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 insulinogenic index) at extremely persuasive levels of statistical significance ($P = 2.1 \times 10^{-71}$). Defects in insulin processing and insulin secretion were seen in glucose-raising allele carriers at *TCF7L2*, *SCL30A8*, *GIPR*, and *C2CD4B*. Abnormalities in early insulin secretion were suggested in glucose-raising allele carriers at *MTNR1B*, *GCK*, *FADS1*, *DGKB*, and *PROX1* (lower insulinogenic 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 physiological 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*, *CRY2*, *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 glycemic 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

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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 in or near the loci listed above on dynamic physiologic measures of insulin processing, secretion, and sensitivity in order to provide insights on how these variants influence glucose levels and, in some cases, increase type 2 diabetes risk.

RESEARCH DESIGN AND METHODS

Cohort descriptions. The cohorts included in this study contributed a total of 29,084 unique individuals (supplementary Table 1, available in an online appendix at http://diabetes.diabetesjournals.org/cgi/content/full/db09-1568/DC1). All participants were white adults from Europe or the U.S. and free of diabetes assessed by clinical diagnosis, diabetes treatment, or FG ≥7 mmol/l. As in our previous discovery datasets (1,2), we decided to retain individuals with impaired fasting glucose or impaired glucose tolerance because we wished to avoid truncating the glucose distribution at such low levels that it would effectively eliminate individuals with the greatest genetic predisposition to hyperglycemia, i.e., the most informative segment of the population. Local research ethic committees approved all 14 studies, and all participants gave informed consent.

Phenotype descriptions. We studied the following glycemic phenotypes: *1*) circulating levels of fasting intact proinsulin (adjusted for fasting insulin), as a reflection of insulin processing, insulin secretion, β-cell mass/function, or a combination thereof (n = 17,402); *2*) the insulinogenic index, defined as [(insulin at 30 min) − (insulin at 0 min)]/[(glucose at 30 min) − (glucose at 0 min)], as a measure of glucose-stimulated insulin secretion (n = 15,399) and also indicative of β-cell mass/function (12); *3*) insulin sensitivity measured with standard intravenous methods (glucose uptake divided by steady-state insulin concentration [M/I] derived from euglycemic-hyperinsulinemic clamp [n = 2,250]) (13), insulin sensitivity index (S_I) from FSIGT (n = 575) (14), and steady-state plasma glucose (SSPG) from the insulin suppression test (n = 370) (15), combined after standardization (Z score transformation, N = 3,195); and *4*) four OGTT-derived measures of insulin sensitivity, namely the Stumvoll (8), Matsuda (9), Belfiore (10), and Gutt (11) indices (supplementary Table 3) (n = 15,554–15,999). In secondary analyses, we additionally studied associations with split proinsulin (n = 3,934) and C-peptide (n = 7,158).

We confirmed the potential utility of the OGTT-derived insulin sensitivity indices by examining correlations with euglycemic clamp–derived insulin sensitivity (M/I) in nondiabetic participants in the Uppsala Longitudinal Study of Adult Men (ULSAM) and the Relationship between Insulin Sensitivity and Cardiovascular risk (RISC) studies (supplementary Table 4); these ranged from 0.67 to 0.76 in ULSAM and 0.36 to 0.49 in RISC.

Other quantitative trait measurements. Glucose was measured in whole blood, plasma or serum, or a combination of these. Whole blood glucose levels were corrected to plasma glucose using a correction factor of 1.13. Concentrations of insulin, proinsulin, and C-peptide were estimated from plasma or serum as described in supplementary Table 1 for each of the cohorts.

Statistical methods. We tested associations with quantitative glycemic phenotypes for the lead SNPs reported as most significantly associated within 19 regions identified by the recent MAGIC fasting glucose and insulin, and 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 $<1 \times 10^{-6}$ or $<1 \times 10^{-4}$ in studies with genome-wide data, or <0.01 in direct genotyping studies) were excluded from analyses (supplementary Table 1). Call rates for directly genotyped SNPs exceeded 90%; information content r^2hat >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 M/I, S_I, 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 (pmol/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 genotype and imputation uncertainty into account, except in the Framingham Heart Study where both genotyped and imputed SNPs were analyzed using the lmekin function 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/Metal/index.html) and GWAMA (http://www.well.ox.ac.uk/gwama/index.shtml) software. Heterogeneity was assessed using the Q statistics.

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 [$P < 5 \times 10^{-8}$]). However, we have focused specifically on the results with P values $<10^{-3}$.

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	Alleles (effect*/ other)		Insulin processing, secretion and/or β-cell function	Insulin secretion	Insulin sensitivity				
				Proinsulin†	Insulinogenic index†	Insulin sensitivity intravenous measures‡	Stumvoll†§	Matsuda†§	Belfiore†§	Gutt†§
n				17,402	15,399	3,195	15,789	15,554	15,802	15,999
Loci implicated in abnormal insulin processing										
rs7944584	*MADD*	A/T	Beta (SE)	0.10 (0.0059)	0.0072 (0.01)	−0.024 (0.028)	−0.011 (0.005)	−0.02 (0.0078)	−0.0085 (0.0038)	−0.0076 (0.0048)
			$P_{age+sex}$	2.07×10^{-71}	0.47	0.38	0.033	0.0097	0.024	0.11
rs17271305	*VPS13C*	G/A	Beta (SE)	−0.018 (0.007)	0.019 (0.013)	−0.022 (0.039)	0.0041 (0.0055)	−0.0034 (0.0097)	−0.0011 (0.0047)	−0.0012 (0.0078)
			$P_{age+sex}$	0.016	0.13	0.58	0.46	0.72	0.81	0.88
Loci associated with higher proinsulin and lower insulin secretion										
rs7903146	*TCF7L2*	T/C	Beta (SE)	0.045 (0.0066)	−0.057 (0.011)	−0.01 (0.026)	0.011 (0.0052)	0.022 (0.0084)	0.011 (0.004)	−0.0052 (0.0057)
			$P_{age+sex}$	4.08×10^{-12}	2.00×10^{-7}	0.70	0.041	0.0082	0.0053	0.36
rs13266634	*SLC30A8*	C/T	Beta (SE)	0.031 (0.0067)	−0.035 (0.011)	−0.04 (0.027)	0.0047 (0.0049)	0.013 (0.0083)	0.0072 (0.0038)	−0.003 (0.0057)
			$P_{age+sex}$	2.74×10^{-6}	0.0012	0.14	0.34	0.12	0.055	0.6
rs10423928	*GIPR*	A/T	Beta (SE)	0.031 (0.0062)	−0.079 (0.011)	0.033 (0.032)	0.016 (0.0055)	0.029 (0.0085)	0.025 (0.0044)	−0.0099 (0.0051)
			$P_{age+sex}$	6.23×10^{-7}	2.10×10^{-13}	0.30	0.0029	0.00077	1.00×10^{-8}	0.053
rs11071657	*C2CD4B*	A/G	Beta (SE)	0.017 (0.0059)	−0.019 (0.01)	0.016 (0.028)	−0.0033 (0.0058)	0.0026 (0.0081)	0.0017 (0.0043)	−0.0001 (0.0049)
			$P_{age+sex}$	0.0037	0.063	0.56	0.56	0.74	0.70	0.99
Loci associated with abnormalities in early insulin secretion										
rs10830963	*MTNR1B*	G/C	Beta (SE)	0.0074 (0.0054)	−0.081 (0.009)	0.046 (0.024)	−0.0079 (0.0045)	−0.0091 (0.0071)	0.0021 (0.0034)	−0.012 (0.0043)
			$P_{age+sex}$	0.17	2.30×10^{-19}	0.057	0.077	0.20	0.55	0.0064
rs4607517	*GCK*	A/G	Beta (SE)	0.0026 (0.0072)	−0.048 (0.013)	0.0015 (0.033)	−0.0079 (0.0065)	−0.0053 (0.0099)	0.0029 (0.0048)	−0.023 (0.0058)
			$P_{age+sex}$	0.72	2.22×10^{-4}	0.96	0.22	0.59	0.54	8.13×10^{-5}
rs174550	*FADS1*	T/C	Beta (SE)	−0.0005 (0.0051)	−0.028 (0.0086)	0.011 (0.023)	0.0078 (0.0043)	0.005 (0.0067)	0.0048 (0.0033)	−0.0013 (0.0042)
			$P_{age+sex}$	0.93	0.0013	0.65	0.069	0.46	0.15	0.77
rs2191349	*DGKB*	T/G	Beta (SE)	0.0057 (0.0049)	−0.023 (0.0083)	0.0046 (0.029)	0.005 (0.0041)	0.014 (0.0064)	0.0038 (0.0032)	0.0021 (0.0041)
			$P_{age+sex}$	0.25	0.006	0.87	0.22	0.031	0.23	0.61
rs340874	*PROX1*	C/T	Beta (SE)	0.0069 (0.0051)	−0.02 (0.0083)	0.0062 (0.023)	−0.0042 (0.0042)	−0.0002 (0.0064)	0.0013 (0.0032)	−0.0031 (0.0041)
			$P_{age+sex}$	0.18	0.015	0.78	0.31	0.98	0.68	0.44
rs560887	*G6PC2*	C/T	Beta (SE)	−0.0017 (0.0073)	0.052 (0.013)	−0.045 (0.025)	0.0044 (0.0068)	−0.011 (0.0098)	−0.009 (0.0045)	−0.0052 (0.0059)
			$P_{age+sex}$	0.81	4.98×10^{-5}	0.069	0.52	0.26	0.044	0.38
Loci associated with reduced insulin sensitivity										
rs780094	*GCKR*	C/T	Beta (SE)	0.003 (0.0054)	0.015 (0.0094)	−0.034 (0.023)	−0.017 (0.0051)	−0.032 (0.0075)	−0.011 (0.0036)	0.00 (0.0045)
			$P_{age+sex}$	0.57	0.12	0.14	0.0011	2.89×10^{-5}	0.0033	0.99
rs35767	*IGF1*	G/A	Beta (SE)	0.0002 (0.0067)	−0.0061 (0.011)	−0.02 (0.03)	0.0016 (0.0054)	−0.022 (0.0084)	−0.0095 (0.0041)	−0.016 (0.0052)
			$P_{age+sex}$	0.98	0.57	0.50	0.77	0.010	0.020	0.0021

TABLE 1
Continued

No obvious effects on insulin processing, secretion, or sensitivity										
rs11708067	*ADCY5*	A/G	Beta (SE)	0.016 (0.0063)	−0.014 (0.011)	−0.0095 (0.027)	0.0051 (0.0054)	−0.0009 (0.0082)	−0.0031 (0.004)	−0.016 (0.0049)
			$P_{age+sex}$	0.013	0.20	0.73	0.34	0.91	0.44	0.0012
rs10885122	*ADRA2A*	G/T	Beta (SE)	−0.017 (0.0076)	−0.018 (0.013)	−0.005 (0.033)	−0.0095 (0.0062)	−0.0077 (0.0098)	−0.0011 (0.0047)	−0.0051 (0.006)
			$P_{age+sex}$	0.029	0.15	0.88	0.13	0.43	0.81	0.40
rs11605924	*CRY2*	A/C	Beta (SE)	−0.0053 (0.0049)	−0.014 (0.0083)	−0.013 (0.022)	−0.0076 (0.0041)	−0.0079 (0.0065)	−0.0027 (0.0031)	−0.0098 (0.004)
			$P_{age+sex}$	0.28	0.098	0.55	0.067	0.23	0.38	0.015
rs11920090	*SLC2A2*	T/A	Beta (SE)	−0.0112 (0.0073)	0.028 (0.012)	−0.021 (0.033)	0.0009 (0.0061)	−0.0007 (0.0096)	−0.0031 (0.0046)	−0.0095 (0.006)
			$P_{age+sex}$	0.13	0.022	0.54	0.88	0.94	0.50	0.11
rs7034200	*GLIS3*	A/C	Beta (SE)	0.018 (0.0063)	0.0008 (0.011)	0.0027 (0.028)	−0.0015 (0.0048)	0.0006 (0.0079)	−0.0043 (0.0037)	−0.0036 (0.005)
			$P_{age+sex}$	0.0050	0.94	0.92	0.75	0.94	0.24	0.48

*Per-allele betas (SEs) are shown at the upper lines; *P* values from age- and sex-adjusted analyses are shown at the lower lines. Effect allele was defined as the 2-h glucose–raising alleles for *GIPR* rs10423928 and *VPS13C* rs17271305; the fasting insulin–raising allele for *IGF1* rs35767; and the fasting glucose–raising allele for all other SNPs. †We used naturally log-transformed trait values for proinsulin, insulinogenic index, and the insulin sensitivity indices. ‡Insulin sensitivity measured with standard intravenous methods (M/I derived from euglycemic-hyperinsulinemic clamp [13]; S_I from FSIGT [14]; and SSPG from the insulin suppression test [15]) and combined after Z-score transformation. §The insulin sensitivity indices are defined in supplementary Table 3.

tional BMI adjustment). 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 ($P = 2.1 \times 10^{-71}$); 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 2.0×10^{-7}, respectively), *SLC30A8* rs13266634 ($P = 2.7 \times 10^{-6}$ and 0.0012) and *GIPR* rs10423928 ($P = 6.2 \times 10^{-7}$ and 2.1×10^{-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. 1*A–D*). Except for an association between the *GIPR* rs10423928 and higher insulin sensitivity as assessed by the Belfiore ($P = 1.0 \times 10^{-8}$), Matsuda ($P = 0.0008$), and Stumvoll ($P = 0.003$) indices, the other associations of these SNPs with measures of insulin sensitivity were very modest ($P = 0.01 - 0.05$) 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 ($P = 2.3 \times 10^{-19}$), *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* ($P = 8.1 \times 10^{-5}$) 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

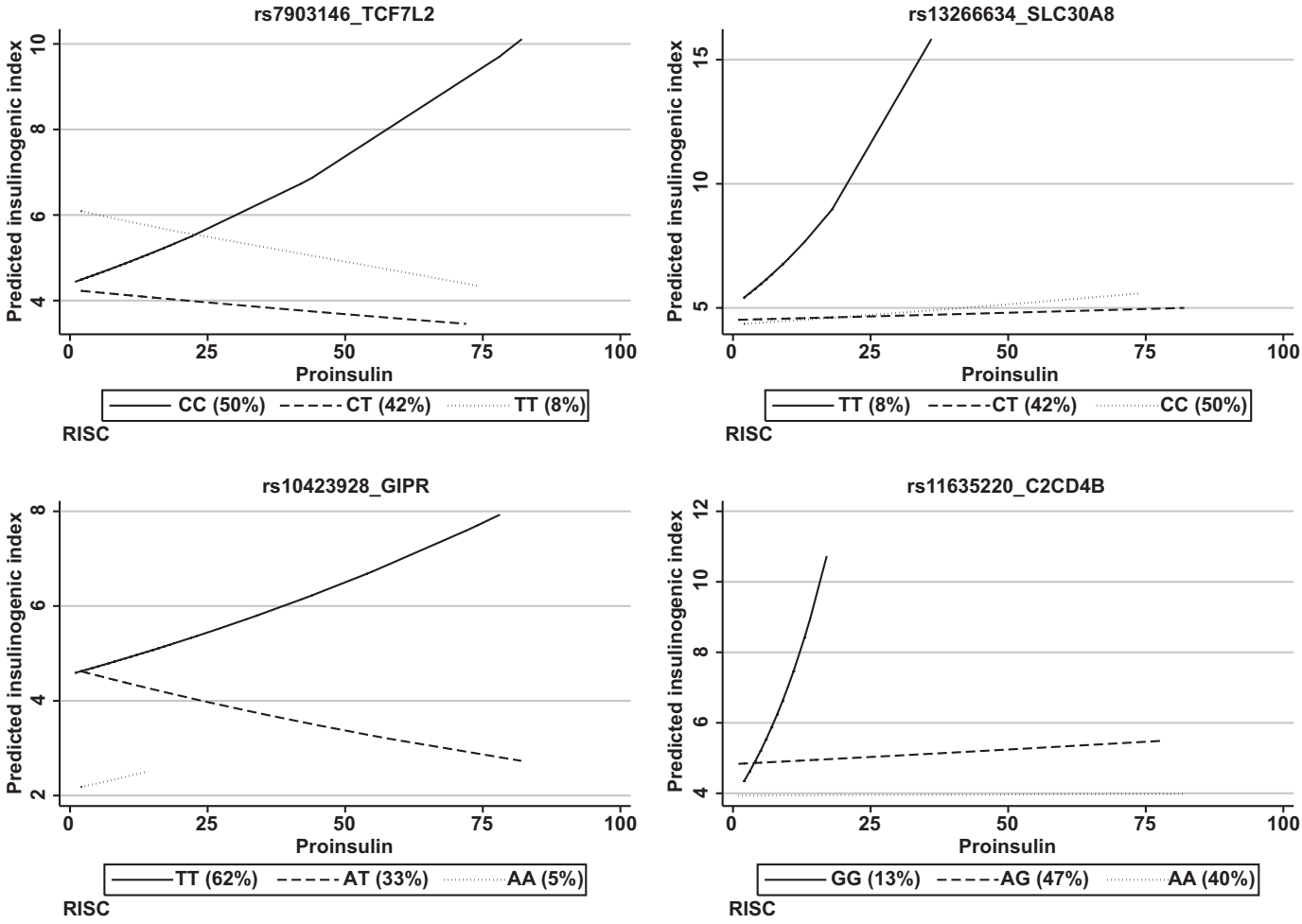

FIG. 1. Insulin secretion and proinsulin in the RISC study (n = 1,319) by genotype at *TCF7L2* rs7903146, *SLC30A8* rs13266634, *GIPR* rs10423928, and *C2CD4B* rs11071657. Proinsulin processing can be studied by plotting the relation between early insulin secretion (measured by insulinogenic index from OGTT) and proinsulin. The three curves in each plot represent the regression lines of the logarithm of estimated insulin secretion as a linear function of the proinsulin level for all participants, distributed according to the genotypes. In all four plots, the relationship between the insulinogenic index and 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, indicating the active secretion of insulin precursors in lieu of mature insulin.

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 FG-raising allele at *GCKR* rs780094 was associated with lower insulin sensitivity by the Stumvoll (P = 0.001), Matsuda ($P = 2.9 \times 10^{-5}$), and Belfiore (P = 0.003) indices, whereas the fasting insulin–raising allele at *IGF1* rs35767 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 (r^2 = 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 population (21). In our MAGIC meta-analysis of ~14,000 individuals, 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 processing, 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 glucose metabolism. Our results demonstrate that these genetic loci influence glycemic regulation by diverse pathways (supplementary Fig. 3).

Loci implicated in abnormal insulin processing. The glucose-raising allele at *MADD* was associated with elevated 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 *MTNR1B*, *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 *MTNR1B* (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 *MTNR1B* 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). *MTNR1B* 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 *MTNR1B* 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 secretory 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 is 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.

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NOTE

Additional acknowledgments can be found in the online appendix at http://diabetes.diabetesjournals.org/cgi/content/full/db09-1568/DC1.

APPENDIX

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