Detailed Physiologic Characterization Reveals Diverse Mechanisms for Novel Genetic Loci Regulating Glucose and Insulin Metabolism in Humans

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OBJECTIVE—Recent genome-wide association studies have revealed loci associated with glucose and insulin-related traits. We aimed to characterize 19 such loci using detailed measures of insulin processing, secretion, and sensitivity to help elucidate their role in regulation of glucose control, insulin secretion and/or action.

RESEARCH DESIGN AND METHODS—We investigated associations of loci identified by the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) with circulating proinsulin, measures of insulin secretion and sensitivity from oral glucose tolerance tests (OGTTs), euglycemic clamps, insulin suppression tests, or frequently sampled intravenous glucose tolerance tests in nondiabetic humans (n = 29,084).

RESULTS—The glucose-raising allele in MADD was associated with abnormal insulin processing (a dramatic effect on higher proinsulin levels, but no association with insulinoergic index) at extremely persuasive levels of statistical significance (P = 2.1 × 10⁻⁷). Defects in insulin processing and insulin secretion were seen in glucose-raising allele carriers at TCF7L2, SLC30A8, GIPR, and C2CD4B. Abnormalities in early insulin secretion were suggested in glucose-raising allele carriers at MTNR1B, GCK, FADS1, DGKB, and PROX1 (lower insulinoergic index; no association with proinsulin or insulin sensitivity). Two loci previously associated with fasting insulin (GCKR and IGF1) were associated with OGTT-derived insulin sensitivity indices in a consistent direction.

CONCLUSIONS—Genetic loci identified through their effect on hyperglycemia and/or hyperinsulinemia demonstrate considerable heterogeneity in associations with measures of insulin processing, secretion, and sensitivity. Our findings emphasize the importance of detailed physiologic characterization of such loci for improved understanding of pathways associated with alterations in glucose homeostasis and eventually type 2 diabetes. Diabetes 59:1266–1275, 2010

A recent meta-analysis of genome-wide association studies of fasting glycemic traits in nondiabetic individuals conducted by the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) has reported the discovery of nine new loci associated with fasting glucose (FG) (in or near ADCY5, MADD, ADRA2A, CR2Y, FADS1, PROX1, SLC2A2, GLIS3, and C2CD4B) and one locus associated with fasting insulin levels (IGF1) (1). The same study showed effects on FG for seven previously published glucose and/or type 2 diabetes loci G6PC2, MTNR1B, GCK, DGKB, GCKR, SLC30A8, and TCF7L2. Another recent MAGIC meta-analysis, published back-to-back with the aforementioned study, identified two additional novel loci (GIPR and VPS13C) associated with 2-h glucose after an oral glucose tolerance test (OGTT) (2). In complementary case-control analyses, an increased risk of type 2 diabetes was demonstrated at genome-wide significance for carriers of the glucose-raising risk alleles in or near the new glycomic loci ADCY5, PROX1, GCK, DGKB, GCKR, as well as the known type 2 diabetes loci MTNR1B, SLC30A8, and TCF7L2 (1). This is a powerful demonstration of how analyses of continuous metabolic traits in healthy individuals can lead to the discovery of previously unsuspected type 2 diabetes susceptibility genes. Detailed
physiological characterization of each locus may help elucidate their role in regulation of glucose levels, insulin secretion and/or action, and identify potential pathways involved in type 2 diabetes pathogenesis.

The insulin-processing pathway follows several canonical steps in the synthesis and secretion of peptide hormones. Proinsulin is produced in the endoplasmic reticulum and packaged into secretory vesicles in the Golgi apparatus. Several proteases cleave proinsulin into mature insulin and C-peptide. In normoglycemic individuals, higher intact proinsulin levels are associated with elevated glucose levels (3,4), increased insulin secretion, and insulin resistance. In prospective studies, higher intact proinsulin has been positively associated with an increased risk of type 2 diabetes (5). Circulating proinsulin can thus be considered as a measure of β-cell mass or function, insulin processing, insulin secretion, or a combination of these.

Impaired insulin secretion and hepatic and peripheral insulin resistance contribute to the pathogenesis of type 2 diabetes (6). Glucose-stimulated insulin secretion can be assessed using the insulinogenic index, which is derived from an OGTT and is strongly correlated with more sophisticated measures of insulin secretion (7). The euglycemic-hyperinsulinemic clamp technique, the insulin suppression test, and the frequently sampled intravenous glucose tolerance test (FSIGT) provide accurate measures of insulin sensitivity but are difficult to implement in the context of large-scale epidemiological studies. Several indices derived from multiple-point OGTT data correlate well with clamp-assessed sensitivity and have been suggested as more practical surrogate measures (8–11).

Genetic loci associated with glycemic traits have modest effect sizes (1,2), suggesting that individual studies are likely to be underpowered to detect associations with detailed physiologic characteristics. We therefore established a consortium of 14 studies with detailed measures of circulating proinsulin (9 studies), glucose and insulin at a minimum of three time points during a standard 75-g OGTT (9 studies), FSIGT (1 study), insulin suppression test (1 study), and/or euglycemic-hyperinsulinemic clamps (2 studies). We sought to investigate systematically the effects of single nucleotide polymorphisms (SNPs) previously associated with FG, fasting insulin, and/or 2-h glucose meta-analyses (1,2). Alternative proxy SNPs (showing maximal linkage disequilibrium [LD] with the index SNP in the European CEU HapMap sample) were selected for each locus to allow for differences in genotyping capacities of various platforms (supplementary Table 2). In samples where initial genotyping of an index SNP failed, a proxy SNP in strong LD with the original SNP was genotyped whenever possible. Markers that failed Hardy-Weinberg equilibrium (exact P value <1×10^-6 or 1×10^-8 in studies with GWAMA and zinc-thione meta-analyses, <0.01 in direct genotyping studies) were excluded from analyses (supplementary Table 1). Call rates for directly genotyped SNPs exceeded 90%; information content r² >0.3 for MACH-imputed (16) or proper-info >0.4 for IMPUTE-inferred (17) SNPs were required for SNP inclusion in analysis. In samples where more than one SNP was genotyped within the same region and the index SNP was not available, the proxy SNP with the higher call rate and stronger LD was selected.

In addition to diabetes or nonwhite ethnicity, some studies applied additional exclusion criteria as detailed in supplementary Table 1. In each cohort, we used natural log-transformed trait values for fasting proinsulin, insulinogenic index, Stumvoll, Matsuda, Belfiore, and Gutt insulin sensitivity indices, fasting split proinsulin and C-peptide, and Z score transformed values for M1, S1, and SSPG as the dependent variables in linear regression models that included terms for age, sex, study site (if applicable), geographical covariates (if applicable), and age squared (Framingham only) to assess the association of additively coded genotypes with trait values. Analyses were performed with and without adjustment for BMI. Analyses of proinsulin and split proinsulin were additionally adjusted for natural log-transformed fasting insulin (nmol/L).

Data were available from 14 independent studies, including 3 with directly genotyped and imputed genome-wide data and 11 with de novo genotyping data. Association testing was performed using STATA 10.1 (Stata, College Station, TX) or SAS 9 (SAS Institute, Cary, NC) software for directly genotyped SNPs and using SNPTEST (17) or MERLIN (18) software that takes linkage disequilibrium functions from the R kinship package (R Foundation for Statistical Computing, Vienna, Austria, 2007) to account for familial correlation. We performed inverse variance fixed-effects meta-analyses using METAL (http://www.sph.umich.edu/csg/abecasis/Meta/index.html) and GWAMA (http://www.well.ox.ac.uk/gwama/index.shtml) software. Heterogeneity was assessed using the Q statistic.

We report nominal P values without adjustment for multiple testing given the high prior probabilities for associations with the examined phenotypes (all loci have already been associated with at least one glycemic phenotype at genome-wide levels of statistical significance [P < 5×10^-8]). However, we have focused specifically on the results with P values <1×10^-4.

RESULTS

Based on the results observed for the different traits, we organized loci displaying similar patterns into groups based on the presumed mechanism of action in Table 1 (age- and sex-adjusted) and supplementary Table 5 (addi-
TABLE 1
Associations of 19 SNPs previously associated with fasting glucose, fasting insulin, and/or 2-h glucose on detailed physiologic measures of insulin processing, secretion, and sensitivity

| SNP       | Nearest gene | Loci implicated in abnormal insulin processing | Loci associated with higher proinsulin and lower insulin secretion | Loci associated with abnormalities in early insulin secretion | Loci associated with reduced insulin sensitivity |
|-----------|--------------|-----------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|------------------------------------------------|
| rs7944584 | MADD         | rs7944584 A/T                                 | rs17271305 G/A                                               | rs903146 TCF7L2 T/C                                           | rs8709063 MTN1B G/C                                |
| rs17271305| VPS13C       | rs17271305 G/A                               | rs1326634 SLC30A8 C/T                                        | rs10423928 A/T                                               | rs4607517 GCK A/G                                  |
| rs1083063 | CD24b        | rs1083063 C/T                                 | rs1083063 C/T                                                | rs1083063 C/T                                                | rs1083063 C/T                                    |
| rs10423928| GIPR         | rs10423928 A/T                                | rs10423928 A/T                                               | rs10423928 A/T                                               | rs10423928 A/T                                    |
| rs11071657| C2CD4B       | rs11071657 A/G                                | rs11071657 A/G                                               | rs11071657 A/G                                               | rs11071657 A/G                                    |
| rs4607517 | GCK          | rs4607517 G/A                                 | rs4607517 G/A                                                | rs4607517 G/A                                                | rs4607517 G/A                                     |
| rs174550  | FADS1        | rs174550 C/T                                  | rs174550 C/T                                                 | rs174550 C/T                                                 | rs174550 C/T                                     |
| rs560887  | G6PC2        | rs560887 C/T                                  | rs560887 C/T                                                 | rs560887 C/T                                                 | rs560887 C/T                                     |

**Alleles**
- **A**: allele associated with higher proinsulin and lower insulin secretion
- **T**: allele associated with abnormalities in early insulin secretion
- **G**: allele associated with reduced insulin sensitivity

**Proinsulin**
- Proinsulin is a measure of proinsulin levels in the blood.

**Insulinogenic index**
- The insulinogenic index is a measure of insulin response to oral glucose tolerance test (OGTT).

**Insulin sensitivity**
- Insulin sensitivity measures the ability of the body to utilize insulin.

**Insulin sensitivity measures‡**
- Stumvoll§
- Matsuda§
- Belfiore§
- Gutt§

**Allele Frequencies**
- **n**: number of individuals
- **P**: p-value

**Association of SNPs withphysiologic measures**
- **Beta (SE)**: coefficient and standard error of the association between SNP and physiologic measure
- **P_abg**: p-value for the association between SNP and physiologic measure

**Note:**
- Intravenous insulin measures are not included in the table due to space limitations.
Continued

No obvious effects on insulin processing, secretion, or sensitivity

The influence of BMI adjustment on genetic associations was generally minor and specifically noted when relevant.

**Loci implicated in abnormal insulin processing.** Failing β-cells are expected to show diminished insulin secretion, while compensatory increases in circulating proinsulin denote the β-cell’s attempt to maintain euglycemia (19). Therefore, genetic differences in fasting proinsulin levels (adjusted for fasting insulin) without a concomitant effect on insulinogenic index suggest abnormal insulin processing. The most striking association occurred between the FG-raising allele at MADD rs7944584 and higher fasting proinsulin levels ($P = 2.1 \times 10^{-7}$); its lack of association with the insulinogenic index suggests an effect of this locus on insulin processing (supplementary Figs. 1 and 2). Less significant effects of this allele on lower OGTT-derived insulin sensitivity measures ($P = 0.01 - 0.03$) were also observed. Consistent with the above, MADD rs7944584 was strongly associated with higher fasting split proinsulin (supplementary Table 6), but not with fasting C-peptide (supplementary Table 7). The 2-h glucose-raising allele at VPS13C rs17271305 was modestly associated with lower fasting proinsulin levels ($P = 0.02$), but not associated with measures of insulin secretion or action.

**Loci associated with higher proinsulin and lower insulin secretion.** Several genetic variants were associated with indices of β-cell dysfunction, i.e., higher fasting proinsulin levels and a lower insulinogenic index, including the glucose-raising alleles at TCF7L2 rs7903146 ($P = 4.1 \times 10^{-12}$ and $2.0 \times 10^{-7}$ respectively), SLC30A8 rs13266634 ($P = 2.7 \times 10^{-6}$ and 0.0012) and GIPR rs10423928 ($P = 6.2 \times 10^{-7}$ and $2.1 \times 10^{-13}$). A trend was also seen for the FG-raising allele at C2CD4B rs11071657 associating with higher fasting proinsulin levels ($P = 0.004$) and lower insulinogenic index ($P = 0.06$). At these loci the relationship between the insulinogenic index and fasting proinsulin levels was linear for carriers of the protective allele, whereas carriers of the risk alleles failed to demonstrate an increase in insulinogenic index in proportion to rising proinsulin levels (Fig. 1A-D). Except for an association between the GIPR rs10423928 and higher insulin sensitivity as assessed by the Belfiore ($P = 1.0 \times 10^{-3}$), Matsuda ($P = 0.0008$), and Stumvoll ($P = 0.003$) indices, the other associations of these SNPs with measures of insulin sensitivity were very modest ($P < 10^{-3}$) and/or inconsistent. TCF7L2 rs7903146 was the only locus in this group associated with lower C-peptide levels (supplementary Table 7). We note that although the VPS13C and C2CD4B loci are physically close to each other (101 kb apart), LD between the two index SNPs is relatively weak ($r^2 = 0.28$ based on CEU HapMap).

**Loci associated with abnormalities in early insulin secretion.** A subset of other variants showed association between FG-raising alleles and lower insulinogenic index without an association with fasting proinsulin levels: MTNR1B rs10830963 ($P = 2.3 \times 10^{-16}$), GCK rs4607517 ($P = 2.2 \times 10^{-4}$), FADS1 rs174550 ($P = 0.001$), DGKB rs2191349 ($P = 0.006$), and PROX1 rs340874 ($P = 0.02$). The FG-raising alleles at GCK ($P = 8.1 \times 10^{-3}$) and MTNR1B ($P = 0.006$) were also associated with a lower Gutt index, but not with any of the other insulin sensitivity measures.

The FG-raising allele at G6PC2 rs560887 was associated with a higher insulinogenic index ($P = 5.0 \times 10^{-5}$), a
finding previously reported by others (20). It was also
weakly associated with lower insulin sensitivity measured
by intravenous techniques in BMI-adjusted analyses (P =
0.02) (supplementary Table 5).

**Loci associated with reduced insulin sensitivity.** The
glucose-raising allele at GCKR rs780094 was associated with
lower insulin sensitivity by the Stumvoll (P =
0.001), Matsuda (P = 2.9 × 10−3), and Belfiore (P = 0.003)
indices, whereas the fasting insulin–raising allele at IGF1
rs55767 was associated with lower insulin sensitivity by the
Matsuda (P = 0.01), Belfiore (P = 0.02), and Gutt (P =
0.002) indices. GCKR rs780094 was also associated with
increased C-peptide levels (supplementary Table 7).

**Loci without obvious effects on insulin processing,
secretion, or sensitivity.** Five of the examined loci—
ADCY5 rs11708067, ADRA2A rs10885122, CRY2 rs11605924,
SLC2A2 rs11920090, and GLIS3 rs7034200—did not show
any apparent associations with any of the examined
phenotypes (Table 1). We note that the ADRA2A SNP
rs10885122, previously associated with fasting glucose (1)
and assayed here, is 202 kb away from and uncorrelated
with rs553668 (r² = 0.003 in CEU HapMap). The A allele at
rs553668 has been recently associated with type 2 diabetes
and reduced insulin secretion in a Scandinavian popula-
tion (21). In our MAGIC meta-analysis of ~14,000 individ-
uals, the A allele at rs553668 is nominally associated with
higher β-cell function by homeostasis model assessment
(P = 0.003) and higher fasting insulin (P = 0.02), but
shows no association with fasting glucose (P = 0.21).

**DISCUSSION**
In this report we investigated the effects of 19 SNPs
previously associated with FG, fasting insulin, and/or 2-h
glucose on multiple physiologic measures of insulin pro-
cessing, secretion, and sensitivity in 14 cohorts with over
29,000 unique participants. For at least 12 of these SNPs,
this is the first report to study their associations with such
comprehensive physiologic measures of insulin and glu-
cose metabolism. Our results demonstrate that these ge-
netic loci influence glycemic regulation by diverse
pathways (supplementary Fig. 3).

**Loci implicated in abnormal insulin processing.** The
glucose-raising allele at MADD rs7903146 was associated with
increased fasting proinsulin (adjusted for fasting insulin), but
not with insulin secretion. The dramatic effect size on
fasting proinsulin levels (two- to 10-fold that of other loci) seems out of proportion with its modest elevation of FG and an otherwise unremarkable impact on other glycemic measures, suggesting that this locus is associated with an isolated insulin processing defect without a major impairment of insulin secretory capacity. It is therefore not surprising that despite the effects of this locus on FG and fasting proinsulin levels, it has a negligible influence on type 2 diabetes risk (1). MADD encodes a death domain–containing adaptor protein, which interacts with the death domain of tumor necrosis factor-α receptor 1 and propagates apoptotic signals (22); however, if functional variants in MADD were involved in mechanisms leading to β-cell damage, one would expect to have seen a concomitant deterioration of β-cell function. The isolated proinsulin association raises the possibility that other genes in the region may contain a causal variant (in LD with rs7944584), which is functionally responsible for the observed insulin processing defect. Nearby genes include PACSIN3, which encodes a protein involved in vesicle formation, transport, and endocytosis whose transcript is relatively abundant in the human pancreas (23); ARFGAP2, which has been implicated in vesicular trafficking between the Golgi and the endoplasmic reticulum (24); and SLC39A13, which encodes a zinc transporter (25).

**Loci associated with higher proinsulin and lower insulin secretion.** The glucose-raising variants at TCF7L2, SLC30A8, GIPR, and C2CD4B were all associated with increased fasting proinsulin levels and decreased insulinogenic index. The relationship between the insulinogenic index and fasting proinsulin was linear for carriers of the protective allele at TCF7L2 and SLC30A8, whereas carriers of the risk alleles failed to demonstrate an increase in insulinogenic index in proportion to rising proinsulin levels, indicating an active secretion of insulin precursors in lieu of mature insulin. This has several potential explanations: 1) reduced β-cell mass through either diminished proliferation or enhanced apoptosis resulting in increased β-cell stress in the face of increased insulin demand; 2) an impairment in the molecular processing from proinsulin to insulin; or 3) defective vesicle trafficking. In sum, all these possibilities could manifest themselves by the exocytosis of more preprotein products and lower secretion of insulin in response to glucose. TCF7L2 encodes a nuclear receptor for β-catenin involved in the Wnt signaling pathway; the association of SNP rs7903146 in this gene with type 2 diabetes is now well established as the strongest common genetic determinant of type 2 diabetes yet described. Here we confirm the previously reported associations of this variant with measures of impaired insulin secretion and with fasting proinsulin levels (rev. in 26). Current evidence suggests that TCF7L2 causes an impairment in insulin secretion by affecting insulin granule exocytosis and β-cell responsiveness to incretins (perhaps by downregulation of glucagon-like peptide 1 receptors); incretin resistance may in turn diminish β-cell mass. Our data support any of the above mechanisms.

**GIPR** encodes the receptor for glucose-dependent insulinotropic polypeptide (GIP, also known as gastric inhibitory polypeptide), another incretin hormone. Interaction of GIP with its receptor on the β-cells increases cAMP levels and intracellular calcium, which enhances exocytosis of insulin-containing granules, mostly during the later response to oral glucose (20–120 min) (27). Individuals with type 2 diabetes and their relatives have an impaired insulinotropic effect of GIP (28), perhaps due to defective or reduced number of GIP receptors in β-cells (29). A common variant in GIPR was associated with 2-h glucose in a prior MAGIC meta-analysis (2), as well as a lower insulinogenic index and a lower ratio of insulin to glucose area under the curve during an OGTT; in this study we have replicated the insulinogenic index result and shown an association of the same allele with higher fasting proinsulin levels. The effect of this variant on reducing both early and late insulin secretion may explain the perceived improvement in insulin sensitivity by OGTT-derived measures, which is driven by lower insulin levels throughout the OGTT. These observations are fully consistent with the known mechanisms described above.

**SLC30A8** encodes the zinc transporter, ZnT8, which co-localizes with insulin in the β-cell and is important in the storage and maturation of insulin within cytoplasmic granules (30). ZnT8-null mice have impaired glucose tolerance and decreased insulin secretion in vivo (31). Furthermore, mice carrying a SLC30A8 exon three deletion had lower plasma insulin levels, and islets from these mice showed decreased zinc content and lower glucose–stimulated insulin secretion (32). Here we confirm previous reports that carriers of the risk genotype at SLC30A8 exhibit abnormalities in insulin secretion (33) and increased circulating proinsulin (34). Thus, variants in both TCF7L2 and SLC30A8 affect FG, proinsulin levels, and insulin secretion and, in doing so, increase type 2 diabetes risk.

We provided biologic mechanisms to explain the associations we observed between variation in these loci and abnormal insulin processing or elevated proinsulin levels. However, many different biologic conditions can result in abnormal insulin processing and regulation of proinsulin levels. Therefore, in the absence of experiments to directly test these mechanisms, we view these associations as hypothesis-generating for future studies to formally test these mechanisms.

**Loci associated with abnormalities in early insulin secretion.** Genetic defects in pathways primarily involved in insulin secretion are expected to cause higher glucose levels. Of all examined loci, the glucose-raising alleles of SNPs at MTN1R1B, FADS1 and DGKB, and GCK showed an association with lower insulinogenic index, but no significant association with fasting proinsulin or insulin sensitivity. Thus, these loci seem to influence insulin secretory capacity without affecting insulin processing or inducing significant β-cell stress, which would result in higher circulating proinsulin.

Our results confirm that the glucose-raising allele in MTN1R1B (encoding the melatonin receptor 1B) is associated with lower insulin secretion after oral or intravenous glucose challenge (35–37). We did not see a significant association of MTN1R1B with fasting proinsulin levels, which is in line with the observation in the Tübingen Family Study (37) but in contrast with the Helsinki Birth Cohort results (36). MTN1R1B is expressed in human islets and co-localizes with insulin; melatonin inhibits insulin secretion by rat insulinoma cells (36,37). It is therefore possible that genetic variation in MTN1R1B enhances β-cell responsiveness to melatonin. Fatty acid metabolism may also play a role in early insulin secretion. FADS1 encodes fatty acid desaturase 1, a key enzyme in the metabolism of unsaturated (ω-3 and ω-6) fatty acids. These lipid moieties play a major role in the stability of cellular membranes, but fatty acid desatu-
rases can also convert polyunsaturated fatty acids into cell signaling metabolites. Polymorphisms in FADS1 that are strongly correlated with the FG-associated SNP have been associated with FADS1 mRNA expression levels in the liver (1) and differences in cell membrane or circulating fatty acid profiles (38,39). The type of fatty acids influences glucose-stimulated insulin secretion in incubated pancreatic islet (40) and in perfused pancreas (41). Insulin secretion differs in response to oral challenges varying in their fatty acid composition (42,43). Thus, a plausible mechanism by which insulin secretion function is reduced without the need to postulate reduced β-cell mass or survival can also be envisioned for this locus.

DGKB encodes for diacylglycerol kinase β, which is a member of a family of intracellular lipid kinases that phosphorylate diacylglycerols. Within the β-cell, diacylglycerols are implicated in the intracellular pathways of parasympathetic stimulation of insulin secretion, which is activated by meal intake through the vagus nerve (44). If a DGKB variant influences the β-cell response to neural stimulation via a second messenger pathway, it can also do so without affecting β-cell integrity and thus show no association with fasting proinsulin levels.

GCK encodes glucokinase, which phosphorylates glucose to glucose-6-phosphate and is thus the rate-limiting enzyme for glucose sensing in β-cells. Loss-of-function mutations in GCK are responsible for maturity-onset diabetes of the young (MODY) 2, a syndrome characterized by mild fasting hyperglycemia and glucose intolerance due to reduced sensitivity of insulin secretion to changes in glycemia, resulting in an impaired secretory response (45). Non-MODY GCK variants have been associated with FG levels in multiple cohorts (46), an association that reached genome-wide significance in MAGIC (35).

The G6PC2 FG-raising allele was associated with a higher insulinogenic index. This is consistent with observations in obese children, where another SNP in the same locus was associated with both increased FG and higher insulinogenic index (47), and in Mexican Americans, where the FG-raising allele was also associated with increased FG and OGTT 30-min insulin change (48). G6PC2 encodes glucose-6-phosphatase, catalytic 2, which catalyzes glucose-6-phosphate dephosphorylation, thereby opposing the action of GCK in the β-cell. The observation that risk allele carriers have a higher FG and yet a higher insulinogenic index is in contrast with the results obtained for GCK and may explain why this variant shows a flat-to-slightly protective effect on type 2 diabetes (1).

Thus, a simple elevation of the glucostatic set point does not provide a fully satisfactory explanation. An alternative is that balance between GCK and G6PC2 activities may be affected by genetic variation resulting in changes in pulsatile insulin secretion, which could interfere with normal insulin signaling between the pancreas and insulin-sensitive tissues. This hypothesis is supported by two lines of evidence. First, GCK and G6PC2 regulate the rate-limiting step of glycolysis, and oscillations in glycolysis have been shown to be correlated with oscillations in insulin secretion in vitro (49,50). Second, recent animal studies showing that disruption of pulsatile insulin secretion results in a loss of efficiency in insulin action at the liver, leading to modest hepatic insulin resistance and increased hepatic glucose output (51). These changes would then cause the observed compensatory rise in insulin secretion.

Loci associated with insulin resistance. FG-raising alleles at GCKR and IGF1 have previously been shown to be associated with insulin resistance by homeostasis model assessment (1). In the present study, we confirm this observation using dynamic indices not restricted to glucose and insulin measured in the fasting state. Both GCKR and IGF1 are strongly expressed in the liver, and could thus contribute to development of hepatic insulin resistance. GCKR encodes glucokinase regulatory protein, which inhibits glucokinase in the liver; the index SNP in strong LD with the missense variant P446L, whose FG-raising allele inhibits glucokinase activity in the presence of physiological concentrations of fructose-6 phosphate (52), thus leading to increased hepatic glucose production. IGF1 encodes the insulin-like growth factor I (IGF-I), which has significant structural homology with insulin. Circulating IGF-I can bind to insulin receptors and stimulate glucose transport in fat and muscle while decreasing hepatic glucose output, thus lowering blood glucose while suppressing insulin secretion (53). However the role of IGF-I, and especially polymorphisms in or near IGF1, in glucose homeostasis and insulin sensitivity is not well understood.

Despite state-of-the-art methods and the large sample size to date, we found little evidence of the examined SNPs being convincingly associated with insulin sensitivity. This could reflect a smaller sample size for the intravenous insulin sensitivity analyses (n = 3,195) than for the analyses of insulin secretion, and hence lower statistical power. It is well established that measures of β-cell function show stronger heritability than measures of insulin action, the latter being subject to large day-to-day variation. And while insulin sensitivity measures are correlated, differences among them do exist that increase heterogeneity and reduce power (54). Although the correlation between intravenous and OGTT-derived measures of insulin resistance is high (supplementary Table 4), the discrepancy in results among these measures may reflect differences in the genetic contribution to the correlation (55). In addition, biological reasons may explain the lack of associations with insulin sensitivity, including trait heterogeneity (i.e., constructed by multiple components with presumably different genetic determinants, such as hepatic glucose output and peripheral glucose uptake) or the SNP selection since these SNPs were chosen from analyses of FG, fasting insulin, and 2-h glucose, traits that might be more strongly associated with insulin processing and secretion than with peripheral insulin sensitivity. Regardless, these results suggest that care must be exerted when comparing association results that use differing measures of insulin sensitivity and highlight that their underlying genetic physiology requires further study.

Limitations. Because our studies are conducted in free-living humans, our mechanistic inferences are limited by the measures derived from human subjects in vivo and the assumptions contained therein. In the absence of appropriate cellular or animal models, we cannot offer conclusive proof of mechanism at the molecular level. Furthermore, a strong association with one specific measure does not preclude a weaker association with a different measure, and therefore a complex interplay between various processes involved in insulin secretion and action may be operational. Glucose itself (even in the nondiabetic range studied here) may affect the variables under consideration; however, because these variants were discovered by their association with glucose levels, it did not seem advisable to remove the contribution of glucose to the traits under study by statistical adjustment.
Finally, we emphasize that the SNPs genotyped here are simply associated with the traits under consideration and thus may be correlated with but not represent the causal variants, nor lie in the biologically relevant genes.

**Conclusion.** We have undertaken a detailed physiologic characterization of 19 genetic loci recently identified through associations with FG or insulin and/or 2-h glucose and demonstrate considerable heterogeneity in the associations of these loci with measures of insulin processing, secretion, and sensitivity. Our findings emphasize the importance of detailed physiological characterization of such loci for improved understanding of mechanisms by which newly discovered loci might influence glucose physiology and type 2 diabetes risk.

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**DISCLOSURES**

J.C.F. has received consulting honoraria from Publicis Healthcare, Merck, bioStrategies, XOMA, and Daiichi-Sankyo, and has been a paid invited speaker at internal scientific seminars hosted by Pfizer and Alnylam Pharmaceuticals. L.G. has been a consultant for and served on advisory boards for sanofi-aventis, GlaxoSmithKline, Novartis, Merck, Tethy Bioscience, and XOMA and has received lecture fees from Lilly and Novartis. I.B. and her husband own stock in GlaxoSmithKline and Incyte. No other potential conflicts of interest relevant to this article were reported.

**AUTHOR CONTRIBUTIONS**

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**NOTE**

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**APPENDIX**

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