; Patterson et al. 2004; Smith et al. 2004). Thus, admixed populations such as these can provide unique insight into potential genetic factors that may underlie complex disease traits that differ by ancestry. By analyzing the association between genetic ancestry and these traits we may determine how promising these traits are for admixture mapping.

We used a panel of ancestry informative markers to estimate genetic ancestry in a large cohort of individuals who self-reported their race as "Black" and participated in the Health, Aging, and Body Composition (Health ABC) study. We evaluated the association between genetic ancestry and several adipocytokines and adipocytokine soluble receptors, including CRP, TNF-α, PAI-1, IL-6, leptin, adiponectin, interleukin-6 soluble receptor (IL-6 SR), interleukin-2 soluble receptor (IL-2 SR), TNF-α soluble receptor I (TNF-α SR I), and TNF-α soluble receptor II (TNF-α SR II). We also evaluated whether the distribution of adiposity mediated the association between ancestry and adipocytokines, and whether the addition of other potential mediator variables associated with the metabolic syndrome further changed these associations.

Materials and methods

Study subjects

The Health ABC study enrolled a total of 3,075 well functioning older men and women aged 70–79 between April 1997 and June 1998. The participants were a sample of Medicare beneficiaries residing in the areas surrounding Pittsburgh, PA and Memphis, TN. Inclusion criteria for the study were that participants had to report no difficulty in walking 1/4 mile, climbing ten steps, or performing basic activities of daily living. Exclusion criteria included any individual requiring assistive devices for ambulation, having difficulty performing activities of daily living, having life-threatening illness or having a diagnosis of cancer that required treatment within the past 3 years, or planning to leave the area within 3 years. Since ancestry informative markers were typed only on 1,241 Black participants with available DNA, including 534 men and 707 women, our analyses were restricted to this sub-sample. While African-American is the generally accepted terminology in admixture studies, we use the term "Black American" to refer to these particular study subjects since the terms "White American" and "Black American" were used at the time of study enrollment.

Data collection and variable definitions

Questionnaire variables collected at the time of the baseline visit included self-report race, age, sex, education level, and income. Aspirin use, statin use, and non-steroidal anti-inflammatory use were obtained via a medication inventory at the same time. Prevalent coronary heart disease (CHD) was obtained at the baseline visit by self-report and hospitalization records and included history of coronary artery bypass graft (CABG), percutaneous transluminal coronary

angioplasty (PTCA), myocardial infarction, or angina plus use of anti-anginal medication. Prevalent diabetes was determined by self-report, use of anti-diabetic medications, fasting plasma glucose >126 mg/dl, or 2-h oral glucose tolerance test >200 mg/dl. Systolic and diastolic blood pressures were measured by manual sphygmomanometer with participants in a seated position.

Weight and height were measured on a standard balance beam scale to the nearest 0.1 kg and by a stadiometer to the nearest 0.1 cm, respectively. Body mass index (BMI) was calculated as body weight in kilograms divided by height in meters squared. Abdominal visceral fat was assessed by computed tomography (CT) scans using Somatom Plus 4 (Siemens, Erlangen, Germany), Picker PQ 2000S (Marconi Medical Systems, Cleveland, OH, USA), or a 9800 Advantage scanner (General Electric, Milwaukee, WI, USA) with standardized protocols and was measured at the L4–L5 vertebrae. Using ILD development software (RSI Systems, Boulder, CO, USA), visceral and abdominal fat areas were calculated by multiplying the number of pixels by the pixel area for each type of fat and these measures are expressed in units of cm^2. By using the internal abdominal wall fascial plane, visceral fat was differentiated visually from other fats such as subcutaneous fat. Of the 1,241 Black participants, a total of 27 were missing abdominal visceral fat.

After an overnight fast of at least 8 h, participants had venipuncture performed at the baseline visit. Serum samples were stored, frozen at −70°C, at McKesson BioServices, Rockville, MD, USA. CRP was measured by colorimetric competitive ELISA (For CRP: Macy laboratory, University of Vermont, Burlington, VT) with an intra-assay coefficient of variation (CV) of 5.14%. Both adiponectin and leptin were measured by radioimmunoassay (Linco Research, St. Charles, MO, USA) and had a CV of 1.8–3.6 and 3.7–7.5%, respectively. IL-6 and TNF-α were measured by solid phase ELISA (R&D Systems, Minneapolis, MN, USA) with CVs of 13.1–18.1% for IL-6 and 14.8–14.9% for TNF-α. PAI-1 was measured by a two-site ELISA (For PAI-1: Collen laboratory, Katholieke Universitat Leuven, Leuven, Belgium) with a CV of 3.47%. IL-2 SR, IL-6 SR, TNF-α SR I, and TNF-α SR II were all measured by ultra-sensitive ELISA (R&D Systems) and had CVs of 5.0–6.7, 7.8–8.4, 3.5–5.2, and 4.3–4.9%, respectively. A total of 100 participants were missing leptin, 61 IL-6, 17 PAI-1, 85 TNF-α, 2 adiponectin, and 4 CRP. The soluble receptors, including TNF-α SR I, TNF-α SR II, IL-6 SR, and IL-2 SR, were assayed on roughly half of the cohort. Triglyceride and HDL levels were measured using a colorimetric assay (Orthoclinical Diagnostics, Raritan, NJ, USA). Fasting plasma glucose was measured using automated glucose oxidase reaction (YSI 2300 Glucose Analyzer, Yellow Springs, OH, USA) and fasting serum insulin was measured by radioimmunoassay (Pharmacia, Uppsala, Sweden).

Genotype frequencies in ancestral populations were estimated using 120 sub-Saharan Africans from Senegal, Ghana, Cameroon, and Botswana, and 78 European Americans from Chicago and Baltimore (Smith et al. 2004). Forty-one single nucleotide polymorphisms (SNPs) that are useful as ancestry informative markers (Smith et al. 2004) were typed for each of the 1,241 participants using the MassARRAY system (Sequenom, San Diego, CA, USA). Six of the 41 markers had high discordance rates, so 35 of the 41 markers were used in the final analysis. Using a 5% blind duplicate sample, the overall concordance rate for these 35 markers was 99.8%. Four participants were excluded from the analysis due to discrepancies between self-reported and genetically identified race status.

Statistical analysis

Estimation of ancestry

We used a maximum likelihood approach to estimate genetic ancestry. Briefly, the likelihood of ancestry based on each individual's genotype was assessed using the ancestral population allele frequencies. The sum of the log-likelihoods of individual ancestry from all of the markers was maximized as a function of that person's ancestry, allowing ancestry to take a range between 0 and 1 (Chakraborty et al. 1986). Using STRUCTURE (Pritchard et al. 2000), we determined a 2-population model, where the proportions of African and European ancestry for each individual sum to 1, was the most appropriate.

Tests of Hardy–Weinberg proportions (HWE) were carried out using Haploview Version 3.2 (Barrett et al. 2005). Markers not in HWE were not excluded from further analyses, as it is expected in populations with substructure that there may be deviations from HWE, especially at ancestry informative markers, and that this would tend to favor excess homozygosity (Wittke-Thompson et al. 2005). Tests of LD among markers were carried out using EMLD, September 2003 Version (MD Anderson Cancer Center, Houston, TX, USA). Since the markers are physically unlinked, then LD between pairs of these markers should be due to heterogeneity in individual ancestry, which provides a complimentary analysis of evidence of genetic substructure and/or recent admixture (Choudhry et al. 2006).

Statistical modeling

Unadjusted associations of baseline covariates, adiposity measures, and adipocytokines with European ancestry by quartile were assessed using chi-square, ANOVA, or, for variables whose distribution appeared skewed, the Kruskal–Wallis test. We log transformed the adipocytokine levels to meet assumptions about normality for multivariate

analyses. In addition, BMI and abdominal visceral fat were divided by their standard deviations to more directly compare associations with these secondary predictors.

Multivariate linear regression was used to examine the adjusted associations of percent European ancestry and adipocytokines, entering ancestry as a linear predictor variable. To obtain an estimate of the association between ancestry and the adipocytokine levels that was independent from variation of ancestry in our dataset, we divided the ancestry variable by its standard deviation. Since the adipocytokines were natural log transformed, the transformation $100 \times (e^{\beta}\text{-}1)$ was used for the beta coefficients to obtain the percent decrease or increase in the adipocytokine per standard deviation increase in European ancestry. In stages, we examined the unadjusted models, models adjusted for covariates, models adjusted for covariates and adiposity measures, and models adjusted for covariates, adiposity measures plus additional potential mediator variables to examine the potential mediation effects of these variables on the relationship between percent European ancestry and adipocytokines. Potential interactions of the ancestry and gender were assessed in each adipocytokine model. All covariates were included in the final models, regardless of significance. *P*-values of <0.05 were considered statistically significant. SAS Version 8.2 (SAS Institute, Cary, NC, USA) was used for all modeling analyses.

Results

Fifteen of the 35 markers are not in Hardy–Weinberg Equilibrium (HWE) (Table 1) with all 15 demonstrating excess homozygosity compared with the expected proportions under HWE. We also tested for allelic association between markers. Since these markers are either on different chromosomes or far from each other on physical and genetic maps, the LD between them is a function of their ancestry informativeness and of how recent the admixture has occurred. Remarkably, we found that 549 of 595 pairs of markers were in significant LD although the r^2-values were all <0.20, reflecting modest associations (Supplementary Table 1). The mean individual European ancestry was 22.1 ± 15.3%. We found significantly higher European ancestry among participants from Pittsburgh (25.1 ± 16.7%) compared with participants from Memphis (18.7 ± 12.8%), $P < 0.001$.

Univariate associations for European ancestry by quartile for abdominal visceral fat, CRP, TNF-α, PAI-1, and IL-6 SR were either statistically significant or marginally statistically significant. Abdominal visceral fat, PAI-1, and IL-6 SR increased with increasing European ancestry quartiles, while CRP decreased with increasing quartiles of European ancestry (Table 2). In unadjusted linear regression models, ancestry was significantly associated with PAI-1, CRP, and adiponectin (Table 3). Each standard deviation increase in European ancestry was associated with a 9.4% (4.5, 14.5) increase in PAI-1, a 7.4% (−11.9, −2.5) decrease in CRP, and a 4.1% (0.26, 8.0) increase in adiponectin. Leptin, TNF-α, and IL-6 were not significantly associated with ancestry in unadjusted models. IL-6 SR also had a significant relationship with ancestry in unadjusted models, with a one standard deviation increase in ancestry corresponding to a 4.1% (2.2, 6.0) increase in IL-6 SR (Table 4).

In multivariate models adjusted for age, site, gender, education, income, prevalent CHD, prevalent diabetes, statin use, aspirin use, and NSAID use, the relationships of ancestry per standard deviation and CRP, leptin, adiponectin, and PAI-1, were attenuated, while the relationships of ancestry with TNF-α SR I, TNF-α SR II, and IL-2 SR were strengthened (Tables 3, 4). The addition of BMI and abdominal visceral fat to these multivariate models with the above covariates strengthened the association between European ancestry and adiponectin and CRP levels (Tables 3, 4). A standard deviation increase in ancestry was associated with a 4.7% (0.76, 8.8) increase in adiponectin, and a 7.6% (−12.6, −2.2) decrease in CRP (Table 3) when adiposity measures were added. The association between ancestry and PAI-1 was substantially attenuated by the addition of adiposity measures (Table 3). With the addition of traits associated with the metabolic syndrome including systolic blood pressure, diastolic pressure, HDL cholesterol, triglycerides, fasting glucose, and fasting insulin to these models, the associations of ancestry with adiponectin became stronger, with more modest strengthening effects on CRP, IL-6 SR, TNF-α SR I and TNF-α SR II (Table 3, 4). The association between ancestry and PAI-1 was further attenuated with the addition of the above mediators (Table 3). The effect of the addition of the metabolic syndrome traits was mainly due the addition of HDL to the models in the case of adiponectin, while fasting insulin had a greater effect in the soluble receptor models (results not shown). The associations of ancestry with adiponectin, CRP, IL-2 SR, IL-6 SR, and TNF-α SR II were all statistically significant in the final stage of adjustment. We found no evidence that the association of European ancestry and any adipocytokine or adipocytokine soluble receptor differed by gender.

Discussion

European ancestry was associated with a number of adipocytokines and adipocytokine soluble receptors in the Health ABC cohort. Overall, higher European ancestry was significantly associated with higher levels of adiponectin, IL-2 SR, IL-6 SR, and TNF-α SR II and lower levels of CRP in models adjusted for a variety of potential confounders and

Table 1 Chromosome locations, allele frequencies, and Hardy–Weinberg (HWE) equilibrium

Ancestry informative marker	Location		Reference/variant allele	African variant allele frequency	European variant allele frequency	HWE *P*-value
	Chromosome	Hg17 position				
rs734908	1	53656058	G/T	0.01	0.75	0.0360
rs6662385	1	72885596	C/T	0.04	0.73	0.3280
rs2814778	1	155987756	C/T	0.00	1.00	0.1900
rs2339475	2	29911441	A/G	0.98	0.26	<0.0001
rs2060447	2	163426060	C/T	0.94	0.06	<0.0001
rs1525760	4	117492984	C/T	0.05	0.88	0.5620
rs1530044	4	166785016	A/T	0.11	0.90	0.0220
rs930072	5	36701828	C/T	0.05	0.87	0.0050
rs3024354	6	6237429	G/T	0.04	0.82	0.0700
rs1454436	6	156941024	C/T	0.07	0.74	<0.0001
rs2965404	7	21522742	C/T	0.06	0.83	0.0650
rs1011024	7	98842052	A/G	0.97	0.12	0.0070
rs1861141	7	153498093	A/G	0.17	0.94	0.9890
rs7865808	9	2903620	A/G	0.17	0.98	0.0260
rs2301550	9	20344796	A/G	0.88	0.03	0.0270
rs803733	9	122912433	C/T	0.99	0.12	0.1520
rs4242762	10	3797541	C/G	0.05	0.62	0.7050
rs2394931	10	74336071	A/G	0.14	0.97	0.0010
rs9418990	10	135226847	A/G	0.89	0.18	0.9999
rs680273	11	64296211	C/G	0.93	0.09	0.6260
rs10791800	11	105628804	G/T	0.99	0.22	0.0070
rs959354	11	129514791	A/G	0.83	0.07	0.6790
rs4759816	12	129980322	A/G	0.84	0.05	0.9030
rs913607	13	105708805	C/G	0.90	0.20	0.0010
rs1956485	14	25933230	C/T	0.88	0.16	0.3600
rs3825663	14	89499502	C/T	0.14	0.92	0.3660
rs1426654	15	46213776	A/G	0.97	0.01	0.1060
rs1374092	15	70595482	A/C	0.86	0.08	0.0660
rs7201030	16	75081293	A/G	0.04	0.89	0.0280
rs959071	17	19082819	C/T	0.94	0.15	0.1260
rs1941141	18	7620982	A/G	0.97	0.19	<0.0001
rs4436849	18	28809444	A/C	0.75	0.01	0.7420
rs11878536	19	47115315	C/G	0.13	0.94	0.3800
rs6034866	20	17551728	A/G	0.05	0.92	0.4280
rs4821667	22	36154581	A/C	0.03	0.77	0.0020

mediators. As the associations were adjusted for covariates and adiposity, the adiposity measures generally strengthened the relationship between percent European ancestry and the adipocytokines, with the exception of PAI-1 and TNF-α. Associations of European ancestry and the adipocytokine soluble receptors were not altered by the addition of adiposity measures. Addition of further potential mediator variables associated with the metabolic syndrome (i.e., systolic blood pressure, diastolic blood pressure, HDL cholesterol, triglycerides, fasting glucose, and fasting insulin) in the multivariate models resulted in stronger associations of European ancestry with adiponectin, CRP, IL-6 SR, TNF-α SR I, and TNF-α SR II showing evidence of negative confounding by these variables. More specifically, HDL cholesterol drives the change in ancestry-adiponectin association. This is consistent with the strong mediation effect of HDL on the association between adiponectin and CHD seen in analyses of the Health ABC data comparing Blacks and Whites (Kanaya et al. 2006b). Diabetes was more prevalent among persons with greater African ances-

Table 2 Characteristics of Black participants in Health ABC at baseline, 1997–1998 by quartile of percent European ancestry

	Quartile 1 admixture	Quartile 2 admixture	Quartile 3 admixture	Quartile 4 admixture	*P*-value*
	≤10.8%	10.9–19.3%	19.4–30.2%	≥30.3%	
	n = 313	*n* = 308	*n* = 308	*n* = 308	
Age, years	73.3 ± 2.8	73.4 ± 3.0	73.3 ± 2.8	73.7 ± 2.9	0.2781
Gender					
Male	143 (45.7)	126 (40.9)	143 (46.4)	121 (39.3)	0.1971
Female	170 (54.3)	182 (59.1)	165 (53.6)	187 (60.7)	
Study site					
Memphis, TN	170 (54.3)	178 (57.8)	135 (43.8)	99 (32.1)	<0.0001
Pittsburgh, PA	143 (45.7)	130 (42.2)	173 (56.2)	209 (67.9)	
Prevalent CHD	81 (25.9)	72 (23.4)	78 (25.3)	77 (25.0)	0.9024
Prevalent diabetes	104 (33.2)	95 (30.8)	88 (28.6)	80 (26.0)	0.2313
Current aspirin use	84 (26.8)	84 (27.3)	111 (36.0)	113 (36.7)	0.0069
Current NSAID use	72 (23.0)	70 (22.7)	70 (22.6)	63 (20.5)	0.8320
Current statin use	24 (7.7)	36 (11.7)	33 (10.7)	44 (14.3)	0.0797
Fasting plasma glucose, mg/dl	115.7 ± 56.5	107.3 ± 32.8	109.6 ± 38.7	107.8 ± 38.4	0.3310
Fasting insulin, μIU/ml	8.7 ± 4.9	9.8 ± 12.0	9.5 ± 6.5	8.9 ± 5.9	0.7864
Trigylcerides, mg/dl	116.7 ± 53.1	106.9 ± 43.4	118.4 ± 65.1	133.4 ± 103.2	0.0004
HDL cholesterol, mg/dl	57.6 ± 17.9	58.8 ± 18.2	57.1 ± 16.6	55.1 ± 17.2	0.0639
Systolic blood pressure, mmHg	140.7 ± 23.5	139.8 ± 22.0	138.0 ± 22.0	138.2 ± 21.8	0.3655
Diastolic blood pressure, mmHg	74.0 ± 13.2	73.7 ± 12.2	73.4 ± 12.2	73.5 ± 11.9	0.9475
BMI, kg/m^2	28.8 ± 5.3	28.7 ± 6.1	28.7 ± 5.3	28.4 ± 4.9	0.8104
Abdominal visceral fat, cm^2	127.7 ± 61.4	126.4 ± 62.1	128.8 ± 57.2	137.2 ± 67.4	0.1340
CRP, mg/l	3.8 ± 4.6	3.7 ± 4.5	3.6 ± 6.1	3.2 ± 3.9	0.0868
TNF-α, pg/ml	3.4 ± 2.0	3.1 ± 1.4	3.3 ± 2.1	3.4 ± 1.6	0.0407
Adiponectin, μg/ml	9.0 ± 6.1	10.0 ± 6.9	9.5 ± 6.2	9.6 ± 5.7	0.2639
PAI-1, ng/ml	26.4 ± 26.7	26.9 ± 23.5	26.9 ± 21.7	30.8 ± 23.0	0.0057
IL-6, pg/ml	2.7 ± 2.2	2.8 ± 2.1	2.5 ± 2.1	2.5 ± 1.9	0.2852
Leptin, ng/ml	15.3 ± 12.2	15.3 ± 12.8	14.8 ± 11.2	15.2 ± 10.8	0.8715
IL-6 soluble receptor, pg/ml	30,956.5 ± 7,243.2	31,836.7 ± 7,962.6	33,296.2 ± 8,321.4	34,058.2 ± 7,749.1	0.0012
IL-2 soluble receptor, pg/ml	1,228.4 ± 916.1	1,198.4 ± 642.5	1,263.3 ± 677.9	1,256.2 ± 496.2	0.1604
TNF I soluble receptor, pg/ml	1,588.9 ± 777.4	1,551.6 ± 661.5	1,511.9 ± 557.2	1,494.0 ± 449.4	0.9444
TNF II soluble receptor, pg/ml	3,433.5 ± 917.3	3,375.0 ± 915.5	3,422.9 ± 852.8	3,491.0 ± 846.9	0.6291

**P*-values obtained using chi-square, ANOVA or Kruskal–Wallis tests where appropriate

try. Controlling for diabetes, fasting insulin level strengthened the association between admixture and the TNF-α soluble receptors. Since TNF-α has a relatively short half-life, the receptors are used as a surrogate for TNF-α activation. TNF-α has been associated with increased level of insulin resistance (Steinburg et al. 2006). However, in this cross-sectional context, it is unclear whether the stronger association of TNF soluble receptors with European ancestry represents a treatment effect or a potential genetic effect. Interestingly, adjustment for metabolic syndrome characteristics for PAI-1 further attenuated this association, suggesting that the metabolic variables may lie in the causal pathway between ancestry and PAI-1.

Estimates of European ancestry in our cohort are consistent with other studies among African Americans (Parra et al. 1998; Tang et al. 2006). For example, Parra et al. (1998) observed around 20% European ancestry for African American individuals from Pittsburgh and around 12% in Charleston, SC. We observed about 25% European ancestry on average for Blacks residing in Pittsburgh, and about 19% for Blacks in Memphis, a statistically significant difference. Previous studies have also demonstrated significant variation among different regions in the US (Parra et al. 1998; Reiner et al. 2005).

Our analysis of individual genetic ancestry found a substantial range of individual ancestry in the population.

Table 3 Percent difference (95% CI) in adipocytokines per standard deviation increase of European ancestry

	PAI-1	*P*	Adiponectin	*P*	Leptin	*P*	CRP	*P*	IL-6	*P*	TNF-α	*P*
Unadjusted	9.4 (4.5, 14.5)	0.0001	4.1 (0.26, 8.0)	0.04	4.2 (−2.2, 11.1)	0.20	−7.4 (−11.9, −2.5)	0.004	−2.6 (−6.4, 1.2)	0.18	2.3 (−0.40, 5.0)	0.10
+Covariates[a]	5.8 (0.75, 11.2)	0.02	2.3 (−1.7, 6.4)	0.26	−1.5 (−7.2, 4.6)	0.63	−5.2 (−10.5, 0.38)	0.07	−0.24 (−4.5, 4.2)	0.91	2.6 (−0.45, 5.6)	0.10
+Adiposity[b]	2.4 (−2.3, 7.3)	0.33	4.7 (0.76, 8.8)	0.02	−3.7 (−8.3, 1.1)	0.13	−7.6 (−12.6, −2.2)	0.006	−1.8 (−6.1, 2.6)	0.41	1.6 (−1.4, 4.7)	0.30
+Mediators[c]	0.87 (−4.0, 6.0)	0.73	7.1 (3.0, 11.3)	0.0008	−2.4 (−7.6, 3.0)	0.37	−8.6 (−14.1, −2.8)	0.004	−2.4 (−7.0, 2.3)	0.31	1.0 (−2.2, 4.3)	0.53

[a] Includes age, site, gender, education, income, prevalent CHD, prevalent diabetes, statin use, aspirin use, and NSAID use

[b] BMI and abdominal visceral fat added

[c] Fasting plasma glucose, fasting insulin, systolic blood pressure, diastolic blood pressure, triglycerides, and HDL cholesterol added

Variation in individual percent ancestry measurements such as these is partially due to statistical error in the measurement of individual ancestry, as well as to true differences in percent ancestry among individuals. However, even without the use of ancestry estimation estimates, there is substantial evidence from our data of heterogeneity in the genetic background of individuals. LD between markers on different chromosomes provides a non-parametric test of heterogeneity in individual ancestry (Choudhry et al. 2006). Our study found that 549 of 595 marker pairs are in significant LD at the nominal level ($P < 0.05$). This is much higher than the expected number of pairs under the null hypothesis (~5% or ~30 pairs of markers). The much higher than expected rate of association is most likely due to the informativeness of the markers we chose, the large sample size, and also to the heterogeneity of individual ancestry within our dataset. Deviations from HWE can also be used to suggest evidence of substructure, and in particular, evidence of non-random mating within the population. Fifteen of 35 markers were not in HWE and, as would be expected in populations with this substructure, we found excess homozygosity with these markers.

Adiposity measures, in particular visceral adiposity, generally strengthened the relationship of admixture with each adipocytokine. Previous analyses of our data demonstrate that European ancestry is associated with abdominal visceral fat (Hsueh et al. 2006). The strong effect of visceral fat on the associations we observed is due to the fact that visceral fat is associated with greater percent European ancestry. Visceral fat is known to be associated with higher levels of adipocytokines. This complex relationship suggests that in Black Americans with greater percent European ancestry, visceral fat volume is greater, and that the difference in visceral fat volume is associated with adipocytokine levels. In particular, we find a stronger association between higher adiponectin and European ancestry and between lower leptin and European ancestry after adjustment for visceral fat. Since the presence of greater abdominal visceral fat is associated with lower adiponectin and higher leptin, the association between higher adiponectin, lower leptin, and European ancestry is only revealed after adjusting for visceral fat. But, as noted above, these associations may still be confounded by unmeasured non-genetic factors that correlate with ancestry so this association may reflect genetic or non-genetic factors.

Previous studies have found adipocytokine levels to differ by race and gender groups. Khera et al. (2005) found race and gender differences in levels of CRP, with Blacks having higher CRP levels than Whites, and men having higher CRP levels than women. A study by Festa et al. (2003) found PAI-1 levels were lower in Blacks than in Whites. Hulver et al. (2004) found the same association in another study. Prior studies of Health ABC participants

Table 4 Percent difference (95% CI) in soluble receptors per standard deviation increase in European ancestry

	IL-2 SR	*P*	IL-6 SR	*P*	TNF-α SR I	*P*	TNF-α SR II	*P*
Unadjusted	3.1 (−0.22, 6.6)	0.07	4.1 (2.2, 6.0)	<0.001	0.07 (−2.5, 2.7)	0.96	0.86 (−1.1, 2.9)	0.40
+Covariates[a]	5.2 (1.3, 9.2)	0.009	3.8 (1.8, 5.8)	0.0002	1.8 (−1.2, 4.8)	0.24	2.1 (−0.18, 4.4)	0.07
+Adiposity[b]	5.2 (1.2, 9.4)	0.01	3.8 (1.7, 5.9)	0.0003	1.5 (−1.5, 4.7)	0.32	1.9 (−0.42, 4.3)	0.11
+Mediators[c]	5.6 (1.3, 10.1)	0.01	4.7 (2.4, 7.0)	<0.0001	2.4 (−0.95, 5.9)	0.16	2.9 (0.27, 5.5)	0.03

[a] Includes age, site, gender, education, income, prevalent CHD, prevalent diabetes, statin use, aspirin use, and NSAID use

[b] BMI and abdominal visceral fat added

[c] Fasting plasma glucose, fasting insulin, systolic blood pressure, diastolic blood pressure, triglycerides, and HDL cholesterol added

have compared Blacks and Whites and have also demonstrated differences in some of these markers. Ruhl et al. (2004) found leptin concentrations were higher in women than in men and higher in Blacks than in Whites using Health ABC data. Visser et al. (2002) found higher levels of IL-6 among Black men compared to White men, and Black women compared to White women using data from participants of the Health ABC study while the pattern with TNF was opposite. Kanaya et al. (2006b) found adiponectin concentrations were lower in Blacks than in Whites. Our analyses of ancestry within Blacks in the Health ABC study are consistent with data from the Health ABC study and in the other studies that have compared adipocytokines between these groups. Specifically, we found increasing European ancestry significantly associated with lower CRP levels and higher adiponectin levels. For some markers (IL-6, TNF-α, and leptin) we found no significant association, although the direction of association for these markers was consistent with the direction of association seen in previous studies comparing Blacks and Whites.

Our study is also consistent with the recent genetic admixture study that found a trend, although non-significant, toward lower levels of CRP with higher percent of European ancestry (Reiner et al. 2005); however, this study did not address the possible change in association between CRP and admixture by adjusting for adiposity measures. We found the CRP-ancestry trend to be significant, and observed a mediation of this association by adiposity measures.

Although our study includes a large sample size with a panel of markers that are highly informative for ancestry and validated measures of adipocytokines, some limitations should be considered. A relatively small number (35) of ancestry informative markers was used to obtain the percent European ancestry estimates which can lead to error in the ancestry estimates a (Risch et al. 2002); Rosenberg et al. 2003). However, since the error in measuring individual ancestry is random with respect to the outcomes in our study, this should tend to bias our results toward the null hypothesis. Thus, any of the associations observed in our study are likely an underestimate of the true association. Another potential limitation is the small number of participants with European ancestry ≥50%, 79. More participants with European ancestry ≥50% are needed to more accurately assess the associations of ancestry and adipocytokines in populations with a wider and more varied distribution of ancestry. For instance, some complex traits may exhibit non-linear associations with percent ancestry, especially if gene–gene interactions are present (Chakraborty et al. 1986). In addition, studies in other admixed populations with a different mean of ancestry (e.g., US Latinos) may be useful to determine whether the associations identified in this study are consistently identified in people with mostly European but some African ancestry. In Health ABC, ancestry informative markers were not typed on the White participants in the cohort; however, based on prior studies the proportion of African ancestry in this group is likely to be <5% (Reiner et al. 2005). Furthermore, Native American ancestry was not measured, but based on previous studies the percent Native American ancestry among Black Americans also averages <3% (Shriver et al. 2003). Since this study includes only older Black Americans aged 70–79, our results may not generalize to younger populations who often have much lower levels of adipocytokines. As African ancestry among most African Americans is West African, our markers were based on ancestral populations from that location. Thus, we cannot generalize our results to populations of mixed East African and European descent.

Phenotypes that are known to be different across ethnic groups and to be associated with ancestry in an admixed population such as African Americans are particularly good candidates for admixture mapping. Admixture mapping has recently been used to identify genetic loci for hypertension (Zhu et al. 2005), multiple sclerosis (Reich et al. 2005), and prostate cancer (Freedman et al. 2006). Based on our results, soluble IL-6 receptor, soluble IL-2 receptor, adiponectin, and CRP levels may be candidate phenotypes for admixture mapping. We might expect to identify a locus or loci with an allele/s with higher European ancestry associated with soluble IL-6 receptor, soluble IL-2 receptor and adiponectin levels and a locus with an allele(s) with higher African ancestry

associated with CRP levels. In addition, the effect of the loci for adiponectin and CRP may be more easily detected in studies that account for abdominal visceral fat.

An alternative explanation for our findings may be that the association between ancestry and these adipocytokines and adipocytokine soluble receptors is due to non-genetic confounders, which were not adequately characterized in this study. Although we adjusted for many of the known correlates adipocytokine levels, it is possible that other differences in treatment or additional socioeconomic factors for which we do not have information may underlie some of the observed associations. CRP, for example, has previously been shown to be associated with measures of SES, in particular, income level (Reiner et al. 2005). Our data showed a strong relationship between ancestry and study site, with a mean admixture proportion higher in Pittsburgh than in Memphis, as identified by others as well (Parra et al. 1998). This site association suggests there may be other contributing factors not yet accounted for and the association of ancestry with treatment with aspirin and statins indicates that there are biomedical and behavioral factors that affect this analysis. Thus, it is possible that measurement and adjustment for additional environmental confounders may reduce the associations we observed. Only by identification of particular genetic variants associated with these adipocytokines that explain the association between the adipocytokines and outcomes can we be more certain that genetic factors underlie the observed associations.

Levels of these adipocytokines are associated with many complex diseases such as diabetes and cardiovascular disease. However, it is not known whether these associations are truly causal. Studies identifying the genes and genetic variants that underlie these associations may help to resolve controversy about the causal contribution of these predictive markers, and should increase our understanding of these complex traits.

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