African Ancestry and Its Correlation to Type 2 Diabetes in African Americans: A Genetic Admixture Analysis in Three U.S. Population Cohorts

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters

Citation

Published Version
doi:10.1371/journal.pone.0032840

Citable link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:9393258

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA
African Ancestry and Its Correlation to Type 2 Diabetes in African Americans: A Genetic Admixture Analysis in Three U.S. Population Cohorts

Ching-Yu Cheng1,4,5,6, David Reich7,10, Christopher A. Haiman9, Arti Tandon7,10, Nick Patterson10, Selvin Elizabeth1,2, Ermeg L. Akylbekova11, Frederick L. Brancati11,2,3, Josef Coresh1,2, Eric Boerwinkle12, David Altshuler8,10,13, Herman A. Taylor14, Brian E. Henderson9, James G. Wilson15, W. H. Linda Kao1,3

1Department of Epidemiology, Johns Hopkins University, Baltimore, Maryland, United States of America, 2Welch Center for Prevention, Epidemiology and Clinical Research, Johns Hopkins University, Baltimore, Maryland, United States of America, 3Department of Medicine, Johns Hopkins University, Baltimore, Maryland, United States of America, 4Saw Swee Hock School of Public Health, and Department of Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore, 5Duke-NUS Graduate Medical School, Singapore, Singapore, 6Singapore Eye Research Institute, Singapore, Singapore, 7Department of Genetics, Harvard Medical School, Boston, Massachusetts, United States of America, 8Department of Medicine, Harvard Medical School, Boston, Massachusetts, United States of America, 9Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, California, United States of America, 10Program in Medical and Population Genetics, Broad Institute of Harvard and M.I.T., Cambridge, Massachusetts, United States of America, 11Jackson Heart Study Analysis Group, Jackson State University, Jackson, Mississippi, United States of America, 12Human Genetics Center, University of Texas Health Science Center at Houston, Houston, Texas, United States of America, 13Center for Human Genetic Research and Diabetes Unit, Department of Medicine, Massachusetts General Hospital, Boston, Massachusetts, United States of America, 14Jackson State University, Tougaloo College, and the University of Mississippi Medical Center, Jackson, Mississippi, United States of America, 15Department of Physiology and Biophysics, The University of Mississippi Medical Center, Jackson, Mississippi, United States of America

Abstract

The risk of type 2 diabetes is approximately 2-fold higher in African Americans than in European Americans even after adjusting for known environmental risk factors, including socioeconomic status (SES), suggesting that genetic factors may explain some of this population difference in disease risk. However, relatively few genetic studies have examined this hypothesis in a large sample of African Americans with and without diabetes. Therefore, we performed an admixture analysis using 2,189 ancestry-informative markers in 7,021 African Americans (2,373 with type 2 diabetes and 4,648 without) from the Atherosclerosis Risk in Communities Study, the Jackson Heart Study, and the Multiethnic Cohort to 1) determine the association of type 2 diabetes and its related quantitative traits with African ancestry controlling for measures of SES and 2) identify genetic loci for type 2 diabetes through a genome-wide admixture mapping scan. The median percentage of African ancestry of diabetic participants was slightly greater than that of non-diabetic participants (study-adjusted difference = 1.6%, P < 0.001). The odds ratio for diabetes comparing participants in the highest vs. lowest tertile of African ancestry was 1.33 (95% confidence interval 1.13–1.55), after adjustment for age, sex, study, body mass index (BMI), and SES. Admixture scans identified two potential loci for diabetes at 12p13.31 (LOD = 4.0) and 13q14.3 (Z score = 4.5, P = 6.6 × 10−6). In conclusion, genetic ancestry has a significant association with type 2 diabetes above and beyond its association with non-genetic risk factors for type 2 diabetes in African Americans, but no single gene with a major effect is sufficient to explain a large portion of the observed population difference in risk of diabetes. There undoubtedly is a complex interplay among specific genetic loci and non-genetic factors, which may both be associated with overall admixture, leading to the observed ethnic differences in diabetes risk.


Editor: Stephen L. Atkin, Postgraduate Medical Institute & Hull York Medical School, University of Hull, United Kingdom

Received November 9, 2011; Accepted January 31, 2012; Published March 16, 2012

Copyright: © 2012 Cheng et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: The ARIC Study was carried out as a collaborative study supported by the National Heart, Lung, and Blood Institute (NHLBI) contracts N01-HC-55015, N01-HC-55016, N01-HC-55018, N01-HC-55019, N01-HC-55020, N01-HC-55021 and N01-HC-55022, with support for this analysis by the National Institute of Diabetes and Digestive and Kidney Diseases R21DK073482 and R01DK084107 (JGW) from the NHLBI and contracts N01-HC-95170, N01-HC-95171, and N01-HC-95172 from the NHLBI and the National Institute of Minority Health and Health Disparities. The MEC Study was supported by the National Cancer Institute grants CA63464 and CA54281. DR is supported by a Burroughs Wellcome Career Development Award in the Biomedical Sciences and DR, NP and AT were supported by NIH grant U01-HG004168. FB was supported by NIH grant P60DK079637. The funding sources played no role in design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: wkao@jhsp.edu
Introduction

Approximately 13% of the U.S. adults have type 2 diabetes [1], representing a significant burden on public health in the United States. Type 2 diabetes is approximately twice as prevalent in African Americans as in European Americans. In the Multiethnic Cohort (MEC), this racial/ethnic difference persisted after stratification by body mass index (BMI) [2]. Data from the National Health and Nutrition Examination Survey (NHANES) confirm the substantial racial disparity in diabetes across the U.S. [1,3]. In the Atherosclerosis Risk in Communities (ARIC) Study, African Americans are twice as likely as whites to develop incident type 2 diabetes—a disparity which persists even after extensive adjustment for socioeconomic status (SES) and behavioral risk factors [4]. This persistent disparity suggests that genetic factors may contribute to ethnic differences in susceptibility to type 2 diabetes.

Despite remarkable efforts in the past three years that have led to the discovery of more than 30 susceptibility loci for type 2 diabetes and related quantitative traits [5–18], there has been only one genome-wide association study of type 2 diabetes in African Americans [19]. The disparity in diabetes prevalence between African Americans and European ancestry makes diabetes an attractive phenotype to study by admixture mapping, a method that systematically scans the genomes of groups of recently admixed individuals (e.g., African Americans) to search for genetic loci where persons with a disease or trait, in aggregate, have more (or less) African ancestry than their genome-wide average. Admixture mapping and subsequent fine-mapping studies have been successful in identifying genetic variants for other complex phenotypes, including prostate cancer [20,21], end stage renal disease [22], white blood cell count [23,24], and circulating levels of interleukin 6 soluble receptor [25].

Given the observed ethnic/racial disparities in diabetes prevalence, we hypothesized that some diabetes susceptibility alleles are present at higher frequency in African Americans than in European Americans, resulting in association between genetic ancestry and diabetes risk that is independent of its association with other non-genetic risk factors for type 2 diabetes. Thus we sought 1) to establish the association of genetic ancestry with diabetes and related quantitative traits in African Americans, after accounting for the non-genetic risk factors, and 2) to identify diabetes susceptibility loci by conducting a genome-wide admixture mapping scan. To maximize power to detect genetic association, we performed a pooled analysis of 7,021 African-American participants, including 2,373 diabetic cases, from three U.S. population cohorts, including the ARIC Study, the Jackson Heart Study (JHS), and the MEC study.

Results

Characteristics of participants

The characteristics and genetic ancestry of the 7,021 African Americans (including 2,373 with type 2 diabetes) included in the study are shown in Table 1 and Figure S1. The overall median global African ancestry was 83.7% (interquartile range, 76.2%–88.7%). African ancestry distributions were different among the three cohorts, with MEC participants having a lower average percentage of African ancestry ($P<0.001$). Diabetic participants tended to have higher BMI, lower education level and lower family income, compared to non-diabetic participants (Table S1).

Association of ancestry with diabetes

Pooling the three cohorts together, the median percentage of African ancestry of diabetic participants was 1.6% greater than that of non-diabetic participants ($P<0.001$, adjusted for study). The odd ratios (ORs) for diabetes were higher with increasing tertiles of percentage of African ancestry ($P<0.001$). Diabetic participants tended to have higher BMI, lower education level and lower family income, compared to non-diabetic participants (Table S1).

Table 1. Genetic African ancestry by participant characteristics and study.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ARIC</th>
<th>JHS</th>
<th>MEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>African Ancestry, Median (IQR), %</td>
<td>African Ancestry, Median (IQR), %</td>
<td>African Ancestry, Median (IQR), %</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>2285 (100)</td>
<td>3185 (100)</td>
<td>1551 (100)</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21–39</td>
<td>237 (7.4)</td>
<td>84.1 (79.4–87.9)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>40–59</td>
<td>1519 (47.7)</td>
<td>83.9 (77.9–88.3)</td>
<td>627 (40.4)</td>
</tr>
<tr>
<td>≥60</td>
<td>1429 (44.9)</td>
<td>84.2 (77.4–89.2)</td>
<td>924 (59.6)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>1210 (38.0)</td>
<td>83.8 (77.6–88.2)</td>
<td>1001 (64.5)</td>
</tr>
<tr>
<td>Women</td>
<td>1975 (62.0)</td>
<td>84.2 (78.2–88.8)</td>
<td>550 (35.5)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>403 (12.6)</td>
<td>84.0 (78.2–88.8)</td>
<td>277 (17.8)</td>
</tr>
<tr>
<td>25–&lt;30</td>
<td>1053 (33.1)</td>
<td>83.8 (77.0–88.5)</td>
<td>682 (44.0)</td>
</tr>
<tr>
<td>≥30</td>
<td>1729 (54.3)</td>
<td>84.3 (78.3–88.7)</td>
<td>592 (38.2)</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>829 (26.0)</td>
<td>85.3 (79.5–89.3)</td>
<td>913 (58.9)</td>
</tr>
<tr>
<td>No</td>
<td>2356 (74.0)</td>
<td>83.6 (77.3–88.3)</td>
<td>638 (41.1)</td>
</tr>
</tbody>
</table>

ARIC, the Atherosclerosis Risk in Communities Study; JHS, the Jackson Heart Study; MEC, the Multiethnic Cohort; IQR, interquartile range; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared).

*P* value was generated from the Wilcoxon rank-sum test or the Kruskal-Wallis test. doi:10.1371/journal.pone.0032840.t001
Table 2. Odds ratio of diabetes by genetic African ancestry.

<table>
<thead>
<tr>
<th>Study and Model</th>
<th>African Ancestry</th>
<th>Excess Odds Explained, %a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tertile 1</td>
<td>Tertile 2</td>
</tr>
<tr>
<td>ARIC, JHS and MEC combined</td>
<td></td>
<td></td>
</tr>
<tr>
<td>African ancestry, %</td>
<td>&lt;79.4</td>
<td>79.4–87.2</td>
</tr>
<tr>
<td>Diabetes, yes/no, No.</td>
<td>774/1574</td>
<td>763/1612</td>
</tr>
<tr>
<td>Model 1, baseb</td>
<td>1 [Reference]</td>
<td>1.25 (1.10–1.43)</td>
</tr>
<tr>
<td>Model 2, BMIC</td>
<td>1 [Reference]</td>
<td>1.21 (1.06–1.39)</td>
</tr>
<tr>
<td>ARIC and JHS combined</td>
<td></td>
<td></td>
</tr>
<tr>
<td>African ancestry, %</td>
<td>&lt;80.5</td>
<td>80.5–87.5</td>
</tr>
<tr>
<td>Diabetes, yes/no, No.</td>
<td>296/1088</td>
<td>255/1000</td>
</tr>
<tr>
<td>Model 1, baseb</td>
<td>1 [Reference]</td>
<td>1.35 (1.16–1.57)</td>
</tr>
<tr>
<td>Model 2, BMIC</td>
<td>1 [Reference]</td>
<td>1.32 (1.17–1.70)</td>
</tr>
<tr>
<td>Model 3, SESd</td>
<td>1 [Reference]</td>
<td>1.27 (1.09–1.49)</td>
</tr>
<tr>
<td>Model 4, BMISEx</td>
<td>1 [Reference]</td>
<td>1.26 (1.07–1.48)</td>
</tr>
</tbody>
</table>

BMI, individuals in the second and third tertiles were still, respectively, 1.21 and 1.4 times more likely to have diabetes than their counterparts in the first tertile (Model 2 in Table 2).

To determine whether the observed excess odds of diabetes with increasing African ancestry might further be explained through the association between genetic ancestry and other non-genetic risk factors, such as measures of SES, we constructed additional models using only ARIC and JHS, where these data were available. Univariately, measures of SES were associated with both genetic ancestry (Table S2) and diabetes (Table S3). Even after adjusting for the three SES indicators, individuals in the second and third tertiles of African ancestry were about 1.27 and 1.37 times more likely to have diabetes than those in the first tertile (Model 3 in Table 2). The three SES indicators together accounted for about 22% of the excess odds of diabetes with increasing African ancestry. Collectively, BMI and the three SES measures explained about 30% of the excess odds of diabetes observed in individuals in the third tertile. The associations between increasing African ancestry and greater odds of diabetes were also evident in models based on restricted cubic splines (Figure 1).

Association of ancestry with diabetes-related traits

In ARIC and JHS only, we assessed the association of African ancestry to hemoglobin A1c (HbA1c), fasting glucose and insulin level, and insulin resistance, which was estimated by the homeostasis model assessment (HOMA-IR) (Table S4 and Table S5). A total of 4,880 participants had measurements on HbA1c, and 5,037 had fasting glucose and insulin level. Greater African ancestry was significantly correlated with higher HbA1c (P<0.001, see also Figure S2). However, African ancestry accounts for only 0.7% of the variance in HbA1c levels after adjustment for age, sex and study, while SES alone accounted for a slightly higher proportion of variance of 1.1%. After excluding individuals who were receiving diabetes treatment (because such treatment directly affects trait levels), the effects of ancestry generally became weaker (Table 3). The other three traits, fasting glucose, insulin and HOMA-IR levels, were also positively associated with African ancestry (Table 3).

![Figure 1. Odds ratio of type 2 diabetes by African ancestry in the ARIC and JHS studies.](https://doi.org/10.1371/journal.pone.0032840.g001)
Table 3. Mean difference in the levels of diabetes-related quantitative traits by genetic African ancestry after excluding participants receiving diabetes treatment.

<table>
<thead>
<tr>
<th>Trait and Model</th>
<th>African Ancestry</th>
<th>P Value for Trend</th>
<th>Effect Explained, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tertile 1</td>
<td>Tertile 2</td>
<td>Tertile 3</td>
</tr>
<tr>
<td>Hemoglobin A&lt;sub&gt;1c&lt;/sub&gt; (% (n = 4100))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1, base&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 [Reference]</td>
<td>0.10 (0.02–0.18)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.10 (0.02–0.18)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Model 2, BMI&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0 [Reference]</td>
<td>0.08 (0.01–0.16)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.09 (0.01–0.17)&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Model 3, SES&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0 [Reference]</td>
<td>0.07 (−0.01–0.16)</td>
<td>0.07 (−0.01–0.16)</td>
</tr>
<tr>
<td>Model 4, BMI+SES&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0 [Reference]</td>
<td>0.06 (−0.01–0.14)</td>
<td>0.06 (−0.02–0.14)</td>
</tr>
<tr>
<td>Glucose, mg/dL (n = 4423)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1, base&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 [Reference]</td>
<td>1.20 (−0.89–3.28)</td>
<td>1.57 (−0.51–3.65)</td>
</tr>
<tr>
<td>Model 2, BMI&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0 [Reference]</td>
<td>0.93 (−1.14–2.99)</td>
<td>1.16 (−0.91–3.22)</td>
</tr>
<tr>
<td>Model 3, SES&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0 [Reference]</td>
<td>0.48 (−1.62–2.58)</td>
<td>0.73 (−1.38–2.84)</td>
</tr>
<tr>
<td>Model 4, BMI+SES&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0 [Reference]</td>
<td>0.30 (−1.80–2.38)</td>
<td>0.41 (−1.68–2.51)</td>
</tr>
<tr>
<td>Insulin, mU/L (n = 4423)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1, base&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 [Reference]</td>
<td>0.87 (0.12–1.62)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.68 (−0.07–1.43)</td>
</tr>
<tr>
<td>Model 2, BMI&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0 [Reference]</td>
<td>0.58 (−0.11–1.27)</td>
<td>0.23 (−0.46–0.93)</td>
</tr>
<tr>
<td>Model 3, SES&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0 [Reference]</td>
<td>0.81 (0.06–1.57)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.60 (−0.16–1.36)</td>
</tr>
<tr>
<td>Model 4, BMI+SES&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0 [Reference]</td>
<td>0.60 (−0.10–1.29)</td>
<td>0.25 (−0.46–0.95)</td>
</tr>
<tr>
<td>HOMA-IR (n = 4423)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1, base&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 [Reference]</td>
<td>0.28 (0.05–0.52)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.23 (−0.00–0.47)</td>
</tr>
<tr>
<td>Model 2, BMI&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0 [Reference]</td>
<td>0.20 (−0.02–0.42)</td>
<td>0.10 (−0.12–0.32)</td>
</tr>
<tr>
<td>Model 3, SES&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0 [Reference]</td>
<td>0.24 (−0.00–0.47)</td>
<td>0.17 (−0.07–0.41)</td>
</tr>
<tr>
<td>Model 4, BMI+SES&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0 [Reference]</td>
<td>0.17 (−0.05–0.40)</td>
<td>0.07 (−0.15–0.30)</td>
</tr>
</tbody>
</table>

BMI: body mass index (calculated as weight in kilograms divided by height in meters squared); SES: socioeconomic status (including education, income, and occupation).

<sup>a</sup>Tertiles 1, 2 and 3 of African ancestry are <80.3%, 80.3%–87.3% and >87.3%, respectively, for hemoglobin A<sub>1c</sub>, and <80.2%, 80.2%–87.3% and >87.3%, respectively, for glucose, insulin, and HOMA-IR.

<sup>b</sup>Effects explained is defined as (β<sub>2</sub>−β<sub>1</sub>)/(1−β<sub>1</sub>), where β<sub>1</sub> is the regression coefficient in traits in Model 1; β<sub>2</sub> is the regression coefficient after adjustment for covariates in each model.

<sup>c</sup>Model 1: Mean difference (95% confidence interval) is adjusted for age, sex, and study.

<sup>d</sup>Model 2: Mean difference (95% confidence interval) is adjusted for covariates in Model 1 and BMI.

<sup>e</sup>Model 3: Mean difference (95% confidence interval) is adjusted for covariates in Model 1 and SES.

<sup>f</sup>Model 4: Mean difference (95% confidence interval) is adjusted for covariates in Model 1, BMI, and SES.

P<0.05, as compared to the reference tertile.

doi:10.1371/journal.pone.0032840.t003

Admixture scans

We conducted genome-wide admixture scans on type 2 diabetes in the 7,021 African Americans (Figure 2 and Table S6). In the diabetic cases, we detected an admixture association in diabetic cases at 12p13.31 with a locus-specific LOD of 4.0, just reaching the threshold for suggestiveness. The 12p13.31 peak was also supported by a case-control Z score of −4.2 (nominal P<3.5×10<sup>−5</sup>), which was marginally genome-wide significant. At this locus, diabetic cases had lower European ancestry (i.e., higher African ancestry) than non-diabetic controls. The second strongest association between greater African ancestry and type 2 diabetes, and to map susceptibility loci for type 2 diabetes. With 2,373 cases with type 2 diabetes and 4,648 controls, we found that greater African ancestry was significantly associated with type 2 diabetes and HbA<sub>1c</sub> values even after adjustment for BMI and markers of SES, including education, income, and occupation. Despite the significant association between greater African ancestry and type 2 diabetes, no major locus for diabetes could be detected by our admixture scans, using the more powerful locus-genome statistic.

Our results show that there is 30% to 40% increase in odds of type 2 diabetes among participants in the highest (>87.5%) vs. the lowest (<80.5%) tertile of African ancestry, even after adjustment for measures of SES and/or BMI. Our restricted cubic spline models also support this extrapolation, implying that genetic ancestry is a major independent determinant of the observed ancestry before and after adjustment, but the associations were not statistically significant (Table 3 and Table S5).

Discussion

We have conducted a large-scale admixture genetic analysis in more than 7,000 African Americans to determine the association of African ancestry with type 2 diabetes and to map susceptibility loci for type 2 diabetes. With 2,373 cases with type 2 diabetes and 4,648 controls, we found that greater African ancestry was significantly associated with type 2 diabetes and HbA<sub>1c</sub> values even after adjustment for BMI and markers of SES, including education, income, and occupation. Despite the significant association between greater African ancestry and type 2 diabetes, no major locus for diabetes could be detected by our admixture scans, using the more powerful locus-genome statistic.

Our results show that there is 30% to 40% increase in odds of type 2 diabetes among participants in the highest (>87.5%) vs. the lowest (<80.5%) tertile of African ancestry, even after adjustment for measures of SES and/or BMI. Our restricted cubic spline models also support this extrapolation, implying that genetic ancestry is a major independent determinant of the observed P=6.6×10<sup>−5</sup>, exceeding the level of nominal genome-wide significance. At the 13q14.3 locus, the diabetic cases had higher European ancestry than the non-diabetic controls.

Value for Trend Effect Explained, %

P<0.05, as compared to the reference tertile.
disparity in diabetes risk between the two ethnic groups. We note that in our study, markers of SES (education, income, and occupation) account for only a modest proportion (~22%) of the excess odds of type 2 diabetes due to ancestry. The results contrast to previous findings in Hispanic Americans [26], where the association between their non-European ancestry and type 2 diabetes is also significant, but where SES appears to be a much greater confounder, as adjustment for it significantly attenuated the association signal. It is also worth noting that the previous study had less power than our study as it used fewer individuals and fewer ancestry informative markers [26].

Insulin resistance and β-cell dysfunction are known to be major factors in the pathogenesis of type 2 diabetes. Evidence from epidemiological studies indicates that African Americans tend to be more insulin resistant and have greater insulin responses to glucose than European Americans [27–33]. Our results showed a positive (but statistically non-significant) correlation between African ancestry and HOMA-IR, in line with one earlier study that demonstrated that children with greater African ancestry had lower insulin sensitivity and a higher acute insulin response [34]. In genome-wide scans to date, the majority of the genetic variants for type 2 diabetes identified in European-derived populations appeared to be related to impaired insulin secretion [6,17,35], while only IRS1 has been unequivocally associated with insulin resistance [18]. However, a limitation is that these studies have been carried out largely in Europeans. It will be interesting to explore whether the loci associated to type 2 diabetes in African Americans are also associated with impaired insulin secretion, once genome-wide association studies of sufficient power are carried out.

Previous genome-wide scans for type 2 diabetes in African-descent populations have been extremely limited, and there has only been one study using admixture-based approaches. In the GENNID (Genetic of NIDDM) Study, using markers from a linkage panel in 1,450 African Americans, the strongest admixture association was found on chromosome 12 (90 cM), but no loci achieved genome-wide significance [36]. In our large population with a high number of ancestry informative markers, the two most interestingly loci using were at 12p13.31 and 13q14.3. While neither of these loci was significant by our locus-genome statistic which has the most statistical power, the case-control Z score at both loci exceeded the threshold for genome-wide significance, which makes these loci of interest for further study. An attractive candidate gene at the 12p13.31 locus is glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which is a key enzyme in the glycolytic pathway and is known to affect insulin receptor signaling [37]. The 12p13.31 locus has been found to be associated with type 1 diabetes in previous genome-wide association studies in European-derived populations [15], but neither of the two loci has been associated with type 2 diabetes in either African Americans or Europeans.

An interesting feature of our admixture scanning results is that diabetes risk at 12p13.31 and 13q14.3 were associated with ancestry in opposite directions. At 13q14.3, greater European ancestry is associated with a higher risk of diabetes, opposite to the direction of the overall epidemiological association, a phenomenon that we documented for the first time in a study of obesity loci [38]. These two loci, together with the other modest admixture signals on chromosome 12 and 1, and the absence of significant signals in locus-genome statistic elsewhere in the genome, suggest no evidence for a large genetic effect for type 2 diabetes that is racially/ethnically differentiated, such as that at the MTHFR/ApoL1 locus for non-diabetic end stage renal disease [22,39]. Thus multiple loci modest effects may, in aggregate, explain the apparent difference in genetic risk for type 2 diabetes between African Americans and European Americans.

Our study has important limitations. Despite the fact that our study size far exceeds previous genome scans for type 2 diabetes risk loci in African Americans, statistical power remains an important concern. We carried out simulation studies to examine the power of our study to detect a genomic locus of elevated African ancestry [40]. With a total of 2,373 diabetic cases, we expect to have 80% power to detect a 1.8-fold increased risk of type 2 diabetes per allele for alleles that are ancestry informative between Europeans and West Africans but less power for weaker ORs. A second limitation is that we used BMI as the only measure of adiposity. Including some other measure, such as waist circumference, might further attenuate the diabetes-ancestry association. In our previous analysis, however, we found that BMI, but not waist circumference, was significantly correlated with genetic ancestry after adjustment for SES [41], suggesting that confounding by waist circumference would have a minimal effect on results. Third, as in many studies involving SES, we were not able to fully assess SES and made inferences about SES based on education, income, and occupation, which, although are strong markers for SES, are still imperfect [42]. For example, SES may also be correlated with other diabetes risk factors, such as diet and lifestyle, and historical socioeconomic factors could in theory
interact in complex ways with African ancestry to influence diabetes risk, making the associations among them even more complicated.

In summary, in community-based populations with more than 7,000 African Americans, we found that genetic ancestry is significant associated with type 2 diabetes above and beyond the effects of markers of SES, and we detected several suggestive loci that may harbor genetic variants modulating diabetes risk. These results suggest that in African Americans, genetic ancestry has a significant effect on the risk of type 2 diabetes that are independent of the contribution of SES, but that no single locus with a major effect explains a large portion of the observed disparity in diabetes risk between African Americans and European Americans. In addition, they suggest that genetic measured African ancestry contributes to the risk of type 2 diabetes via both genetic and non-genetic pathways. The effect of ancestry on any individual locus in the genome is likely to be modest, but in aggregate, differences in ancestry may contribute substantially to the observed ethnic disparity in risk of type 2 diabetes.

Materials and Methods

Ethics statement
This study was conducted according to the principles expressed in the Declaration of Helsinki. All data collections were carried out according to protocols approved by Johns Hopkins Bloomberg School of Public Health Institutional Review Board for the study of human subjects. Written informed consent was obtained from all participants.

Study populations
The individuals enrolled in the present study came from three studies: the ARIC, JHS and MEC studies (Table 1). A detailed description of the three studies as well as the numbers of participants that were analyzed after applying various data quality filters are presented in Text S1. A brief description of each study is provided here.

The ARIC study is a prospective epidemiologic study that examines clinical and subclinical atherosclerotic disease in a cohort of 15,792 persons, including 4,266 self-reported African Americans, aged 45 to 64 years at their baseline examination from 1987 to 1989. The sampling procedure and methods used in ARIC have been described in detail elsewhere [43]. A total of 2,285 African-American participants from the ARIC study were included in the current analysis.

The JHS is a long-term, community-based observational study of cardiovascular disease and its risk factors in 5,301 self-identified African Americans recruited between 2000 and 2004 from three counties surrounding Jackson, Mississippi [44,45]. The study includes persons aged 35–84 who did not have diabetes and were derived from the same population-based cohort of 15,792 persons, including 4,266 self-reported African Americans recruited between 1993 and 1996 in Hawaii and Los Angeles, California, of whom 16.3% were African Americans [47]. Potential cohort members were identified primarily through Department of Motor Vehicles’ license files and, additionally for African Americans, Health Care Financing Administration data files. Participants were between the ages of 45 and 75 years at the time of recruitment. A total of 1,551 African-American participants from the MEC study, selected for a diabetes case-control study, were included in this analysis.

Diabetes and related traits
Information on body weight and height was collected in all three studies. In ARIC and JHS, anthropometry was performed during the clinical visit in the fasting state with an empty bladder by certified technicians. Body mass index was calculated as weight (in kg)/height (in meters) squared. In MEC, BMI was calculated using self-reported weight and height. The ARIC Study and JHS also have measurements of other diabetes-related quantitative traits, including fasting serum glucose and insulin concentrations, and HbA1c. Participants were asked to fast for at least 12 hours before morning blood collection. Blood samples were collected into vacuum tubes containing serum-separator gel (glucose, insulin) or EDTA (HbA1c). Specimens were then processed and analyzed in the ARIC and JHS Central Laboratories at University of Minnesota [48,49]. Serum glucose and insulin were measured by the hexokinase method [49,50] and by radioimmunooassay [49], respectively. HbA1c was assayed with Tosoh HPLC instruments [48,49]. The present analysis used data from the baseline examination in all three cohorts, except that in ARIC HbA1c was measured in stored whole blood samples from the second clinical visit [48]. Insulin resistance was estimated by the homeostasis model assessment (HOMA-IR) as fasting plasma glucose [mmol/l] times fasting serum insulin [mU/L] divided by 22.5.

Type 2 diabetes was defined as the presence of any one of the following at the baseline examination in the ARIC and JHS studies: 1) fasting glucose ≥7.0 mmol/l (126 mg/dl); 2) non-fasting glucose ≥11.1 mmol/l (200 mg/dl); 3) hemoglobin A1c ≥6.5% [31]; 4) current use of diabetic medication; or 5) a positive response to the question “Has a doctor ever told you that you had diabetes (sugar in the blood)?” In addition, diabetic individuals in ARIC or JHS who reported age of diagnosis younger than 30 years were excluded. In the MEC, diabetic individuals were defined as those who indicated on the baseline or follow-up questionnaires that they had a history of diabetes, and were taking medication for diabetes at the time of blood draw. The question did not differentiate between type 1 diabetes and type 2 diabetes, and thus we expect a small fraction (<10%) of the respondents to have type 1 diabetes [52]. In the ARIC and JHS study, non-diabetic controls were defined as African-American participants who did not have diabetes and were derived from the same populations as the diabetic cases. In the MEC, non-diabetic controls were from a group of samples who neither had a history of diabetes nor were taking medication for diabetes and had been specifically genotyped as part of previous admixture scans for prostate cancer [20] and hypertension [53].

Socioeconomic status
Information on three SES indicators, including personal education level, occupation, and family income, was collected during the baseline interview in the ARIC and JHS study. For the purpose of this analysis, education level was categorized into four groups: 1) less than high school; 2) high school graduate or high school-level General Educational Development credential; 3) some college; or 4) college completed, or some graduate or professional school. Income level was categorized as affluent, upper-middle, lower-middle, or poor based on total combined family income, family size, and poverty levels in each year when the interview was conducted. Some participants (9.5% and 13.8% in ARIC and JHS, respectively) did not provide their income information and were coded as a separate category (missing). A more detailed description of the assessment of income is presented in Text S1. Occupations were categorized according to the criteria of the 1980 U.S. census into six groups: managerial and professional specialty;
We used ANCESTRYMAP [40] to search for genomic regions associated with an increased percentage of either European or African ancestry. The ANCESTRYMAP software provided two statistics: a locus-genome statistic and a case-control statistic. A locus-genome statistic was obtained in cases by calculating the likelihood of the genotyping data at the SNPs at the locus under a risk model and comparing it to the likelihood of the genotyping data at the SNPs at the locus assuming that the locus is unassociated with the phenotype [40]. We tested 6 pre-specified European ancestry risk models ranging from 0.7 to 1.3. To accumulate evidence of association in these models, we averaged the ratio of these two likelihoods emerging from each model at each point in the genome, taking the log10 of this likelihood ratio to produce a locus-specific LOD score. We considered a locus-specific LOD score for association at a particular locus of >5 as genome-wide significant and >4 as suggestive [58]. To obtain an assessment of the evidence for a risk locus anywhere in the genome, we averaged the likelihood ratio for association across all loci in the genome, and took the log10 to obtain a “genome-wide score” [58]. We interpreted a genome-wide score >2 as significant, and >1 as suggestive.

A case-control statistic was calculated by comparing locus-specific deviations in European ancestry in cases versus controls at each locus across the genome [40]. For loci identified by the case-control statistic, the level of genome-wide significance was defined as a Z score >4.06 or <-4.06, corresponding to an uncorrected nominal P<5×10^{-5}, or a corrected nominal P<0.05 after conservatively correcting for 1,000 hypotheses tested (approximately equals the number of independent chromosomal segments assigned to either African or European ancestry).

Supporting Information

Figure S1 Histograms of African ancestry in the African American participants by study and diabetes status. Percentages of African ancestry were estimated using subsets of 2,189 ancestry-informative SNPs. The grey bars represent the diabetic participants; the blue bars represent the non-diabetic participants.

(DOC)

Figure S2 Scatterplot of hemoglobin A1c and percentage of African ancestry in the ARIC and JHS studies. The solid line in the figure is a lowess smoother.

(DOC)

Table S1 Characteristics of ARIC and JHS study participants by diabetes status.

(DOC)

Table S2 Genetic African ancestry by socioeconomic status in African Americans in the ARIC and JHS studies (n = 5470).

(DOC)

Table S3 Association of diabetes and socioeconomic status in African Americans in the ARIC and JHS studies.

(DOC)

Table S4 Genetic African ancestry by diabetes-related quantitative traits in African Americans in the ARIC and JHS studies.

(DOC)

Table S5 Mean difference in the levels of diabetes-related quantitative traits by genetic African Ancestry.

(DOC)
Table S6  Summary of the Admixture scans on type 2 diabetes results by chromosome. (DOC)

Text S1  Supplementary Methods: study populations, assessment of income, genotyping and quality filters, estimating genetic ancestry with Hidden Markov Models, and calculating contribution of covariates to the observed association. (DOC)

Acknowledgments
The authors thank the staff and participants of the ARIC, JHS, and MEC studies.

Author Contributions
Conceived and designed the experiments: CYC DR CAH JW WHK. Performed the experiments: DR CAH EB JW. Analyzed the data: CYC AT NP. Contributed reagents/materials/analysis tools: DR CAH SE ELA FLB JG EB DA HAT BEH JW WHK. Wrote the paper: CYC DR WHK.

References


