Supplement Material

Soluble CD14: genome-wide association analysis and relationship to cardiovascular risk and mortality in the older adults

Reiner et al; Soluble CD14 in older adults

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Measurement of plasma biomarkers

Phlebotomy was performed on the morning of enrollment after an 8–12-h fast. IL-6 and CRP were measured in baseline samples using commercial (Quantikine IL-6, R&D Systems, Minneapolis, MN, USA) and validated in-house high-sensitivity enzyme-linked-immunosorbent-assays (ELISA), respectively [1]. Baseline sCD14 was measured by commercial ELISA (R&D Systems, Minneapolis, MN, cat # CD140) in 4,609 EA and 819 AA. The inter-assay coefficients of variation ranged from 5.32% to 12.36%.

Genotyping

Genome-wide genotyping was performed on 2,952 EA and 528 AA unrelated (excluding 1st and 2nd degree relatives) CHS participants free of baseline CVD and with sCD14 measurements using the Illumina 370CNV platform. The genome-wide genotype data for 312,883 SNPs were augmented to 2.2 million SNPs by imputing unmeasured, autosomal SNPs with reference genotype data from HapMap2 and HapMap3 CEU (release 22 or 24, build 36) using MaCH [2].

We performed fine-mapping of the *CD14* locus in 3,950 EA (3,660 with sCD14 measured) and 792 AA (683 with sCD14 measured) participants who were genotyped using the custom gene-centric IBCv2 genotyping array [3] that contains high SNP marker density and linkage disequilibrium (LD) coverage for various cardiovascular, metabolic, and inflammation-related genes, including *CD14*. IBCv2 array used a "cosmopolitan" tagging approach to capture the genetic diversity across candidate genes in the multiple ethnic populations represented in the HapMap, including both Europeans and West Africans [3]. Tag SNPs were selected to capture known variation with MAF>0.02 and an r^2 of at least 0.8 in HapMap populations.

Ascertainment of clinical events

Details of event ascertainment and adjudication during CHS follow-up have been published [4]. Participants, family members, or other previously identified informants reported new cardiovascular events during semiannual contacts by telephone or at a clinic visit. Medical records were obtained to confirm the diagnosis, and events were adjudicated by a physician review panel. Criteria included history of chest pain, cardiac enzyme levels, and serial ECGs for potential MI events, and onset of symptoms, duration of deficits, and findings on computed tomography or MRI for potential stroke events. Adjudicated clinical CVD events and mortality occurring through June 30, 2008 were available, which allowed for a maximum of 20 years of follow-up. Primary clinical endpoints for this analysis were (1) all cause mortality; (2) incident non-procedure-related fatal or nonfatal myocardial infarction (MI); (3) incident fatal or nonfatal coronary heart disease (CHD), which is defined broadly as non-fatal or fatal MI, as well as coronary artery angioplasty or bypass grafting, and angina; and (4) incident fatal or nonfatal stroke.

Estimation of sCD14 phenotypic variance explained by genome-wide SNPs

Using the Illumina 370CNV genotyping array, we estimated the proportion of phenotypic variance of sCD14 explained by genetic relationships between individuals within a population by applying the Genome-wide Complex Trait Analysis (GCTA) method to CHS EA [5,6]. The GCTA method first calculates the genetic relationship matrix (GRM) between all pairs of individuals from all autosomal SNPs on genome-wide genotyping array. The sCD14 phenotypic variance explained by all SNPs was then estimated by the restricted maximum likelihood approach (REML) as a random effect in the mixed linear model. Stringent quality control measures were applied to the 312,883 SNPs from the Illumina 370CNV array prior to GCTA analysis, in order to control for ascertainment bias and any artifacts introduced into the data by

the genotyping process. These included using only genotyped SNPs, and filtering SNPs and samples based on MAF <0.01, SNP genotype missing rate <0.05, Hardy-Weinberg *P*<0.01, individual missing rate <0.01, and individuals who had pairwise relatedness greater than 0.025 [5]. This resulted in a final data set of N=2571 EA individuals. The GRMs were incorporated into an restricted maximum likelihood (REML) analysis that estimates of the proportion of phenotypic variance explained by all genotyped SNPs. The REML analysis was adjusted for age, sex, and eigenvectors 1–10 from principal component analyses to account for possible confounding by population substructure.

REFERENCES

1. Macy EM, Hayes TE, Tracy RP. Variability in the measurement of C-reactive protein in healthy subjects: implications for reference intervals and epidemiological applications. Clin Chem. 1997;43:52-8.
2. Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR. MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. Genet Epidemiol. 2010;34:816-34.
3. Keating BJ, Tischfield S, Murray SS, Bhangale T, Price TS, Glessner JT, Galver L, Barrett JC, Grant SF, Farlow DN, Chandrupatla HR, Hansen M, Ajmal S, Papanicolaou GJ, Guo Y, Li M, Derohannessian S, de Bakker PI, Bailey SD, Montpetit A, Edmondson AC, Taylor K, Gai X, Wang SS, Fornage M, Shaikh T, Groop L, Boehnke M, Hall AS, Hattersley AT, Frackelton E, Patterson N, Chiang CW, Kim CE, Fabsitz RR, Ouwehand W, Price AL, Munroe P, Caulfield M, Drake T, Boerwinkle E, Reich D, Whitehead AS, Cappola TP, Samani NJ, Lusis AJ, Schadt E, Wilson JG, Koenig W, McCarthy MI, Kathiresan S, Gabriel SB, Hakonarson H, Anand SS, Reilly M, Engert JC, Nickerson DA, Rader DJ, Hirschhorn JN, Fitzgerald GA. Concept, design and implementation of a cardiovascular gene-centric 50 k SNP array for large-scale genomic association studies. PLoS One. 2008;3:e3583.
4. Ives DG, Fitzpatrick AL, Bild DE, Psaty BM, Kuller LH, Crowley PM, Cruise RG, Theroux S. Surveillance and ascertainment of cardiovascular events. The Cardiovascular Health Study. Ann Epidemiol 1995;5:278-85.
5. Lee SH, Wray NR, Goddard ME, Visscher PM. Estimating missing heritability for disease from genome-wide association studies. Am. J. Hum. Genet. 2011;88:294-305.
6. Yang J, Lee SH, Goddard ME, Visscher PM. GCTA: a tool for genome-wide complex trait analysis. Am J Hum Genet. 2011;88:76-82.

Supplemental Table I. Characteristics of CHS participants with soluble CD14 measured

	European Americans	**African Americans**
Total (N)	4609	819
Female sex (N, %)	2608 (56.6)	520 (63.5)
Age (years, SD)	72.8 (5.6)	72.7 (5.5)
Current smoking (N, %)	530 (11.5)	128 (15.7)
Type 2 Diabetes (N, %)	1295 (28.2)	308 (37.9)
Hypertension (N, %)	1907 (41.2)	507 (62.0)
BMI (kg/m2, SD)	26.3 (4.5)	28.6 (5.6)
Systolic Blood Pressure (mm Hg, SD)	136 (21)	142 (23)
Diastolic Blood Pressure (mm Hg, SD)	70 (11)	75 (11)
LDL-C (mg/dL, SD)	130 (35)	128(36)
HDL-C (mg/dL, SD)	54 (16)	58 (16)
Triglycerides (mg/dL, median, IQR)	125 (95 - 169)	102 (79 - 135)
Carotid IMT (mm, SD)	1.45 (0.57)	1.38 (0.52)
ABI (median, IQR)	1.09 (1.01 - 1.17)	1.06 (0.93 - 1.15)
sCD14 (ng/mL, SD)	1662 (348)	1515 (406)
C-reactive protein (mg/L, median, IQR)	1.83 (0.93 - 3.25)	2.56 (1.25 - 5.72)
Fibrinogen (mg/dL, SD)	320 (65)	345 (74)
Interleukin-6 (pg/mL, median, IQR)	1.68 (1.15 - 2.56)	1.87 (1.26 - 2.84)

SD=standard deviation
IMT = intima-medial thickness
ABI = ankle brachial index
IQR - inter-quartile range

Supplemental Table II: Risk of CVD outcomes and mortality associated with soluble CD14 and other inflammation biomarkers

Mortality	**sCD14**	**CRP**	**IL-6**	**Fibrinogen**
Q5 v. Q1	1.27 (1.11, 1.45)	0.99 (0.85, 1.15)	1.56 (1.56, 1.81)	0.92 (0.80, 1.06)
P linear	0.0001	0.81	4.2 x 10-11	0.62
1 SD	1.11 (1.06, 1.16)	1.00 (0.95, 1.05)	1.17 (1.12, 1.23)	0.99 (0.94, 1.04)
P cont	3.4 x 10-6	0.95	8.7 x 10-12	0.72
MI	**sCD14**	**CRP**	**IL-6**	**Fibrinogen**
Q5 v. Q1	0.92 (0.73, 1.17)	1.11 (0.84, 1.47)	1.02 (0.91, 1.59)	1.35 (1.05, 1.74)
P linear	0.67	0.32	0.018	0.010
1 SD	0.96 (0.89, 1.05)	1.01 (0.92, 1.12)	1.11 (1.02, 1.21)	1.10 (1.01, 1.20)
P cont	0.39	0.78	0.017	0.034
CHD	**sCD14**	**CRP**	**IL-6**	**Fibrinogen**
Q5 v. Q1	1.03 (0.86, 1.23)	1.42 (1.15, 1.75)	1.31 (1.07, 1.61)	1.09 (0.90, 1.32)
P linear	0.64	0.001	0.006	0.27
1 SD	1.01 (0.95, 1.07)	1.14 (1.06, 1.23)	1.08 (1.01, 1.15)	1.01 (0.95, 1.08)
P cont	0.73	0.0004	0.022	0.75
Stroke	**sCD14**	**CRP**	**IL-6**	**Fibrinogen**
Q5 v. Q1	1.15 (0.92, 1.44)	1.13 (0.88, 1.47)	1.43 (1.10, 1.86)	0.76 (0.60, 0.97)
P linear	0.077	0.80	0.004	0.11
1 SD	1.08 (1.00, 1.17)	1.02 (0.93, 1.11)	1.11 (1.02, 1.20)	0.95 (0.87, 1.04)
P cont	0.041	0.71	0.013	0.28

Adjusted for age, sex, race, smoking, diabetes, LDL, hypertension, SBP, baseline CVD, carotid IMT, and ankle-brachial index, and the remaining biomarkers. Mortality was additionally adjusted for baseline CVD and cystatin.

sCD14 cutpoints: 284-1356; 1356-1521; 1521-1682; 1682-1881; 1881-4030.
CRP cutpoints: 0.07-0.78; 0.79-1.50; 1.51-2.40; 2.41-4.29; 4.30-119.
IL-6 cutpoints: 0.24-1.06; 1.06-1.47; 1.47-1.97; 1.98-2.90; 2.90-44.
Fibrinogen cutpoints: 109-269; 270-302;303-330;331-366;367-872.

SUPPLEMENTAL TABLE III: Association of sCD14-associated SNPs with incident CVD outcomes and mortality in EA and AA

	MI				CHD				Stroke				Mortality			
	AA (n=90)		EA (n=754)		AA (n=174)		EA (n=1,325)		AA (n=102)		EA (n=814)		AA (n=435)		EA (n=3,271)	
SNP	HR [95%CI]	P	HR [95%CI]	P	HR [95%CI]	P	HR [95%CI]	P	HR [95%CI]	P	HR [95%CI]	P	HR [95%CI]	P	HR [95%CI]	P
rs778584	1.08 (0.78 - 1.50)	0.62	0.90 (0.80 - 1.01)	0.065	1.15 (0.92 - 1.43)	0.23	0.91 (0.83 - 0.99)	0.025	1.33 (0.98 - 1.80)	0.066	0.94 (0.84 - 1.05)	0.25	1.12 (0.97 - 1.29)	0.13	0.98 (0.93 - 1.04)	0.50
rs4914	0.79 (0.43 - 1.46)	0.45	1.02 (0.86 - 1.20)	0.85	0.86 (0.57 - 1.31)	0.49	1.09 (0.96 - 1.23)	0.18	1.63 (1.02 - 2.62)	0.042	1.05 (0.89 - 1.24)	0.56	0.89 (0.68 - 1.15)	0.38	0.99 (0.91 - 1.07)	0.73
rs5744455	1.05 (0.63 - 1.76)	0.84	1.05 (0.92 - 1.20)	0.44	1.03 (0.73 - 1.47)	0.87	1.04 (0.94 - 1.14)	0.49	0.96 (0.57 - 1.61)	0.88	0.93 (0.81 - 1.06)	0.28	0.81 (0.62 - 1.05)	0.11	0.99 (0.92 - 1.05)	0.66
rs5744451	1.26 (0.76 - 2.10)	0.37	NA	NA	1.02 (0.67 - 1.56)	0.91	NA	NA	0.87 (0.57 - 1.48)	0.62	NA	NA	1.11 (0.87 - 1.42)	0.38	NA	NA
rs1063412	1.15 (0.71 - 1.88)	0.57	1.07 (0.95 - 1.20)	0.30	1.04 (0.74 - 1.47)	0.81	1.06 (0.98 - 1.15)	0.16	0.92 (0.57 - 1.47)	0.73	0.91 (0.80 - 1.02)	0.11	0.95 (0.74 - 1.22)	0.70	0.99 (0.93 - 1.05)	0.77

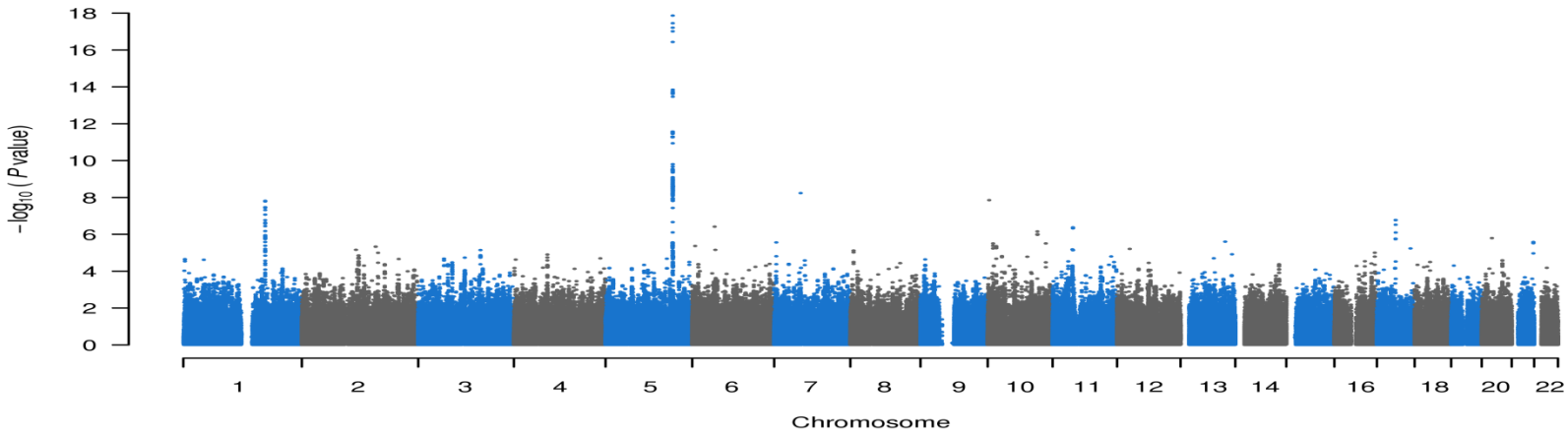
Adjusted for age, sex, smoking, diabetes, BMI, hypertension, and eigenvectors 1- 10.

SUPPLEMENTAL TABLE IV: Association of sCD14-associated SNPs with incident CHD and stroke in EA and AA from the Women's Health Initiative

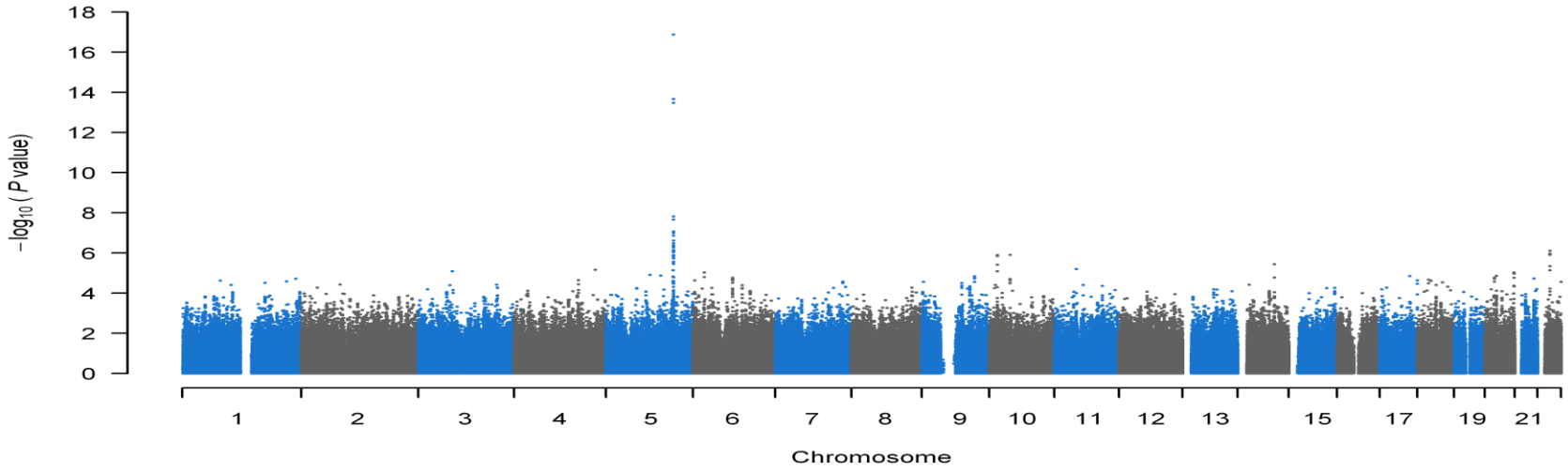
	CHD				Stroke			
	AA (n=296)		EA (n=3,042)		AA (n=251)		EA (n=2,222)	
SNP	OR [95%CI]	P	OR [95%CI]	P	OR [95%CI]	P	OR [95%CI]	P
rs778584	0.97 (0.73 - 1.27)	0.81	0.97 (0.90 - 1.05)	0.43	1.21 (0.91 - 1.60)	0.18	0.98 (0.90 - 1.06)	0.58
rs4914	1.16 (0.70 - 1.90)	0.57	1.08 (0.96 - 1.22)	0.18	1.17 (0.71 - 1.90)	0.54	1.01 (0.88 - 1.15)	0.93
rs5744455	1.09 (0.71 - 1.69)	0.68	0.97 (0.89 - 1.07)	0.57	1.04 (0.69 - 1.57)	0.84	1.04 (0.94 - 1.14)	0.49
rs5744451	1.11 (0.73 - 1.69)	0.63	NA	NA	1.09 (0.71 - 1.66)	0.70	NA	NA

Adjusted for age, sex, smoking, diabetes, BMI, hypertension, and eigenvectors 1- 10.

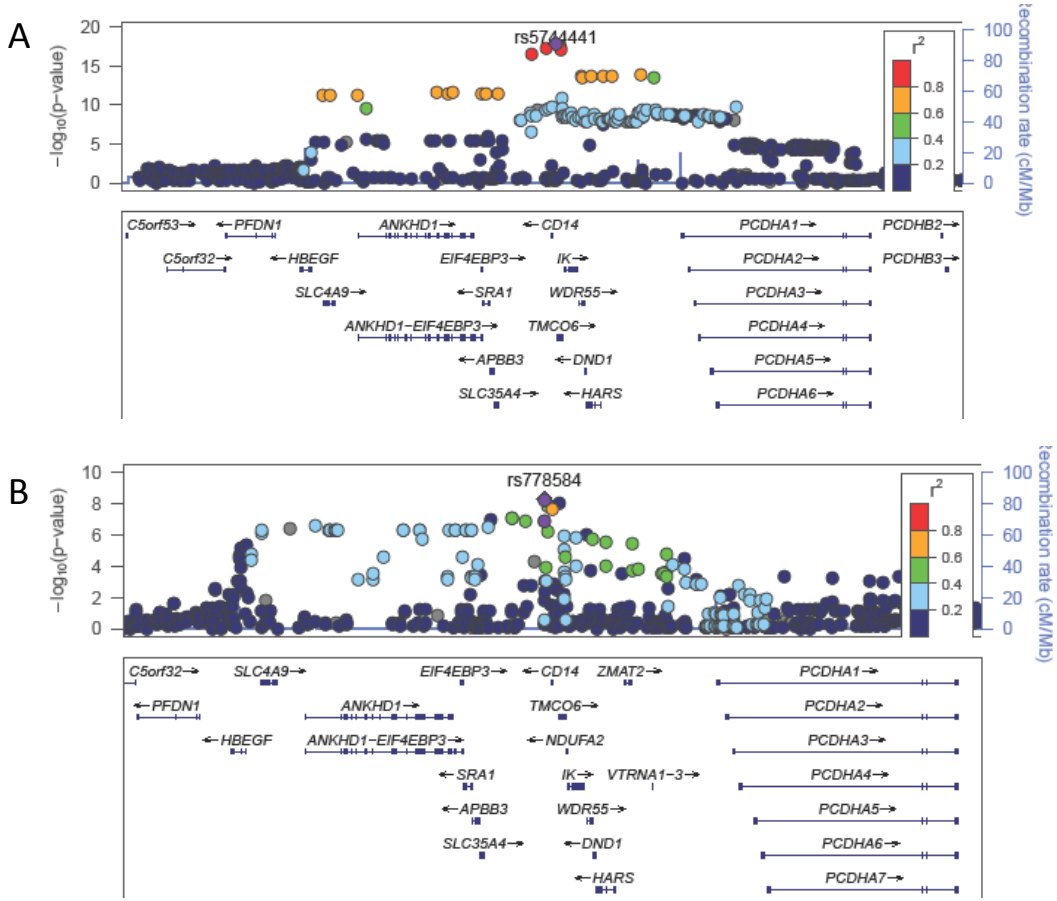
Supplemental Figure I. Manhattan Plot of soluble CD14 GWAS in European Americans



Supplemental Figure II. Manhattan Plot of soluble CD14 GWAS in African Americans

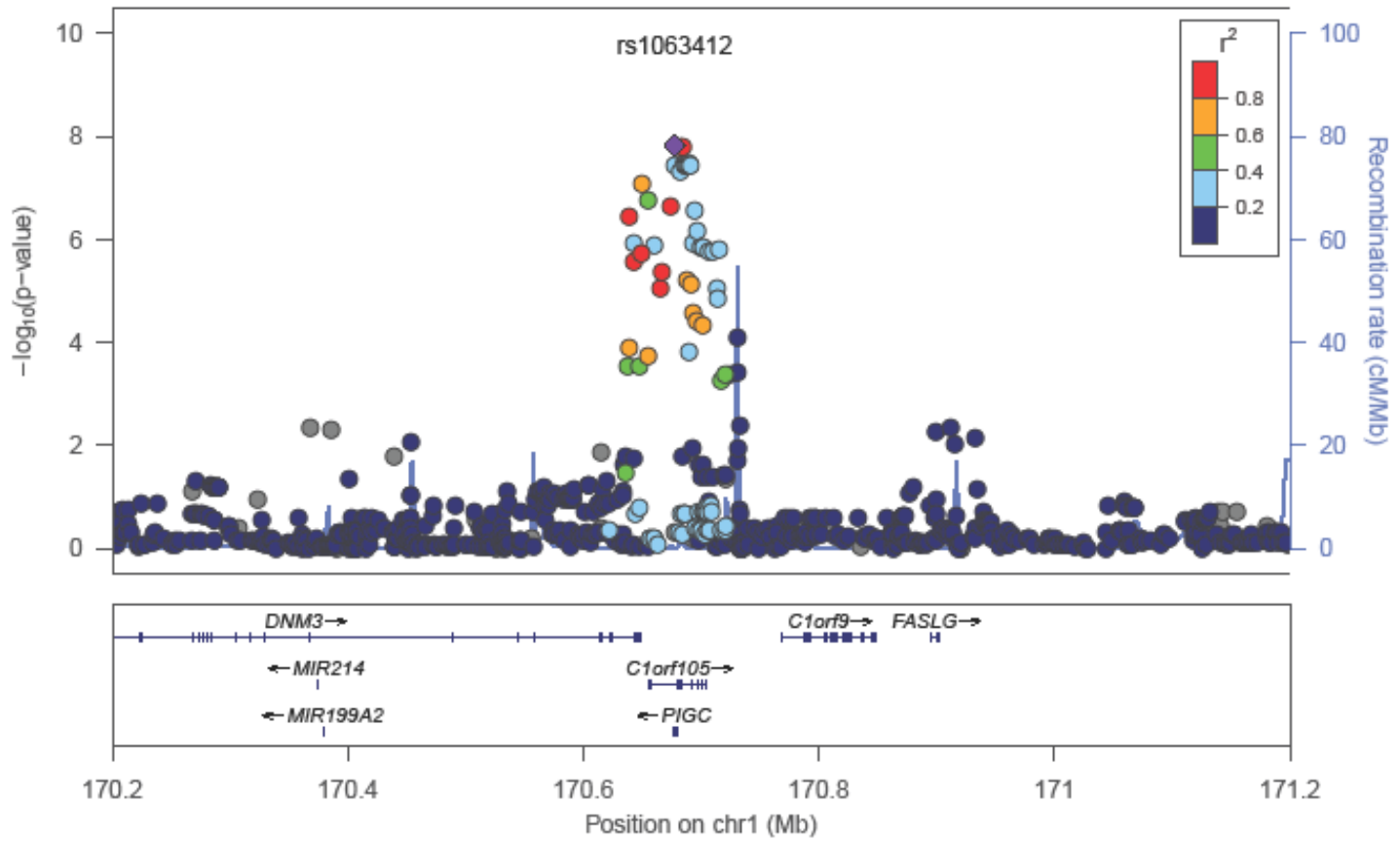


Supplemental Figure III: Regional association plots for chromosome 5q31.



Shown are soluble CD14 regional association plots generated using LocusZoom for the chromosome 5q31 locus in (**A**) European Americans; (**B**) African Americans. The color of each single nucleotide polymorphism (SNP) indicates the level of pairwise linkage disequilibrium (LD) based on r-squared relative to the lead SNP in the region. r-squared values were calculated from HapMap CEU for European Americans and YRI for African Americans. SNPs with missing LD information are shown in gray.

Supplemental Figure IV: Regional association plot for chromosome 1q24 in European Americans.



Shown is the soluble CD14 regional association plots generated using LocusZoom the chromosome 1q24 locus in European Americans. The color of each single nucleotide polymorphism (SNP) indicates the level of pairwise linkage disequilibrium (LD) based on r-squared relative to the lead SNP in the region. r-squared values were calculated from HapMap CEU. SNPs with missing LD information are shown in gray.

Supplemental Figure V. Regional association plot of 1q24 region in African Americans