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Effects of Genetic Variants Previously Associated with Fasting Glucose and Insulin in the Diabetes Prevention Program

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Abstract

Common genetic variants have been recently associated with fasting glucose and insulin levels in white populations. Whether these associations replicate in pre-diabetes is not known. We extended these findings to the Diabetes Prevention Program, a clinical trial in which participants at high risk for diabetes were randomized to placebo, lifestyle modification or metformin for diabetes prevention. We genotyped previously reported polymorphisms (or their proxies) in/near G6PC2, MTNR1B, GCK, DGKB, GCKR, ADCY5, MADD, CRT2, ADR2A, PFKP, GIPR, GLIS3, C2C4DB, IGF1, and IRS1 in 3,548 Diabetes Prevention Program participants. We analyzed variants for association with baseline glycemic traits, incident diabetes and their interaction with response to metformin or lifestyle intervention. We replicated associations with fasting glucose at MTNR1B (P<0.001), G6PC2 (P=0.002) and GCKR (P=0.001). We noted impaired β-cell function in carriers of glucose-raising alleles at MTNR1B (P<0.001), and an increase in the insulinoenic index for the glucose-raising allele at G6PC2 (P<0.001). The association of MTNR1B with fasting glucose and impaired β-cell function persisted at 1 year despite adjustment for the baseline trait, indicating a sustained deleterious effect at this locus. We also replicated the association of MADD with fasting proinsulin levels (P<0.001). We detected no significant impact of these variants on diabetes incidence or interaction with preventive interventions. The association of several polymorphisms with quantitative glycemic traits is replicated in a cohort of high-risk persons. These variants do not have a detectable impact on diabetes incidence or response to metformin or lifestyle modification in the Diabetes Prevention Program.

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* A list of Diabetes Prevention Program Research Group investigators is provided in the Appendix S1.
Introduction

Glucose homeostasis is tightly regulated. Control of its variation in non-diabetic individuals is influenced by familial factors, many of which are presumed to be heritable [1,2]. In searching for genetic determinants of quantitative glycemic traits, candidate gene and genome-wide association studies (GWAS) conducted in populations of European descent have identified associations of fasting glucose with genetic variants in or near the genes that encode glucokinase (GCK; [3]), the glucose-6-phosphatase catalytic subunit (G6PC2; [4,5]) and the melatonin receptor 1b (MTNR1B; [6,7]). The Meta-Analysis of Glucose and Insulin-related traits Consortium (MAGIC) recently performed a global meta-analysis of 21 GWAS cohorts followed by replication in 26 studies, totaling >122,000 non-diabetic individuals for fasting glucose and >48,000 non-diabetic individuals for fasting insulin [8]. These efforts confirmed the GCK, G6PC2 and MTNR1B associations, and uncovered associations of fasting glucose with single nucleotide polymorphisms (SNPs) in or near DGKB, GCKR, ADCY3, MADD, CR1Y, ADRA2A, FADS1, PROX1, SLC2A2, GLIS3, C2CD4B and the type 2 diabetes genes TCF7L2 and SLC30A8. In addition, SNPs in or near IGF1, GCKR and perhaps IRSI have been found to influence fasting insulin concentrations, a surrogate for insulin resistance. Of these loci, only GCK, MTNR1B, DGKB, GCKR, ADCY3 and PROX1 (besides TCF7L2 and SLC30A8) were associated with type 2 diabetes at genome-wide significance levels, with several others (but not all) showing a consistent trend but not meeting the same stringent statistical threshold. This work has illustrated that genetic associations with quantitative intermediate traits may lead to the discovery of type 2 diabetes loci, but also that not all genetic loci that influence fasting glucose levels in healthy individuals necessarily contribute to type 2 diabetes pathogenesis.

The MAGIC investigators have also performed more detailed characterization of the mechanisms of glucose regulation influenced by these loci in white individuals [9]. In the Third National Health and Nutrition Examination Survey (NHANES III), a genetic risk score constructed with the glucose-raising alleles was shown to have consistent effects in other ethnic groups representative of the US population [10]. The Gene × Lifestyle interactions And Complex traits Involved in Elevated disease Risk (GLACIER) investigators showed that several of these loci associate with impaired fasting glucose (IFG) cross-sectionally and prospectively, and some have a progressively deleterious effect on fasting glucose [11]. Shortly thereafter, the Whitehall II investigators reported that a genetic risk score constructed with these variants was strongly associated with fasting glucose and remained stable over time [12]. Finally, we have recently shown that different genetic variants influence type 2 diabetes risk at distinct stages of the normoglycemia to IFG to type 2 diabetes progression, with MTNR1B and GCK exerting their effects preferentially in the normoglycemia to IFG transition [13].

To understand why some loci raise fasting glucose but do not increase type 2 diabetes risk, it is critical to establish whether their glucose-raising effects remain evident in the setting of impaired glucose tolerance (IGT), as glycemic context may modulate the strength of the genetic effect [13]. Furthermore, the impact of these loci on the prospective development of diabetes has not yet been reported. Finally, establishing whether and how distinct preventive interventions modulate these effects may facilitate the clinical translation of these findings and illuminate the specific genes and mechanisms by which these loci affect glycemic homeostasis. We concentrated on SNPs associated with fasting glucose, rather than those associated with 2-hour glucose [14], because 1) the two 2-hour glucose SNPs that are not already captured by fasting glucose-associated variants (GIPR and VPS13C) have no detectable impact on type 2 diabetes [15], 2) the ascertainment of DPP participants by the strict IGT definition is likely to bias the distribution of 2-hour glucose alleles, 3) longitudinal changes in 2-hour glucose among carriers of the 2-hour glucose-raising alleles have already been reported in a better suited population cohort [16], and 4) evidence obtained by the MAGIC investigators argues against an interaction of known 2-hour glucose loci with physical activity or body mass index (BMI) (Robert Scott, personal communication). We therefore genotyped the fasting glucose-associated SNPs in the multi-ethnic cohort of the Diabetes Prevention Program (DPP), and analyzed their relationships with glycemic measures at baseline and one year, the development of diabetes, and their potential interaction with preventive interventions on diabetes incidence.

Methods

The Diabetes Prevention Program

The DPP study design and baseline characteristics of the participants have been described previously [17,18]. Briefly, the DPP was designed to test whether intensive lifestyle modification or pharmacologic interventions with metformin or troglitazone prevent or delay the onset of diabetes in individuals at high risk. The trial, conducted from 1996 to 2001 in 27 US-based medical centers, included 3,234 participants randomized to intensive lifestyle modification (goal 7% weight loss and >150 min/week of physical activity), metformin (850 mg twice daily), or placebo; the fourth arm, comprising 585 additional participants randomized to troglitazone, was terminated early because of concerns with hepatotoxicity. For enrollment, participants had to have a fasting glucose between 95–125 mg/dL and IGT (2-hour glucose between 140–199 mg/dL after a 75-gram oral glucose tolerance test [OGTT]). Of the total 3,819 DPP participants, 3,548 had DNA and consented to genetic investigation; 56.4% were of European descent, 20.2% African American, 16.8% Hispanic, 4.3% Asian and 2.4% American Indian by self-report. Their mean age was 51 years and mean BMI was 34.0 kg/m². The primary endpoint (diabetes incidence, ascertained biannually and confirmed on a second occasion) was reached in nearly 33% of participants randomized to the placebo arm after a mean of 3.2 years of follow-up; there was a 58% reduction of diabetes incidence in the lifestyle intervention group and a 31% reduction in the metformin group compared to placebo [19]. For the purposes of this study, participants randomized to troglitazone were excluded, leaving a total of 2,890 individuals with valid genotypes for analysis. Institutional Review Board approval was obtained by each participating site, and all participants included in this report provided written informed consent for the main study and for subsequent genetic investigations.

Quantitative Glycemic Traits

We calculated the insulin sensitivity index (ISI) as 22.5/[(fasting insulin × fasting glucose)/100.01]; the ISI is the reciprocal of insulin resistance calculated by homeostasis model assessment (HOMA-IR) [20]. We estimated insulin secretion by the insulinoic index using the formula [(insulin at 30 min)/(insulin at 0 min)]/(glucose at 30 min)/(glucose at 0 min)]. The oral disposition index (Dlo) was calculated as 1/(fasting insulin × insulinoic index) [21]. We studied genetic associations with these measures at baseline and at 1 year; we chose one year because changes in weight were most
pronounced at that time point, and it contained the highest number of participants with available measures.

**SNP Selection and Genotyping**

We genotyped the index SNPs associated with fasting glycemic traits reported by the MAGIC investigators [8]. Where assay design failed we selected proxies based on linkage disequilibrium $r^2 = 0.915$. DNA was extracted from peripheral blood leukocytes and quantitated as previously described [22]. 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 [23].

**Statistical Analyses**

We used Cox proportional hazards regression models with genotype, intervention and their interactions as the independent variables predicting time to diabetes over mean 3.2 years follow-up. We adjusted for gender, age at enrollment, ethnicity, treatment arm, and baseline BMI. For the quantitative glycemic traits, we employed generalized mixed models to test additive effects of genotype on baseline log-transformed quantitative traits, and on the same traits after one year of intervention adjusted for the baseline value, age, sex, self-reported ethnicity, BMI and treatment arm. We note that these SNPs have been associated with glycemic traits at genome-wide levels of significance, and therefore their prior probability of true effects is many orders of magnitude higher than the genome average. As our analyses represent further characterization of each of these established loci, we selected a value threshold of 0.05. Finally, we also tested for any evidence of epistatic interactions between the $MTNR1B$ SNP rs10830963 and the G6PC2 SNP rs573225, both of which have significant effects on fasting glucose in the DPP, by including appropriate interaction terms at baseline and one year.

**Results**

**Baseline Associations**

The SNPs genotyped, their chromosomal location, the nearest gene and their allele frequencies in the five DPP ethnic groups are shown in Table 1. Allele frequencies were comparable to those previously reported by MAGIC in Europeans [8] and NHANES III in non-Hispanic whites, African Americans and US Hispanics [10].

| Table 1. SNPs genotyped and their allele frequencies by ethnic group. |
|---------------------------------------------------------------|
| **SNP** | **Chromosome** | **Position (NCBI 36)** | **Nearest gene** | **Alleles (effect/other)** | **White (n = 1,617)** | **African-American (n = 592)** | **Hispanic (n = 475)** | **Asian (n = 125)** | **American Indian (n = 81)** |
| rs340874 | 1 | 184833918 | PROX1* | C/T | 55.9 | 19.8 | 41.2 | 42.3 | 35.4 |
| rs573225 | 2 | 161653734 | G6PC2 | A/G | 71.7 | 91.8 | 85.4 | 90.3 | 92.0 |
| rs11708067 | 3 | 120438894 | ADCY5* | A/G | 79.4 | 85.9 | 77.3 | 91.2 | 70.0 |
| rs11920090 | 3 | 168087406 | SLC2A2 | T/A | 87.1 | 67.3 | 86.6 | 91.1 | 94.4 |
| rs2191349 | 7 | 14947780 | DKK8* | T/G | 55.7 | 57.9 | 48.1 | 64.0 | 24.1 |
| rs197793 | 7 | 44131132 | GCK* | T/A | 19.5 | 23.7 | 32.3 | 21.6 | 48.1 |
| rs7034200 | 9 | 4244098 | GLI5 | A/C | 48.9 | 64.2 | 57.3 | 46.0 | 65.6 |
| rs10885122 | 10 | 106670840 | ADRA2A | G/T | 88.0 | 35.6 | 84.3 | 86.8 | 88.9 |
| rs11605924 | 11 | 45579933 | CRY2 | A/C | 49.3 | 87.0 | 47.7 | 68.0 | 51.9 |
| rs7944584 | 11 | 47035421 | MADD | A/T | 71.7 | 95.0 | 83.7 | 90.4 | 98.1 |
| rs174550 | 11 | 57899714 | FADS1 | T/C | 68.0 | 91.4 | 43.5 | 55.2 | 11.1 |
| rs10830963 | 11 | 88799685 | MTNR1B* | G/C | 28.8 | 9.0 | 22.7 | 41.2 | 24.1 |
| rs11071657 | 15 | 39256547 | C2CD4B | A/G | 64.4 | 86.9 | 53.7 | 70.0 | 37.0 |

* Allele frequencies (%)

| Fastinnglucose | Alleles | Allele frequencies (%)
|-----------------|-------------|
| rs340874 | C/T | 55.9/19.8 |
| rs573225 | A/G | 71.7/91.8 |
| rs11708067 | A/G | 79.4/85.9 |
| rs11920090 | T/A | 87.1/67.3 |
| rs2191349 | T/G | 55.7/57.9 |
| rs197793 | T/A | 19.5/23.7 |
| rs7034200 | A/C | 48.9/64.2 |
| rs10885122 | G/T | 88.0/35.6 |
| rs11605924 | A/C | 49.3/87.0 |
| rs7944584 | A/T | 71.7/95.0 |
| rs174550 | T/C | 68.0/91.4 |
| rs10830963 | G/C | 28.8/9.1 |
| rs11071657 | A/G | 64.4/86.9 |

| Fasting insulin | Alleles | Allele frequencies (%)
|-----------------|-------------|
| rs4675095 | A/T | 93.3/98.5 |
| rs855228 | G/T | 84.3/40.9 |

| Fasting glucose and insulin | Alleles | Allele frequencies (%)
|-----------------------------|-------------|
| rs780094 | C/T | 59.6/81.7 |

*Allele frequencies correspond to the effect allele. Gene names: PROX1, prospero homeobox 1; G6PC2, glucose-6-phosphatase catalytic 2; ADCY5, adenylate cyclase 5; SLC2A2, solute carrier family 2, member 2; DKK8, diacylglycerol kinase, beta 90 kDa; GCK, glucokinase; GLI5, GLI family zinc finger 3; ADRA2A, adrenergic, alpha-2A, receptor; CRY2, cryptochrome 2; MADD, MAP-kinase activating death domain; FADS1, fatty acid desaturase 1; MTNR1B, melatonin receptor 1B; C2CD4B, C2 calcium-dependent domain containing 4B; IRS1, insulin receptor substrate 1; G6PC2, glucose-6-phosphatase; GCKR, glucokinase regulator.

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## Table 2. Nominal genotypic associations with quantitative traits at baseline.

| SNP       | Nearest gene | Alleles (effect/other) | Trait       | LS Means (95% CI) | Additive P value | Pairwise P values |
|-----------|--------------|------------------------|-------------|-------------------|------------------|------------------|
| rs573225  | G6PC2        | A/G                    | FG (mg/dL)  | AA 106.7 (106.2–107.2) | 0.002            | AA vs AG 0.002   |
|           |              |                        |             | AG 105.6 (104.9–106.3) |                  |                  |
|           |              |                        |             | GG 105.8 (104.5–107.1) |                  |                  |
|           |              |                        | Fins (μU/mL)| AA 24.44 (23.65–25.26) | 0.006            | AA vs AG 0.31    |
|           |              |                        |             | AG 24.97 (23.87–26.11) |                  |                  |
|           |              |                        |             | GG 27.71 (25.56–30.05) |                  |                  |
|           |              |                        | Ins Index   | AA 1.25 (1.20–1.31)   | 0.002            | AA vs AG 0.16    |
|           |              |                        |             | AG 1.20 (1.13–1.28)   |                  |                  |
|           |              |                        |             | GG 1.04 (0.92–1.17)   |                  |                  |
|           |              |                        | ISI         | AA 0.153 (0.15–0.161) | 0.03             | AA vs AG 0.62    |
|           |              |                        |             | AG 0.154 (0.147–0.161)|                  |                  |
|           |              |                        |             | GG 0.138 (0.127–0.15) |                  |                  |
|           |              |                        | Dlo         | AA 0.049 (0.047–0.051)| <0.001           | AA vs AG 0.03    |
|           |              |                        |             | AG 0.046 (0.043–0.049)|                  |                  |
|           |              |                        |             | GG 0.037 (0.033–0.042)|                  |                  |
| rs11708067| ADCYS        | A/G                    | Fins (μU/mL)| AA 24.05 (23.24–24.88) | 0.001            | AA vs AG 0.001   |
|           |              |                        |             | AG 25.85 (24.79–26.95) |                  |                  |
|           |              |                        |             | GG 25.29 (23.12–27.67) |                  |                  |
|           |              |                        | ISI         | AA 0.158 (0.153–0.164) | 0.004            | AA vs AG 0.003   |
|           |              |                        |             | AG 0.148 (0.141–0.154) |                  |                  |
|           |              |                        |             | GG 0.151 (0.138–0.166) |                  |                  |
| rs11920090| SLC2A2       | T/A                    | Dlo         | AA 0.042 (0.037–0.049) | 0.006            | AA vs AT 0.27    |
|           |              |                        |             | AT 0.046 (0.043–0.049) |                  |                  |
|           |              |                        |             | TT 0.049 (0.047–0.051) |                  |                  |
| rs7944584 | MADD         | A/T                    | Proins (pmol/L)| AA 16.4 (15.9–16.92) | <0.001           | AA vs AT <0.001  |
|           |              |                        |             | AT 14.98 (14.34–15.65) |                  |                  |
|           |              |                        |             | TT 13.53 (12.46–14.68) |                  |                  |
| rs174550  | FADS1        | T/C                    | Fins (μU/mL)| TT 23.78 (22.83–24.78) | 0.008            | TT vs CT 0.06    |
|           |              |                        |             | CT 24.97 (23.96–26.03) |                  |                  |
|           |              |                        | ISI         | CC 25.52 (24.25–26.86) |                  |                  |
|           |              |                        |             | CC 0.149 (0.141–0.157) | 0.01             | TT vs CT 0.09    |
|           |              |                        |             | CT 0.153 (0.146–0.159) |                  |                  |
|           |              |                        |             | TT 0.160 (0.153–0.167) |                  |                  |
| rs10830963| MNWR1B       | G/C                    | FG (mg/dL)  | GG 108.7 (107.6–109.9) | <0.001           | GG vs CG 0.02    |
|           |              |                        |             | CG 107.3 (106.7–108.0) |                  |                  |
|           |              |                        | Proins (pmol/L)| GG 15.88 (14.80–17.03) | 0.009            | GG vs CG 0.66    |
|           |              |                        |             | CC 105.6 (105.1–106.2) |                  |                  |
|           |              |                        |             | CG 105.0 (104.5–105.5) |                  |                  |
|           |              |                        | Ins Index   | GG 11.7 (10.5–12.9)   | 0.01             | GG vs CG 0.74    |
| rs855228  | IGF1         | T/C                    | FG (mg/dL)  | TT 106.1 (105.5–106.7) | 0.01             | TT vs CT 0.37    |
|           |              |                        |             | CT 106.4 (105.8–107.0) |                  |                  |
| rs780094  | GCKR         | C/T                    | FG (mg/dL)  | CC 107.7 (106.6–108.7) |                  |                  |
|           |              |                        |             | CC 106.8 (106.2–107.3) | 0.001            | CC vs CT 0.12    |
|           |              |                        |             | CC 106.3 (105.6–106.9) |                  |                  |

FG, fasting glucose; Fins, fasting insulin; Ins Index, insulinogenic index; ISI, insulin sensitivity index; Dlo, oral disposition index; Proins, fasting proinsulin adjusted for fasting insulin. To convert glucose mg/dL to mmol/L, divide by 18.01. To convert insulin μU/mL to pmol/L, multiply by 6.0.

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Fasting Glucose and Insulin Variants in the DPP
Table 3. Associations with quantitative traits at one year.

| SNP         | Nearest gene | Alleles (effect/other) | FG P int | FG P assoc | Fins P int | Fins P assoc | Proins P int | Proins P assoc | Ins Index P int | Ins Index P assoc | ISI P int | ISI P assoc | DIo P int | DIo P assoc |
|-------------|--------------|------------------------|----------|-----------|-----------|-------------|--------------|---------------|----------------|----------------|-----------|------------|----------|------------|
| rs340874    | PROX1        | C/T                    | 0.81     | 0.47      | 0.90      | 0.15        | 0.99         | 0.08          | 0.42           | 0.99          | 0.89      | 0.14       | 0.63     | 0.22       |
| rs573225    | G6PC2        | A/G                    | 0.96     | 0.17      | 0.08      | 0.88        | 0.34         | 0.91          | 0.77           | 0.20          | 0.11      | 0.66       | 0.81     | 0.08       |
| rs11708067  | ADCY3        | A/G                    | 0.80     | 0.27      | 0.22      | 0.98        | 0.31         | 0.85          | 0.46           | 0.32          | 0.20      | 0.80       | 0.86     | 0.52       |
| rs11920090  | SLCA2        | T/A                    | 0.88     | 0.49      | 0.24      | 0.53        | 0.79         | 0.38          | 0.59           | 0.98          | 0.25      | 0.49       | 0.69     | 0.89       |
| rs2191349   | DGKB         | T/G                    | 0.79     | 0.41      | 0.04      | –           | 0.09         | 0.80          | 0.50           | 0.55          | 0.07      | 0.84       | 0.84     | 0.99       |
| rs917793    | GCK          | T/A                    | 0.07     | 0.12      | 0.39      | 0.12        | 0.08         | 0.31          | 0.86           | 0.23          | 0.24      | 0.08       | 0.42     | 0.07       |
| rs7034200   | GLIS3        | A/C                    | 0.72     | 0.98      | 0.02      | –           | 0.25         | 0.56          | 0.94           | 0.03          | –        | 0.13       | 0.64     | –          |
| rs10885122  | ADRA2A       | G/T                    | 0.82     | 0.14      | 0.20      | 0.60        | 0.16         | 0.46          | 0.29           | 0.18          | 0.25      | 0.44       | 0.31     | 0.03       |
| rs11605924  | CRV2         | A/C                    | 0.20     | 0.56      | 0.13      | 0.46        | 0.76         | 0.80          | 0.40           | 0.28          | 0.13      | 0.40       | 0.21     | 0.58       |
| rs7944584   | MADD         | A/T                    | 0.04     | –         | 0.73      | 0.80        | 0.63         | 0.30          | 0.11           | 0.10          | 0.63      | 0.83       | 0.36     | 0.29       |
| rs174550    | FADS1        | G/C                    | 0.58     | 0.20      | 0.87      | 0.09        | 0.73         | 0.23          | 0.76           | 0.97          | 0.86      | 0.07       | 0.64     | 0.65       |
| rs10830963  | MTNR1B       | A/C                    | 0.68     | 0.003     | 0.17      | 0.37        | 0.27         | 0.41          | 0.69           | 0.002         | 0.16      | 0.90       | 0.96     | 0.08       |
| rs11071657  | C2CD4B       | A/G                    | 0.04     | –         | 0.96      | 0.42        | 0.55         | 0.97          | 0.38           | 0.09          | 0.98      | 0.54       | 0.35     | 0.048      |
| rs4675095   | IRS1         | A/T                    | 0.21     | 0.99      | 0.67      | 0.26        | 0.68         | 0.59          | 0.53           | 0.55          | 0.71      | 0.30       | 0.62     | 0.62       |
| rs855228    | IGF1         | T/C                    | 0.39     | 0.60      | 0.15      | 0.27        | 0.52         | 0.87          | 0.36           | 0.72          | 0.15      | 0.24       | 0.63     | 0.75       |
| rs780094    | GCKR         | C/T                    | 0.57     | 0.76      | 0.07      | 0.22        | 0.25         | 0.38          | 0.17           | 0.92          | 0.09      | 0.26       | 0.43     | 0.28       |

FG, fasting glucose; Fins, fasting insulin; Ins Index, insulinogenic index; ISI, insulin sensitivity index; DIo, oral disposition index; Proins, fasting proinsulin adjusted for fasting insulin. P int denotes the P value for the genotype x intervention interaction test; P assoc denotes the P value for the main effect association in the full cohort when P int > 0.05.

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Table 4. Levels of quantitative glycemic traits at one year by genotype and treatment arm at loci with a nominally significant interaction.

| SNP gene | Alleles (effect/other) | Trait | Placebo LS Means (95% CI) | P values | Metformin LS Means (95% CI) | P values | Lifestyle LS Means (95% CI) | P values |
|----------|------------------------|-------|---------------------------|----------|-----------------------------|----------|-----------------------------|---------|
| rs2191349| T/G                    | Fins  | GG 24.71 (22.96–26.59)    | 0.99     | GG 22.62 (21.02–24.34)     | 0.21     | GG 18.43 (17.03–19.94)     | 0.99     |
|          |                        |       | DGKB (µU/mL)              |          | TT 25.71 (24.00–27.54)     | 0.99     | TT 20.41 (19.03–21.88)     | 0.21     |
| rs7034200| A/C                    | Fins  | AA 24.86 (23.27–26.55)    | 0.99     | AA 22.54 (21.06–24.12)     | 0.12     | AA 18.01 (16.76–19.36)     | 0.28     |
|          |                        |       | GLIS3 (µU/mL)             |          | CC 25.07 (23.18–27.12)     | 0.99     | CC 20.45 (19.00–22.01)     | 0.05     |
| rs7944584| A/T                    | FG    | AA 106.8 (105.5–108.1)    | 0.99     | AA 102.4 (101.2–103.5)     | 0.99     | AA 102.1 (101.0–103.2)     | 0.99     |
|          |                        |       | MADD (mg/dL)              |          | TT 104.3 (102.6–106.1)     | 0.99     | TT 102.7 (101.1–104.2)     | 0.99     |
|          |                        |       | C2CD48 (mg/dL)            |          | AG 105.9 (104.5–107.4)     | 0.99     | AG 102.7 (101.4–104.0)     | 0.99     |

P values for pairwise comparisons between genotypic groups are shown, with groups separated by a “/”. Fins, fasting insulin (µU/mL); ISI, insulin sensitivity index; FG, fasting glucose (mg/dL). To convert glucose mg/dL to mmol/L, divide by 18.01. To convert insulin µU/ml to pmol/L, multiply by 6.0.

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We tested associations of these SNPs with baseline fasting glucose, fasting insulin, fasting proinsulin adjusted for fasting insulin, the insulinogenic index, the ISI and the DIo in this multiethnic cohort of individuals with IGT. We replicated associations with fasting glucose at \textit{G6PC2} (\(P = 0.002\)), \textit{MTNR1B} (\(P = 0.001\)) and \textit{GCKR} (\(P = 0.001\)). We also replicated associations of the glucose-raising allele with reduced insulinogenic index at \textit{MTNR1B} and increased insulinogenic and disposition indices at \textit{G6PC2}. We again noted a strong association of \textit{MADD} with fasting proinsulin levels, adjusted for concomitant insulin (\(P = 0.001\)). All nominally significant (\(P < 0.05\)) associations and corresponding trait distributions are shown in Table 2.

**Table 5.** Diabetes incidence by genotype at each locus, in the overall cohort and stratified by treatment arm.

| SNP        | Nearest gene | Alleles | \(\text{SNP}^*\) Tx | Treatment adjusted HR (95% CI) | \(\text{P}-\text{value}\) | PLACEBO HR (95% CI) | \(\text{P}-\text{value}\) | METFORMIN HR (95% CI) | \(\text{P}-\text{value}\) | LIFESTYLE HR (95% CI) | \(\text{P}-\text{value}\) |
|------------|--------------|---------|----------------------|-------------------------------|---------------------------|---------------------|---------------------|----------------------|---------------------|-----------------------|---------------------|
| rs340874   | PROX1*       | C (vs T) | N                    | 0.88 (0.78–0.98)              | 0.02                      | 0.85 (0.71–1.01)    | 0.06                | 0.92 (0.75–1.12)    | 0.39                | 0.86 (0.68–1.08)    | 0.20                |
| rs573225   | G6PC2        | A (vs G) | N                    | 1.11 (0.97–1.27)              | 0.14                      | 0.96 (0.77–1.19)    | 0.70                | 1.18 (0.94–1.47)    | 0.15                | 1.27 (0.98–1.64)    | 0.07                |
| rs11708067 | ADCYS*       | A (vs G) | N                    | 1.06 (0.92–1.23)              | 0.38                      | 1.04 (0.84–1.28)    | 0.73                | 1.08 (0.84–1.35)    | 0.60                | 1.10 (0.82–1.47)    | 0.51                |
| rs11920090 | SLC2A2       | T (vs A) | N                    | 1.02 (0.88–1.19)              | 0.75                      | 1.08 (0.86–1.33)    | 0.56                | 1.00 (0.77–1.30)    | 0.99                | 0.99 (0.71–1.37)    | 0.93                |
| rs2191349  | DGKB*        | T (vs G) | N                    | 1.06 (0.94–1.18)              | 0.34                      | 1.05 (0.88–1.27)    | 0.56                | 1.10 (0.90–1.33)    | 0.34                | 1.01 (0.80–1.27)    | 0.96                |
| rs917793   | GCK*         | T (vs A) | N                    | 0.96 (0.84–1.10)              | 0.59                      | 0.87 (0.70–1.07)    | 0.20                | 1.14 (0.90–1.44)    | 0.29                | 0.92 (0.69–1.22)    | 0.56                |
| rs70342000 | GLU5         | A (vs C) | N                    | 1.00 (0.89–1.12)              | 1.00                      | 0.90 (0.75–1.08)    | 0.22                | 1.04 (0.85–1.27)    | 0.68                | 1.15 (0.91–1.47)    | 0.25                |
| rs10885122 | ADRA2A       | G (vs T) | N                    | 1.03 (0.91–1.16)              | 0.63                      | 1.01 (0.84–1.22)    | 0.89                | 1.03 (0.84–1.28)    | 0.76                | 1.08 (0.84–1.39)    | 0.56                |
| rs11605924 | CRV2         | A (vs C) | N                    | 1.01 (0.90–1.12)              | 0.91                      | 0.93 (0.78–1.10)    | 0.40                | 1.06 (0.88–1.28)    | 0.56                | 1.09 (0.87–1.37)    | 0.47                |
| rs7944584  | MADD         | A (vs T) | N                    | 0.93 (0.80–1.08)              | 0.29                      | 0.86 (0.69–1.08)    | 0.20                | 0.89 (0.69–1.14)    | 0.35                | 1.11 (0.84–1.47)    | 0.47                |
| rs174550   | FADS1        | T (vs C) | N                    | 0.94 (0.83–1.05)              | 0.26                      | 0.95 (0.80–1.14)    | 0.57                | 0.98 (0.80–1.19)    | 0.81                | 0.86 (0.67–1.09)    | 0.21                |
| rs10830963 | MTNR1B*      | G (vs C) | N                    | 1.07 (0.94–1.22)              | 0.29                      | 1.20 (0.98–1.47)    | 0.07                | 1.01 (0.80–1.26)    | 0.95                | 0.95 (0.73–1.24)    | 0.69                |
| rs11071657 | C2CD4B       | A (vs G) | N                    | 0.93 (0.83–1.05)              | 0.26                      | 0.93 (0.77–1.12)    | 0.43                | 0.92 (0.75–1.14)    | 0.42                | 0.96 (0.75–1.22)    | 0.72                |
| rs4675095  | IRS1         | A (vs T) | N                    | 0.96 (0.78–1.18)              | 0.68                      | 0.93 (0.67–1.30)    | 0.69                | 1.16 (0.84–1.59)    | 0.37                | 0.71 (0.44–1.15)    | 0.17                |
| rs855228   | IGF1         | T (vs C) | N                    | 1.09 (0.97–1.23)              | 0.14                      | 1.04 (0.87–1.25)    | 0.66                | 1.16 (0.95–1.43)    | 0.15                | 1.12 (0.88–1.43)    | 0.38                |
| rs780094   | GCKR*        | C (vs T) | N                    | 0.96 (0.85–1.08)              | 0.48                      | 0.91 (0.75–1.10)    | 0.33                | 1.01 (0.82–1.23)    | 0.93                | 0.96 (0.75–1.22)    | 0.72                |

*Loci previously associated with type 2 diabetes. Effect allele denotes the allele associated with higher glucose or insulin levels in MAGIC. There are no significant SNP \times treatment interactions. One nominally significant \(P\) value for association with diabetes incidence is not consistent with the expected direction of effect.

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**Figure 1.** Effect of genotype at \textit{MTNR1B} rs10830963 on glycemic traits at baseline and one year. Fasting glucose is shown in panel (a) and the insulinogenic index is shown in panel (b). Because no significant SNP \times intervention interaction was found, the full cohort was analyzed in aggregate. Fasting glucose is higher (\(P = 0.003\)) and the insulinogenic index is lower (\(P = 0.002\)) in carriers of the G risk allele after one year, even after adjustment for the corresponding baseline levels. Least-square means (± 95% CI) are shown. To convert glucose mg/dL to mmol/L, divide by 18.01. doi:10.1371/journal.pone.0044424.g001
Associations at One Year

We tested whether the metformin or lifestyle preventive interventions interacted with each SNP to modulate quantitative glycemic traits at one year. We adjusted one-year traits for the corresponding baseline trait, to indicate change in each variable during active treatment. Where no nominally significant interaction with treatment was found, SNP main effects on the one-year trait were tested in the whole cohort with an adjustment for treatment arm; if an interaction was detected at $P<0.05$, analyses were stratified by treatment arm (Table 3). Nominally significant interactions were found for DGKB and fasting insulin, GLIS3 and both fasting insulin and ISI, and both MADD and C2CD4B and fasting glucose. Least-square means for each genotype group and the corresponding pairwise comparisons are shown in Table 4. At MTNR1B, the glucose-raising allele continued to have a significant main effect on raising fasting glucose and lowering the insulinogenic index at one year (Figure 1). Because one-year traits are adjusted for the baseline level, this effect is indicative of a worsening deleterious effect of this locus on β-cell function. We further explored the concordant effects of SNPs at MTNR1B and G6PC2 on fasting glucose but discordant effects for insulinogenic index by testing for epistatic interactions between the two on fasting glucose at baseline and one year: the interaction terms were not statistically significant.

Diabetes Incidence

We tested whether the metformin or lifestyle preventive interventions interact with each SNP on the risk of developing diabetes during 3.2 years of mean follow-up. As no nominal interactions were found, the effects of each SNP on diabetes incidence were evaluated in the full cohort while adjusting for treatment arm; stratified analyses are also shown (Table 5). The only nominal association with diabetes incidence was found for the glucose-lowering allele at PROX1 ($P=0.02$), in a direction opposite to that reported in case-control analyses in MAGIC, where the C allele increased type 2 diabetes risk (odds ratio 1.07 [95% CI 1.05–1.09], $P=7.2 \times 10^{-10}$) [8].

Discussion

The MAGIC investigators reported a number of loci that influence fasting glucose and fasting insulin levels in non-diabetic populations of European descent; only a few of the loci were also associated with type 2 diabetes at genome-wide levels of significance [8]. The authors speculated that it is not the mere elevation in fasting glucose, but how fasting glucose is raised, that determines overall β-cell dysfunction and future type 2 diabetes risk. However, whether these loci exert their action on fasting glucose in the initial stages of diabetes progression (e.g. from normoglycemia to impaired glucose regulation) or later (e.g. from IGT to type 2 diabetes) is not known. In the GLACIER cohort, eleven loci (including the known type 2 diabetes genes TCP7L2 and SLC30A8) were nominally associated with IFG cross-sectionally, and MTNR1B and G6PC2 were also associated with development of IFG in longitudinal analyses [11]. We have recently shown that among type 2 diabetes-associated loci, risk alleles at MTNR1B, GCKR and SLC30A8 confer a stronger rate of progression from normoglycemia to IFG than from IFG to type 2 diabetes [13]. Here we extend these findings by testing these SNPs from the IGT to type 2 diabetes transition, and by assessing their effects on quantitative glycemic traits at baseline and one year in a multiethnic cohort of persons with IGT.

We have demonstrated that the three loci with the strongest reported effect on fasting glucose (MTNR1B, GCKR and G6PC2) have consistent effects in the DPP. All three were known to be associated with fasting glucose prior to the MAGIC GWAS meta-analysis [4,5,6,7,26,27,28]. Power may have been limiting to detect the other reported associations [24].

We have also confirmed that the glucose-raising allele at MTNR1B is associated with a reduced insulinogenic index, as measured during the initial phase of insulin secretion during an OGTT [9,29]. As shown by Lysсенко and coworkers, the deleterious effects of this allele on β-cell function persist over time; while they noted such worsening over 24 years of follow-up [29], here we see such effects over a much shorter time span (one year). In GLACIER a similar non-significant trend was noted over 10 years of follow-up [11], although a consistent effect was not detected in the Whitehall II study [12]. Because MTNR1B does increase risk of type 2 diabetes [8], this pattern of sustained deterioration suggests that identifying these individuals early in their glycemic progression may be beneficial in prevention efforts.

In contrast, the glucose-raising allele at G6PC2 is associated with superior β-cell function on dynamic testing; this has been shown previously [9,30], and is consistent with the role of this gene product in regulating hepatic glucokinase and its null effect on type 2 diabetes risk [8]. We found no evidence in support of a non-additive interaction between MTNR1B and G6PC2 on fasting glucose at baseline or one year. The strong effect of the MADD locus on fasting proinsulin levels is also confirmed [9,31]; because this association is adjusted for concomitant insulin levels, it reflects an increased secretion of insulin precursors out of proportion to the degree of basal insulin resistance. The other nominal associations newly reported here do not withstand correction for the multiple statistical tests performed, and should be considered hypothesis-generating requiring confirmation in independent studies.

In summary, the strongest effects of genetic loci on fasting glucose in non-diabetic individuals of European descent are also evident in a multiethnic cohort with IGT. The deleterious influence of the glucose-raising allele at MTNR1B on β-cell function appears to worsen with time, and this effect is evident in as short a time as one year. Genetic testing may identify a subset of patients with IGT more likely to respond to preventive interventions [32].

Supporting Information

Appendix S1  DPP Research Group. (DOC)

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Author Contributions

Conceived and designed the experiments: JCF KAJ PWF ARS WCK. Performed the experiments: JCF JBM. Analyzed the data: KAJ JCF CCM WCK. Contributed reagents/materials/analysis tools: KM EH RG SEK RFA WCK. Wrote the paper: JCF. Reviewed and edited the manuscript: KM EH RG SEK RFA WCK JCF KAJ PWF ARS JBM CCM DD.
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