Extension of Type 2 Diabetes Genome-Wide Association Scan Results in the Diabetes Prevention Program

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Extension of Type 2 Diabetes Genome-Wide Association Scan Results in the Diabetes Prevention Program

Allan F. Moore,‡ Kathleen A. Jablonski,§ Jarred B. McAteer,† Richa Saxena,¶ Toni I. Pollin,∥ Paul W. Franks,¶ Robert L. Hanson,§ Alan R. Shuldiner,∥ William C. Knowler,§ David Altshuler,†,‡,¶,¶,¶ and Jose C. Florez†,‡,¶,¶ for the Diabetes Prevention Program Research Group

OBJECTIVE—Genome-wide association scans (GWASs) have identified novel diabetes-associated genes. We evaluated how these variants impact diabetes incidence, quantitative glycemic traits, and response to preventive interventions in 3,548 subjects at high risk of type 2 diabetes enrolled in the Diabetes Prevention Program (DPP), which examined the effects of lifestyle intervention, metformin, and troglitazone versus placebo.

RESEARCH DESIGN AND METHODS—We genotyped selected single nucleotide polymorphisms (SNPs) in or near diabetes-associated loci, including EXT2, CDKAL1, CDKN2A/B, IGF2BP2, HHEX, LOC387761, and SLC30A8 in DPP participants and performed Cox regression analyses using genotype, intervention, and their interactions as predictors of diabetes incidence. We evaluated their effect on insulin resistance and secretion at 1 year.

RESULTS—None of the selected SNPs were associated with increased diabetes incidence in this population. After adjustments for ethnicity, baseline insulin secretion was lower in subjects with the risk genotype at HHEX rs1111875 (P = 0.01); there were significant differences in baseline insulin sensitivity. Both at baseline and at 1 year, subjects with the risk genotype at LOC387761 had paradoxically increased insulin secretion; adjustment for self-reported ethnicity abolished these differences. In ethnicity-adjusted analyses, we noted a nominal differential improvement in β-cell function for carriers of the protective genotype at CDKN2A/B after 1 year of troglitazone treatment (P = 0.01) and possibly lifestyle modification (P = 0.05).

CONCLUSIONS—We were unable to replicate the GWAS findings regarding diabetes risk in the DPP. We did observe genotype associations with differences in baseline insulin secretion at the HHEX locus and a possible pharmacogenetic interaction at CDKN2A/B.

The recent implementation of genome-wide association scans (GWASs) as an investigative tool has resulted in a qualitative leap in identifying diabetes-related genes (1,2). These surveys, which are agnostic to candidate genes, can cover ~80% of common human genome variants with current technology, thus providing unprecedented insight into the genetic architecture of type 2 diabetes. In 2007, the first published type 2 diabetes GWAS confirmed the importance of TCF7L2 on diabetes incidence (odds ratio [OR] 1.65, P < 1.0 × 10⁻⁵) and identified several new type 2 diabetes loci, SLC30A8 (1.26, P = 5.0 × 10⁻⁵), HHEX (1.21, P = 9.1 × 10⁻⁵), LOC38771 (1.14, P = 2.9 × 10⁻⁵), and EXT (1.26, P = 1.2 × 10⁻⁴) (3). SLC30A8 encodes a zinc transporter protein that carries zinc from the cytoplasm into insulin secretory vesicles within the pancreatic β-cell, an important step in insulin synthesis and secretion (4). HHEX is essential for the development of the pancreas and liver and is a target of the Wnt signaling pathway (5).

After the initial GWAS publication, four other high-density scans were published simultaneously by different groups, confirming many of the initial findings. In addition to replicating the prior associations of TCF7L2, HHEX, and SLC30A8, investigators from Iceland identified CDK5 regulatory subunit-associated protein 1-like 1 (CDKAL1) as another potential diabetes-related gene (OR 1.2, P = 1.8 × 10⁻⁵) (6). This gene is hypothesized to lead to β-cell degeneration by modulating CDK5/CDK5R1 activity. The Diabetes Genetics Initiative, the Wellcome Trust Case Control Consortium, and the Finland–U.S. Investigation of Type 2 Diabetes Genetics Initiative published GWASs that were combined in a preliminary meta-analysis of >30,000 samples (7–9). Again, the above findings were confirmed, and novel diabetes loci in or near IGF2BP2...
that associated with type 2 diabetes as a categorical trait.

The DPP is a multicenter trial that examined whether lifestyle modification or metformin therapy prevents the development of diabetes in individuals with risk factors for type 2 diabetes, including elevated fasting glucose, impaired glucose tolerance, overweight or obesity, and sedentary lifestyle. The DPP randomly assigned 3,819 subjects at high risk of diabetes to lifestyle modification (≥7% weight loss and ≥150 min of physical exercise weekly), metformin (850 mg twice daily), troglitazone (400 mg daily), or placebo. The troglitazone arm with 585 participants was stopped early because of medication-related hepatotoxicity. The primary end point was the development of diabetes confirmed by two consecutive measures of glucose. The trial was conducted at 27 centers, all of which obtained institutional review board approval. The distribution of self-reported ethnicities among the 3,548 participants in this genetic study was 56.4% Caucasian, 20.2% African American, 16.8% Hispanic, 4.3% Asian, and 2.4% American Indian. The mean age was 51 years, and the mean BMI was 34.0 kg/m². There was a range of BMI values for each ethnic group, with Asian/Pacific Islanders having the lowest (29.5 kg/m²) followed by Hispanics (33.1 kg/m²), Caucasians (34.1 kg/m²), African Americans 35.3 kg/m², and American Indians (35.4 kg/m²). After an average of 3 years of follow-up, the lifestyle modification group had a 58% reduction in diabetes incidence, whereas the metformin group had a 31% reduction compared with placebo.

**SNP selection and genotyping.** We selected SNPs that had been highly associated with type 2 diabetes at high levels of statistical significance in previous high-density (300,000–500,000) GWAS analyses (5,7,9). DNA was extracted from peripheral blood leukocytes with standard methods. Genotyping was carried out by allele-specific primer extension of multiplex amplified products and detection using matrix-assisted laser desorption ionization time-of-flight mass spectrometry on a Sequenom iPLEX platform (20,21). The mean genotyping success rate was 99.5%. All SNPs were in Hardy-Weinberg equilibrium (HWE) within each self-reported ethnic group. We genotyped one SNP per gene for all genes with the exception of HHEX and EXT2 for which multiple SNPs were tested. In HHEX, the SNPs rs7903103 and rs1111875 are in linkage disequilibrium in the HapMap CEU population (*P* < 0.05) but not the YRI population (*P* > 0.05). Three SNPs rs2231132, rs1113132, and rs1113131 are in linkage disequilibrium for both the YRI and CEU populations in the HapMap Project: all three SNPs are in perfect linkage disequilibrium (*P* < 0.001, *D’* = 1.0) in both populations. SNP selection and genotyping.

**Quantitative glycemic measures.** Baseline and annual OGTTs were performed on all subjects who had not developed diabetes before the 1-year examination and used to calculate the insulin sensitivity index (ISI) and insulinogenic index. The ISI, the reciprocal of insulin resistance by homeostasis model assessment was calculated as 22.5[(fasting insulin (μU/ml) - (glucose at 30 min) (mg/dl)] (23). Quantitative traits were measured at baseline and 1 year. We elected to analyze quantitative traits at 1 year for two reasons: first, the greatest effect on weight loss was observed in the lifestyle modification group at year 1, and second, a substantial number of subjects did not complete the OGTT on the 3rd year (because they had either developed diabetes already or because the trial ended before they reached that time point) (19).

**Statistical analysis.** The primary end point of this study was the time to development of diabetes. We examined genotype (under an additive model), intervention, and genotype-by-intervention interactions as independent predictors of time to onset of diabetes using Cox regression models. We also developed an aggregate risk genotype score for each subject. For each of the 10 SNPs studied, if the genotype was homozygous for the high-risk allele, we assigned one point; if heterozygous, one-half point; and if homozygous for the low-risk allele, zero points. We divided the score into quartiles and ran a Cox regression testing for the relationship between the score category and the incidence of diabetes. Baseline glycemic variables, the insulinogenic index, and the ISI were log transformed for non-normality, and the geometric means were compared across genotype groups (high-risk homozygotes, heterozygotes, and low-risk homozygotes) by ANCOVA (F test). We compared the glycemic variables at 1 year using ANCOVA models with the independent variables of genotype and treatment group adjusted for the baseline glycemic variable with interaction terms of genotype and treatment. All analyses were repeated after adjusting for sex, age, self-reported ethnicity, and BMI. P values were
TABLE 1 Risk allele frequencies

<table>
<thead>
<tr>
<th>Marker SNP</th>
<th>Gene region</th>
<th>Alleles*</th>
<th>Risk allele frequency</th>
<th>Reference groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Caucasian</td>
<td>African American</td>
</tr>
<tr>
<td>rs1111875</td>
<td>HHEX</td>
<td>C/T</td>
<td>2.001</td>
<td>716</td>
</tr>
<tr>
<td>rs7923837</td>
<td>HHEX</td>
<td>G/A</td>
<td>63.4</td>
<td>90.3</td>
</tr>
<tr>
<td>rs11037909</td>
<td>EXT2</td>
<td>T/C</td>
<td>72.9</td>
<td>84.5</td>
</tr>
<tr>
<td>rs7340878</td>
<td>EXT2</td>
<td>T/C</td>
<td>72.8</td>
<td>89.3</td>
</tr>
<tr>
<td>rs1113132</td>
<td>EXT2</td>
<td>C/G</td>
<td>73.1</td>
<td>90.0</td>
</tr>
<tr>
<td>rs13266634</td>
<td>SLC30A8</td>
<td>C/T</td>
<td>71.0</td>
<td>89.3</td>
</tr>
<tr>
<td>rs7480010</td>
<td>LOC387761</td>
<td>G/A</td>
<td>28.5</td>
<td>83.1</td>
</tr>
<tr>
<td>rs10811661</td>
<td>CDKN2A/B</td>
<td>T/C</td>
<td>84.8</td>
<td>93.9</td>
</tr>
<tr>
<td>rs1470579</td>
<td>IGF2BP2</td>
<td>C/A</td>
<td>32.6</td>
<td>73.1</td>
</tr>
<tr>
<td>rs7754840</td>
<td>CDKAL1</td>
<td>C/G</td>
<td>32.7</td>
<td>56.8</td>
</tr>
</tbody>
</table>

Data are percent. *Putative risk allele listed first. †French. ‡Scandinavian. 3. CEU, European ancestry. YRI, African ancestry.

were adjusted for multiple comparisons across genotypes within each SNP using the Holm procedure (24).

We calculated power for predicting diabetes incidence using the methods of Hsieh and Lavori (25). We assumed HWE within each ethnic group and an additive genetic model. Assuming there are no gene-treatment interactions, these calculations show that the overall DPP cohort has 83% power to detect the previously reported effect size of −1.2 for a SNP of 10% frequency, whereas the placebo, lifestyle modification, and metformin arms have 53, 34, and 44% power, respectively. The DPP has inadequate power for detecting an incidence in the placebo arm (hazard ratio 1.21, 95% CI 1.12–1.31, P = 0.001), whereas the placebo, lifestyle modification, and metformin arms have 53, 34, and 44% power, respectively. The DPP has inadequate power for detecting an effect size of <1.1 (Supplementary Table 1, available in an online appendix at http://dx.doi.org/10.1210/diabetes.2007-0590).

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Three hypotheses are tested in the current project. We hypothesized the following: 1) variants in the genes identified by the recently completed GWAS will be associated with the development of type 2 diabetes incidence prospectively; 2) quantitative traits measuring insulin secretion (insulinogenic index) and insulin sensitivity (ISI) will vary by genotype at the same diabetes-associated loci; and 3) genetic variation in these regions will affect response to metformin, troglitazone, or the lifestyle intervention as reflected in diabetes incidence or related quantitative traits. To a large extent, the first two hypotheses represent confirmation of previous robust findings; therefore, an overall P value of 0.05 was considered statistically significant. On the other hand, the interaction with behavioral and pharmacological interventions represents a novel exploration, and therefore, the nominal P values should be interpreted after taking into account the number of independent variants and the three DPP interventions examined.

RESULTS

Diabetes incidence. Risk allele frequencies by ethnicity are listed in Table 1. The risk allele frequencies were higher in African American participants compared with those of European ancestry, with the greatest disparity in allele frequency between ethnicities at LOC387761 and IGF2BP2. Risk allele frequencies by ethnicity were compared with the Hap Map European cohort (CEU), HapMap Yoruba cohort (YRI), and the cohorts studied in the original GWAS (3,7). Generally, the risk alleles appeared to be enriched among the DPP white participants compared with the reference populations (HapMap CEU or the GWAS original cohorts) and among the DPP African American participants compared with their white counterparts. We did not observe a significant association of any of the reported variants with diabetes incidence in either the overall cohort or the placebo group (Supplementary Table 2). Adjustments for self-reported ethnicity or BMI did not alter the results (Table 2 and Supplementary Table 3). For CDKN2A/B rs10811661, we noted a nominally significant interaction between genotype and intervention; thus, we stratified diabetes incidence analyses by treatment arm. For this SNP, a nonsignificant effect on diabetes incidence in the placebo arm (hazard ratio 1.21, P = 0.13) was attenuated in the metformin and lifestyle intervention arms (Table 2). Analysis of the aggregate risk genotype score identified a nominally significant increase in the incidence of diabetes for those with a risk allele score of 7.5–9 compared with those with a score of <3.5 (P = 0.04).

Baseline quantitative traits. In the crude analyses, we noted apparent associations of genotype at EXT2 rs3740878 and LOC387761 rs7480010 with baseline β-cell function, as measured by the insulinogenic index (Supplementary Table 4). The EXT2 rs3740878 risk T allele was nominally associated with reduced insulin secretion in carriers of the high-risk genotype compared with those with the low-risk genotype (P = 0.01); this difference may have been driven in part by a compensatory response to the borderline higher insulin resistance of CC homozygotes. The insulin response at LOC387761 rs7480010 was paradoxical: The high-risk G allele was associated with higher insulin secretion (P < 0.001), whereas insulin resistance remained relatively constant in all genotypic groups. When adjusted for self-reported ethnicity, however, the above associations at both LOC387761 and EXT2 were abolished. Following such adjustments, the HHEX rs1111875 high-risk genotype was associated with decreased insulin secretion (P = 0.01) (Table 3); however, this association was lost after adjustment for BMI (P = 0.13). The other SNPs tested did not demonstrate any other significant differences in baseline β-cell function in either crude or adjusted analyses (Table 3 and Supplementary Table 4).

Both HHEX SNPs, rs7923837 and rs1111875, were nominally associated with decreased insulin sensitivity in carriers of the risk genotype in crude analyses (P = 0.05 and 0.03, respectively). Again, these associations disappeared when adjusted for self-reported ethnicity. None of the other SNPs tested predicted differences in insulin sensitivity by genotype in either crude or adjusted analyses (Table 3 and Supplementary Table 4).

Follow-up quantitative traits. Before adjustment for ethnicity, an unexpected higher level of insulin secretion in carriers of the risk genotype at LOC387761 rs7480010 was again noted in all treatment arms at 1 year, with nominal statistical significance in the combined group (P < 0.001) and placebo arm (P = 0.01). Similarly, a paradoxical trend toward higher insulin secretion in participants with the IGF2BP2 rs1470579 high-risk genotype when compared with the low-risk genotype at baseline
was maintained at 1 year in the overall cohort \((P = 0.03)\), the metformin arm \((P = 0.02)\) and possibly the troglitazone arm \((P = 0.06)\) (Supplementary Table 5). Adjustments for self-reported ethnicity abolished all significant differences in insulin secretion by genotype at LOC387761 rs7480010. Also, the only nominally significant differences at \(IGF2BP2\) rs1470579 occurred in the lifestyle arm: higher adjusted ISI values were seen in high-risk genotype subjects compared with low-risk genotype subjects \((P = 0.02)\), with a corresponding compensatory change in insulin secretion \((P = 0.03)\) (Table 4). The analysis was repeated by adjusting quantitative glycemic traits for BMI, and no significant associations were identified.

A nominally significant interaction between genotype and treatment arm on insulin secretion was seen at \(CDKN2A/B\) rs10811661 in crude \((P = 0.03)\), ethnicity-adjusted \((P = 0.04)\), and BMI-adjusted \((P = 0.04)\) analyses. In ethnicity-adjusted analyses, we noted a nominal differential improvement in \(\beta\)-cell function for carriers of the protective genotype at \(CDKN2A/B\) after 1 year of troglitazone treatment \((P = 0.01)\) and lifestyle modification \((P = 0.05)\). These results persisted when adjusted for BMI alone in both the troglitazone \((P = 0.03)\) and lifestyle modification \((P = 0.02)\) arms.

We detected one nominally significant interaction between genotype at \(HHHEX\) rs79238327 and treatment arm on insulin sensitivity at 1 year in the crude analysis \((P = 0.006)\). Treatment with metformin and troglitazone improved the insulin sensitivity of carriers of the high-risk genotype at \(HHHEX\) rs79238327 to a greater extent than in those who carried the low-risk genotype, an effect that was not seen in the lifestyle arm (Supplementary Table 5). There were no significant interactions between genotype and treatment arm on insulin sensitivity after adjustments for ethnicity.

### DISCUSSION

We were unable to validate the individual association of previously reported genetic variants identified by GWAS with diabetes incidence in the DPP; however, when taken as an aggregate, we identified a nominally significant increase in diabetes incidence for those with a risk allele score of 7.5–9 compared with those with a score of \(<3.5\) \((P = 0.04)\). Given the higher prior probability of these diabetes-associated variants, this nominal \(P\) value is of interest even in the context of multiple hypotheses testing and illustrates a potential strategy for combining a full complement of diabetes-associated variants in risk prediction as additional loci are identified.

The DPP is a unique cohort that differs from the case-control design used in the prior GWAS studies detailed above. Participants in the DPP were at relatively high-risk at baseline (as evidenced by the 11% per year development of diabetes in the placebo group) and were presumably at a relatively late stage in the pathogenesis of disease. The DPP population is very homogeneous in their risk of type 2 diabetes; thus, as there is a smaller phenotypic difference between DPP participants who develop diabetes and those who do not (compared with the case-control designs of the published GWAS), the role of genetic variation is more difficult to ascertain. This limitation is supported by the apparently higher frequency of the risk alleles in the DPP white cohort compared with the reference groups from the HapMap CEU population and original GWAS cohorts.
The DPP is also limited in its ability to replicate the GWAS findings given its interventional design, in which a majority of participants received either a medication or lifestyle modification designed to prevent diabetes, again reducing the number of incident cases and thus our ability to observe the effect of genetic variation at multiple diabetes-related genes. In addition, the DPP cohort is multiethnic, introducing divergent allele frequencies and additional population differences that may increase population heterogeneity. The impact of these newly identified variants is quite modest, and our power calculations show that the cohort examined here only has marginal power to detect such effect sizes. Furthermore, positive gene-treatment interactions may have reduced our power even further. Nevertheless, in previous work, we have been able to convincingly replicate the association of relatively powerful genetic factors such as TCF7L2 with diabetes, illustrating that the DPP is an appropriate cohort to study genetic variants of high enough frequency and/or with strong effects (16). The SNPs examined here were marker SNPs chosen from prior GWASs and are not known to represent causal mutations. Further fine mapping of these gene regions will be required in larger, better-powered studies to identify potential causal variants, because the current study is underpowered for such an analysis. Finally, the analysis of two of the genetic loci investigated, EXT2 and LOC387761, was largely exploratory because these loci have not been reproducibly associated with type 2 diabetes and associated traits in more recent studies.

Interestingly, in unadjusted analyses of variants at both LOC387761 and IGF2BP2, carriers of the presumed high-risk genotypes had paradoxically higher insulin secretion levels at baseline and 1 year. The LOC387761 finding is consistent with results recently reported by Palmer et al. (15) in the IRAS-FS, in which Hispanic Americans with the risk variant at LOC387761 had apparently higher acute insulin response \((P = 0.005)\) and disposition index \((P = 0.04)\) than low-risk genotype carriers (these results were not replicated in the African American cohort). When we adjusted for ethnicity, however, the associations of genotype at LOC387761 with insulin secretion were abolished, as were most of the IGF2BP2 associations (see below). The disparate results of our crude and adjusted analysis underscores the critical role ethnicity may play in confounding genetic association studies, particularly in admixed populations. Genetic loci with allele frequencies that diverge significantly across populations are particularly susceptible to confounding by ethnicity when tested for association with phenotypes whose prevalence also differs across populations. In such a scenario, a particular variant may simply be a marker for ancestry rather than truly associated with the trait under study. In the DPP, although diabetes incidence did not differ significantly across the five ethnic groups (19), baseline quantitative glycemic traits did (26). LOC387761 rs7480010 and IGF2BP2 rs1470579 SNPs have dramatically different allele frequencies in white and black populations (Table 1), which may allow genotype-phenotype associations to be confounded by genotype-ethnicity associations. Further studies, powered for stratified analyses of minority populations and adjusted for possible population substructure with the use of ancestry informative markers, will be required for investigators to fully understand the role genetic variants at these two loci play in individual ethnic groups. With regard to LOC387761, given the failure of other groups to replicate the association of this locus with type 2 diabetes (3,6,8,9) and the disappearance of statistical significance in our results once ethnicity is taken into consideration, the role of this locus in disease pathogenesis remains unclear.

We identified significant differences in insulin secretion by genotype at HHEX at baseline and 1-year follow-up; however, adjusting the results for BMI abolished the effect, emphasizing the role BMI plays in modulating the impact of this genetic variant. Other investigators have also identified differences in insulin secretion by genotype at HHEX, including Pascoe et al. (12), who found a significant decrease in 30-min insulin response in subjects...
### TABLE 4
Follow-up quantitative traits adjusted for ethnicity

<table>
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<tr>
<th></th>
<th>Placebo</th>
<th>Lifestyle</th>
<th>Metformin</th>
<th>Troglitazone</th>
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<tr>
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| Type 2 diabetes GWAS results extended in DPP

**Ins Index**

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<tr>
<td>rs10811661</td>
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</tr>
<tr>
<td>CDKN2A/B ISI</td>
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</tbody>
</table>

P-values and 95% confidence intervals are provided in parentheses.

*Continued on following page*
with the HHEX risk variant; 2) Grarup et al. (13), who found that the risk variant of HHEX was associated with a decreased acute insulin response after OGTT or tolbutamide challenge; and 3) Staiger et al. (14), who showed that the risk variant of HHEX was associated with decreased insulin secretion after OGTT or intravenous glucose challenge.

We did not replicate the associations of several other diabetes-related variants with insulin secretion documented by others (6,12–15). We may have been underpowered to replicate these previous findings because of a smaller effect of these genes on insulin secretion and sensitivity when compared with those identified by the original GWAS investigations. An alternative explanation is that in these participants at high risk for diabetes, pathological changes had already taken place that obscured the effect of single genetic variants on these physiological parameters.

We identified a single genomic region with a possible genotype-intervention interaction. The previously reported impairment in β-cell function in carriers of the high-risk genotype at CDKN2A/B rs10811661 when compared with the alternative genotypes was augmented by treatments that improved insulin sensitivity: subjects with the low-risk genotype at CDKN2A/B improved β-cell function to a greater extent than those with the high-risk genotype after treatment with troglitazone and possibly lifestyle modification for 1 year, suggesting that they may have benefited more from these interventions. This interaction was identified in both crude and ethnically adjusted analysis. More scientific investigation on the biological consequences of genotypic variation at CDKN2A/B will be required to determine why subjects with the high-risk genotype, who had decreased insulin secretion, benefited less from metformin or lifestyle modification than low-risk genotype subjects. Although this is one of the first reports of a potential pharmacogenetic interaction with one of the newly identified type 2 diabetes gene regions, this finding is limited by the modest nominal P values obtained here, the multiple tests performed, and the unclear mechanism of action. Independent confirmation of these complex gene-environment interactions is needed.

In summary, although we were unable to replicate the findings of the original GWAS scans in our smaller, prediabetic population, our quantitative trait analysis confirms differences in insulin secretion by genotype at HHEX and CDKN2A/B. This study also emphasizes the important role ancestry may play at the diabetes-associated SNPs in LOC387761 and IGF2BP2, which have dramatically different allele frequencies in populations of European and African ancestry. Finally, we have identified a potential genotype-intervention interaction at CDKN2A/B; however, this hypothesis-generating finding needs to be confirmed by additional studies. Further studies are required to better understand the differences in insulin dynamics that result from variants at these and the other diabetes-associated genes identified to date.

ACKNOWLEDGMENTS

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We gratefully acknowledge the commitment and dedication of all participants in the DPP, without whom this work would not have been possible.

REFERENCES