Common Variants of the Novel Type 2 Diabetes Genes *CDKAL1* and *HHEX/IDE* Are Associated With Decreased Pancreatic β-Cell Function

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OBJECTIVE— Type 2 diabetes is characterized by impaired pancreatic β-cell function and decreased insulin sensitivity. Genome-wide association studies have identified common, novel type 2 diabetes susceptibility loci within the *FTO*, *CDKAL1*, *CDKN2A/CDKN2B*, *IGF2BP2*, *HHEX/IDE*, and *SLC30A8* gene regions. Our objective was to explore the relationships between the diabetes-associated alleles and measures of β-cell function and whole-body insulin sensitivity.

RESEARCH DESIGN AND METHODS— A total of 1,276 healthy subjects of European ancestry were studied at 19 centers. Indexes of β-cell function (including 30-min insulin response and glucose sensitivity) were derived from a 75-g oral glucose tolerance test, and whole-body insulin sensitivity (M/I) was assessed by hyperinsulinemic-euglycemic clamp. Genotype/phenotype relationships were studied by linear trend analysis correcting for age, sex, and recruitment center.

RESULTS— *CDKAL1* and *HHEX/IDE* diabetes-associated alleles were both associated with decreased 30-min insulin response (both $P = 0.0002$) and decreased pancreatic β-cell glucose sensitivity ($P = 9.86 \times 10^{-5}$ and 0.009, respectively), and these relationships remained after correction for M/I. The *FTO* susceptibility allele showed a weak but consistent association with increased adiposity, which in turn was linked to a decrease in M/I. However, none of the other novel diabetes susceptibility alleles were associated with insulin sensitivity.

CONCLUSIONS— *CDKAL1* and *HHEX/IDE* diabetes-associated alleles are associated with decreased pancreatic β-cell function, including decreased β-cell glucose sensitivity that relates insulin secretion to plasma glucose concentration. We confirmed the association between the *FTO* allele and increased adiposity, but none of the other novel susceptibility alleles were associated with whole-body insulin sensitivity. ***Diabetes*** **56: 3101–3104, 2007**

Type 2 diabetes is a complex trait characterized by decreased insulin secretion and decreased insulin action at target tissues. Knowledge of the molecular mechanisms and the underlying genetic architecture remains incomplete, although recent progress has been made through the genome-wide association (GWA) analyses (1–6). As well as confirming the involvement of *TCF7L2*, *PPARG*, and *KCNJ11*, the GWAs have detected novel type 2 diabetes susceptibility loci within or close to the *FTO* (fat mass and obesity associated), *CDKAL1* (cyclin-dependent kinase 5 regulatory subunit associated protein 1-like 1), *CDKN2A/CDKN2B* (encoding the tumor suppresors $p15^{INK4b}$ and $p16^{INK4a}$, respectively), *IGF2BP2* (IGF2 binding protein 2), *HHEX/IDE* (homeobox, hematopoietically expressed/insulin degrading enzyme), and *SLC30A8* (zinc transporter) genes. With the exception of *FTO*, which alters diabetes risk through increased adiposity (6), it is not known how variation within these genes increases diabetes susceptibility, although initial work suggests that *CDKAL1* and *SLC30A8* might act through altered pancreatic β-cell function (1–3).

The purpose of this study was to explore the relationship between the replicated susceptibility variants and detailed phenotypes of pancreatic β-cell function and whole-body insulin sensitivity. To do this, we used the RISC (Relationship between Insulin Sensitivity and Cardiovascular Disease) cohort, which comprises nondiabetic healthy European subjects recruited as part of a long-term study of insulin sensitivity and cardiovascular disease (7). A major benefit of using this healthy, disease-free cohort is that it avoids the secondary metabolic effects of the established diabetic state, which can confound and obscure the relationship between the gene variants and indexes of pancreatic β-cell function and insulin action.

RESEARCH DESIGN AND METHODS

Table 1 summarizes the key phenotypic data for the men and women, confirming that this was a cohort of healthy subjects. The study recruitment methods and inclusion/exclusion criteria have been previously described (7). Briefly, healthy men and women of European ancestry, aged between 30 and 60 years, were recruited from 19 centers in 14 European countries. Subjects with diabetes, hypertension, or dyslipidemia were excluded (7). The analysis presented in this study is based on 1,276 subjects who had passed the

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GWA, genome-wide association; OGTT, oral glucose tolerance test; RISC, Relationship between Insulin Sensitivity and Cardiovascular Disease; SNP, single nucleotide polymorphism.

TABLE 1
Key characteristics for male and female subjects in the RISC cohort

	Male	Female
n	575	701
Age (years)	43 ± 8	44 ± 8
BMI (kg/m^2)	26.4 ± 3.5	24.9 ± 4.3
Fat mass (kg)	19.0 ± 8.1	22.7 ± 9.2
Fat-free mass (kg)	64.9 ± 7.1	44.9 ± 4.4
Waist-to-hip ratio	0.92 ± 0.08	0.82 ± 0.09
Fasting blood glucose (mmol/l)	5.2 ± 0.5	4.9 ± 0.6
Impaired glucose tolerance (%)	4	5
Triglycerides (mmol/l)	1.1 ± 1.7	0.9 ± 1.6
Cholesterol (mmol/l)	4.9 ± 0.9	4.8 ± 0.9
HDL (mmol/l)	1.2 ± 0.3	1.6 ± 0.4
Systolic blood pressue (mmHg)	122.2 ± 10.8	113.7 ± 12.6
Diastolic blood pressue (mmHg)	76.5 ± 7.6	72.8 ± 8.1
M/I (μmol · min^{-1} · kg$_{ffm}$$^{-1}$ · nmol/l^{-1})	109 (79–151)	138 (105–187)
30-min insulin response (pmol/mmol)	26.5 (17.7–39.8)	26.3 (18.6–79.4)
β-Cell glucose sensitivity (pmol · min^{-1} · m^{-2} · mmol/l^{-1})	101 (74–139)	119 (82–175)

Data are means ± SD or geometric mean (25th–75th quartile). ffm, fat-free mass.

eligibility criteria and had completed genotype data. Local ethics committee approval was obtained by each recruitment center, and written consent was obtained from all participants.

Subjects underwent detailed anthropometric assessment and a 75-g oral glucose tolerance test (OGTT) with blood sampling before and 30, 60, 90, and 120 min after the oral glucose load. Fat mass was determined as the difference between body weight and fat-free mass determined by bioimpedance (Tanita International Division, Yiewsley, U.K.). On a separate day within 1 month of the OGTT, subjects underwent a hyperinsulinemic-euglycemic clamp as previously reported (7). To ensure consistency across study centers, the clamp procedure was standardized and each center underwent prestudy training. Clamp data were then transferred and analyzed at the RISC coordinating center (Pisa, Italy) and quality assured against preset criteria. These were as follows: clamped glucose levels within 20% of target (fasting glucose concentration) and coefficient of variation (CV) of ≤15%, as well as avoidance of hypoglycemia (glucose <3.5 mmol/l). Insulin sensitivity was assessed as the mean glucose infusion rate over the last 40 min of the clamp, corrected for the mean plasma insulin levels achieved during the same period (M/I).

Pancreatic β-cell function was assessed using the OGTT data. The 30-min insulin response (8) was calculated as the ratio of the insulin concentration increment to the 30-min glucose concentration (30-min insulin − 0-min insulin/30-min glucose). Indexes of β-cell function parameters were derived from mathematical analysis of plasma glucose and C-peptide, using C-peptide deconvolution (9) and a model previously illustrated (10,11). In short, the model describes the relationship between glucose concentration and insulin secretion (ISR). It includes an ISR versus plasma glucose concentration dose-response curve (modulated by a potentiation factor accounting for effects due to hyperglycemia exposure and incretins) and a parameter describing early insulin release (rate sensitivity, marker of first-phase secretion). The key parameter is β-cell glucose sensitivity, i.e., the slope of dose-response curve. An OGTT-derived measure of insulin sensitivity was also calculated as previously described (12).

Samples were processed and stored locally before transfered to the central assay laboratories and analyzed as previously reported (7). Genomic DNA was extracted using a Nucleon BACC2 kit (Tepnel Life Sciences, Manchester, U.K.). Single nucleotide polymorphisms (SNPs) were selected as those best representing the recently reported type 2 diabetes associations and for which there were robust assays. All SNPs were genotyped by KBiosciences (3,6), except the Pro12Ala variant of *PPARG*, which was genotyped by PCR–restriction fragment–length polymorphism (13). A haplotype formed by the T-alleles of rs10757283 and rs10811661 in the *CDKN2A/2B* region was associated with type 2 diabetes more strongly than the individual SNPs (3). We therefore used unphased software (14) to assess the association between this haplotype and the traits under investigation.

Variables with a positive skewed distribution (M/I, 30-min insulin response, and all model parameters from the β-cell model) were log transformed to normalize distributions. These data are presented as the median (interquartile range).

As all of the novel type 2 diabetes risk alleles conformed to an additive inheritance pattern (3), linear trend analysis was first performed to test for associations between SNP genotypes and selected phenotypes after adjusting for age, sex, and recruitment center. If an SNP displayed a nonadditive inheritance pattern for a given phenotype, general linear model analysis was used to test for association. For all analyses, $P \leq 0.01$ was considered statistically significant. We calculated that the cohort, for example, had 80% power at $P = 0.01$ to detect differences of 0.13 and 0.18 SDs per allele for minor allele frequencies of 0.44 and 0.30, respectively.

RESULTS

The genotype frequencies for the eight SNPs and the Pro12Ala variant are summarized in the online appendix supplementary Table 1 (available at http://dx.doi.org/10.2337/db07-0634), and all were in Hardy-Weinberg equilibrium. Although all analyses were corrected for recruitment center, we compared genotype frequencies between subjects from north and south European recruitment centers. There were small but significant differences for rs564398, rs9939609, and rs1801282 (all $P < 0.05$).

Table 2 summarizes the relationships between the diabetes-associated alleles and the key phenotypic traits, namely whole-body insulin sensitivity (M/I) and two indexes of β-cell function (30-min insulin response and β-cell glucose sensitivity). The key findings are that the diabetes risk alleles for SNPs rs10946398 and rs1111875 of the *CDKAL1* and *HHEX/IDE* gene regions, respectively, showed significant associations with the measures of β-cell function. For *CDKAL1*, the diabetes risk allele was associated with decreased glucose sensitivity ($P = 9.86 \times 10^{-5}$) and decreased 30-min insulin response ($P = 0.0002$). These associations remained significant after adjustment for M/I ($P = 0.0005$ and 0.006, respectively). For *HHEX/IDE*, the diabetes risk allele was associated with decreased 30-min insulin response ($P = 0.0002$) and a trend for decreased glucose sensitivity ($P = 0.009$). Again, these associations remained after adjustment for M/I ($P = 0.0005$ and 0.01, respectively). None of the other variants, including the *CDKN2A/2B* risk haplotype, showed an association with any measures of β-cell function.

We included the *PPARG* Pro12Ala variant as a "positive control" based on previous reports of an association between this variant and insulin sensitivity (15,16). This observation was replicated by general linear model analysis that showed a borderline significant association between the Ala allele and increased M/I ($P = 0.026$), which remained after correction for BMI ($P = 0.006$). In light of the previously reported relationship between *FTO* and BMI, we specifically examined the relationship between rs9939609 and three measures of adiposity (Table 2). There was an association between the diabetes susceptibility allele and increased adiposity, which was not strongly significant (BMI $P = 0.022$) but was consistent with the effect sizes previously reported (6). We also found an association between the allele and decreased insulin sensitivity ($P = 0.023$), but this was no longer evident after correction for BMI. None of the other novel diabetes susceptibility alleles or the *CDKN2A/2B* risk haplotype showed a significant association with whole-body insulin sensitivity. There were no significant associations between

TABLE 2
Relationships between diabetes susceptibility alleles and measures of β-cell function (30-min insulin response and β-cell glucose sensitivity) and whole-body insulin sensitivity (M/I)

Genomic region/ variant	Genotype 1/2	Trait	11	12	22	*P*
rs10757283	C/**T**	30-min ins resp	25.7 (17.8–37.2)	26.3 (18.6–38.9)	26.3 (16.6–38.9)	0.678
CDKN2B		Glucose sens	111 (78–156)	114 (80–158)	114 (76–160)	0.336
		M/I	133 (93–186)	127 (91–176)	124 (86–170)	0.077
rs10811661	**T**/C	30-min ins resp	25.7 (17.4–37.8)	28.2 (20.0–38.9)	28.8 (15.8–38.9)	0.216
CDKN2B		Glucose sens	115 (79–157)	109 (78–164)	120 (78–157)	0.504
		M/I	130 (94–179)	123 (89–173)	146 (101–181)	0.293
rs564398	**A**/G	30-min ins resp	26.3 (17.4–38.9)	26.9 (18.6–38.0)	25.1 (17.0–36.3)	0.367
CDKN2B		Glucose sens	113 (77–149)	114 (79–169)	107 (71–147)	0.413
		M/I	123 (91–173)	129 (92–176)	134 (92–189)	0.065
rs10946398	A/**C**	30-min ins resp	28.2 (19.5–39.8)	25.1 (17.4–36.3)	24.5 (15.8–36.3)	**0.0002**
CDKAL1		Glucose sens	119 (83–170)	111 (77–155)	102 (68–140)	**9.86×10^{-5}**
		M/I	126 (86–172)	129 (95–180)	117 (89–178)	0.108
rs1111875	**G**/A	30-min ins resp	25.1 (17.0–35.5)	26.0 (18.2–39.8)	29.5 (20.0–39.8)	**0.0002**
HHEX/IDE		Glucose sens	107 (72–154)	117 (81–156)	120 (82–175)	**0.009**
		M/I	127 (91–175)	128 (91–176)	132 (95–184)	0.135
rs13266634	**C**/T	30-min ins resp	25.7 (17.4–37.5)	27.0 (19.5–38.9)	25.7 (18.4–38.9)	0.128
SLC30A8		Glucose sens	112 (76–155)	114 (80–159)	125 (92–186)	0.072
		M/I	128 (92–179)	126 (89–167)	153 (96–196)	0.330
rs4402960	G/**T**	30-min ins resp	26.9 (17.8–38.0)	25.1 (18.2–38.0)	28.5 (20.8–39.1)	0.540
IGF2BP2		Glucose sens	115 (80–162)	111 (76–154)	118 (80–158)	0.067
		M/I	131 (92–177)	126 (91–176)	126 (87–160)	0.204
rs9939609	**A**/T	30-min ins resp	25.1 (18.0–37.2)	26.6 (17.5–38.9)	26.3 (19.1–38.0)	0.690
FTO		Glucose sens	111 (72–147)	113 (79–158)	114 (81–159)	0.312
		M/I	123 (89–167)	127 (88–177)	134 (100–182)	0.023
		BMI	25.5 (23.4–28.4)	25.1 (22.8–28.1)	24.6 (22.4–27.4)	0.022
		WHR	0.88 (0.80–0.94)	0.86 (0.79–0.93)	0.85 (0.78–0.92)	**0.009**
		Fat mass	21.3 (14.2–27.9)	19.7 (14.7–25.6)	19.3 (14.4–24.6)	0.035
Pro12Ala (rs1801282)	**Pro**/Ala	30-min ins resp	22.4 (18.6–28.2)	24.5 (16.6–34.7)	26.9 (18.6–39.8)	0.028
PPARG		Glucose sens	136 (80–164)	107 (75–149)	115 (79–160)	0.612
		M/I	127 (93–176)	125 (87–179)	168 (134–210)	0.212

Data are median (25th–75th quartile). *P* values for linear trend analysis adjusted for age, sex, and recruitment center. Fat mass is measured in kilograms. Risk allele/variants are in boldface. 30-min ins resp, 30-min insulin response (pmol/mmol); Glucose sens, β-cell glucose sensitivity (pmol · min^{-1} · m^{-2} · mmol/l^{-1}); M/I, whole-body insulin sensitivity (μmol · min^{-1} · kg_{ffm}^{-1} · nmol/l^{-1}). ffm, fat-free mass.

the OGTT-derived measure of insulin sensitivity and the diabetes susceptibility alleles (online supplementary Table 2).

We assessed whether any of the SNPs showed evidence of interacting with BMI to influence 30-min insulin response, β-cell glucose sensitivity, and M/I by regression analysis adjusting for the usual covariates. There was no evidence of interactive effects (all $P > 0.05$).

DISCUSSION

Recent GWAs have identified novel type 2 diabetes susceptibility loci within or close to the genes encoding *FTO*, *CDKAL1*, *CDKN2A/CDKN2B*, *IGF2BP2*, *HHEX/IDE*, and *SLC30A8* (1–6). The purpose of this study was to examine the relationships between the diabetes-associated alleles and measures of β-cell function and whole-body insulin sensitivity.

Three key findings have emerged from this study. First, the type 2 diabetes–associated alleles of the SNPs located within the *CDKAL1* and *HHEX/IDE* gene regions were associated with impaired β-cell function. Both these SNPs showed clear associations with increased type 2 diabetes risk in GWA studies of subjects of European ancestry, with odds ratios (ORs) of 1.12 and 1.13 for *CDKAL1* and *HHEX/IDE*, respectively (3–5). For each gene, the type 2 diabetes susceptibility allele was associated with decreased 30-min insulin response and decreased β-cell glucose sensitivity. These relationships remained after correction for whole-body insulin sensitivity (M/I), in keeping with primary defects of β-cell function rather than secondary changes in response to altered insulin sensitivity. It is worth noting, however, that these are not direct measures of insulin secretion but provide indirect indexes of β-cell function. The measure of β-cell glucose sensitivity represents the mean slope of the dose-response relationship between insulin secretion determined from C-peptide kinetics and plasma glucose concentration. Decreased β-cell glucose sensitivity therefore indicates decreased insulin secretion over the glucose concentration range achieved during the OGTT. We recently investigated predictors of progression to abnormal glucose tolerance in a high-risk nondiabetic cohort and found that decreased glucose sensitivity was the strongest independent predictor when all the other model parameters and the early insulin response were included (10). Our results support those of Steinthorsdottir et al. (2) who showed that variation within *CDKAL1* was similarly associated with type 2 diabetes and decreased early insulin response. As previously reported (2–5), the role of *CDKAL1* in pancreatic β-cell function remains unknown, although we found that it is strongly expressed in human adult pancreatic islets relative to other tissues (3).

The susceptibility allele at rs1111875 within the *HHEX/IDE* gene region was shown to be associated with type 2

diabetes in both our own and other GWA studies (1–5). *HHEX* is highly expressed in pancreatic islet tissue (3), and failure of ventral pancreas development is a feature of *HHEX*-null mice (17). Conditional knockdown of *HHEX* in pancreatic β-cells will help to define its role in β-cell differentiation and function. It is important to note, however, that the association signal is a region of extended linkage disequilibrium that includes other genes, including *IDE* and *KIF11*, that encode the insulin-degrading enzyme and kinesin-interacting factor, respectively (3).

We have found no relationship between the indexes of β-cell function and the susceptibility alleles in the other novel gene regions (*CDKN2A/CDKN2B*, *IGF2BP2*, and *SLC30A8* genes). This is somewhat surprising, as some of the risk alleles had ORs close to those of *CDKAL1* and *HHEX/IDE* (3). Other possible explanations are that these genes do not impact upon the phenotypes that we have examined or, perhaps, that the effect of the risk alleles is strongly age dependent, as ours is a relatively young cohort (Table 1).

The second finding is that we confirmed that the *FTO* susceptibility allele is associated with increased adiposity, and this was consistent across all three measures of adiposity (BMI, waist-to-hip ratio, and fat mass). The *FTO* susceptibility allele was also weakly associated with decreased insulin sensitivity, but this was explained through the increased adiposity.

The third finding is that none of the other novel susceptibility alleles were associated with altered whole-body insulin sensitivity. This is despite the fact that the RISC cohort is one of the largest collections of healthy subjects, all of European ancestry, to be assessed for whole-body insulin sensitivity using the gold standard hyperinsulinemic-euglycemic clamp technique. We acknowledge, however, that the exclusion criteria may have served to compress the distribution of insulin sensitivity across the cohort and limited the scope to detect associations between this phenotype and the diabetes susceptibility alleles. Nonetheless, we were able to show that homozygous Ala allele carriers of the *PPARG* Pro12Ala variant have greater whole-body clamp-derived insulin sensitivity compared with Pro allele carriers, in keeping with the findings of a similar study that assessed whole-body insulin sensitivity by hyperinsulinemic-euglycemic clamp (16). This would suggest, therefore, that if any of the other novel diabetes susceptibility variants do influence insulin sensitivity, then their effects must be comparatively weak and will require a larger cohort to be detected.

In conclusion, we have shown that the *CDKAL1* and *HHEX/IDE* susceptibility alleles are associated with decreased pancreatic β-cell function and have confirmed the relationship between variation in *FTO* and increased adiposity. However, none of the other novel diabetes susceptibility alleles were associated with altered whole-body insulin sensitivity.

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