Published in final edited form as:

Circ Cardiovasc Genet. 2014 August ; 7(4): 505–513. doi:10.1161/CIRCGENETICS.113.000386.

Comparative Analysis of Metabolic Syndrome Components in over 15,000 African Americans Identifies Pleiotropic Variants: Results from the PAGE Study

Cara L. Carty, PhD[1], **Samsiddhi Bhattacharjee, PhD**[2,3], **Jeff Haessler, MS**[1], **Iona Cheng, PhD**[4], **Lucia A. Hindorff, MPH, PhD**[5], **Vanita Aroda, MD**[6], **Christopher S. Carlson, PhD**[1], **Chun-Nan Hsu, PhD**[7], **Lynne Wilkens, PhD**[8], **Simin Liu, MD, ScD**[9], **Elizabeth Selvin, PhD, MPH**[10], **Rebecca Jackson, MD**[11], **Kari E. North, PhD**[12], **Ulrike Peters, PhD**[1], **James S. Pankow, PhD**[13], **Nilanjan Chatterjee, PhD**[2], and **Charles Kooperberg, PhD**[1]

[1]Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA

[2]National Cancer Institute

[3]National Institute of Biomedical Genomics, Kalyani, WB, India

[4]Cancer Prevention Institute of California, Fremont, CA

[5]National Human Genome Research Institute, National Institutes of Health, Bethesda, MD

[6]MedStar Health Research Institute, Hyattsville, MD

[7]University of Southern California, Marina del Rey, CA

[8]University of Hawaii Cancer Center, Honolulu, HI

[9]Brown University, Providence, RI

[10]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

[11]Ohio State University, Columbus, OH

[12]University of North Carolina, Chapel Hill, NC

[13]University of Minnesota, Minneapolis, MN

Abstract

Background—Metabolic syndrome (MetS) refers to the clustering of cardio-metabolic 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 (AA) from the 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).

Correspondence: Cara L. Carty, Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave N. / M3-A410, Seattle, WA 98109, Tel: 206-667-2873, Fax: 206-667-4142, ccarty@fhcrc.org.

Conflict of Interest Disclosures: None.

Unlike conventional models which lack power when associations for MetS components are null or have opposite effects, ASSET uses one-sided tests to detect positive and negative associations for components separately and combines tests accounting for correlations among components. With ASSET, we identify 27 SNPs in 1 glucose and 4 lipids loci (*TCF7L2, LPL, APOA5, CETP, LPL, 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 AA-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; e.g. 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 AA. 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.

Keywords

metabolic syndrome; population studies; high-density lipoprotein cholesterol; genetic variation; hyperglycemia; ASSET; PAGE Study; African Americans; cardio-metabolic traits; Metabochip

---

Metabolic Syndrome (MetS) is the designation used for the clustering of cardio-metabolic 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 and cardiovascular disease, and all-cause mortality.[1–4] Prevalence of MetS varies by race and sex; in the US, it is higher in African American women than men, and higher in Hispanics than in other race/ethnicity groups.[5] Overall, the age-adjusted prevalence ranges from 16–36% in the US and has been increasing in recent years,[5] yet the etiology of MetS is not well-understood. While 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.

As per the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP-III) criteria,[4, 8] MetS requires the presence of least three of the five following components: central obesity, high blood pressure, elevated triglycerides, low high density lipoprotein cholesterol (HDL), or fasting hyperglycemia; use of medication to treat the prior four conditions also qualifies. The heritability of MetS, as defined above, is estimated to be approximately 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 three components may not cluster in all MetS cases, and SNPs may be associated with one MetS component and not with another. This complexity has prompted the use of novel methods such as factor analysis or gene network analysis to better 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 African Americans and investigate whether the identified genetic variants show evidence of pleiotropy (i.e. a genetic variant influencing multiple traits), and thus may explain some of the correlated architecture of MetS traits.

Using the Metabochip genotyping array[12] in African Americans from the Population Architecture using Genomics and Epidemiology (PAGE) Study, we contrast SNP association findings from ASSET with those from a) logistic regression modeling MetS as a binary outcome, and b) meta-analysis of the 5 individual components of MetS, coded as binary variables. Unlike the traditional meta-analysis which may lack power when associations for each of the components are heterogeneous (i.e. null, or have effects in opposite directions), ASSET uses one-sided tests to detect positive and negative results 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 African American (AA) adults from the Atherosclerosis Risk in Communities (ARIC) and Women's Health Initiative (WHI) who are part of the Population Architecture using Genomics and Epidemiology (PAGE) Study.[13] Briefly, the ARIC study is a population-based cohort of non-Hispanic white and AA men and women who were between 45–64 years of age in 1987-89 and recruited from four U.S. communities: Forsyth County, North Carolina; Jackson, Mississippi; suburban areas of Minneapolis, Minnesota; and Washington County, Maryland.[14] A subset of the ARIC cohort, n= 3,340 ARIC AA with available Metabochip genotyping were included in these analyses. The WHI is a study of the etiology and prevention of chronic diseases in 161,838 postmenopausal women aged 50–79 years at recruitment (1993-98) from 40 clinical centers in the US.[15, 16] WHI consists of an observational study, two 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 NCEP-ATP-III guidelines requiring the presence of ≥ 3 of the following : waist circumference > 102cm in men or 88 cm in women; triglyceride levels ≥ 150 mg/dl or treatment for dyslipidemia; HDL levels < 40 mg/dl in men or <50mg/dL in women or treatment for dyslipidemia; blood pressure levels ≥ 130/85 mmHg or treatment for hypertension; and fasting plasma glucose levels ≥ 100 mg/dL or treatment for diabetes.[8, 17] Practically, these guidelines differ from the NCEP-ATP-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 non-missing data for the majority of components (three of five) at baseline were included as MetS cases or controls in analyses; for example, an individual having three of five components but missing data for the other two, was included as a MetS case, and an individual not meeting criteria for three of the five components, even if the other two 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 0.5cm. 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 absence of the condition in individuals with non-missing data for that component. The ASSET methodology, as described below, includes any participant with baseline data for at least one of the five MetS components.

Genotyping

The Metabochip is a high density genotyping array designed for fine-mapping GWAS-identified regions for cardio-metabolic 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 ≥ 1e-6. The following sample criteria were applied: sample call rate ≥ 0.95; no excessive heterozygosity (|F| > .35); no over-relatedness (≤ 100 1st and 2nd degree relatives); sample not an ancestry outlier (≤ 6 standard deviations in 1st 10 PCA); and among estimated 1st degree relative pairs, the 1st 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 a) SNPs and MetS as a binary outcome, and b) SNPs and individual MetS components coded as binary variables based on the modified NCEP-ATP-III guidelines. All models were adjusted for age, sex (except WHI) and global ancestry using principal components. Determination of ancestry principal components (PCs) was performed with EIGENSOFT[21, 22] as described in Buyske *et al*. (supplemental methods).[19] In both ARIC and WHI, models were adjusted for the first two 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 five 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 five 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 (i.e. 0.05/169,196 SNPs tested). For investigation of pleiotropy for SNPs that were significant in the ASSET models, i.e. 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*., ASSET[11] 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 due to overlapping participants (since 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 two directions using a chi-square 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 AA were included in the ASSET analysis; these individuals had Metabochip genotype data and baseline data for at least one MetS component. A subset of those individuals, n=12,574, had sufficient non-missing data for the classification of 5,507 MetS cases (43.7%) and 7,067 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 female 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 LD with each other and not

independent, we restrict further discussion to the most significant SNPs, i.e. 'top SNPs', in each locus and to other significant SNPs that are in low LD (r^2<0.2 in AA) with these top SNPs for a total of 11 SNPs in 5 loci. Only one 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 two SNPs were also significant in the ASSET analysis.

Pleiotropy

Of the top ASSET SNPs: rs12721054/*APOC1* was associated with all five 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 Figure 1.

Validation

We investigated MetS traits associations for the 27 SNPs significant in the AA ASSET models in a large, independent population of Hispanic Americans, n=5172. Characteristics of the population are presented in Supplementary Table 1. 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 three 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/#of loci.) Pleiotropy was less evident in the Hispanic population, though overall, trends were consistent with AA. 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

While the clinical utility 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 approximately 5% in 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 pathways,[29–31] though 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 (T2D), including the intronic SNP that we identified (rs7903146) which was recently reported in African Americans.[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 SNPs, 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 GWA studies of MetS have had variable success in identifying reproducible, common genetic mechanisms underlying MetS. Instead, these studies have generally identified genes from specific pathways which may be strongly associated with 1 or 2 of the MetS components, but not necessarily 3 or more.[29, 30] Similarly, most factor analysis and principal component (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*. identified variants in the diverse genes (*APOC1*, *BRAP*, and *PLCG1*) associated with multiple domains in European-descent individuals.[33] Specifically, rs4420638/*APOC1* was associated with elevated plasma glucose, atherogenic 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 AA, and is only modestly correlated with rs4420638, r^2=0.39 in AA. (rs4420683 failed our SNP QC so we are unable to assess its association in AA) Interestingly, rs12721054 is potentially a novel African-descent variant. It is essentially monomorphic in HapMap-3 CEU and rare in other HapMap-3 populations, e.g. minor allele frequency=1% in HapMap-3 Mexicans. It was recently reported to be associated with triglycerides in an AA GWAS of lipid traits.[34] We found two 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 AA, though neither is in high LD with rs12721054/ *APOC1* in AA (Supplemental Figure 1). 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 AA for triglyceride and glucose components and additionally, the waist circumference component for rs780094 (ASSET P-value= 7.4e-6) and for the nearby *GCKR* variants: rs780093/intron (ASSET P-value=8.3e-6) and rs1260326/missense (ASSET P-value=7.1e-7). In our study, rs780094 was also weakly associated with a fourth component, HDL levels (Supplemental Figure 2). With component effects in opposite directions and one decidedly null component, rs780094 provides another interesting example of the utility 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], rs7499892/*CETP* [40]) or T2D (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 one component (i.e. low HDL) and decreased risk of having another component (i.e. high waist circumference). *TCF7L2*, an important locus for diabetes, 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 one *LPL* SNP was significantly associated with MetS in the logistic regression analysis.

While we have mentioned limitations of the conventional approaches, the ASSET method also has limitations. It clusters associations into three categories: positive, null and negative associations. For some SNP-trait associations, this categorization may be arbitrary and not scientifically meaningful, i.e. 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. Additionally, the two-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 cardio-metabolic 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 cardio-metabolic traits in GWAS) is limited. One challenge of the MetS outcome is that it requires non-missing 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, though it is important to emphasize that in this case, these inferences may not be shared among individuals in the population, i.e. ecological fallacy.[43] In other words, while a SNP may be associated with 3 or more 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, i.e. the *TCFL2* variants. We acknowledge that common underlying cardio-metabolic 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.[44]) An additional limitation impacts our pleiotropy inferences; namely, the potential misclassification error inherent in the MetS definition. For example, a user of lipid-lowering medication due to LDL dyslipidemia would automatically be classified as having at least two MetS components (HDL and triglycerides) even if he/she had normal HDL and triglyceride levels. Consistent

with ATP III criteria in which the presence of T2D does not preclude a diagnosis of MetS,[8] type 2 diabetics 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 use of dyslipidemia medication, even if his/her lipid values are not particularly high, which could create a spurious pleiotropic relationship between glucose, HDL, and triglycerides. Although two *LPL* variants were associated with these three 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, approximately 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 non-differential with respect to genotype and any potential bias to be towards 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 AA. With the exception of the potentially AA-specific *APOC1* variant which 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 a number of pleiotropic variants in AA, many demonstrating antagonistic pleiotropy for cardio-metabolic traits important for MetS. We also report SNP associations for these important cardiometabolic traits in a population with a high burden of MetS, Hispanics, though pleiotropy findings in the Hispanic population were attenuated, perhaps due to reduced power in the smaller sample. However, if confirmed, such information on pleiotropy, and particularly, alleles with opposing effects, may be clinically important, as 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] Additionally, 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The PAGE consortium thanks the staff and participants of all PAGE studies for their important contributions. The authors thank the 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://cleo.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Long%20List.pdf

Funding Sources: (a) This work is 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 Epidemiologic Architecture of Genes Linked to Environment (EAGLE)), U01HG004802 (to the Multi-Ethnic Cohort (MEC)), U01HG004790 (to the Women's Health Initiative (WHI)), and U01HG004801 (to the PAGE Coordinating Center), and their respective NHGRI ARRA supplements. The contents of this paper are solely the

responsibility of the authors and do not necessarily represent the official views of the NIH. The complete list of PAGE members can be found at http://www.pagestudy.org.

(b) The data and materials included in this report result from collaboration between the following studies. Funding support for the “Epidemiology of putative genetic variants: The Women’s Health Initiative” study is provided through the NHGRI PAGE program (U01HG004790 and its NHGRI ARRA supplement). The WHI program is funded by the National Heart, Lung, and Blood Institute, National Institutes of Health, U.S. Department of Health and Human Services through contracts HHSN268201100046C, HHSN268201100001C, HHSN268201100002C, HHSN268201100003C, HHSN268201100004C, and HHSN271201100004C. Funding support for the Genetic Epidemiology of Causal Variants Across the Life Course (CALiCo) program is provided through the NHGRI PAGE program [U01HG004803 and its NHGRI ARRA supplement]. The following CALiCo studies contributed to this manuscript and are funded by the following agencies: The Atherosclerosis Risk in Communities (ARIC) Study is carried out 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, N01-HC-55022].

Assistance with phenotype harmonization, SNP 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 ARRA 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].

References

1. Ballantyne CM, Hoogeveen RC, McNeill AM, Heiss G, Schmidt MI, Duncan BB, et al. Metabolic syndrome risk for cardiovascular disease and diabetes in the ARIC study. Int J Obes. 2008; 32(Suppl 2):S21–S24.
2. Sattar N, McConnachie A, Shaper AG, Blauw GJ, Buckley BM, de Craen AJ, et al. Can metabolic syndrome usefully predict cardiovascular disease and diabetes? Outcome data from two prospective studies. Lancet. 2008; 371:1927–1935. [PubMed: 18501419]
3. Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, et al. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. JAMA. 2002; 288:2709–2716. [PubMed: 12460094]
4. Alberti KG, Zimmet P, Shaw J. The metabolic syndrome--a new worldwide definition. Lancet. 2005; 366:1059–1062. [PubMed: 16182882]
5. Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. JAMA. 2002; 287:356–359. [PubMed: 11790215]
6. Huang PL. A comprehensive definition for metabolic syndrome. Dis Model Mech. 2009; 2:231–237. [PubMed: 19407331]
7. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet. 2005; 365:1415–1428. [PubMed: 15836891]
8. Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation. 2004; 109:433–438. [PubMed: 14744958]
9. Teran-Garcia M, Bouchard C. Genetics of the metabolic syndrome. Appl Physiol Nutr Metab. 2007; 32:89–114. [PubMed: 17332787]
10. Zabaneh D, Chambers JC, Elliott P, Scott J, Balding DJ, Kooner JS. Heritability and genetic correlations of insulin resistance and component phenotypes in Asian Indian families using a multivariate analysis. Diabetologia. 2009; 52:2585–2589. [PubMed: 19763535]
11. Bhattacharjee S, Rajaraman P, Jacobs KB, Wheeler WA, Melin BS, Hartge P, et al. A subset-based approach improves power and interpretation for the combined analysis of genetic association studies of heterogeneous traits. Am J Hum Genet. 2012; 90:821–835. [PubMed: 22560090]
12. Voight BF, Kang HM, Ding J, Palmer CD, Sidore C, Chines PS, et al. The metabochip, a custom genotyping array for genetic studies of metabolic, cardiovascular, and anthropometric traits. PLoS Genet. 2012; 8:e1002793. [PubMed: 22876189]

13. Matise TC, Ambite JL, Buyske S, Carlson CS, Cole SA, Crawford DC, et al. The next PAGE in understanding complex traits: design for the analysis of Population Architecture Using Genetics and Epidemiology (PAGE) study. Am J Epidemiol. 2011; 174:849–859. [PubMed: 21836165]
14. The ARICInvestigators. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. Am J Epidemiol. 1989; 129:687–702. [PubMed: 2646917]
15. Anderson GL, Manson J, Wallace R, Lund B, Hall D, Davis S, et al. Implementation of the Women's Health Initiative study design. Ann Epidemiol. 2003; 13:S5–17. [PubMed: 14575938]
16. The Women's Health Initiative Study Group. Design of the Women's Health Initiative clinical trial and observational study. Control Clin Trials. 1998; 19:61–109. [PubMed: 9492970]
17. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 2009; 120:1640–1645. [PubMed: 19805654]
18. Genuth S, Alberti KG, Bennett P, Buse J, Defronzo R, Kahn R, et al. Follow-up report on the diagnosis of diabetes mellitus. Diabetes Care. 2003; 26:3160–3167. [PubMed: 14578255]
19. Buyske S, Wu Y, Carty CL, Cheng I, Assimes TL, Dumitrescu L, et al. Evaluation of the metabochip genotyping array in African Americans and implications for fine mapping of GWAS-identified loci: the PAGE study. PLoS ONE. 2012; 7:e35651. [PubMed: 22539988]
20. Liu EY, Buyske S, Aragaki AK, Peters U, Boerwinkle E, Carlson C, et al. Genotype imputation of Metabochip SNPs using a study-specific reference panel of ~4,000 haplotypes in African Americans from the Women's Health Initiative. Genet Epidemiol. 2012; 36:107–117. [PubMed: 22851474]
21. Patterson N, Price AL, Reich D. Population structure and eigenanalysis. PLoS Genet. 2006; 2:e190. [PubMed: 17194218]
22. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet. 2006; 38:904–909. [PubMed: 16862161]
23. R Development Core Team. A language and environment for statistical computing. 2008
24. Berglund G, Elmstahl S, Janzon L, Larsson SA. The Malmo Diet, Cancer Study. Design and feasibility. J Intern Med. 1993; 233:45–51. [PubMed: 8429286]
25. Kahn R. Metabolic syndrome--what is the clinical usefulness? Lancet. 2008; 371:1892–1893. [PubMed: 18501420]
26. Monda KL, North KE, Hunt SC, Rao DC, Province MA, Kraja AT. The genetics of obesity and the metabolic syndrome. Endocr Metab Immune Disord Drug Targets. 2010; 10:86–108. [PubMed: 20406164]
27. Park YW, Zhu S, Palaniappan L, Heshka S, Carnethon MR, Heymsfield SB. The metabolic syndrome: prevalence and associated risk factor findings in the US population from the Third National Health and Nutrition Examination Survey, 1988–1994. Arch Intern Med. 2003; 163:427–436. [PubMed: 12588201]
28. Joy T, Hegele RA. Genetics of metabolic syndrome: is there a role for phenomics? Curr Atheroscler Rep. 2008; 10:201–208. [PubMed: 18489847]
29. Kristiansson K, Perola M, Tikkanen E, Kettunen J, Surakka I, Havulinna AS, et al. Genome-wide screen for metabolic syndrome susceptibility Loci reveals strong lipid gene contribution but no evidence for common genetic basis for clustering of metabolic syndrome traits. Circ Cardiovasc Genet. 2012; 5:242–249. [PubMed: 22399527]
30. Zabaneh D, Balding DJ. A genome-wide association study of the metabolic syndrome in Indian Asian men. PLoS ONE. 2010; 5:e11961. [PubMed: 20694148]
31. Kraja AT, Vaidya D, Pankow JS, Goodarzi MO, Assimes TL, Kullo IJ, et al. A bivariate genome-wide approach to metabolic syndrome: STAMPEED consortium. Diabetes. 2011; 60:1329–1339. [PubMed: 21386085]
32. Ng MC, Saxena R, Li J, Palmer ND, Dimitrov L, Xu J, et al. Transferability and fine mapping of type 2 diabetes loci in African Americans: the Candidate Gene Association Resource Plus Study. Diabetes. 2013; 62:965–976. [PubMed: 23193183]

33. Avery CL, He Q, North KE, Ambite JL, Boerwinkle E, Fornage M, et al. A phenomics-based strategy identifies loci on APOC1, BRAP, and PLCG1 associated with metabolic syndrome phenotype domains. PLoS Genet. 2011; 7:e1002322. [PubMed: 22022282]
34. Coram MA, Duan Q, Hoffmann TJ, Thornton T, Knowles JW, Johnson NA, et al. Genome-wide characterization of shared and distinct genetic components that influence blood lipid levels in ethnically diverse human populations. Am J Hum Genet. 2013; 92:904–916. [PubMed: 23726366]
35. Orho-Melander M, Melander O, Guiducci C, Perez-Martinez P, Corella D, Roos C, et al. Common missense variant in the glucokinase regulatory protein gene is associated with increased plasma triglyceride and C-reactive protein but lower fasting glucose concentrations. Diabetes. 2008; 57:3112–3121. [PubMed: 18678614]
36. Sparso T, Andersen G, Nielsen T, Burgdorf KS, Gjesing AP, Nielsen AL, et al. The GCKR rs780094 polymorphism is associated with elevated fasting serum triacylglycerol, reduced fasting and OGTT-related insulinaemia, and reduced risk of type 2 diabetes. Diabetologia. 2008; 51:70–75. [PubMed: 18008060]
37. Aulchenko YS, Ripatti S, Lindqvist I, Boomsma D, Heid IM, Pramstaller PP, et al. Loci influencing lipid levels and coronary heart disease risk in 16 European population cohorts. Nat Genet. 2009; 41:47–55. [PubMed: 19060911]
38. Smith EN, Chen W, Kahonen M, Kettunen J, Lehtimaki T, Peltonen L, et al. Longitudinal genome-wide association of cardiovascular disease risk factors in the Bogalusa heart study. PLoS Genet. 2010:6.
39. Kettunen J, Tukiainen T, Sarin AP, Ortega-Alonso A, Tikkanen E, Lyytikainen LP, et al. Genome-wide association study identifies multiple loci influencing human serum metabolite levels. Nat Genet. 2012; 44:269–276. [PubMed: 22286219]
40. Zemunik T, Boban M, Lauc G, Jankovic S, Rotim K, Vatavuk Z, et al. Genome-wide association study of biochemical traits in Korcula Island, Croatia. Croat Med J. 2009; 50:23–33. [PubMed: 19260141]
41. Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, Lango H, et al. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. Science. 2007; 316:1336–1341. [PubMed: 17463249]
42. Park YM, Province MA, Gao X, Feitosa M, Wu J, Ma D, et al. Longitudinal trends in the association of metabolic syndrome with 550 k single-nucleotide polymorphisms in the Framingham Heart Study. BMC Proc. 2009; 3(Suppl 7):S116. [PubMed: 20017981]
43. Robinson WS. Ecological correlations and the behavior of individuals. Am Sociol Rev. 1950; 15:351–357.
44. Solovieff N, Cotsapas C, Lee PH, Purcell SM, Smoller JW. Pleiotropy in complex traits: challenges and strategies. Nat Rev Genet. 2013; 14:483–495. [PubMed: 23752797]
45. Dumitrescu L, Carty CL, Taylor K, Schumacher FR, Hindorff LA, Ambite JL, et al. Genetic determinants of lipid traits in diverse populations from the population architecture using genomics and epidemiology (PAGE) study. PLoS Genet. 2011; 7:e1002138. [PubMed: 21738485]
46. Sivakumaran S, Agakov F, Theodoratou E, Prendergast JG, Zgaga L, Manolio T, et al. Abundant pleiotropy in human complex diseases and traits. Am J Hum Genet. 2011; 89:607–618. [PubMed: 22077970]

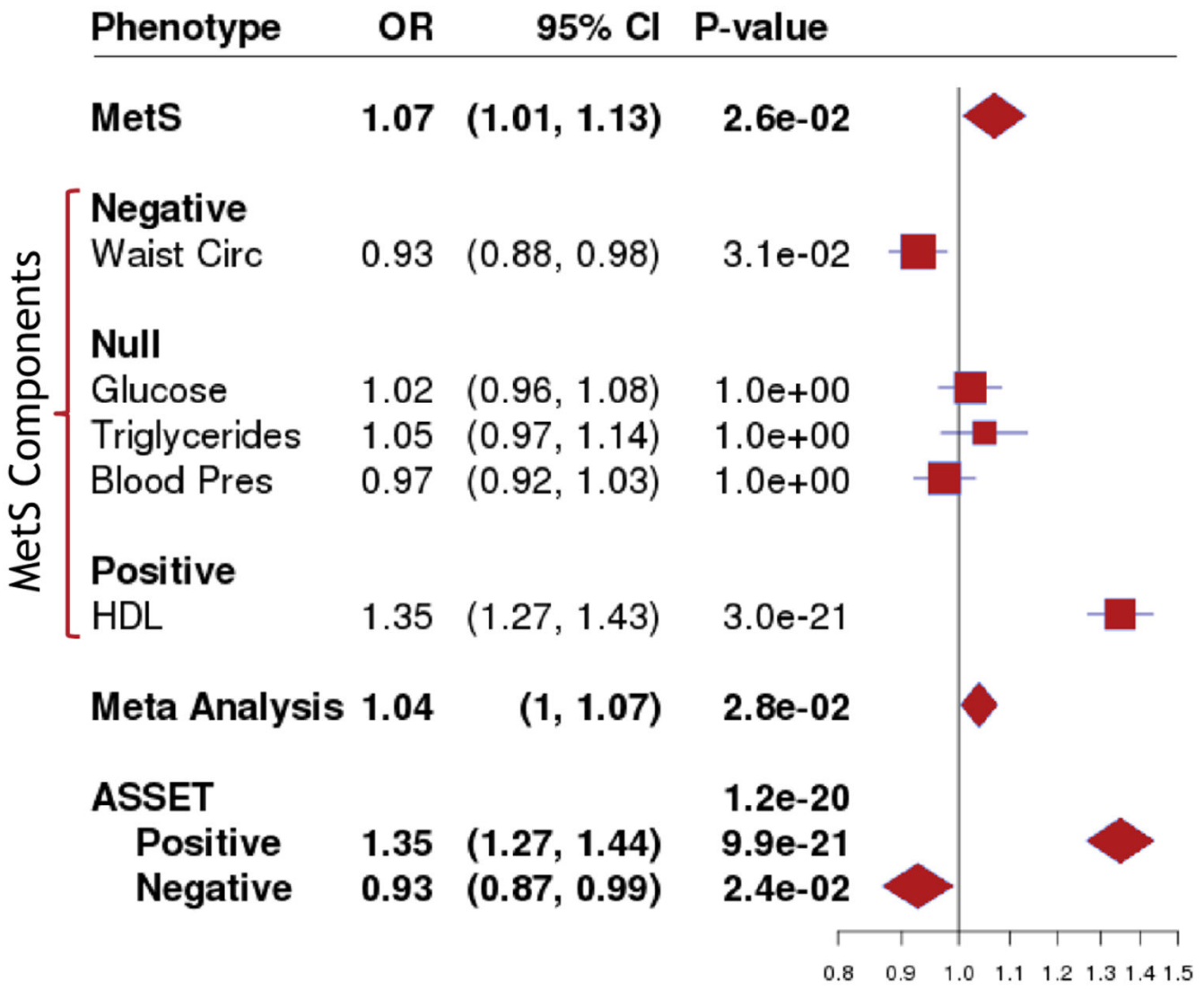

Figure 1.

Plot of ASSET top SNP (rs247616) contrasting results from different methods Results from ASSET, logistic regression models of MetS and MetS individual components, and the meta-analysis of MetS components for rs247616-G are plotted. Note: P-values for MetS components already include correction for multiple testing. The rs247616-G allele is strongly associated with higher odds of low HDL levels, OR=1.35 (95%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-value=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 due to null component effects and effects in opposite directions.

Table 1

Baseline characteristics of metabolic syndrome cases and controls, and all 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 in years	54.3 ±5.6	53.1 ± 5.9	53.5 ± 5.9	62.3 ± 6.9	61.1 ± 7.2	61.5 ± 7.1
Female, 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 med†	1042 (84.9)	615 (31.1)	1736 (53.3)	2890 (67.6)	732 (14.4)	3760 (39.7)

*percentage reflects proportion of MetS Cases, MetS Controls or ASSET totals with non-missing data, respectively

†med=medications

Table 2

Significant results from all models in PAGE African Americans

				Model P-values in PAGE 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	3.1e-2	1.8e-3	5.5e-8	0.14	0.11

				Model P-values in PAGE AA			LD‡	
Region* (Chr)	**SNP†**	**Annotation**	**CA/CAF**	**MetS Log Reg**	**Components Meta-Analysis**	**ASSET**	**AA**	**EA**
LDL.1 (19)	**rs12721054 ∥**	***APOC1*/3` UTR**	**G/0.12**	**2.3e-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

LD=linkage disequilibrium; AA=African Americans; Chr=chromosome; CA=coded allele; CAF=coded allele frequency; Log Reg= logistic regression; EA= individuals of European ancestry.

*The Metabochip consists of 257 finely-mapped regions based on GWAS hits for cardio-metabolic and anthropometric traits; regions listed are arbitrarily numbered.

†The SNP with the lowest P-value in each Metabochip region is bolded.

‡LD estimates reflect r^2 values between SNP and the bolded SNP 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.

#While these SNPs are not highly correlated with the bolded SNP in their region, they are highly correlated with each other in AA (r^2>0.6).

Table 3

ASSET and pleiotropy findings in PAGE 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					↑	↑			↓‡	

		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**
	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					↑				↓‡	

CAF=coded allele frequency; AA=African Americans; HA= Hispanic Americans

*Bolded SNPs reflect significant replication in ASSET, at P<0.05

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

‡Component P-value significant before multiple testing correction (i.e. P<0.05), but not after correction.

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