No Interactions Between Previously Associated 2-Hour Glucose Gene Variants and Physical Activity or BMI on 2-Hour Glucose Levels


Gene–lifestyle interactions have been suggested to contribute to the development of type 2 diabetes. Glucose levels 2 h after a standard 75-g glucose challenge are used to diagnose diabetes and are associated with both genetic and lifestyle factors. However, whether these factors interact to determine 2-h glucose levels is unknown. We meta-analyzed single nucleotide polymorphism (SNP) × BMI and SNP × physical activity (PA) interaction regression models for unknown. We meta-analyzed single nucleotide polymorphism (SNP) × BMI and SNP × physical activity (PA) interaction regression models for five SNPs previously associated with 2-h glucose. It is perhaps unlikely that top loci from genome-wide association studies or BMI (β = 0.06–0.12 mmol/allele, P ≤ 1.53 × 10−7), but no significant interactions were found with PA (P > 0.18) or BMI (P ≥ 0.04). In this large study of gene–lifestyle interaction, we observed no interactions between genetic and lifestyle factors, both of which were associated with 2-h glucose. It is perhaps unlikely that top loci from genome-wide association studies will exhibit strong subgroup-specific effects, and may not, therefore, make the best candidates for the study of interactions.

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Glucose levels 2 h after a 75-g glucose challenge are used to diagnose diabetes and are associated with cardiovascular morbidity and mortality even below the diabetic threshold (1). A large number of type 2 diabetes–associated genetic variants have now been identified (2), and recent genome-wide meta-analyses identified five loci that were associated with postchallenge glucose at genome-wide levels of significance (3). Previously identified single nucleotide polymorphisms (SNPs) in TCFTL2 and GCKR were associated with 2-h glucose levels, as were newly identified loci in ADCY5, GIPR, and VPS13C. Risk alleles at each of these loci conferred elevated 2-h glucose levels with effect sizes ranging from 0.07 to 0.11 mmol/L per allele (3), although with some heterogeneity.

Age, BMI, and physical inactivity are all associated with glycemia and are key risk factors for type 2 diabetes (4–6). Glucose levels at 2 h appear more susceptible to age- and lifestyle-mediated increases than fasting glucose levels. For example, physical activity (PA) levels have been shown to be inversely associated with 2-h glucose but not with fasting glucose (7,8). Differences in 2-h glucose between individuals at either end of the PA spectrum are appreciable, with the most active individuals having a mean 2-h glucose level ~1 mmol/L lower than those with low PA levels (7). Furthermore, lifestyle intervention trials including prescribed PA have been effective in decreasing the incidence of diabetes in individuals with impaired glucose tolerance at baseline (9,10). However, it is unclear whether these responses to PA are homogenous among those with genetically conferred elevations in 2-h glucose levels or whether genetic effects are similar across lifestyle strata. Identification of gene–lifestyle interactions will offer valuable insight into the etiologic processes leading to disease and the biologic pathways by which lifestyle modification can reduce the risk of diabetes.

Although gene–lifestyle interactions are suggested as being important in the etiology of type 2 diabetes, few consistently replicated examples have been identified (11) and methodologic difficulties limit the opportunity for literature-based meta-analyses (12). The association of 2-h glucose with lifestyle and genetic factors makes it a good trait for the study of gene–lifestyle interaction. Furthermore, the heterogeneity observed in the association between SNPs and 2-h glucose (3) is potentially attributable to factors such as gene–lifestyle interaction. Therefore, we investigated the presence of gene–lifestyle interactions at these five 2-h glucose-associated loci (in or near GCKR, ADCY5, TCFTL2, VPS13C, and GIPR) by meta-analyzing SNP×PA and SNP×BMI interactions on 2-h glucose in up to 54,884 individuals from 22 studies.

RESEARCH DESIGN AND METHODS

Participating cohort characteristics. We meta-analyzed results from up to 22 Meta-Analyses of Glucose and Insulin Related Traits Consortium (MAGIC) studies (3) comprising up to 54,884 individuals. Study descriptive are detailed in Supplementary Table 1. Participants with known diabetes, those with fasting glucose ≥ 7 mmol/L, and individuals with a BMI < 18.5 kg/m² were excluded. All studies were cross-sectional except for Atherosclerosis Risk in Communities Study (ARIC) where PA data were available at the visit ~3 years before 2-h glucose measurement.

Lifestyle exposure classification. Study-specific details of the measurement of PA are in Supplementary Table 1. Where a quantitative measure of PA was available, individuals below the first quintile were classified as inactive and the remainder as active (i.e., >20 inactive and 80% active). In studies where PA data were categorical, the proportion of inactive individuals was dependent on the questionnaire used and reported in Supplementary Table 1. Inactive individuals were coded as 0 and active individuals as 1 in the analyses. BMI was treated as a continuous variable in the primary analyses.

Genotyping and statistical analysis. Genotyping methods are reported in Supplementary Table 1 and have been described in detail previously (3). Analysis of each SNP was performed using linear regression models testing the association of each SNP with BMI, adjusted for age, sex, fasting glucose, and PA as a continuous variable, and any necessary study-specific variables. We also examined the association of each SNP with BMI, adjusted for...
RESULTS
Study descriptives are reported in Supplementary Table 1. Inactive individuals had a higher 2-h glucose (β = 0.22 mmol/L [95% CI 0.13–0.31], P = 1.65 × 10⁻⁶) and BMI (β = 0.73 kg/m² [0.51–0.95], P = 1.42 × 10⁻¹⁰) than active individuals. Higher BMI was also associated with higher 2-h glucose levels (β per 1 kg/m² = 0.086 mmol/L [0.08–0.10], P = 1.04 × 10⁻⁴⁷). SNP effects were consistent with those reported previously in overlapping studies (Fig. 1A) (3). SNP×PA and SNP×BMI interactions on 2-h glucose. Figure 1B shows the difference in SNP effect on 2-h glucose between inactive and active individuals (SNP×PA P = 0.18 for interaction). Likewise, we did not observe any significant interaction effects when analyses were limited to those studies showing association between PA and 2-h glucose (SNP×PA P ≥ 0.1 for interaction). Figure 1C shows the difference in SNP effect on 2-h glucose per 10 kg/m². Again, no statistically significant interaction effects were observed after correction for multiple testing (five tests for each hypothesis: α = 0.01), although rs1260326 in GCKR reached nominal levels of statistical significance (albeit with very small interaction effects). BMI-stratified results for rs1260326 showed that SNP effects were largest in the 30 to 34.9 kg/m² group (Supplementary Fig. 1), although few individuals at >35 kg/m² makes the smaller effect in this stratum difficult to interpret.

Association of SNPs with BMI. As can be seen from Fig. 2, TCF7L2 rs12243326 and GIPR rs10423928 were both associated with BMI: the alleles associated with increased 2-h glucose were associated with lower BMI. The TCF7L2 and GIPR SNPs were both associated with a 0.11 kg/m² lower BMI per allele (95% CI −0.17 to −0.04 and −0.20 to −0.03, respectively; Fig. 2). These findings were directionally consistent with those from previous meta-analyses by the GIANT consortium (13) (rs12243326 P = 5.7 × 10⁻⁴; rs10423928 P = 1.9 × 10⁻⁷).

DISCUSSION
Each of the gene variants investigated in the current study was robustly associated with 2-h glucose levels, as reported previously in overlapping studies (3). However, we observed

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FIG. 1. A: Effect of SNP is shown on 2-h glucose. The β-coefficient is the magnitude of the observed association. B: Shows the SNP×PA interaction effect in which the β-coefficient is the difference in SNP association effect between inactive and active individuals. Inactive individuals were coded as 0 and active individuals a 1; therefore, a value of 0 for the interaction coefficient reflects equivalent SNP effect in inactive and active strata, whereas a positive value reflects a larger SNP effect in active individuals. C: The SNP×BMI interaction is shown. Here, the β-coefficient is the difference in SNP effect per 10 kg/m² difference in BMI. A positive value reflects a larger SNP effect in those with higher BMI. The 2-h glucose-raising allele in A is always the effect allele.
Previous studies have reported gene–lifestyle interactions, although often based on small sample sizes without independent replication (11). In light of the small effect sizes of most complex disease-associated SNPs, large sample sizes are important to investigate interactions (14). However, despite the large sample size in the current study, no significant interactions were observed between 2-h glucose-associated SNPs and established lifestyle correlates. Notably, however, in 6 of the 10 interaction meta-analyses we performed (i.e., 5 SNPs and interaction with PA or BMI), at least 1 individual study would have shown a significant interaction had it been studied in isolation. However, had we considered studies individually, for 200 tests of interaction we would have observed only nine interactions at $P_{\text{adj}} = 0.05$, spread among a range of studies. We suggest that these associations reflect type I errors. Such a finding further supports our use of large sample sizes or independent replication to reduce the potential for type I error.

Although the variety of subjective measures and dichotomous classification of PA is an important limitation of the current study, inactive individuals had a higher 2-h glucose and a higher BMI than active individuals, suggesting the validity of our PA classification. Another factor may contribute to the absence of interactions: the SNPs we selected arose as top SNPs associated with 2-h glucose levels from a genome-wide meta-analysis (3). Although heterogeneity of associations was observed for \(ADCY5\), \(TCF7L2\), and \(GIPR\) (3), these SNPs had the strongest association $P$ values in the genome by virtue of their effect size relative to the variation in effect size among samples. One may not, therefore, expect significant variation in genetic effect between subgroups of the population. A similar approach to ours was recently used to investigate interactions between breast cancer–associated genes and risk-altering lifestyle exposures and also failed to detect significant interactions between them (15), although there was a suggestion that a physically active lifestyle attenuated genetic predisposition to obesity (16).

Although it has been suggested that the search for interactions should be informed by biologic plausibility (11), experience from the study of genetic main effects, where hypothesis-generating discovery approaches revolutionized the field (2), suggests that such an approach, not limited to those SNPs with extremely significant main effects, may be valuable in detecting gene–lifestyle interactions. Such approaches have been proposed and efforts are underway, but whether current analytic methods will yield success in the genome-wide search for gene–lifestyle interactions remains unclear.

Data from large-scale trials, such as the Finnish Diabetes Prevention Study (DPS) and Diabetes Prevention Program (DPP), have shown suggestions of differential response to intervention by genotype (17–22), although not always reaching statistical significance for interaction (17–19). However, lifestyle interventions in such studies often contain numerous lifestyle modifications, making interpretation of any interaction difficult, whereas large-scale genotype-stratified lifestyle intervention trials are not feasible. Therefore, prospective nested approaches will likely offer the most efficient approach for the study of gene–lifestyle interaction (23), allowing standardized measures of lifestyle at baseline and also the opportunity to study large numbers of individuals. Refined and standardized lifestyle exposure measurement will also represent a valuable alternative to straightforward increases in sample size (24).

Variants in \(TCF7L2\) have previously been associated with diabetes (2,17) and a number of related traits (3,25). The diabetogenic, glucose-raising allele was previously associated with lower BMI, although not conclusively (26), and principally in individuals with diabetes (27,28). Here, we replicate this association in a larger sample size of participants without diabetes, where the glucose-raising allele at rs12243326 was associated with a 0.11 kg/m$^2$–lower BMI (Fig. 2). Similarly, we report that the glucose-raising allele at
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