# Analysis of Metabolic Syndrome Components in >15 000 African Americans Identifies Pleiotropic Variants

## Results From the Population Architecture Using Genomics and Epidemiology Study

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***Background***—Metabolic syndrome (MetS) refers to the clustering of cardiometabolic risk factors, including dyslipidemia, central adiposity, hypertension, and hyperglycemia, in individuals. Identification of pleiotropic genetic factors associated with MetS traits may shed light on key pathways or mediators underlying MetS.

***Methods and Results***—Using the Metabochip array in 15 148 African Americans from the Population Architecture using Genomics and Epidemiology (PAGE) study, we identify susceptibility loci and investigate pleiotropy among genetic variants using a subset-based meta-analysis method, ASsociation-analysis-based-on-subSETs (ASSET). Unlike conventional models that lack power when associations for MetS components are null or have opposite effects, Association-analysis-based-on-subsets uses 1-sided tests to detect positive and negative associations for components separately and combines tests accounting for correlations among components. With Association-analysis-based-on-subsets, we identify 27 single nucleotide polymorphisms in 1 glucose and 4 lipids loci (*TCF7L2*, *LPL*, *APOA5*, *CETP*, and *APOC1/APOE/TOMM40*) significantly associated with MetS components overall, all $P<2.5e{-}7$, the Bonferroni adjusted $P$ value. Three loci replicate in a Hispanic population, n=5172. A novel African American-specific variant, rs12721054/*APOC1*, and rs10096633/*LPL* are associated with ≥3 MetS components. We find additional evidence of pleiotropy for *APOE*, *TOMM40*, *TCF7L2*, and *CETP* variants, many with opposing effects (eg, the same rs7901695/*TCF7L2* allele is associated with increased odds of high glucose and decreased odds of central adiposity).

***Conclusions***—We highlight a method to increase power in large-scale genomic association analyses and report a novel variant associated with all MetS components in African Americans. We also identify pleiotropic associations that may be clinically useful in patient risk profiling and for informing translational research of potential gene targets and medications. **(*Circ Cardiovasc Genet*. 2014;7:505-513.)**

**Key Words:** African continental ancestry group ■ genetic pleiotropy ■ genetic variation ■ high-density lipoprotein cholesterol ■ Hispanic Americans ■ hyperglycemia ■ metabolic syndrome

Metabolic syndrome (MetS) is the designation used for the clustering of cardiometabolic risk factors within an individual, including dyslipidemia, central obesity, high blood pressure, and insulin resistance. Individuals with MetS are at increased risk of chronic diseases, including diabetes mellitus and cardiovascular disease, and all-cause mortality.[1–4] Prevalence of MetS varies by race and sex; in the United States, it is higher in African American (AA) women than in AA men, and higher in Hispanics than in other race/ethnicity groups.[5] Overall, the age-adjusted prevalence ranges from 16% to 36% in the United States and has been increasing in recent years,[5] yet the cause of MetS is not well understood. Although insulin resistance, obesity, abnormal adipose tissue metabolism, and endothelial dysfunction are key contributors,[6,7] elucidation of the precise MetS pathophysiology is complicated by the relatedness of these pathways and potentially common underlying mediators. Investigation of genetic factors associated with several MetS component traits may shed light on key pathways or mediators underlying the syndrome as a whole and also aid in genetic risk prediction of MetS.

**Clinical Perspective on p 513**

Received October 16, 2013; accepted May 23, 2014.

**The Data Supplement is available at http://circgenetics.ahajournals.org/lookup/suppl/doi:10.1161/CIRCGENETICS.113.000386/-/DC1.**

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As per the National Cholesterol Education Program Adult Treatment Panel III criteria,[4,8] MetS requires the presence of ≥3 of the 5 following components: central obesity, high blood pressure, elevated triglycerides, low high-density lipoprotein (HDL) cholesterol, or fasting hyperglycemia; use of medication to treat the former 4 conditions also qualifies. The heritability of MetS, as defined above, is estimated to be ≈30% although heritability of the individual components is generally higher and may vary by ethnicity.[9,10] Genetic analyses of MetS are complicated by the inherently heterogeneous definition of MetS. For example, the same 3 components may not cluster in all MetS cases, and single nucleotide polymorphisms (SNPs) may be associated with 1 MetS component and not with another. This complexity has prompted the use of novel methods, such as factor analysis or gene network analysis, to characterize the genetic architecture of MetS. Applying a recently described method, ASsociation analysis based on subSETs (ASSET),[11] we sought to identify MetS susceptibility loci in AAs and investigate whether the identified genetic variants show evidence of pleiotropy (ie, a genetic variant influencing multiple traits), and thus may explain some of the correlated architecture of MetS traits.

In AAs, using the Metabochip genotyping array[12] from the Population Architecture using Genomics and Epidemiology (PAGE) study, we contrast SNP association findings from ASSET with those from (1) logistic regression modeling MetS as a binary outcome and (2) meta-analysis of the 5 individual components of MetS, coded as binary variables. Unlike the traditional meta-analysis that may lack power when associations for each of the components are heterogeneous (ie, null or have effects in opposite directions), ASSET uses 1-sided tests to detect positive and negative associations for the components separately and combines the tests taking into account correlations among the components, and correction for these multiple tests. Significant results from ASSET are tested in an independent Hispanic population.

## Methods

### Study Population

The study population includes 15 148 AA adults from the Atherosclerosis Risk in Communities (ARIC) and Women's Health Initiative (WHI), who are part of the PAGE study.[13] Briefly, the ARIC study is a population-based cohort of non-Hispanic white and AA men and women who were between 45 and 64 years of age in 1987 to 1989 and recruited from 4 US communities: Forsyth County, NC; Jackson, MI; suburban areas of Minneapolis, MN; and Washington County, MD.[14] A subset of the ARIC cohort, n=3340 ARIC AAs with available Metabochip genotyping were included in these analyses. The WHI is a study of the cause and prevention of chronic diseases in 161 838 postmenopausal women aged 50 to 79 years at recruitment (1993–1998) from 40 clinical centers in the United States.[15,16] WHI consists of an observational study, 2 clinical trials of postmenopausal hormone therapy (estrogen alone or estrogen plus progestin), a calcium and vitamin D supplement trial, and a dietary modification trial. A subset of the baseline WHI cohort, n=11 808 AA women with Metabochip genotyping data or imputed genotypes were included in these analyses. The independent validation population includes 5172 self-reported Hispanic/Latina women from the WHI that were genotyped on the Metabochip as a part of the PAGE study. All study protocols were approved by institutional review boards at the participating institutions, and all included participants gave informed consent.

### Outcomes

MetS was defined in men and women according to the American Heart Association/National Heart Lung and Blood Institute modified National Cholesterol Education Program Adult Treatment Panel III guidelines requiring the presence of ≥3 of the following: waist circumference >102 cm in men or 88 cm in women; triglyceride levels ≥150 mg/dL or treatment for dyslipidemia; HDL levels <40 mg/dL in men or <50 mg/dL in women or treatment for dyslipidemia; blood pressure levels ≥130/85 mm Hg or treatment for hypertension; and fasting plasma glucose levels ≥100 mg/dL or treatment for diabetes mellitus.[8,17] Practically, these guidelines differ from the National Cholesterol Education Program Adult Treatment Panel III guidelines for the elevated fasting glucose criterion; the threshold is reduced from 110 to 100 mg/dL, which corresponds to the recently modified American Diabetes Association criteria for impaired fasting glucose.[18] MetS controls had ≤2 components. Only individuals having nonmissing data for the majority of components (3 of 5) at baseline were included as MetS cases or controls in analyses; for example, an individual having 3 of 5 components but missing data for the other 2 was included as a MetS case, and an individual not meeting criteria for 3 of the 5 components, even if the other 2 were missing, was included as a control. In both ARIC and WHI, blood lipids and glucose were measured in fasting samples. Using a tape measure, waist circumference was measured at the level of the natural waist in a horizontal plane to the nearest 0.5 cm. Resting blood pressure was measured using standard protocols. Baseline medication use was assessed using a combination of self-report and a medication inventory in ARIC and WHI. For analyses involving MetS components, binary variables were created using the above criteria to reflect either the presence or the absence of the condition in individuals with nonmissing data for that component. The ASSET methodology, as described below, includes any participant with baseline data for ≥1 of the 5 MetS components.

### Genotyping

The Metabochip is a high-density genotyping array designed for fine-mapping genome-wide association study (GWAS)–identified regions for cardiometabolic and anthropometric traits (including genes previously associated with blood lipids and glucose, waist circumference, and blood pressure) and also includes genetic variants to capture ancestral diversity.[12] Our analysis includes all polymorphic SNPs with minor allele frequency ≥0.001 and passing stringent quality control (QC; n=169 196 SNPs). Genotyping QC procedures have been described previously,[19] but briefly, the following SNP QC criteria were applied: GenomeStudio GenTrain Score ≥0.6 and cluster separation score ≥0.4, call rate ≥0.95, replicate errors ≤2, HapMap discordance ≤1/30, and exact Hardy–Weinberg equilibrium $P \geq 1e{-}6$. The following sample criteria were applied: sample call rate ≥0.95; no excessive heterozygosity ($|F| > 0.35$); no over-relatedness (≤100 first- and second-degree relatives); sample not an ancestry outlier (≤6 SD in first 10 principal component [PC] analysis); and among estimated first-degree relative pairs, the first-degree relatives with lower call rates were dropped. A proportion of the WHI AA samples (54%) had high-quality imputed genotype data that were included in these analyses. QC methods for the imputation are described in Liu et al.[20]

### Analysis

SNPs were coded using additive genetic models (0/1/2). Logistic regression was used to test for study-specific associations between (1) SNPs and MetS as a binary outcome and (2) SNPs and individual MetS components coded as binary variables based on the modified National Cholesterol Education Program Adult Treatment Panel III guidelines. All models were adjusted for age, sex (except WHI), and global ancestry using principal components. Determination of ancestry PCs was performed with EIGENSOFT,[21,22] as described in Buyske et al[19] (Methods in the Data Supplement). In both ARIC and WHI, AA models were adjusted for the first 2 ancestry PCs. For each trait, the results between ARIC and WHI were combined using a fixed-effect meta-analysis using inverse-variance weighting (no notable heterogeneity was observed). We then combined the results for the 5 traits in ASSET (http://www.bioconductor.org/packages/devel/bioc/

html/ASSET.html) using R software.[23] ASSET uses a fixed-effect meta-analysis on an adaptively selected combination of the traits. As a comparison of the ASSET results, we performed straightforward fixed-effect meta-analysis between the 5 traits. In the discovery phase to identify MetS susceptibility loci using either the binary MetS outcome or the ASSET method, significance was defined as $P<2e{-}7$ (ie, 0.05/169 196 SNPs tested). For investigation of pleiotropy for SNPs that were significant in the ASSET models, that is, looking at SNP associations for the individual components, we used a nominal significance threshold for each component, $P<0.05$.

### ASSET

Proposed by Bhattacharjee et al,[11] ASSET is a suite of statistical tools designed for pooling association signals across multiple traits (or studies) when true effects may exist only in a subset of the traits and could be in opposite directions. The method explores all possible subsets of traits and evaluates fixed-effect meta-analysis–type test statistics for each subset. The final test statistic is obtained by maximizing the subset-specific test statistics over all possible subsets and then evaluating its significance after efficient adjustment for multiple testing, taking into account the correlation between test statistics across different subsets because of overlapping participants (because multiple phenotypes may be available on participants). The method not only returns a *P* value for the overall evidence of association of a SNP across traits but also outputs the best subset containing the traits that contributed to the overall association signal. The resulting test is much more powerful than considering all combinations of traits and using a Bonferroni correction. For detection of association signals with effects in opposite directions, ASSET allows subset searches separately for positively and negatively associated traits and then combines association signals from 2 directions using a $\chi^2$ test statistic.

### Validation

For the validation of significant SNPs in the Hispanic population, we used a nominal threshold of significance, $P<0.05$.

## Results

Overall, 15 148 AAs were included in the ASSET analysis; these individuals had Metabochip genotype data and baseline data for ≥1 MetS component. A subset of those individuals, n=12 574, had sufficient nonmissing data for the classification of 5507 MetS cases (43.7%) and 7067 controls (56.2%). In both ARIC and WHI, the triglycerides component was most frequently missing, with 4% and 24% missing, respectively. Among those who could be classified, 38% of ARIC participants met MetS criteria, and 46% of WHI women had MetS (Table 1). In ARIC, MetS cases were more likely to be women than controls. In both ARIC and WHI, the high triglycerides component was the least prevalent component in MetS cases and also was rare (≈5%) in controls.

### Discovery

A total of 27 SNPs were significant ($P<2e{-}7$) in the 2-sided ASSET models (Table 2). Given that many SNPs in each Metabochip locus are in high linkage disequilibrium with each other and not independent, we restrict further discussion to the most significant SNPs, that is, top SNPs, in each locus and to other significant SNPs that are in low linkage disequilibrium ($r^2<0.2$ in AAs) with these top SNPs for a total of 11 SNPs in 5 loci. Only 1 SNP was significant in the logistic regression analysis (rs10096633/*LPL*) or in the meta-analysis of MetS components (rs12721054/*APOC1*). As indicated in Table 2, these 2 SNPs were also significant in the ASSET analysis.

### Pleiotropy

Of the top ASSET SNPs, rs12721054/*APOC1* was associated with all 5 MetS components; rs10096633/*LPL* was associated with glucose, triglycerides, and HDL; rs3135506/*APOA5* was associated with triglycerides and HDL; rs7901695/*TCF7L2* was associated with waist circumference and glucose; and rs247616/*CETP*, rs7412/*APOE* and rs61679753/*TOMM40* were associated with waist circumference and HDL (Table 3). Several top SNPs in *CETP* were significantly associated with only the HDL component in ASSET: rs7205692, rs247619, rs6499862, and rs7499892. Pleiotropy results for other significant SNPs are shown in Table 3. As an illustration of the ASSET method, results for rs247616 from the ASSET, logistic regression model of MetS, and the meta-analysis of MetS components are plotted in the Figure.

### Validation

We investigated MetS traits associations for the 27 SNPs significant in the AA ASSET models in a large, independent

**Table 1. Baseline Characteristics of African American Metabolic Syndrome Cases and Controls, and All African American Individuals in the ASSET Analyses**

| | ARIC | | | WHI | | |
|---|---|---|---|---|---|---|
| Characteristic | Cases (n=1229) | Controls (n=1979) | ASSET (n=3340) | Cases (n=4278) | Controls (n=5088) | ASSET (n=11 808) |
| Mean age±SD, y | 54.3±5.6 | 53.1±5.9 | 53.5±5.9 | 62.3±6.9 | 61.1±7.2 | 61.5±7.1 |
| Women, n (%)* | 861 (70.1) | 1142 (57.8) | 2094 (62.7) | 4278 (100.0) | 5088 (100.0) | 11, 808 (100.0) |
| MetS components, n (%)* | | | | | | |
| Low HDL/lipid med | 816 (67.2) | 285 (14.5) | 1104 (34.6) | 2868 (67.0) | 651 (12.8) | 3559 (38.9) |
| High triglycerides/lipid med | 521 (42.9) | 73(3.7) | 596 (18.7) | 2113 (49.4) | 252 (5.0) | 2378 (26.5) |
| High waist circumference | 1062 (86.4) | 831 (42.0) | 1981 (59.5) | 3576 (83.6) | 1782 (35.0) | 6698 (56.9) |
| High blood pressure/ hypertension med | 854 (69.7) | 695 (35.2) | 1615 (48.6) | 3928 (91.8) | 2905 (57.1) | 8521 (72.2) |
| High glucose/diabetes mellitus med | 1042 (84.9) | 615 (31.1) | 1736 (53.3) | 2890 (67.6) | 732 (14.4) | 3760 (39.7) |

ARIC indicates Atherosclerosis Risk in Communities; ASSET, Association analysis based on subsets; HDL, high-density lipoprotein; med, medications; MetS, metabolic syndrome; and WHI, Women's Health Initiative.

*Percentage reflects proportion of MetS cases, MetS controls or ASSET totals with nonmissing data, respectively.

**Table 2. Significant Results From All Models in African Americans**

| | | | | Model *P* Values in AA | | | LD‡ | |
|---|---|---|---|---|---|---|---|---|
| Region* (Chr) | SNP† | Annotation | CA/CAF | MetS Log Reg | Components Meta-Analysis | ASSET | AA | EA |
| HDL.1 (8) | rs10096633§‖ | *LPL*/near 3′ UTR‖ | C/0.59‖ | 4.5e−8‖ | 1.1e−5‖ | 1.5e−9‖ | 1.0‖ | 1.0‖ |
| | rs326 | *LPL*/intron | T/0.46 | 5.1e−5 | 9.0e−4 | 8.4e−9 | 0.58 | 0.32 |
| | rs13702 | *LPL*/3′ UTR | A/0.50 | 3.1e−6 | 2.0e−4 | 3.0e−8 | 0.68 | 0.33 |
| | rs15285 | *LPL*/3′ UTR | G/0.50 | 4.1e−6 | 2.3e−4 | 7.5e−8 | 0.67 | 0.33 |
| T2D.1 (10) | rs7901695‖ | *TCF7L2*/intron‖ | C/0.45‖ | 4.8e−1‖ | 6.3e−1‖ | 1.0e−7‖ | 1.0‖ | 1.0‖ |
| | rs7903146 | *TCF7L2*/intron | G/0.71 | 2.5e−1 | 4.9e−1 | 1.1e−7 | 0.49 | 0.84 |
| | rs4506565 | *TCF7L2*/intron | A/0.45 | 3.9e−1 | 7.3e−1 | 1.3e−7 | 0.99 | 0.96 |
| HDL.2 (11) | rs3135506‖ | *APOA5*/ missense‖ | C/0.94‖ | 3.7e−4‖ | 3.5e−4‖ | 1.8e−7‖ | 1.0‖ | 1.0‖ |
| HDL.3 (16) | rs247616‖ | *CETP*/near-5′‖ | G/0.74‖ | 2.6e−2‖ | 2.8e−2‖ | 1.2e−20‖ | 1.0‖ | 1.0‖ |
| | rs247617 | *CETP*/near-5′ | A/0.26 | 2.6e−2 | 2.7e−2 | 1.3e−20 | 1.0 | 0.99 |
| | rs183130 | *CETP*/near-5′ | G/0.74 | 2.7e−2 | 2.8e−2 | 1.4e−20 | 1.0 | 1.0 |
| | rs4783961 | *CETP*/near-5′ | G/0.57 | 2.0e−2 | 1.4e−3 | 9.4e−18 | 0.46 | 0.48 |
| | rs711752 | *CETP*/intron | G/0.73 | 3.7e−2 | 5.2e−2 | 5.1e−17 | 0.65 | 0.57 |
| | rs17231520 | *CETP*/near-5′ | G/0.93 | 1.1e−2 | 6.2e−2 | 9.1e−15 | 0.21 | <0.01 |
| | rs34065661 | *CETP*/missense | C/0.93 | 1.2e−2 | 6.4e−2 | 1.2e−14 | 0.21 | <0.01 |
| | rs6499862¶ | *CETP*/near-5′ | G/0.70 | 3.4e−2 | 2.6e−3 | 2.6e−11 | 0.16 | 0.11 |
| | rs7499892 | *CETP*/intron | G/0.63 | 5.5e−3 | 2.5e−2 | 2.6e−11 | 0.05 | 0.11 |
| | rs247619 | *CETP*/near-5′ | G/0.94 | 7.1e−3 | 1.7e−2 | 5.1e−11 | 0.19 | <0.01 |
| | rs12446515 | *CETP*/near-5′ | G/0.85 | 2.7e−1 | 1.6e−1 | 1.2e−10 | 0.47 | 0.99 |
| | rs17231506 | *CETP*/near-5′ | G/0.85 | 2.6e−1 | 1.8e−1 | 1.4e−10 | 0.48 | 0.99 |
| | rs56156922 | *CETP*/near-5′ | A/0.85 | 2.6e−1 | 1.8e−1 | 1.8e−10 | 0.47 | 0.99 |
| | rs3764261 | *CETP*/near-5′ | C/0.68 | 1.4e−1 | 4.9e−2 | 7.5e−10 | 0.74 | 0.99 |
| | rs12149545 | *CETP*/near-5′ | G/0.91 | 1.1e−1 | 1.8e−1 | 1.3e−8 | 0.27 | 0.96 |
| | rs7205692¶ | *CETP*/near-5′ | A/0.67 | 3.1−2 | 1.8e−3 | 5.5e−8 | 0.14 | 0.11 |
| LDL.1 (19) | rs12721054‖# | *APOC1*/3′ UTR‖ | G/0.12‖ | 2.3 e−7‖ | 9.7e−8‖ | 2.0e−11‖ | 1.0‖ | 1.0‖ |
| | rs7412¶ | *APOE*/missense | C/0.90 | 5.0e−2 | 8.3e−2 | 4.7e−9 | 0.01 | <0.01 |
| | rs61679753¶ | *TOMM40*/intron | A/0.89 | 1.1e−1 | 2.3e−1 | 6.9e−8 | 0.01 | <0.01 |

AA indicates African American; ASSET, Association analysis based on subsets; EA, European ancestry; CA, coded allele; CAF, coded allele frequency; HDL, high-density lipoprotein; LD, linkage disequilibrium; SNP, single nucleotide polymorphism; and T2D, type 2 diabetes mellitus.

*The Metabochip consists of 257 finely mapped regions based on genome-wide association study hits for cardiometabolic and anthropometric traits; regions listed are arbitrarily numbered.

†The SNP with the lowest *P* value in each Metabochip region is represented by ‖.

‡LD estimates reflect $r^2$ values between SNP and the SNP represented by ‖ in the same Metabochip region in the PAGE AA population and in a European-ancestry population.[24]

SNP was also significant in the §logistic regression analysis of MetS or #meta-analysis of MetS components.

¶Although these SNPs are not highly correlated with the SNP represented by ‖ in their region, they are highly correlated with each other in AA ($r^2$>0.6).

population of Hispanic Americans, n=5172. Characteristics of the population are presented in Table I in the Data Supplement. Of the 5172 Hispanic women, 3734 had sufficient data to be classified into MetS cases (42.6%) and controls (57.4%). In contrast to AA cases, in which high triglycerides was the least prevalent component, in Hispanic cases, high glucose was the least prevalent component, at 60%. Of the 27 SNPs, 17 SNPs from 3 loci (*LPL*, *APOA5*, and *CETP*) were significant in the Hispanic population at *P*<0.05 (Table 3). (Note: these loci also replicated at a more stringent threshold of *P*=0.05/number of loci.) Pleiotropy was less evident in the Hispanic population, although overall, trends were consistent with AAs. None of the SNPs in the *APOC1* or *TCF7L2* loci were significant in Hispanics although the *TCF7L2* SNPs were modestly associated with the waist circumference component.

## Discussion

Although the clinical use of MetS is a subject of ongoing debate,[25] MetS is a highly prevalent condition, and its prevalence is expected to increase along with the growing obesity epidemic.[5] Some propose that obesity is a critical precursor of MetS,[8,26] yet interestingly, MetS is also present among normal-weight individuals (at a prevalence of ≈5% in The Third National Health and Nutrition Examination Survey (1988-94) (NHANES III)),[27] which may suggest that genetic factors unrelated to obesity also play a role in MetS development.[28] Indeed, the majority of MetS genes identified in previous studies are from lipid metabolism

**Table 3. ASSET and Pleiotropy Findings in African Americans and the Validation Hispanic Cohort**

| | | ASSET *P* Value | | CAF | | BP* | | GLUC* | | HDL* | | TG* | | WC* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Region | SNP | AA | HA | AA | HA | AA | HA | AA | HA | AA | HA | AA | HA | AA | HA |
| HDL.1 | rs10096633 | 1.5e−09 | 3.0e−03† | 0.59 | 0.86 | | | ↑ | | ↑ | ↑ | ↑ | ↑ | | |
| | rs326 | 8.4e−09 | 1.1e−02† | 0.46 | 0.68 | | | | | ↑ | ↑‡ | ↑ | ↑ | | |
| | rs13702 | 3.0e−08 | 1.5e−02† | 0.50 | 0.69 | | | | | ↑ | ↑‡ | ↑ | ↑ | | |
| | rs15285 | 7.5e−08 | 4.0e−03† | 0.50 | 0.69 | | | ↑‡ | | ↑ | ↑‡ | ↑ | ↑ | | |
| T2D.1 | rs7901695 | 1.0e−07 | 2.0e−01 | 0.45 | 0.29 | | | ↑ | | | | | | ↓ | ↓‡ |
| | rs7903146 | 1.1e−07 | 1.6e−01 | 0.71 | 0.74 | | | ↓ | | | | | | ↑‡ | ↑‡ |
| | rs4506565 | 1.3e−07 | 3.0e−01 | 0.45 | 0.29 | | | ↑ | | | | | | ↓ | ↓‡ |
| HDL.2 | rs3135506 | 1.8e−07 | 1.3e−11† | 0.94 | 0.88 | | | | | ↓ | ↓ | ↓ | ↓ | | |
| HDL.3 | rs247616 | 1.2e−20 | 2.9e−07† | 0.74 | 0.70 | | | | | ↑ | ↑ | | | ↓ | |
| | rs247617 | 1.3e−20 | 2.3e−07† | 0.26 | 0.30 | | | | | ↓ | ↓ | | | ↑ | |
| | rs183130 | 1.4e−20 | 3.1e−07† | 0.74 | 0.70 | | | | | ↑ | ↑ | | | ↓ | |
| | rs4783961 | 9.4e−18 | 3.4e−01 | 0.57 | 0.51 | | | | | ↑ | | | | | |
| | rs711752 | 5.1e−17 | 1.4e−03† | 0.73 | 0.58 | | | | | ↑ | ↑ | | | ↓ | |
| | rs17231520 | 9.1e−15 | 1.4e−01§ | 0.93 | 0.99 | | | | | ↑ | ↑ | | | | |
| | rs34065661 | 1.2e−14 | 1.4e−01§ | 0.93 | 0.99 | | | | | ↑ | ↑ | | | | |
| | rs7499892 | 2.6e−11 | 5.1e−07† | 0.63 | 0.79 | | ↓‡ | | | ↓ | ↓ | | | | |
| | rs6499862 | 2.6e−11 | 3.5e−02† | 0.70 | 0.83 | | ↓‡ | | | ↓ | ↓ | | | | ↓‡ |
| | rs247619 | 5.1e−11 | 5.7e−02§ | 0.94 | 0.99 | | | | | ↑ | ↑ | | | | |
| | rs12446515 | 1.2e−10 | 4.6e−06† | 0.85 | 0.72 | | | | | ↑ | ↑ | | | ↓ | |
| | rs17231506 | 1.4e−10 | 1.0e−05† | 0.85 | 0.72 | | | | | ↑ | ↑ | | | ↓ | |
| | rs56156922 | 1.8e−10 | 4.9e−06† | 0.85 | 0.72 | | | | | ↑ | ↑ | | | ↓ | |
| | rs3764261 | 7.5e−10 | 5.7e−08† | 0.68 | 0.70 | | | | | ↑ | ↑ | | | | |
| | rs12149545 | 1.3e−08 | 7.4e−06† | 0.91 | 0.73 | | | | | ↑ | ↑ | | | ↓‡ | |
| | rs7205692 | 5.5e−08 | 1.0e−02† | 0.67 | 0.83 | | ↓ | | | ↓ | ↓ | | | | ↓‡ |
| LDL.1 | rs12721054 | 2.0e−11 | 9.6e−02 | 0.12 | 0.01 | ↓‡ | | ↓‡ | | ↓ | | ↓ | | ↓‡ | |
| | rs7412 | 4.7e−09 | 5.2e−01 | 0.90 | 0.96 | | | | | ↑ | | | | ↓‡ | |
| | rs61679753 | 6.9e−08 | 6.5e−01 | 0.89 | 0.97 | | | | | ↑ | | | | ↓‡ | |

AA indicates African American; ASSET, Association analysis based on subsets; BP, blood pressure; CAF, coded allele frequency; GLUC, high glucose; HA, Hispanic Americans; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SNP, single nucleotide polymorphisms; T2D, type 2 diabetes mellitus; TG, high triglycerides; and WC, waist circumference.

*These columns reflect individual MetS components SNP associations. Arrows indicate significantly increased (↑) or decreased (↓) odds of the MetS component; BP, high blood pressure; GLUC, high glucose; HDL, low HDL; TG, high triglyceride; and WC, high waist circumference.

†Indicates significant replication of the SNP in ASSET, at $P<0.05$

‡Component *P* value significant before multiple testing correction (ie, $P<0.05$), but not after correction.

§No overall evidence of association across traits although HDL component was significant.

pathways[29–31] although glucose[30,31] and blood pressure pathways[30] are also implicated. Our study is consistent with these findings; we identified variants in lipid genes (*LPL*, *CETP*, and *APOA5*) and in the transcription factor 7–like 2 gene (*TCF7L2*). *TCF7L2* variants are associated with increased risk of type 2 diabetes mellitus, including the intronic SNP that we identified (rs7903146), which was recently reported in AAs.[32] For these SNPs, we also describe additional associations with other MetS traits. Importantly, we report novel associations with multiple MetS traits for rs12721054/*APOC1*, which may be African specific, and rs61679753/*TOMM40*. The validation of many of the associations in Hispanics, with the exception of the putative African-specific SNP, is supportive of the veracity our findings, especially given that the Hispanic sample has a distinct genetic background from the AA population.

Although family studies suggest that undetected genetic loci may contribute to the clustering of MetS components, candidate gene and GWAS of MetS have had variable success in identifying reproducible, common genetic mechanisms underlying MetS. Instead, these studies have generally identified genes from specific pathways that may be strongly associated with 1 or 2 of the MetS components but not necessarily ≥3.[29,30] Similarly, most factor analysis and PC studies have identified several factors associated with MetS rather than a single common factor.[9] In a PC analysis of MetS and related (inflammation and thrombosis) domains using the IBC-chip genotyping platform, Avery et al[33] identified variants in the diverse genes (*APOC1*, *BRAP*, and *PLCG1*) associated with multiple domains in European-descent individuals. Specifically, rs4420638/*APOC1* was associated with elevated plasma glucose, atherogenic

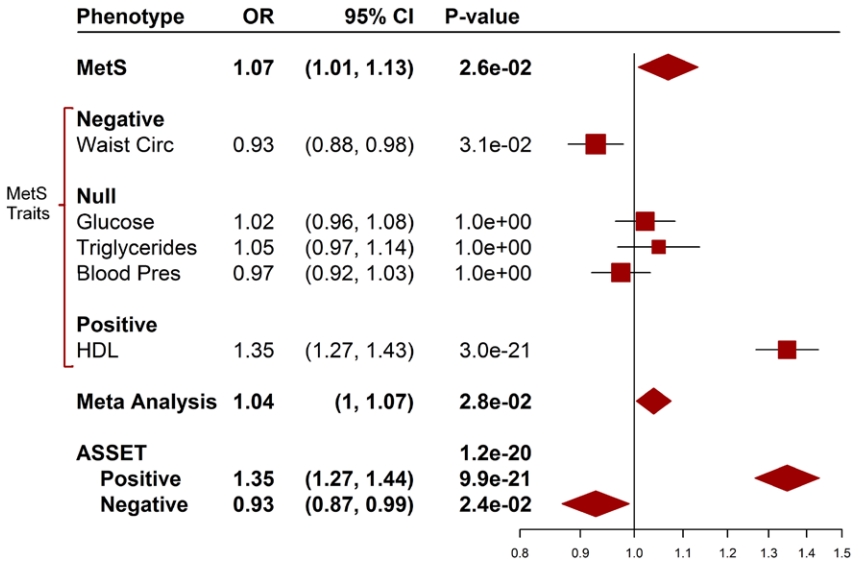


**Figure.** Plot of ASSET top SNP (rs247616) contrasting results from different methods. Results from ASSET, logistic regression models of metabolic syndrome (MetS) and MetS individual components, and the meta-analysis of MetS components for rs247616-G are plotted. *P* values for MetS components already include correction for multiple testing. The rs247616-G allele is strongly associated with higher odds of low high-density lipoprotein (HDL) levels, odds ratio (OR) 1.35 (95% confidence interval [CI], 1.27–1.43; *P*=3e–21), modestly associated with reduced odds of high waist circumference, OR 0.93 (95% CI, 0.88–0.98; *P*=0.031) and not associated with other MetS components (*P*>0.05). The ASSET test takes these component effects in opposite directions into account (hence the low *P*=1.2e–20). In contrast, results from the logistic regression analysis of MetS (*P*=0.026) and the meta-analysis of MetS components (*P*=0.028) are attenuated because of null component effects and effects in opposite directions.

dyslipidemia, vascular inflammation, and central obesity. These *APOC1* results are consistent with our findings although our nearby *APOC1* variant, rs12721054, was associated with all 5 MetS components in AAs and is only modestly correlated with rs4420638, $r^2$=0.39 in AAs (rs4420683 failed our SNP QC so we are unable to assess its association in AAs). Interestingly, rs12721054 is potentially a novel African-descent variant. It is essentially monomorphic in HapMap-3 CEU and rare in other HapMap-3 populations (eg, minor allele frequency=1% in HapMap-3 Mexicans). It was recently reported to be associated with triglycerides in a AA GWAS of lipid traits.[34] We found 2 additional significant SNPs in this gene-rich region: a missense variant in *APOE*, rs7412, and rs61679753, an intronic variant in *TOMM40*; both variants were associated with the HDL and waist circumference components and are correlated in AAs although neither is in high linkage disequilibrium with rs12721054/*APOC1* in AAs (Figure I in the Data Supplement ). Another variant associated with multiple MetS components in the literature is an intronic SNP in the glucokinase regulatory protein (*GCKR*) gene. The rs780094-T allele has been previously associated with increased triglyceride levels and lower glucose levels in European-descent populations.[35,36] Although *GCKR* SNPs did not reach our stringent threshold for statistical significance, we also found strong inverse associations in AAs for triglyceride and glucose components and additionally the waist circumference component for rs780094 (ASSET *P*=7.4e–6) and for the nearby *GCKR* variants: rs780093/intron (ASSET *P*=8.3e–6) and rs1260326/missense (ASSET *P*=7.1e–7). In our study, rs780094 was also weakly associated with a fourth component, HDL levels (Figure II in the Data Supplement). With component effects in opposite directions and 1 decidedly null component, rs780094 provides another interesting example of the use of an approach, such as ASSET, to illustrate relationships between the MetS components.

Overall, many of our top SNPs have been reported in GWAS of lipids traits (rs10096633/*LPL*,[37] rs247616/*CETP*,[38] rs7412/*APOE*,[39] and rs7499892/*CETP*)[40] or type 2 diabetes mellitus (rs7901695/*TCF7L2*)[41] in mainly European-descent populations. The *CETP* association with MetS has been implicated previously.[42] We additionally identified several SNPs with evidence of pleiotropic effects on MetS components. In addition to the *APOC1* variant associated with all components, variants in *APOE*, *TOMM40*, and *CETP* were associated with HDL and waist circumference components, often with effects in opposite directions so that the same allele was associated with increased risk of having 1 component (ie, low HDL) and decreased risk of having another component (ie, high waist circumference). *TCF7L2*, an important locus for diabetes mellitus, was associated with waist circumference and glucose components in opposing directions, which may make it difficult to detect in the context of MetS. *LPL* variants were associated with increased odds of having the triglycerides, HDL, and glucose components. In contrast to the ASSET findings, only 1 *LPL* SNP was significantly associated with MetS in the logistic regression analysis.

Although we have mentioned limitations of the conventional approaches, the ASSET method also has limitations. It clusters associations into 3 categories: positive, null, and negative associations. For some SNP-trait associations, this categorization may be arbitrary and not scientifically meaningful (ie, if no trait has a null association). In cases with no heterogeneity of effects, ASSET may perform similarly to a conventional meta-analysis, but with an additional multiple testing penalty. In addition, the 2-sided ASSET test only provides a *P* value and not an overall effect size.

Our use of the Metabochip array has some advantages; namely, we capture and finely map many of the known cardiometabolic and anthropometric genetic variants identified to date and genotype them on a common platform across studies using a centralized, stringent QC process. However, our ability to identify novel MetS variants or pathways (in gene regions not previously associated with cardiometabolic traits in GWAS) is limited. One challenge of the MetS outcome is that it requires nonmissing data for several variables measured at the same time point. For example, only 83% of our total sample had sufficient data for the MetS analysis. An

advantage of ASSET is that it can be used in samples with missing data to give population level associations with MetS components although it is important to emphasize that in this case, these inferences may not be shared among individuals in the population (ie, ecological fallacy).[43] In other words, although a SNP may be associated with ≥3 components in an ASSET analysis, it should not be inferred that accordingly, the SNP is also associated with MetS in individuals. Also, ASSET may yield significant results in the absence of pleiotropy, such as we saw for rs3764261/*CETP* or identify antagonistic pleiotropy associations inconsistent with clinical MetS (ie, the *TCFL2* variants). We acknowledge that common underlying cardiometabolic mediators may contribute to the observed pleiotropy; SNP effects on multiple traits may not be independent. (For more discussion of mediated pleiotropy see review by Solovieff et al.[44]) An additional limitation affects our pleiotropy inferences, namely, the potential misclassification error inherent in the MetS definition. For example, a user of lipid-lowering medication because of LDL dyslipidemia would automatically be classified as having ≥2MetS components (HDL and triglycerides) even if he or she had normal HDL and triglyceride levels. Consistent with ATP III criteria in which the presence of type 2 diabetes mellitus does not preclude a diagnosis of MetS,[8] individuals with type 2 diabetes mellitus were not excluded from analyses and account for <10% of the total sample. But, similarly, a treated diabetic (and therefore classified as having the glucose component) will likely have more aggressive treatment for cardiovascular disease prevention, such as the use of dyslipidemia medication, even if his or her lipid values are not particularly high, which could create a spurious pleiotropic relationship among glucose, HDL, and triglycerides. Although 2 *LPL* variants were associated with these 3 components, we do not see strong evidence of this scenario in our results; lipids medication use (and potential misclassification) was low at baseline in the WHI and ARIC AA populations, ≈7% and 1%, respectively.[45] More generally, dichotomization of continuous traits into binary components in this analysis also could result in loss of power and misclassification although we expect this misclassification error to be nondifferential with respect to genotype and any potential bias to be toward the null.

In summary, our discovery and validation results support previous genetic findings emphasizing the importance of lipids traits for MetS. Of the 27 significant variants, 24 were associated with the low HDL component in AAs. With the exception of the potentially AA-specific *APOC1* variant that was associated with all MetS components, we did not find strong evidence of a single underlying heritable factor for MetS clustering. This result is consistent with other studies, suggesting that a complex genetic architecture underlies MetS.[9] However, we did identify several pleiotropic variants in AAs, many demonstrating antagonistic pleiotropy for cardiometabolic traits important for MetS. We also report SNP associations for these important cardiometabolic traits in a population with a high burden of MetS, Hispanics, although pleiotropy findings in the Hispanic population were attenuated, perhaps because of reduced power in the smaller sample. However, if confirmed, such information on pleiotropy, and particularly, alleles with opposing effects, may be clinically important, because it could inform translational research of potential gene targets and be useful in risk profiling and in the development, marketing, and prediction of side effects of new medications.[46] In addition, we highlight the value of using new methods to increase power and efficiency in large-scale genomic association analyses and demonstrate challenges related to the use of the MetS construct in large-scale genomic studies.

## Acknowledgments

The Population Architecture using Genomics and Epidemiology (PAGE) consortium thanks the staff and participants of all PAGE studies for their important contributions. We thank the Women's Health Initiative (WHI) investigators and staff for their dedication, and the study participants for making the program possible. A full listing of WHI investigators can be found at https://www.whi.org/researchers/SitePages/WHI%20Investigators.aspx.

## Sources of Funding

This work was supported by the Population Architecture Using Genomics and Epidemiology (PAGE) program which is funded by the National Human Genome Research Institute (NHGRI) (U01HG004803 [to the Genetic Epidemiology of Causal Variants Across the Life Course {CALiCo} program], U01HG004798 [to the Epidemiological Architecture of Genes Linked to Environment], U01HG004802 [to the Multi-Ethnic Cohort], U01HG004790 [to the Women's Health Initiative {WHI}], and U01HG004801 [to the PAGE Coordinating Center], and their respective NHGRI American Recovery and Reinvestment Act supplements. Funding support for the Epidemiology of putative genetic variants: The Women's Health Initiative (WHI) study is provided through the NHGRI PAGE program (U01HG004790 and its NHGRI American Recovery and Reinvestment Act supplement). The WHI program is funded by the National Heart, Lung, and Blood Institute, NIH, US Department of Health and Human Services through contracts HHSN268201100046C, HHSN268201100001C, HHSN268201100002C, HHSN268201100003C, HHSN268201100004C, and HHSN271201100004C. Funding support for the Genetic Epidemiology of CALiCo program is provided through the NHGRI PAGE program (U01HG004803 and its NHGRI American Recovery and Reinvestment Act supplement). The following CALiCo studies contributed to this article and are funded by the following agencies: The Atherosclerosis Risk in Communities study is performed as a collaborative study supported by National Heart, Lung, and Blood Institute contracts (N01-HC-55015, N01-HC-55016, N01-HC-55018, N01-HC-55019, N01-HC-55020, N01-HC-55021, and N01-HC-55022). Assistance with phenotype harmonization, single nucleotide polymorphisms selection and annotation, data cleaning, data management, integration and dissemination, and general study coordination was provided by the PAGE Coordinating Center (U01HG004801-01 and its NHGRI American Recovery and Reinvestment Act supplement). The National Institutes of Mental Health also contributes to the support for the PAGE Coordinating Center. Additional support for this work was provided by the NHGRI through (R01 HG006124).

## Disclosures

None.

## Appendix

From the Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA (C.L.C., J.H., C.S.C., U.P., C.K.); Netaji Subhas Sanatorium, National Institute of Biomedical Genomics, Kalyani, West Bengal, India (S.B.); Division of Genomic Medicine, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD (L.A.H.); Cancer Prevention Institute of California, Fremont (I.C.); MedStar Health Research Institute, Hyattsville, MD (V.A.); Division of Biomedical Informatics, University of California San Diego (C.-N.H.); Biostatistics & Informatics Shared Resource, University of Hawaii Cancer Center, Honolulu (L.W.); Department of Epidemiology, Brown University,

Providence, RI (S.L.); Welch Center for Prevention, Epidemiology and Clinical Research, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD (E.S.); Division of Endocrinology, Diabetes & Metabolism, Department of Internal Medicine, The Ohio State University, Columbus (R.J.); Department of Epidemiology, Carolina Center for Genome Sciences, University of North Carolina, Chapel Hill (K.E.N.); Division of Epidemiology & Community Health, University of Minnesota, Minneapolis (J.S.P.); and Biostatistics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD (N.C.).

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## CLINICAL PERSPECTIVE

Metabolic syndrome (MetS) is characterized by a clustering of cardiometabolic risk factors, including dyslipidemia, central obesity, high blood pressure, and high blood glucose levels. Individuals with MetS have an increased risk of cardiovascular disease, diabetes mellitus, and mortality, yet the cause of MetS is not well understood and its pathophysiology is further complicated by correlations among the MetS traits. Investigation of genetic factors associated with MetS may shed light on key pathways or mediators underlying the syndrome. Applying a new method, ASsociation analysis based on subSETs, we sought to identify MetS susceptibility loci and investigate whether genetic variants with pleiotropic effects account for some of the correlated architecture of MetS traits in a large African American population. A total of 27 variants in 1 glucose and 4 lipids loci (*TCF7L2*, *LPL*, *APOA5*, *CETP*, and *APOC1/APOE/TOMM40*) were significantly associated with MetS traits. Three loci replicated in a Hispanic population. A novel African American-specific variant, rs12721054/*APOC1*, and rs10096633/*LPL* were associated with ≥3 MetS traits. We found additional evidence of pleiotropy for *APOE, TOMM40, TCF7L2*, and *CETP* variants, many with opposing effects (eg, the same rs7901695/*TCF7L2* allele is associated with increased odds of high glucose and decreased odds of central adiposity). Our study highlights a method to increase power in large-scale genomic association analyses and reports a novel variant associated with all MetS components in African Americans. In addition, the pleiotropic associations we identify, particularly the alleles with opposing relationships, may be clinically important, as such information could inform translational research of potential gene targets and medications and be useful in risk profiling of patients.