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(Article begins on next page)
Genome-Wide Association of Body Fat Distribution in African Ancestry Populations Suggests New Loci


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Research Center, Seattle, Washington, United States of America, 46 Department of Medicine, Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts, United States of America, 47 Department of Epidemiology, University of Pittsburgh, Graduate School of Public Health, Pittsburgh, Pennsylvania, United States of America, 48 Department of Social and Preventive Medicine, University at Buffalo, Buffalo, New York, United States of America, 49 Department of Preventive Medicine, University of Tennessee Health Science Center, Memphis, Tennessee, United States of America, 50 Department of Biostatistics, University of Buffalo School of Public Health and Health Professions, New York State Center of Excellence in Bioinformatics and Life Sciences, Buffalo, New York, United States of America, 51 Tufts University, Boston, Massachusetts, United States of America, 52 Department of Biostatistics, University of Washington, Seattle, Washington, United States of America, 53 MedStar Health Research Institute and Georgetown University, Hyattsville, Maryland, United States of America, 54 Department of Epidemiology and Prevention, Public Health Sciences, Wake Forest School of Medicine, Winston-Salem, North Carolina, United States of America, 55 University of California at San Diego Department of Preventive Medicine, La Jolla, California, United States of America, 56 Division of Human Genetics, Children’s Hospital of Philadelphia Research Institute, Philadelphia, Pennsylvania, United States of America, 57 Division of Public Health Sciences, Wake Forest School of Medicine, Winston-Salem, North Carolina, United States of America, 58 Division of Sleep Medicine, Brigham and Women’s Hospital, Boston, Massachusetts, United States of America, 59 Division of Epidemiology and Community Health, University of Minnesota, Minneapolis, Minnesota, United States of America, 60 Health Disparities Research Section, Clinical Research Branch, National Institute on Aging, National Institutes of Health, NIH Biomedical Center, Baltimore, Maryland, United States of America, 61 Center for Public Genomics, Department of Biochemistry and Molecular Genetics and Department of Medicine, University of Virginia, Charlottesville, Virginia, United States of America, 62 Department of Medicine, University of Vermont, Colchester, Vermont, United States of America, 63 Laboratory of Epidemiology, Demography, and Biometry, NIA, Bethesda, Maryland, United States of America, 64 Department of Epidemiology, University of Washington, Seattle, Washington, United States of America, 65 NHLBI’s Framingham Heart Study, Framingham, Massachusetts, United States of America, 66 NHLBI’s Center for Population Studies, Framingham, Massachusetts, United States of America, 67 Division of Endocrinology, Brigham and Women’s Hospital and Harvard Medical School, Boston, Massachusetts, United States of America

Abstract

Central obesity, measured by waist circumference (WC) or waist-hip ratio (WHR), is a marker of body fat distribution. Although obesity disproportionately affects minority populations, few studies have conducted genome-wide association study (GWAS) of fat distribution among those of predominantly African ancestry (AA). We performed GWAS of WC and WHR, adjusted and unadjusted for BMI, in up to 33,591 and 27,350 AA individuals, respectively. We identified loci associated with fat distribution in AA individuals using meta-analyses of GWA results for WC and WHR (stage 1). Overall, 25 SNPs with single genomic control (GC)-corrected p-values $<5.0 \times 10^{-6}$ were followed-up (stage 2) in AA with WC and with WHR. Additionally, we interrogated genomic regions of previously identified European ancestry (EA) WHR loci among AA. In joint analysis of association results including both Stage 1 and 2 cohorts, 2 SNPs demonstrated association, rs2075064 at $LHX2$, $p = 2.24 \times 10^{-8}$ for WC-adjusted-for-BMI, and rs6931262 at $RREB1$, $p = 2.48 \times 10^{-8}$ for WHR-adjusted-for-BMI. However, neither signal was genome-wide significant after double GC-correction ($LHX2$: $p = 6.5 \times 10^{-8}$; $RREB1$: $p = 5.7 \times 10^{-8}$). Six of fourteen previously reported loci for waist in EA populations were significant ($p < 0.05$ divided by the number of independent SNPs within the region) in AA studied here ($TBX15-WARS2, GRB14, ADAMTS9, LY86, RSPO3, ITPR2-SSPN$). Further, we observed associations with metabolic traits: rs13389219 at $GRB14$ associated with HDL-cholesterol, triglycerides, and fasting insulin, and rs13060013 at $ADAMTS9$ with HDL-cholesterol and fasting insulin. Finally, we observed nominal evidence for sexual dimorphism, with stronger results in AA women at the $GRB14$ locus ($p$ for interaction $= 0.02$). In conclusion, we identified two suggestive loci associated with fat distribution in AA populations in addition to confirming 6 loci previously identified in populations of EA. These findings reinforce the concept that there are fat distribution loci that are independent of generalized adiposity.


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individuals, ranging from 31%–76% [12–15] even after accounting for the heritability of levels for WC and WHR in EA and AA ethnicity [10,11] and demonstrate a genetic component. Twin studies established measures of body fat distribution [9] that differ by ethnicity and that are associated with diabetes, hypertension, and heart disease [3–6], even after accounting for generalized adiposity [7,8].

Introduction

Obesity is an important public health problem, leading to epidemic proportions. The prevalence varies by ethnicity, with nearly one-third of European ancestry (EA) and almost one-half of African ancestry (AA) Americans considered obese [1]. Recent studies have suggested that body fat distribution, above and beyond generalized adiposity, is an important metric of metabolic health, as studies have linked higher fat distribution with diabetes, hypertension, and heart disease [9].

Waist circumference (WC) and waist to hip ratio (WHR) are two important body fat distribution metrics. The WHR has been linked with diabetes, hypertension, and heart disease [3–6].

Results

We analyzed genetic loci for waist circumference (WC) and waist hip ratio (WHR) in a large-scale meta-analysis of waist-based traits in AA individuals. We conducted a large-scale meta-analysis of waist-based traits in AA individuals. We analyzed genetic loci for waist circumference (WC) and waist to hip ratio (WHR) in a large-scale meta-analysis of waist-based traits in AA individuals. We analyzed genetic loci for waist circumference (WC) and waist to hip ratio (WHR) in a large-scale meta-analysis of waist-based traits in AA individuals. We analyzed genetic loci for waist circumference (WC) and waist to hip ratio (WHR) in a large-scale meta-analysis of waist-based traits in AA individuals.
Author Summary

Central obesity is a marker of body fat distribution and is known to have a genetic underpinning. Few studies have reported genome-wide association study (GWAS) results among individuals of predominantly African ancestry (AA). We performed a collaborative meta-analysis in order to identify genetic loci associated with body fat distribution in AA individuals using waist circumference (WC) and waist to hip ratio (WHR) as measures of fat distribution, with and without adjustment for body mass index (BMI). We uncovered 2 genetic loci potentially associated with fat distribution: LHX2 in association with WC-adjusted-for-BMI and at RREB1 for WHR-adjusted-for-BMI. Six of fourteen previously reported loci for waist in EA populations were significant in AA studied here (TBX15-WARS2, GRB14, ADAMTS9, LY86, RSPO3, ITPR2-SSPN). These findings reinforce the concept that there are loci for body fat distribution that are independent of generalized adiposity.

Supplementary Table S2 and the Supplementary Materials

Text S1. In our GWAS analysis, we applied single genomic control (GC) correction to avoid the overly conservative double GC correction [22,23] but we also provide double GC-corrected p-values for the joint meta-analysis of stage 1 and stage 2 samples (Table 2).

Stage 1 Genome-Wide Association Analyses for WC and WHR

We conducted genome-wide association analyses for 3.2 million variants, including genotyped and imputed variants, among AA individuals for WC, WC adjusted for BMI (WC-BMI), WHR and WHR adjusted for BMI (WHR-BMI) within each cohort, overall and by sex, and meta-analyzed the results. The Quantile-Quantile and Manhattan plots for all analyses are displayed in Supplementary Figure S1. With concern of overly conservative double GC-correction, we applied single GC-correction p values to select variants for follow-up. Three loci had p < 5 x 10^{-6} under single GC-correction; rs2570467 at PCSK1 with WC, rs1345301 at IL1RL2 with WC, and rs17213965 at MYH11 with WHR-BMI, all in men (n = 5967, 5973, 4398, respectively). Across all traits analyzed, an additional 22 independent SNPs had a single GC-corrected p < 5 x 10^{-6} (Table 2). Heterogeneity tests were examined across all cohorts and none of these 25 SNPs were significant (all p-values > 0.05) in heterogeneity testing after adjusting for multiple testing, indicating that we did not observe statistically different allelic effects for these 25 SNPs across the participating studies.

Stage 2 Analyses and Joint Meta-Analysis of Stage 1 and Stage 2

We carried forward all 25 SNPs with single GC-corrected p < 5 x 10^{-6} from stage 1 and tested their association in Stage 2 with the traits of interest either in the gender-specific or gender-combined data depending on the findings in Stage 1, in up to 10,027 AA individuals with WC and 7,606 AA individuals with WHR. Significance was defined as the joint meta-analysis of stage 1 and stage 2 p-value < 5 x 10^{-6}. Results for these SNPs from discovery, validation and joint analyses are shown in Table 2, and the imputation quality for these SNPs is provided in Supplementary Table S3. Three SNPs with p < 5 x 10^{-6} in the men only analysis of Stage 1 failed to replicate (p > 5 x 10^{-6}, n = 6,000 in stage 1 and n = 3,250 in stage 2) but two of the 25 SNPs carried forward from Stage 1 reached genome-wide significance under single genomic control (GC) in the joint meta-analysis of Stage 1 and Stage 2 data: rs2075064 (LHX2, p = 2.24 x 10^{-7}) in association with WC-BMI, and rs6931262 (RREB1, p = 2.48 x 10^{-6}) in association with WHR-BMI. We note, however, that double GC-corrected p values for these two variants have slightly attenuated p-values: rs2075064 (LHX2, p = 6.5 x 10^{-6}) and rs6931262 (RREB1, p = 5.7 x 10^{-6}), which were no longer genome-wide significant. The regional association plots for these two loci are presented in Figure 1. The lead SNP rs6931262 at RREB1 is 474 kb away from r1294421 at LY86, previously identified in the Genetic Investigation of ANthropomorphic Traits (GIANT) consortium [17] of EA studies in association with WHR-BMI (r^2 = 0.007, D' = 0.093 among YRI Hapmap participants).

Further Characterization of LHX2 and RREB1 Loci

Given the tendency of waist-associated SNPs to exhibit sex-specific effects in samples of EA [17], we first tested the two AA waist loci for evidence of sexual dimorphism (Supplementary Table S4). There was no appreciable difference between the beta coefficients for the lead SNPs at LHX2 or RREB1 in women compared to men in the joint analysis of Stage 1 and Stage 2 samples (p_{sex difference} > 0.46), suggesting little to no sexual dimorphism with respect to these 2 loci.

Next, we tested whether the loci identified in the samples of African ancestry also demonstrate nominal associations in samples of European ancestry. We interrogated the evidence for association, both directional consistency and statistical significance, of these two SNPs in the GIANT consortium meta-analysis of WHR-BMI (n = 77,167 EA participants, http://www.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium) [17]. Neither rs2075064 at LHX2 (p = 0.78) nor rs6931262 at RREB1 (p = 0.13) was statistically significant. The direction of effect for the risk allele was consistent for RREB1 between EA and AA samples, while it was direction-inconsistent for LHX2. However, because linkage disequilibrium patterns with causal SNPs can differ, or allelic heterogeneity can exist across ethnicities, we tested for SNP associations within the 250 kb flanking genomic regions centered at our two top signals to examine whether SNPs in these genomic regions might be associated with WHR-BMI in EA samples. For the LHX2 region, the SNP with the lowest p-value was rs10986172 (MAF = 0.06, p = 2.6 x 10^{-7}, ~30 kb from rs2075064; Figure 2a), which did not reach the Bonferroni-corrected p-value threshold of 6.02 x 10^{-4} (0.05/83 independent tests). For the RREB1 region, the SNP with the lowest p-value in the European Ancestry data was rs9392863 (MAF = 0.26, p = 1.30 x 10^{-3}, ~20 kb from rs6931262, LD with rs6931262: r^2 = 0.005 and D' = 1.00 in HapMap CEU; Figure 2b) which met the Bonferroni-corrected threshold of 6.10 x 10^{-4} (0.05/82) in EA samples. Note that the association for rs9392863 was not significant (p-value = 0.57, LD with rs6931262: r^2 = 0.001 and D' = 1.00 in HapMap YRI) in our AA samples.

Waist circumference may be greater in tall adults. To distinguish the evidence of association with WC-BMI from height, we also tested whether rs2075064 at LHX2 might also be associated with height in the GIANT GWAS of Height (http://www.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium) [23]. No evidence of association was noted (p-value of 0.95).

Local Ancestry Analysis

Recently admixed individuals, such as samples of African ancestry, may have inherited ancestry from more than one ancestral population. However, local ancestry may be confounded with the association signal and lead to spurious association in association analysis. So to further characterize the differences by ancestral groups of our two novel loci (LHX2 and RREB1), we...
performed a sensitivity analysis by additionally adjusting for local ancestry to account for the effect on our trait of interest due to the local ancestry at the tested variant using 5 Stage 1 African ancestry studies. Local ancestry adjustment resulted in similar effect estimates (Supplementary Table S5), suggesting it is unlikely to account for our reported signals.

**Interrogation of Known European WHR Loci in African Ancestry Participants**

Given the association of the *RREB1* locus with WHR-BMI in both AA and EA participants, we next examined fourteen previously published loci in association with WHR-BMI in EA participants [17] in our AA sample (Table 3). Twelve (except rs6861681 and rs1055144) of the fourteen SNPs had the same effect direction with respect to the beta coefficient (binomial distribution p-value = 0.0065), and five demonstrated nominal significance (p < 0.05) in our AA Stage 1 sample (p-value range 1.6 × 10^{-5} to 8.5 × 10^{-4}). We also conduct two-sample t-test to compare the beta coefficients between EA samples and AA samples. None of these fourteen SNPs displayed significant heterogeneity between the two races. We next interrogated the flanking 250 kb genomic regions centered at each of the 14 SNPs in our AA dataset. Of the 14 SNPs, 9 SNPs met the locus-specific Bonferroni corrected threshold in the Stage 1 sample and were carried forward for Stage 2 validation. In the combined Stage 1 and 2 sample, none of these 9 SNPs, 6 remained significant with p-values less than the locus-specific Bonferroni-corrected threshold (0.05 divided by the number of independent SNPs within the

### Table 1. Study sample characteristics.

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample Size WC/Hip</th>
<th>European Ancestry %</th>
<th>Women %</th>
<th>Age (years)</th>
<th>WC (cm)</th>
<th>Hip (cm)</th>
<th>WHR</th>
<th>BMI</th>
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<tbody>
<tr>
<td><strong>Stage 1 Cohorts</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>WHI-SHAre</td>
<td>8138/8128</td>
<td>NA</td>
<td>100</td>
<td>61.6 (7.0)</td>
<td>91.5 (13.4)</td>
<td>111.2 (12.7)</td>
<td>0.82 (0.07)</td>
<td>31.0 (6.4)</td>
</tr>
<tr>
<td>HANDLS</td>
<td>961/961</td>
<td>0.16,0.11/0.22</td>
<td>55.4</td>
<td>48.5 (9.0)</td>
<td>98.5 (17.5)</td>
<td>107.5 (16.4)</td>
<td>0.91 (0.07)</td>
<td>29.9 (8.0)</td>
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<td>MESA/SHAre (Family)</td>
<td>946/946</td>
<td>0.22,0.12/0.30</td>
<td>60.5</td>
<td>58.2 (8.7)</td>
<td>100.6 (16.2)</td>
<td>110.1 (13.2)</td>
<td>0.91 (0.08)</td>
<td>30.7 (6.4)</td>
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<tr>
<td>Health ABC</td>
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<td>57.2</td>
<td>73.4 (2.9)</td>
<td>100.3 (13.8)</td>
<td>NA</td>
<td>NA</td>
<td>28.6 (5.4)</td>
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<td>GENOA</td>
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<td>0.14,0.09/0.21</td>
<td>70.4</td>
<td>56.4 (11.2)</td>
<td>102.7 (17.1)</td>
<td>113.3 (15.0)</td>
<td>0.91 (0.08)</td>
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<td>GeneSTAR</td>
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<td>61.7</td>
<td>42.8 (10.4)</td>
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<td>Family Heart Study</td>
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<td>0.13,0.09/0.19</td>
<td>65.7</td>
<td>53.3 (10.8)</td>
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<td>114.3 (15.7)</td>
<td>0.92 (0.07)</td>
<td>32.7 (7.4)</td>
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<td>HyperGEN</td>
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<td>102.9 (18.5)</td>
<td>114.4 (16.1)</td>
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<td>HUFS</td>
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<td><strong>Stage 2 Cohorts</strong></td>
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<td>CHS</td>
<td>819/820</td>
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<td>BWHHS</td>
<td>1499/1499</td>
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<td>60.9</td>
<td>46.9 (10.1)</td>
<td>82.5 (13.0)</td>
<td>105.4 (12.9)</td>
<td>0.78 (0.09)</td>
<td>28.0 (5.7)</td>
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<td>REGARDS_Diabetes_CASES</td>
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<td>64.7</td>
<td>63.4 (8.7)</td>
<td>104.1(14.5)</td>
<td>NA</td>
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<td>30.2 (7.0)</td>
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<tr>
<td>REGARDS_CONTROLS</td>
<td>1244/0</td>
<td>0.14,0.08/0.22</td>
<td>64.7</td>
<td>63.4 (8.5)</td>
<td>104.2 (14.3)</td>
<td>NA</td>
<td>NA</td>
<td>30.1 (7.2)</td>
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<tr>
<td>SUGAR_Diabetes_CASES</td>
<td>865/855</td>
<td>0.05,0.02/0.09</td>
<td>78.7</td>
<td>54.1 (14.1)</td>
<td>99.5 (16.7)</td>
<td>108.4 (12.5)</td>
<td>0.92 (0.08)</td>
<td>34.1 (7.9)</td>
</tr>
<tr>
<td>SUGAR_CONTROLS</td>
<td>182/181</td>
<td>0.05,0.02/0.11</td>
<td>72.1</td>
<td>54.2 (14.1)</td>
<td>99.5 (16.8)</td>
<td>114.3 (12.6)</td>
<td>0.87 (0.09)</td>
<td>34.7 (6.8)</td>
</tr>
<tr>
<td>MEC Breast Cancer Cases</td>
<td>259/258</td>
<td>NA</td>
<td>100</td>
<td>71.6 (8.6)</td>
<td>96.0 (14.3)</td>
<td>109.1 (14.0)</td>
<td>0.88 (0.10)</td>
<td>28.6 (5.8)</td>
</tr>
<tr>
<td>MEC Breast Cancer Controls</td>
<td>528/527</td>
<td>NA</td>
<td>100</td>
<td>70.9 (8.4)</td>
<td>95.4 (14.4)</td>
<td>110.2 (14.3)</td>
<td>0.87 (0.09)</td>
<td>28.7 (6.0)</td>
</tr>
<tr>
<td>CBCS Breast Cancer Cases</td>
<td>626/626</td>
<td>NA</td>
<td>100</td>
<td>51.3 (11.9)</td>
<td>96.6 (14.2)</td>
<td>113.3 (13.8)</td>
<td>0.85 (0.08)</td>
<td>31.9 (7.2)</td>
</tr>
<tr>
<td>CBCS Breast Cancer Controls</td>
<td>579/579</td>
<td>NA</td>
<td>100</td>
<td>51.8 (11.4)</td>
<td>95.5 (15.1)</td>
<td>114.3 (13.9)</td>
<td>0.84 (0.08)</td>
<td>32.3 (7.5)</td>
</tr>
<tr>
<td>WCHS Breast Cancer Cases</td>
<td>256/259</td>
<td>NA</td>
<td>100</td>
<td>49.8 (9.7)</td>
<td>101.1(16.7)</td>
<td>114.0 (13.9)</td>
<td>0.88 (0.07)</td>
<td>31.9 (7.1)</td>
</tr>
<tr>
<td>WCHS Breast Cancer Controls</td>
<td>237/237</td>
<td>NA</td>
<td>100</td>
<td>49.8 (9.4)</td>
<td>98.7 (15.6)</td>
<td>113.0 (13.7)</td>
<td>0.87 (0.07)</td>
<td>31.3 (7.0)</td>
</tr>
<tr>
<td>MEC Prostate Cancer Cases</td>
<td>542/528</td>
<td>NA</td>
<td>0</td>
<td>73.8 (7.0)</td>
<td>99.9 (12.4)</td>
<td>105.5 (10.9)</td>
<td>0.94 (0.09)</td>
<td>27.1 (4.0)</td>
</tr>
<tr>
<td>MEC Prostate Cancer Controls</td>
<td>877/856</td>
<td>NA</td>
<td>0</td>
<td>69.6 (8.5)</td>
<td>99.4 (12.7)</td>
<td>105.8 (12.0)</td>
<td>0.94 (0.11)</td>
<td>27.3 (3.8)</td>
</tr>
<tr>
<td>MDA Prostate Cancer Cases</td>
<td>153/153</td>
<td>NA</td>
<td>0</td>
<td>59.2 (7.8)</td>
<td>100.5 (13.0)</td>
<td>107.9 (11.3)</td>
<td>0.93 (0.06)</td>
<td>27.5 (1.5)</td>
</tr>
<tr>
<td>MDA Prostate Cancer Controls</td>
<td>228/228</td>
<td>NA</td>
<td>0</td>
<td>59.6 (7.8)</td>
<td>99.7 (12.2)</td>
<td>106.9 (11.2)</td>
<td>0.93 (0.06)</td>
<td>29.1 (5.0)</td>
</tr>
</tbody>
</table>

doi:10.1371/journal.pgen.1003681.t001
Table 2. SNPs associated with waist-related trait at p<5.0E-6 in Stage 1.

<table>
<thead>
<tr>
<th>Trait</th>
<th>SNP</th>
<th>chr</th>
<th>bp (b36)</th>
<th>Gene</th>
<th>All¹</th>
<th>EAF²</th>
<th>beta</th>
<th>SE</th>
<th>P-val</th>
<th>Stage 1</th>
<th>Stage 2</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>P2GC</td>
<td>rs2075064</td>
<td>9</td>
<td>125823668</td>
<td>LHX2</td>
<td>t/c</td>
<td>0.13</td>
<td>-0.08</td>
<td>0.01</td>
<td>5.0E-08</td>
<td>-0.04</td>
<td>0.02</td>
<td>3.2E-02</td>
</tr>
<tr>
<td>WHR_BMI_pooled</td>
<td>rs6931262</td>
<td>6</td>
<td>7162516</td>
<td>RREB1</td>
<td>t/c</td>
<td>0.25</td>
<td>0.07</td>
<td>0.01</td>
<td>5.3E-08</td>
<td>0.04</td>
<td>0.02</td>
<td>4.5E-02</td>
</tr>
<tr>
<td>WC_men</td>
<td>rs6867983</td>
<td>5</td>
<td>55889910</td>
<td>MAP3K1</td>
<td>t/c</td>
<td>0.24</td>
<td>-0.11</td>
<td>0.02</td>
<td>3.5E-07</td>
<td>-0.06</td>
<td>0.03</td>
<td>2.5E-02</td>
</tr>
<tr>
<td>WC_pooled</td>
<td>rs7601155</td>
<td>2</td>
<td>28011986</td>
<td>BRE</td>
<td>t/c</td>
<td>0.13</td>
<td>0.07</td>
<td>0.01</td>
<td>2.9E-07</td>
<td>0.04</td>
<td>0.02</td>
<td>4.6E-02</td>
</tr>
<tr>
<td>WHR_BMI_pooled</td>
<td>rs6931262</td>
<td>6</td>
<td>7162516</td>
<td>RREB1</td>
<td>t/c</td>
<td>0.25</td>
<td>0.07</td>
<td>0.01</td>
<td>5.3E-08</td>
<td>0.04</td>
<td>0.02</td>
<td>4.5E-02</td>
</tr>
<tr>
<td>WC_men</td>
<td>rs6867983</td>
<td>5</td>
<td>55889910</td>
<td>MAP3K1</td>
<td>t/c</td>
<td>0.24</td>
<td>-0.11</td>
<td>0.02</td>
<td>3.5E-07</td>
<td>-0.06</td>
<td>0.03</td>
<td>2.5E-02</td>
</tr>
<tr>
<td>WC_pooled</td>
<td>rs7601155</td>
<td>2</td>
<td>28011986</td>
<td>BRE</td>
<td>t/c</td>
<td>0.13</td>
<td>0.07</td>
<td>0.01</td>
<td>2.9E-07</td>
<td>0.04</td>
<td>0.02</td>
<td>4.6E-02</td>
</tr>
</tbody>
</table>

¹effect allele/other allele.
²effect allele frequency.
³one-side test p-value.
⁴P2GC: double GC-corrected p-value.

doi:10.1371/journal.pgen.1003681.t002
GWAS of Body Fat Distribution in African Ancestry

Figure 1. Regional association plots based on single GC-corrected p-value for LHX2 and RREB1, Stage 1 only. MAF = minor allele frequency. The p-values for the index SNP rs2075064 in LHX2 loci are 5.5E-8, 0.03, and 2.2E-8 for Stage 1, Stage 2 and joint analysis. The p-values for the index SNP rs6931262 at RREB1 loci are 5.3E-8, 0.02 and 2.5E-8 for Stage 1, Stage 2 and joint analysis. The double GC-corrected p-value for the joint analysis are 6.5E-8, 5.7E-8 and 1.8E-6 for rs2075064, rs6931262 and rs1294410, respectively.

doi:10.1371/journal.pgen.1003681.g001
GWAS of Body Fat Distribution in African Ancestry

**A**

rs2075064 in GIANT_WHR

- rs10986172
- MAF: 0.06
- P-value: 0.026

**B**

rs6931262 in GIANT_WHR

- rs9392863
  - $R^2 = 0.005$ and $D' = 1.00$
  - MAF: 0.263
  - P-value: 1.3E-4
flanking region of each index SNP; TBX15-WARS2, GRB14, ADAMTS9, L1I6, RSP03, ITPR2-SSPN, Table 4. Figure 3

Table 3. Examination of index SNPs within known loci in EA in AA for trait WHR ratio adjusted for BMI.

<table>
<thead>
<tr>
<th>Index SNP information</th>
<th>Index SNP Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNP</td>
<td>chr</td>
</tr>
<tr>
<td>SNPs associated with waist-related trait at significant level^3</td>
<td></td>
</tr>
<tr>
<td>rs984222</td>
<td>1</td>
</tr>
<tr>
<td>rs10195252</td>
<td>2</td>
</tr>
<tr>
<td>rs6795735</td>
<td>3</td>
</tr>
<tr>
<td>rs1294421</td>
<td>6</td>
</tr>
<tr>
<td>rs9491966</td>
<td>6</td>
</tr>
<tr>
<td>rs718314</td>
<td>12</td>
</tr>
<tr>
<td>Further SNPs evaluated in follow up but not achieving significance in combined analysis^3</td>
<td></td>
</tr>
<tr>
<td>rs4846567</td>
<td>1</td>
</tr>
<tr>
<td>rs6784615</td>
<td>3</td>
</tr>
<tr>
<td>rs6905288</td>
<td>6</td>
</tr>
<tr>
<td>SNP evaluated but not achieving significance in discovery analysis^3</td>
<td></td>
</tr>
<tr>
<td>rs1011731</td>
<td>1</td>
</tr>
<tr>
<td>rs6881661</td>
<td>5</td>
</tr>
<tr>
<td>rs1055144</td>
<td>7</td>
</tr>
<tr>
<td>rs1443512</td>
<td>12</td>
</tr>
<tr>
<td>rs4823006</td>
<td>22</td>
</tr>
</tbody>
</table>

The index SNPs is from Heid et al, Nature Genetics 2010 [17].
\[^1\]Effect allele/other allele.
\[^2\]Effect allele frequency.
\[^3\]Significance classification refers to the interrogation results of best SNP in Table 4.
\[^4\]p-value of heterogeneity test of beta between EA and AA samples.

doi:10.1371/journal.pgen.1003681.t003

Cross-Trait Associations

Given the evidence for association between waist-based traits and other cardiometabolic risk factors in EA individuals [17], we next examined whether there was similar enrichment in AA individuals (Table 5). rs13389219 at GRB14 was associated with HDL-cholesterol (p = 0.014) [24], triglycerides (p = 0.014) [24], and fasting insulin (p = 0.008) [25], while rs13060013 in ADAMTS9 was associated with HDL-cholesterol (p = 0.0009) [24] and fasting insulin (p = 0.002) [25]. There were nominal associations with related anthropometric traits for rs1936806 at RSP03 in association with BMI [26] (p = 0.003), rs2075064 at LHX2 in association with BMI [26] (p = 0.002), and height (p = 0.02, Christopher Haiman, personal communication).

Discussion

We identified 2 loci at LHX2 and RREB1 with p<5.0×10^{-8} under single GC-correction for waist-based traits in African ancestry individuals, which were not genome-wide significant (p = 6.5×10^{-8} and p = 5.7×10^{-5}) with double-GC correction. Population sub-structure may cause spurious associations in genome-wide association studies and GC factors calculated from

Table 4.

GWAS of Body Fat Distribution in African Ancestry

Figure 2. Regional association plots for LHX2 and RREB1 in GIANT consortium with participants of European ancestry. The blue arrow points to the index SNPs identified from the samples of African ancestry and red arrow points to the best SNPs in GIANT consortium samples of European ancestry.
doi:10.1371/journal.pgen.1003681.g002
variants across the genome are conventionally used to scale the test statistic [27]. However, this method was originally proposed under the hypothesis that only a small number of causal variants underlie complex traits. Recent studies have shown that as the number of causal variants increases, more SNPs (in LD with causal variants) will depart from the null distribution even in the absence of causal variants increases, more SNPs (in LD with causal variants) will depart from the null distribution even in the absence of population sub-structure [22,23]. Furthermore, the GC factor is a function of sample size under a constant phenotypic heritability. Therefore, double GC-correction in a large meta-analysis is likely overly conservative. Thus, we report both single and double GC-corrected p-values.

Table 4. Interrogation of best SNPs with the smallest p-value within known EA loci in AA for trait WHR ratio adjusted for BMI.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Best_SNP</th>
<th>All¹</th>
<th>EAF²</th>
<th>P-val</th>
<th>beta</th>
<th>SE</th>
<th>N²</th>
<th>P⁴</th>
<th>YRI² (D)²</th>
<th>CEU² (D)²</th>
<th>beta</th>
<th>SE</th>
<th>P-val⁶</th>
<th>beta</th>
<th>SE</th>
<th>P-val</th>
<th>P⁺⁺⁺²</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNPs associated with waist-related trait at significant level</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7BKS1-WARS2</td>
<td>rs10923714 a/g</td>
<td>0.29</td>
<td>1.8E-04</td>
<td>0.04</td>
<td>0.01</td>
<td>34</td>
<td>1.5E-03</td>
<td>0.49 (1.00)</td>
<td>0.48 (1.00)</td>
<td>0.03</td>
<td>0.02</td>
<td>6.4E-02</td>
<td>0.04</td>
<td>0.01</td>
<td>6.8E-05</td>
<td>1.1E-04</td>
<td></td>
</tr>
<tr>
<td>GB14</td>
<td>rs13389219 t/c</td>
<td>0.71</td>
<td>4.7E-05</td>
<td>−0.05</td>
<td>0.01</td>
<td>42</td>
<td>1.2E-03</td>
<td>1.00 (1.00)</td>
<td>0.93 (1.00)</td>
<td>−0.02</td>
<td>0.02</td>
<td>2.0E-01</td>
<td>−0.04</td>
<td>0.01</td>
<td>9.5E-05</td>
<td>1.4E-04</td>
<td></td>
</tr>
<tr>
<td>ADAMTS9</td>
<td>rs13060013 a/c</td>
<td>0.22</td>
<td>3.9E-05</td>
<td>0.05</td>
<td>0.01</td>
<td>95</td>
<td>5.3E-04</td>
<td>0.01 (0.45)</td>
<td>0.33 (1.00)</td>
<td>0.02</td>
<td>0.02</td>
<td>1.2E-01</td>
<td>0.04</td>
<td>0.01</td>
<td>3.8E-05</td>
<td>5.9E-05</td>
<td></td>
</tr>
<tr>
<td>LY66</td>
<td>rs1294410 t/c</td>
<td>0.23</td>
<td>6.3E-07</td>
<td>−0.06</td>
<td>0.01</td>
<td>130</td>
<td>3.8E-04</td>
<td>0.39 (0.68)</td>
<td>0.83 (0.96)</td>
<td>−0.02</td>
<td>0.02</td>
<td>1.1E-01</td>
<td>−0.05</td>
<td>0.01</td>
<td>1.0E-06</td>
<td>1.8E-06</td>
<td></td>
</tr>
<tr>
<td>KSPD3</td>
<td>rs1996806 t/c</td>
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<td>1.1E-04</td>
<td>0.04</td>
<td>0.01</td>
<td>24</td>
<td>2.1E-03</td>
<td>0.52 (1.00)</td>
<td>0.88 (1.00)</td>
<td>0.00</td>
<td>0.02</td>
<td>4.4E-01</td>
<td>0.03</td>
<td>0.01</td>
<td>8.1E-04</td>
<td>1.1E-03</td>
<td></td>
</tr>
<tr>
<td>ITPR2-SSPN</td>
<td>rs11048510 c/g</td>
<td>0.23</td>
<td>5.8E-04</td>
<td>0.04</td>
<td>0.01</td>
<td>88</td>
<td>5.7E-04</td>
<td>0.00 (0.25)</td>
<td>0.30 (0.19)</td>
<td>0.04</td>
<td>0.02</td>
<td>1.3E-02</td>
<td>0.04</td>
<td>0.01</td>
<td>4.1E-05</td>
<td>6.0E-05</td>
<td></td>
</tr>
</tbody>
</table>

Further SNPs evaluated in follow up but not achieving significance in combined analysis

| Genes | rs27198767 t/c | 0.29 | 1.1E-04 | 0.04 | 0.01 | 24  | 2.1E-03 | 0.52 (1.00) | 0.88 (1.00) | 0.00 | 0.02 | 4.4E-01 | 0.03 | 0.01 | 8.1E-04 | 1.1E-03 |

SNPs evaluated but not achieving significance in discovery analysis

| Genes | rs1996806 t/c | 0.29 | 1.1E-04 | 0.04 | 0.01 | 24  | 2.1E-03 | 0.52 (1.00) | 0.88 (1.00) | 0.00 | 0.02 | 4.4E-01 | 0.03 | 0.01 | 8.1E-04 | 1.1E-03 |

The index SNPs are from Heid et al, Nature Genetics 2010 [17]. Note that Tables 3 and 4 show different information for the same loci (Table 3 for index SNP and Table 4 for best SNPs with the smallest p-value).

1. effect allele/other allele.
2. effect allele frequency.
3. number of independent (typed) SNPs interrogated in AA sample.
4. Bonferroni p-value threshold (0.05/N3).
5. HapMAP LD information.
6. one-side test p-value.
7. Pval 2GC: double GC-corrected p-value.
8. YRI: dataset.
9. CEU: dataset.
10. beta: effect allele/other allele.
11. SE: standard error.
12. N: number of independent SNPs.
mass in women as compared to men. Abdominal adipose composition may vary more in EA than AA as EA have greater visceral adipose tissue than AA of similar gender. Finally, statistically, for some loci (\textit{RSPO3} and \textit{ADAMTS9}), we cannot rule out the presence of modest gender differences given the relatively small sample sizes as compared with other analyses. This is further reinforced by our power analysis to detect sex-specific associations. We conducted this power analysis for a common variant (specifically, MAF of 0.25 here) with an effect size difference of 0.054, which is derived from the largest effect difference indicated in Table 2 of Heid et al [17]. Using these assumptions, we have only 6.7% power to detect the variant (MAF of 0.25) with a sample size of 23564 in our discovery stage and 10.9% power with combined sample size 33738 from stage 1 and stage 2. This suggests that we have limited power to detect sexual dimorphism if it indeed exists.

It is notable that our top SNP at \textit{RREB1} is within 1 Mb of \textit{LY86}, one of the 14 novel loci identified by the GIANT genome-wide association meta-analysis of WHR [17]. Given the low pairwise linkage disequilibrium and lack of

Figure 3. Regional association plots for all confirmed loci from the GIANT locus interrogation. Each figure is centered by the index SNP (big red) with rs-number and p-value information (stage 1 only); another big rectangle is the best SNP in African Americans, with information including MAF = minor allele frequency; linkage disequilibrium information in HapMap YRI and CEU; \( P_D \), \( P_F \), and \( P_J \) are the single GC-corrected p-value obtained from discovery cohorts only, follow-up cohorts and joint discovery and follow-up data, respectively. Double GC-corrected p-value can be found in Table 4.

doi:10.1371/journal.pgen.1003681.g003
### Table 5. Cross-trait associations for novel loci from Stage 1 + Stage 2 in participants of African ancestry.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Gene</th>
<th>n</th>
<th>p-val</th>
<th>Beta1</th>
<th>Beta2</th>
<th>p-val</th>
<th>Beta1</th>
<th>Beta2</th>
<th>p-val</th>
<th>Beta1</th>
<th>Beta2</th>
<th>p-val</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2075064</td>
<td>LHX2</td>
<td>23564</td>
<td>0.070</td>
<td>2.1E-02</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs6931262</td>
<td>RREB1</td>
<td>23564</td>
<td>0.059</td>
<td>-0.0030</td>
<td>1.0E-01</td>
<td>0.0082</td>
<td>4.0E-01</td>
<td>1.2E-01</td>
<td>0.0217</td>
<td>3.4E-01</td>
<td>7.8E-01</td>
<td>0.0011</td>
</tr>
<tr>
<td>rs10923714</td>
<td>TBX15-WARS2</td>
<td>23564</td>
<td>0.038</td>
<td>-0.0011</td>
<td>2.1E-01</td>
<td>0.0182</td>
<td>3.1E-01</td>
<td>0.0228</td>
<td>1.2E-01</td>
<td>0.0156</td>
<td>1.4E-01</td>
<td>9.4E-01</td>
</tr>
<tr>
<td>rs2075064</td>
<td>DENND1A</td>
<td>23564</td>
<td>0.039</td>
<td>0.0215</td>
<td>6.5E-01</td>
<td>0.0295</td>
<td>3.5E-01</td>
<td>0.0032</td>
<td>1.0E-01</td>
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<td>1.0E-01</td>
<td>9.3E-01</td>
</tr>
<tr>
<td>rs6931262</td>
<td>RREB1</td>
<td>23564</td>
<td>0.051</td>
<td>0.009</td>
<td>3.5E-01</td>
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<td>6.2E-01</td>
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1 effect allele/other allele.
2 effect size based on Stage 1 and Stage 2 combined sample.
doi:10.1371/journal.pgen.1003681.t005

GWAS of Body Fat Distribution in African Ancestry

There are several genes in the region of rs6931262. **RREB1** (Ras-responsive element binding protein 1) participates in Ras signaling and cancer progression in bladder cancer [36], prostate cancer [37], and melanoma [38]. **RREB1** is not known to play a role in adipose tissue, and SNPs in this gene have previously been associated with serum urate levels [39]. Variants in **DENND1A** gene have been associated with polycystic ovarian syndrome in both European ancestry [34] and Chinese women [35]. Taken together, these findings highlight how future studies can further our understanding of how genes in this region may contribute to body fat distribution and related obesity phenotypes.

There are several potential implications of this work. First, these analyses highlight how novel loci for body fat distribution, above and beyond generalized adiposity, can be elucidated by performing GWAS in diverse ethnic populations. Second, we demonstrate some important similarities in the associations among AA as compared to EA individuals with regards to the loci uncovered as well as pleiotropy with other cardio-metabolic phenotypes. Finally, while many of the beta coefficients were similar in women as compared to men, we did uncover modest evidence for sexual dimorphism in the present AA sample.

A major strength of this study is the large sample size of AA participants, representing the largest study to date for waist-based phenotypes in AA. This study has similar limitations to other GWAS performed in AA populations. While the overall sample size was large, the discovery sample was still considerably smaller than those for GWAS meta-analysis conducted in samples of primarily EA populations. In the present analysis, to have 80% power to detect an association that explains 0.1% of the trait variance at a MAF of 0.25 would require 39581 participants. With our largest WC sample size (n = 23564) in discovery stage, we have only 28% power to detect common variant explaining 0.1% of the variance of WC. In addition, GWAS panels such as the Affymetrix 6.0 chip were largely designed based on EA populations and have more limited SNP
coverage for AA samples. For example, one analysis of 76 genes reported that only approximately 43–55% of SNPs were tagged ($r^2 > 0.8$) on the Affymetrix 6.0 panel in YRI samples [40]. Kang and colleagues [41] demonstrated that both local and global ancestry estimates similarly attenuated spurious results due to population stratification in their study of AA ancestry individuals. As with all studies in admixed populations, while association analyses were adjusted for global population structure using principal components, there may be residual population substructure leading to false positive results. Given the minimal attenuation that we observed with local ancestry adjustment, our key findings are unlikely to be spurious. We performed 12 analyses, raising the possibility of false positive findings using standard significance thresholds. PCSK1 is a bona fide locus for obesity [42], yet this SNP failed to replicate in our findings. While we can not rule out power as the reason for the lack of replication, this signal may also have represented a false positive finding in our dataset. Heterogeneity between study samples may limit power, but this is an issue in GWAS and not unique to the present investigation. After double GC correction, our findings did not reach genome-wide significance. However, double GC correction may be overly conservative [22,23]. Finally, a general limitation in GWAS is that coverage of rare (MAF<1%) and low frequency (1%<MAF<5%) variants is poor, and thus associations with rarer variants are likely missed.

GWAS of body fat distribution traits in a large AA sample has revealed two loci likely associated with fat distribution, as well as nominal evidence for association at 6 loci previously identified among EA individuals. These findings highlight the concept that there are loci for fat distribution above and beyond fat distribution in AA and EA individuals, and reinforce the findings using standard significance thresholds. Our key findings are unlikely to be spurious. We started. Stage 2 cohorts with in silico GWAS data were identified with GWAS data at the time the study started. Stage 2 cohorts were part of the CARDIA, CHS, HDS, MESA, and SIGNET (REGARDS, SUGAR), are family studies. The CARDIA consortium (ARIC, CARDIA, CHS, JHS, MESA) consists of several population-based studies that included African ancestry individuals. The WHI study was a clinical trial. HANDLS is a community-based study. Family Heart Study is a multicenter family-based study. GeneSTAR is a prospective study of vascular diseases. GENOA and HYPERGEN are cohorts of sibships enriched for hypertension. Health ABC is a random sample of Medicare beneficiaries in and surrounding Pittsburgh, Pennsylvania, and Memphis, Tennessee. HUFS is a population-based family study in the Washington, D.C. metropolitan area. MEC is a prospective cohort study including 59,000 African American women from across the U.S. CHS is a population-based study of risk factors for CHD and stroke. REGARDS is an observational cohort and SUGAR is a community based family studies focusing on Type 2 Diabetes. Each participating study has obtained institutional review board approval on research involving human subjects and all subjects provided written informed consent. Details regarding each cohort can be found in the Text S1.

Materials & Methods

Phenotype Definition

We analyzed waist-based traits including waist circumference (WC) and waist-hip ratio (WHR), a measure of body fat distribution [43]. Details regarding trait acquisition within each cohort can be found in the Text S1. Individuals less than 20 years of age were excluded from all analyses. Within each cohort, we created two sets of residuals for WC and WHR, one adjusted for age, age$^2$, study site (if applicable) and another additionally adjusted for BMI. Analyses were conducted separately for men and women. The raw residuals were then transformed through an inverse normal function for each subgroup and these transformed residuals were used as our phenotypes in the association analyses. The cohorts with related individuals additionally performed sex-combined analysis. We analyzed four phenotypes: waist circumference (WC), waist circumference adjusted for BMI (WC-BMI), waist hip ratio (WHR) and waist hip ratio adjusted for BMI (WHR-BMI).

Samples

We conducted analysis of WC and WHR in up to 33738 and 27489 AA individuals, respectively. Specifically, the analysis included for WC up to 23,564 individuals and WHR up to 19,744 individuals in stage 1 while 10,174 AA individuals with WC and 7,745 AA individuals with WHR in stage 2. Stage 1 cohorts were part of the CARDIA consortium and other cohorts that were identified with GWAS data at the time the study started. Stage 2 cohorts with in silico GWAS data were identified later. Some participating studies, including CFS, Family Heart Study, GENOA, HUFS, HyperGEN, GeneSTAR, JHS, MESA-family and SIGNET (REGARDS, SUGAR), are family studies. The CARDIA consortium (ARIC, CARDIA, CHS, JHS, MESA) consists of several population-based studies that included African ancestry individuals. The WHI study was a clinical trial. HANDLS is a community-based study. Family Heart Study is a multicenter family-based study. GeneSTAR is a prospective study of vascular diseases. GENOA and HYPERGEN are cohorts of sibships enriched for hypertension. Health ABC is a random sample of Medicare beneficiaries in and surrounding Pittsburgh, Pennsylvania, and Memphis, Tennessee. HUFS is a population-based family study in the Washington, D.C. metropolitan area. MEC is a prospective cohort study including 59,000 African American women from across the U.S. CHS is a population-based study of risk factors for CHD and stroke. REGARDS is an observational cohort and SUGAR is a community based family studies focusing on Type 2 Diabetes. Each participating study has obtained institutional review board approval on research involving human subjects and all subjects provided written informed consent. Details regarding each cohort can be found in the Text S1.

Genotyping and Imputation

Genotype information for each cohort is presented in Supplementary Table S2. As shown in Table 1, the genetic ancestry of our samples, African American, is also partly from European Ancestry. Simply using all YRI sample as reference panel would be inappropriate, given that we generally see an average of 20% CEU admixture. To better capture the genetic structure of our samples, all the genotypes from discovery cohorts were imputed using combined HapMap 1:1 CEU+YRI as reference panel. This imputation has resulted in an allelic concordance rate of 95.6%, which is compatible to rates calculated with the HapMap 2 YRI individuals [44]. Our follow-up stage (Stage 2) included in silico and de novo follow-up cohorts. In-silico follow-up studies similarly use the combined CEU+YRI in HapMap as their reference panel for genotype imputation. Then the expected allele dosage was used in the association analysis to account for the uncertainty introduced by the genotype imputation. More details regarding imputation were in Supplementary Table S2.

Statistical Methods for Discovery (Stage 1)

In each discovery study, genome-wide tests for association between SNPs and phenotypes were conducted separately for men and women using linear regression with principal components adjustment to adjust for global population substructure. In studies from families, men and women were also combined for analyses. Linear mixed effect models, where appropriate, were used to account for the relatedness in family studies.

In addition to study-specific filters, a centralized quality control procedure was performed to extensively examine and check all study-specific results files before meta-analysis. We examined the plausible values for all reported summary statistics to check for potential errors. The genomic control lambda ($\lambda$) value for each set of results was checked for potential p-value inflation. We analyzed SNPs with imputation quality scores greater than 0.3 for studies using MACH or BimBam software, and greater than 0.4 for
studies that used other imputation software such as IMPUTE. Additionally, we filtered out SNPs where the minor allele frequency times the number of subjects was smaller than or equal to 5, to ensure robust estimates.

**Meta-Analysis**

We performed fixed-effects meta-analyses of study-specific genome-wide association results using the inverse-variance weighted approach for the traits described above. Three sets of meta-analyses were conducted for each phenotype using (1) men only results, (2) women only results, and (3) joint men-only and women-only results for studies of unrelated individuals, and sex-combined results for studies with related individuals. The calculated \( k \) genomic control (GC) correction was applied to each cohort’s result. Recent studies showed that under polygenic inheritance, test statistics in large meta-analyses are expected to be elevated even when there is no population sub-structure [22,23]. To avoid an overly conservative adjustment, we focused on the single GC-corrected result. However, we also report the double GC-corrected \( p \)-value for the joint meta-analysis of the stage 1 and stage 2 samples.

**Local Ancestry Analysis**

As a sensitivity analysis, we assessed the impact of local ancestry by including SNP specific local ancestry estimates as a covariate in models for genome-wide significant signals in both the CARE and WHI studies. Locus-specific ancestry (i.e. probabilities of whether an individual has 0, 1, or 2 alleles of African ancestry at each locus) was only available for directly genotyped SNPs and was estimated using a Hidden Markov Model and the local haplotype structure to detect transitions in ancestry along the genome [45]. We considered signals robust to adjustment for local ancestry when the Beta was numerically similar.

**Interrogation of GIANT Loci in the Samples of African Ancestry**

We applied a procedure to evaluate the transferability of association signals across different ethnicities [46]. Specifically, in addition to validating the previously reported index SNPs identified in studies of EA participants [17], we interrogated the surrounding genomic regions in our AA samples. For each reported index EA SNP, we first examined the results in our AA samples and tested for consistency of direction, with respect to the beta coefficients of index SNPs, between EA and AA samples. To accommodate the difference of LD structure across ethnicities, we then interrogated \( \pm 250 \) kb regions around the index SNPs and identified the SNP with the smallest \( p \)-value in AA within the interrogated genomic region. The loci-specific significance threshold was based on Bonferroni correction, defined as 0.05 divided by the number of independent SNPs within an interrogated region. SNPs meeting genome-wide \( p < 5.0 \times 10^{-8} \) or suggestive \( 5.0 \times 10^{-8} < p < 5.0 \times 10^{-6} \) in the Stage 1 meta-analysis were carried forward for follow-up in Stage 2 and joint Stage 1 and 2 meta-analysis. To test the consistency of effect directions between AA and EA samples, a \( p \)-value was calculated based on the cumulative binomial distribution for the observed or more extreme number of variants with a consistent direction.

**Follow-up Analysis (Stage 2) and Joint Analysis of Discovery and Follow-up (Stage 1 and 2) Data**

An analysis approach consistent with the discovery stage (i.e Stage 1), described above, was used for the Stage 2 studies. In this stage, the variants of interest identified from our analysis of Stage 1 and interrogation of previously published EA WHR-BMI loci were followed up in different samples for follow-up meta-analysis and confirming the association. We then conducted additional joint meta-analysis, including studies from both Stage 1 and Stage 2 discovery and follow-up data. In Stage 2 analysis, the replication was defined as having a beta coefficient consistent with the discovery stage; follow-up \( p \)-values are thus represented as one-sided tests. For the joint analysis, we used the standard threshold of \( p \text{-value}<5\times10^{-8} \) for genome-wide significance and a locus-specific Bonferroni corrected threshold for the regions identified by the GIANT consortium.

For newly identified SNPs from both genome-wide association analyses and previous region interrogation analyses, we performed sex-specific association analyses and also tested the difference of meta-analyzed sex-specific beta-estimates \((\beta_{\text{men}} \text{ and } \beta_{\text{women}})\) using the t-statistic

\[
\frac{\beta_{\text{men}} - \beta_{\text{women}}}{\sqrt{SE_{\text{men}}^2 + SE_{\text{women}}^2 - 2rSE_{\text{men}}SE_{\text{women}}}},
\]

where \( r \) is the Spearman rank correlation coefficient between \( \beta_{\text{men}} \) and \( \beta_{\text{women}} \) across all SNPs. Note that we are comparing two parameters and testing whether their difference is equal to zero. This is basically the setting of a two-sample test. Although based on our sample size \( n>5000 \) in combined analysis, the \( Z \)-statistic should work well due to the Central Limit Theory. However, we intended to conservatively use the t-statistic here to calculate the \( p \)-value.

**Interrogation of Novel AA Loci in GIANT**

We also examined the GIANT consortium results [17] for evidence of association for the novel loci identified in the AA samples. We applied similar interrogation procedures detailed in the previous section of the Interrogation of GIANT Loci in the samples of African Ancestry. Briefly, we first looked up the association results for the AA index SNP in GIANT and followed up with the interrogation of its \( \pm 250 \) kb flanking region. The significance was evaluated as 0.05 divided by the number of independent variants within the interrogated region.

**Cross-Trait Analyses**

For the newly identified SNPs from both GWAS and the interrogation analysis we performed cross-trait association analyses of metabolic risk factor and related anthropometric measures, including BMI [26], HDL-cholesterol [24], LDL-cholesterol [24], triglycerides [24], glucose and insulin [25], and height (Christopher Haiman, personal communication), in AA samples for the newly identified SNPs from both genome-wise association analysis and the interrogation analysis.

**Supporting Information**

**Figure S1** Figure S1a – Quantile-quantile and Manhattan plots for genome-wide association for waist circumference and waist circumference adjusted for BMI in sex-specific samples and all samples. Figure S1b – Quantile-quantile and Manhattan plots for genome-wide association for waist-hip ratio and waist-hip ratio adjusted for BMI in sex-specific samples and all samples.

(For Table S1, Table S2, and Table S3, please refer to the supplementary information for the full details.)

**Table S1** Study sample characteristics by gender.

(For Table S2, please refer to the supplementary information for details.)

**Table S2** Genotyping and imputation platforms used by all participating studies.

(For Table S3, please refer to the supplementary information for details.)

**Table S3** Imputation quality at 8 associated loci.
Table S4  Gender-specific association analysis at 8 associated loci. (DOC)
Table S5  Local ancestry analysis for two top variants (rs2075064 at LHX2 and rs6931262 at RREB1) with single GC-corrected p-value<5×10^{-8}. (DOC)

Text S1  Study specific methods. (DOC)

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Author Contributions
Conceived and designed the experiments: ABZ BP CK CSF DAB EKS EWD GJP GLB IBB JIR JRP KCJ LAC LFB LHK LR MKE MZV PAP SBB SL SRK SW STH YVS. Performed the experiments: AFB CAH IBB JMO JRP LAC SL SRK UBY YL. Analyzed the data: ABZ CK CSF DT.Earn EK FG GL JC JCE JSA JZ JCT KEN KL KLM LAC LL LRY LX MAN MKE MKW MRI MS PGJ SL WMC WZ YLS YVS AA ER JD JM UN WM XG JLRJ HMOB. Wrote the paper: APR CSF CTL JCE KCT KEN LAC LFB LL MKK TH WP YW YVS. Study management: AA ABZ BP BVH CK CNR CSR CSC CFY DT DSS EAN EKS EWD GLP GJP HLT IBB JI. JEM JIR JRP KCJ KCT KLM LR LAA MCA MKE MKE MMV MSV SBB SL SRK SN SRP SWB TTH TTH WM TWP YL YVS. Subject recruitment: ABZ BEH BP BVH CAH CRA CP EVB GLB IBB IS JEM JRP KCJ LR MKE MS PJS RCM SBB SL SSS TTH VH WGT. Interpretation of results: AA ABZ APR BM BVH CK CNR CSR CSC CFY DT DSS EAN EKS EWD GLP GJP HLT IBB JI. JEM JIR JRP KCJ KCT KLM LAC LFB LL LRY LX MAN MKE MKE MMV MLN MMS MZV SBB SFAG SL SBS THF TTH WM C XG YL YVS. Critical review of manuscript: AA ABZ APR BM BVH CK CNR CSR CSC DMB DT DSS EAN EWS EWD GLP GJP HLT IBB JI. JEM JIR JRP KCJ KCT KLM LAC LFB LL LRY LX MAN MKE MKE MLN MMS MZV PPG PJS SFAG SL SRP SWS TTH THM WP XG YDIC YL YVS. Bioinformatics: AA ABZ CRD EJF JMA LAC MAN UN WMC YL.

References


