Consistent Directions of Effect for Established Type 2 Diabetes Risk Variants Across Populations

The Population Architecture using Genomics and Epidemiology (PAGE) Consortium

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Common genetic risk variants for type 2 diabetes (T2D) have primarily been identified in populations of European and Asian ancestry. We tested whether the direction of association with 20 T2D risk variants generalizes across six major racial/ethnic groups in the U.S. as part of the Population Architecture using Genomics and Epidemiology (PAGE) Consortium (16,235 diabetes case and 46,122 control subjects from six major U.S. populations). The percentage of positive (odds ratio [OR] > 1 for putative risk allele) associations ranged from 69% in American Indians to 100% in European Americans. Of the nine variants where we observed significant heterogeneity of effect by racial/ethnic group ($P_{\text{heterogeneity}} < 0.05$), eight were positively associated with risk (OR > 1) in at least five groups. The marked directional consistency of association observed for most genetic variants across populations implies a shared functional common variant in each region. Fine-mapping of all loci will be required to reveal markers of risk that are important within and across populations. Diabetes 61:1642–1647, 2012

Over the past decade, genome-wide association studies (GWAS) and candidate gene association studies have been successful in identifying common risk variants for type 2 diabetes (T2D) (1–15). The loci revealed have provided insight into the genetic basis of this common disease, as well as biological pathways important in its pathogenesis. Most of these previously reported risk variants were identified in very large studies or meta-analyses conducted among populations of European and Asian ancestry and have been associated with modest increases in T2D risk (per-allele odds ratios [ORs] between 1.1 and 1.4) (12). Subsequent testing of these well-established variants in other racial and ethnic groups has been limited (12,16–24), and most of the studies have been undersized and underpowered to provide reliable risk estimates and clarity regarding generalizability of the associations in non-European populations. Aggregating results from multiple studies conducted among racially and ethnically diverse populations is one approach to amass an adequate sample size for replicating these modest genetic associations and extend our understanding of T2D genetics to non-European populations. As part of the Population Architecture using Genomics and Epidemiology (PAGE) Consortium, we have tested 20 validated risk variants for association with T2D. These 20 variants represent 18 risk regions and were examined in as many as 16,235 diabetes case and 46,122 control subjects from six major U.S. populations: (European Americans, African Americans, Hispanics, East Asians, Native Hawaiians, and American Indians) from six large population-based studies.

RESEARCH DESIGN AND METHODS

The PAGE Consortium consists of large ongoing population-based studies or consortia (25). The following studies are included in the current study: from the CALiCo (Causal Variants Across the Life Course) consortium, ARIC (the...
Atherosclerosis Risk in Communities Study) (26), CHS (Cardiovascular Health Study) (27), and SHS (Strong Heart Study) (28,29); EAGLE (Epidiologically Architecture of Genes Linked to Environment, based on three National Health and Nutrition Examination Surveys [NHANES]) (30–33); MEC (The Multiethnic Cohort Study) (34). Among the 20 variants evaluated in the current study, the minor allele frequency of each SNP in the discovery populations was at least 5% (Fig. 1). The 20 variants evaluated in the current study were selected from 18 genomic regions found to be significantly associated with risk of T2D in studies published as of September 2009 (Supplementary Table 1). In order to incorporate the T2D information across studies, two case definitions were allowed: self-report and exam based. To be classified as a case subject according to the self-report definition, participants had to report both a previous diagnosis of diabetes and use of medication to treat diabetes. To be classified as a control subject (self-report), participants had to report neither previous diagnosis nor use of diabetes medications. To be classified as a case subject according to the exam-based definition, participants had to either meet the self-report case definition or have a fasting (≥8 h) blood glucose ≥126 mg/dL. To be classified as a control subject (exam based), participants had to be classified as a control subject per the self-report definition and have a fasting blood glucose <126 mg/dL. Both prevalent and incident cases were included. For both definitions, self-reported diabetes diagnosis before age 30 was excluded. Sensitivity analyses in the ARIC study suggested that the magnitude of association between candidate variants and T2D did not differ systematically according to the case definitions we applied (Supplementary Data). Additional study-specific details on the data-collection methods and case definitions can be found in the Supplementary Data.

A total of 16,235 diabetes case and 46,122 control subjects were included in this study (case and control subjects, respectively, by study: ARIC, 1,348/10,978; CHS, 8,504/4,488; SHS, 1,575/1,249; MEC, 6,298/9,980; EAGLE/NHANES, 1,029/4,502; and WHI, 5,120/14,925). None of these studies was involved in the initial discovery efforts of these T2D risk loci. The data from the MEC have previously been reported (37).

**Genotyping.** The 20 variants evaluated in the current study were selected from 18 genomic regions found to be significantly associated with risk of T2D in studies published as of September 2009 (Supplementary Table 1). In the CHS, 859/4,488; SHS, 1,575/1,249; MEC, 6,298/9,980; EAGLE/NHANES, 1,029/4,502; and WHI, 5,120/14,925). None of these studies was involved in the initial discovery efforts of these T2D risk loci. The data from the MEC have previously been reported (37).

Genotyping was conducted in study-specific laboratories using a number of different platforms. Cross-laboratory and cross-platform reproducibility was assessed by genotyping 360 HapMap samples from populations most relevant to PAGE samples in each laboratory. A description of the platforms and quality-control metrics from each study/laboratory is provided in Supplementary Data. The genotype concordance for single nucleotide polymorphisms (SNPs) evaluated in the HapMap samples in more than one laboratory was >95.5% per SNP, with an average concordance of 99.8%.

We excluded results for SNP rs13266634 (SLC30A8) in all populations except European Americans and Hispanics, as there is an adjacent SNP 1 bp away (rs16880462) that has a frequency of 10% in African Americans, 4% in Asians, and 2% in Native Hawaiians (<1% in Hispanics and Europeans) and interferes with genotyping assays, thus resulting in genotype misclassification.

Genetic markers that distinguish the major ancestral populations (African, European, and Asian) were available in three studies. For ARIC, principal components of ancestry were derived from 200,000 SNPs genotyped on a custom array. For WHI (all populations) and MEC (African Americans and Native Hawaiians), ~100 ancestry-informative markers were used in a principal-components analysis to assess major axes of variation (38,39). For a subset of the MEC, Latinos, principal components were derived from markers on the Illumina 2.5M array, and Native Hawaiians ancestry information was not available for the majority of the American Indian (SBS) or East Asian (MEC) samples or samples in EAGLE.

**Statistical analysis.** β values and SEs for each variant were obtained by unconditional logistic regression or Cox proportional hazards regression. For each variant, the allele tested was the allele that was associated with increased risk in previous studies. In each study, models were run separately for each racial/ethnic population and adjusted for sex, age (continuous), and BMI (continuous). Sensitivity analyses were run excluding Native Hawaiians (MEC) and an inverse association of European ancestry and T2D risk in this population. Similarly, in Hispanics the first principal component, which is also a measure of European admixture (and ancestry) in this population, was significantly associated with lower T2D risk (P = 2.1 × 10^-12 in the MEC) (Fig. 2). Adjustment for the first principal component in Hispanics increased the OR and degree of statistical significance for three SNPs that were all less common, although marginally, in Hispanics than in European Americans (rs2237897, KCNQ1; rs4402960, FTO; and rs7803146, TCFT1L2) and diminished significance for rs864745 (JAZF1), which is more common in Hispanics than in European Americans. For the most part, the risk allele frequencies of each population tracked with the risk allele frequency of European Americans (Supplementary Fig. 3). Effect estimates were >1 for >90%–100% of the SNPs across populations (average: 84% (Fig. 1). These variants were significantly associated (P < 0.05) with risk in at least four groups (rs4402960, FTO; rs864745, JAZF1; and rs7803146, TCFT1L2), and of the 17 SNPs evaluated in five or more populations, positive associations were observed with 13 SNPs (OR >1) in at least five groups (Fig. 1). Of the 108
American Indians
Native Hawaiians
East Asians
Hispanics
European Americans

We observed significant heterogeneity of effect by racial/ethnic group for nine SNPs ($P_{\text{heterogeneity}} < 0.05$). However, aside from rs7961581 at TSPAN8, eight of these variants (at TTHADA, IGF2BP2, WFS1, CDKAL1, CDKN2A/CDKN2B [rs2383208], TCF7L2, KCNQ1 [rs2237895], and KCNJ11) were positively associated with risk (OR > 1) in at least five populations (Fig. 1). Thus, even for variants that displayed evidence of significant heterogeneity across population, the direction of effect was generally consistent in the majority of the populations.

**DISCUSSION**

We examined 20 validated risk variants for T2D, representing 18 risk regions, in as many as 16,235 diabetes case and 46,122 control subjects from six major population groups. The vast majority of the variants were positively associated with risk in the five non-European populations. These findings are highly consistent with a previous multiethnic study in the MEC, which contributed a large fraction of the case subjects to this meta-analysis (American Indians 0%, European Americans 11%, African Americans 31%, Hispanics 66%, East Asians 84%, and Native Hawaiians 100%) (37), and suggest that the majority of these variants are likely to be generalized markers of T2D risk across populations.
associations, with the index variants at five loci (WFS1, HHEX, CDKN2A/B, THADA, and KCNQ1) that were found to be significantly associated with risk in at least one of the other non-European populations. The lack of a statistically significant association in African Americans at these loci could be because the risk allele is relatively invariant in populations of African ancestry or low linkage disequilibrium between the index signal and the functional allele. Fine-mapping of these loci, and others such as TCF7L2 in American Indians, where we observed no evidence of significant association (OR 1.08 [95% CI 0.90–1.29]) despite >99% power and despite the suggestion that rs7903146 is the biologically functional variant in African Americans (43) and in genomic studies of open chromatin (44), should be of high priority to extract information about any genetic risk conferred at that locus that may be important for these populations.

This study has a number of limitations. In the design, we allowed for both incident and prevalent diabetes cases as well as different case/control criteria depending on study; however, our sensitivity analysis of the different case groups (Supplementary Data) did not suggest systematic differences in effect sizes based on study design, case definition, or analytic approach. We also had no information about type 1 diabetes in some studies, although case subjects known to be diagnosed before age 30 years were excluded and most participants in these studies were middle-aged or older adults.

This is the largest effort to date to investigate the generalizability of T2D susceptibility variants in the major racial/ethnic groups of the U.S. The consistent patterns of association for these variants provide additional support for the importance of these loci in contributing to T2D risk in multiple populations. Identification of the underlying
biological functional allele(s) in each region, through fine-mapping, will be required to determine the extent to which these regions contribute to racial and ethnic disparities in T2D risk.

ACKNOWLEDGMENTS

The PAGE program is funded by the National Human Genome Research Institute, supported by U01HG004803 (CALiCo [Causal Variants Across the Life Course]), U01HG004798 (EAGLE [Epidemiologic Architecture of Genes Linked to Environment]), U01HG004802 (MEC [Multietnic Cohort]), U01HG004790 (WHI [Women’s Health Initiative]), and U01HG004801 (Coordinating Center).

No potential conflicts of interest relevant to this article were reported.

C.A.H. performed experiments, analyzed data, and wrote the manuscript. M.D.F., K.L.S., P.B., V.S.V., P.W., J.H., and N.F. performed experiments, analyzed data, and contributed to writing the manuscript. K.R.M., B.V.H., R.D.J., J.C.F., L.N.K., S.B., R.J.G., S.L., J.E.M., J.B.M., K.W., K.J.M., S.A.P., P.S., L.R.W., L.A.H., J.L.A., K.E.N., U.P., D.C.C., and L.L.M. contributed materials and to the study design, analysis tools, and interpretation of results and contributed to writing the manuscript. J.S.P. performed the experiments, analyzed data, and wrote the manuscript. C.A.H. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study-specific acknowledgments are listed in the Supplementary Data.

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