Gene Variants in the Novel Type 2 Diabetes Loci *CDC123/CAMK1D*, *THADA*, *ADAMTS9*, *BCL11A*, and *MTNR1B* Affect Different Aspects of Pancreatic β-Cell Function

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OBJECTIVE—Recently, results from a meta-analysis of genome-wide association studies have yielded a number of novel type 2 diabetes loci. However, conflicting results have been published regarding their effects on insulin secretion and insulin sensitivity. In this study we used hyperglycemic clamps with three different stimuli to test associations between these novel loci and various measures of β-cell function.

RESEARCH DESIGN AND METHODS—For this study, 336 participants, 180 normal glucose tolerant and 156 impaired glucose tolerant, underwent a 2-h hyperglycemic clamp. In a subset we also assessed the response to glucagon-like peptide (GLP)-1 and arginine during an extended clamp (n = 123). All subjects were genotyped for gene variants in *JAZF1*, *CDC123/CAMK1D*, *TSPAN8/LGR5*, *THADA*, *ADAMTS9*, *NOTCH2/ADAMS30*, *DCD*, *VEGFA*, *BCL11A*, *HNF1B*, *WFS1*, and *MTNR1B*.

RESULTS—Gene variants in *CDC123/CAMK1D*, *ADAMTS9*, *BCL11A*, and *MTNR1B* affected various aspects of the insulin response to glucose (all $P < 6.9 \times 10^{-3}$). The *THADA* gene variant was associated with lower β-cell response to GLP-1 and arginine (both $P < 1.6 \times 10^{-3}$), suggesting lower β-cell mass as a possible pathogenic mechanism. Remarkably, we also noted a trend toward an increased insulin response to GLP-1 in carriers of *MTNR1B* (P = 0.03), which may offer new therapeutic possibilities. The other seven loci were not detectably associated with β-cell function.

CONCLUSIONS—Diabetes risk alleles in *CDC123/CAMK1D*, *THADA*, *ADAMTS9*, *BCL11A*, and *MTNR1B* are associated with various specific aspects of β-cell function. These findings point to a clear diversity in the impact that these various gene variants may have on (dys)function of pancreatic β-cells. ***Diabetes* 59: 293–301, 2010**

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Genome-wide association (GWA) studies have revealed a large number of novel type 2 diabetes susceptibility loci (1–4). Most of the genes identified during the first wave of GWA study results are shown to affect β-cell function, indicated by lower insulin responses to oral (OGTTs) or intravenous (IVGTTs) glucose tolerance tests (5). By applying the hyperglycemic clamp methodology, considered the gold standard for measurements of β-cell function, we further refined the observed β-cell defects to defects in first- but not second-phase glucose-stimulated insulin secretion (GSIS) (6) or incretin-stimulated secretion (7). This differentiation is of importance to help resolve the pathogenic mechanism of the diabetes loci identified by GWA studies.

More recently the Diabetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium published at least six additional susceptibility loci, *JAZF1*, *CDC123/CAMK1D*, *TSPAN8/LGR5*, *THADA*, *ADAMTS9*, and *NOTCH2/ADAM30* (8), and three putative susceptibility loci, *DCD*, *VEGFA*, and *BCL11A*. Studies using OGTTs have yielded conflicting results on the effects of these new loci on β-cell function and insulin sensitivity. Grarup et al. (9) reported β-cell dysfunction associated with gene variants in *JAZF1*, *TSPAN8/LGR5*, and *CDC123/CAMK1D*. The results for *CDC123/CAMK1D* have only been replicated by Sanghera et al. (10) in Asian Indians but not by three other studies in Caucasians. All of the other three studies also failed to replicate the results for *JAZF1* and *TSPAN8/LGR5* (11–13). Furthermore, gene variants in three other loci have been established as true type 2 diabetes susceptibility loci, *HNF1B*, *WFS1*, and *MTNR1B* (14–19). Although mutations in *HNF1B* are associated with β-cell defects in maturity-onset diabetes of the young, it is unknown whether the type 2 diabetes–associated common single nucleotide polymorphism (SNP) is also associated with reduced β-cell function (14,15). It has been shown that *WFS1* associates with reduced oral (11,13,20–22) but not intravenous glucose-stimulated insulin secretion (22). Schäfer et al. (22) further demonstrated that the *WFS1* gene affects glucagon-like peptide (GLP)-1–stimulated insulin secretion during clamps. For the *MTNR1B* locus, several studies have shown reduced insulin secretion in response to glucose (17–19,23,24).

In this study 180 normal (NGT) and 156 impaired (IGT) glucose tolerant subjects originating from three indepen-

TABLE 1
Clinical characteristics of the individual study samples

	Hoorn*	Utrecht*		NTR Twins*	
	IGT	NGT	IGT	NGT	IGT
n	137	64	12	116	7
Sex (male/female)	64/73	15/49	4/8	58/58	0/7
Age (years)	60.5 ± 8.6	45.9 ± 6.4	49.5 ± 7.7	31.5 ± 6.5	31.2 ± 3.2
BMI (kg/m^2)	28.1 ± 4.0	25.8 ± 3.8	26.7 ± 4.1	24.2 ± 3.5	24.5 ± 3.3
Fasting plasma glucose (mmol/l)	6.3 ± 0.7	4.6 ± 0.4	5.1 ± 0.4	4.6 ± 0.4	4.6 ± 0.6
2-h plasma glucose (mmol/l)	8.8 ± 1.7	5.1 ± 1.0	8.5 ± 1.2	5.2 ± 1.1	8.1 ± 0.3
Fasting plasma insulin (pmol/l)	62 (46–91)	30 (24–42)	66 (42–78)	34 (27–51)	39 (29–60)
First-phase insulin response (pmol/l)	587 (378–895)	885 (644–1,217)	678 (461–909)	814 (589–1,162)	795 (693–1,210)
Second-phase insulin response (pmol/l)	255 (176–354)	260 (191–365)	251 (186–307)	218 (162–358)	217 (210–434)
ISI (μmol · min^{-1} · kg^{-1} · pmol^{-1} · l^{-1})	0.108 (0.068–0.164)	0.190 (0.127–0.282)	0.111 (0.082–0.256)	0.227 (0.152–0.323)	0.123 (0.109–0.183)
DI (μmol · min^{-1} · kg^{-1})	65 (42–92)	172 (103–238)	72 (55–128)	180 (140–234)	138 (82–151)
GLP-1–stimulated insulin release (pmol/l)	NA	NA	NA	1,225 (734–2,587)	848 (577–1,239)
Arginine-stimulated insulin release (pmol/l)	NA	NA	NA	2,188 (1,526–2,973)	1,673 (1,438–1,908)

Data are means ± SD, median (interquartile range), or *n*. *Original population from which the cohort originated (26,28–30). NA, not available.

dent studies in the Netherlands were genotyped for variants in *JAZF1*, *CDC123/CAMK1D*, *TSPAN8/LGR5*, *THADA*, *ADAMTS9*, *NOTCH2/ADAMS30*, *DCD*, *VEGFA*, *BCL11A*, *HNF1B*, *WFS1*, and *MTNR1B*. We tested whether these loci are associated with alterations in β-cell function as assessed by hyperglycemic clamp methodology with, in a subset, two additional secretagogues, namely GLP-1 and arginine. Arginine stimulation during hyperglycemia is a test of (near) maximal insulin secretion and has been proposed as a proxy for β-cell mass (25).

RESEARCH DESIGN AND METHODS

Hyperglycemic clamp cohorts. Participants originated from three independent studies in the Netherlands (26–30). The clinical characteristics of the study sample are given in Table 1. In short we recruited for this study 137 IGT subjects from the Hoorn Study (26,29); 76 subjects (64 NGT/12 IGT) from Utrecht (27,28), and 123 twins and sibs (116 NGT/7 IGT) from the Netherlands Twin Register (NTR) (30). The NTR twin sample includes 66 monozygotic and 28 dizygotic twins as well as 29 of their nontwin sibs recruited from 50 families. Details of the three individual samples have previously been described (6,26–30).

Hyperglycemic clamp procedure. All participants underwent a hyperglycemic clamp at 10 mmol/l glucose for at least 2 h (26,28–30). First-phase insulin secretion was determined as the sum of the insulin levels during the first 10 min of the clamp. Second-phase insulin secretion was determined as the mean of the insulin levels during the last 40 min of the second hour of the clamp (80–120 min). The insulin sensitivity index (ISI) was defined as the glucose infusion rate (*M*, μmol · min^{-1} · kg^{-1}) necessary to maintain the hyperglycemic clamp divided by the plasma insulin concentration (*I*, pmol/l) during the last 40 min of the second hour of the clamp (*M*/*I*, μmol · min^{-1} · kg^{-1} · pmol^{-1} · l^{-1}). Mitrakou et al. (31) compared the ISI determined with a hyperglycemic clamp with insulin sensitivity as determined using the euglycemic-hyperinsulinemic clamp in the same subjects and found a good agreement between the two methods. The disposition index (DI) was calculated by multiplication of first-phase insulin secretion and ISI to quantify insulin secretion in relation to the ambient insulin sensitivity (32,33).

Subjects from the NTR twin sample underwent a modification of the extended clamp using additional GLP-1 and arginine stimulation as described previously by Fritsche et al. (25). GLP-1–stimulated insulin release was measured as the mean incremental area under the curve (160–180 min) after GLP-1 stimulation (1.5 pmol/kg bolus for 1 min at t = 120 min followed by a continuous infusion of 0.5 pmol · kg^{-1} · min^{-1}). Arginine-stimulated acute insulin release was measured by injecting a bolus of 5 g arginine hydrochloride at t = 180 min as described previously (25). The acute insulin response to arginine was calculated as the mean incremental area under the curve from 182–185 min.

Genotyping. Based on the available literature regarding the novel type 2 diabetes genes, we selected gene variants in *JAZF1* (rs864745), *CDC123/CAMK1D* (rs12779790), *TSPAN8/LGR5* (rs7961581), *THADA* (rs7578597), *ADAMTS9* (rs4607103), and *NOTCH2/ADAM30* (rs2641348) (8); the putative type 2 diabetes genes *DCD* (rs1153188), *VEGFA* (rs9472138), and *BCL11A* (rs10490072) (8); and *HNF1B* (rs757210) (14,15), *WFS1* (rs10010131) (16), and *MTNR1B* (rs10830963) (17–19). All SNPs were measured using either the Sequenom platform (Sequenom, San Diego, California) or Taqman SNP genotyping assays (Applied Biosystems, Foster City, California) in all individual subjects. The genotyping success rate was above 96% for all SNPs, and samples measured in duplicate (~5%) were in complete concordance. All genotype distributions obeyed Hardy-Weinberg equilibrium ($P \geq 0.05$) except for *MTNR1B* ($P = 0.01$). SNP genotypes were recoded as 0, 1, or 2, with the 2 genotype as the at-risk genotype reported in the original publications.

Statistics. The effect of the gene variants on the β-cell responses was examined with linear regression assuming an additive model unless otherwise stated. To take into account the family relatedness (i.e., in the twin sample), empirical standard errors were used (using the generalized estimating equations). The analyses of first- and second-phase GSIS, GLP-1, and arginine-stimulated insulin secretion were adjusted for age, sex, BMI, study center, glucose tolerance status (NGT/IGT), and ISI. For the analysis of ISI and DI, ISI was removed from the covariates. All outcome variables were log transformed prior to analysis. In addition to the analysis of the pooled data we also performed a random-effects meta-analysis of the results obtained in the three separate cohorts using Comprehensive Meta-Analysis version 2 software (www.meta-analysis.com). A priori power calculations showed that the design used in this study would allow the detection of a difference in insulin secretion of ~15% (glucose) to 30% (GLP-1, arginine) with 80% power ($\alpha < 0.05$) depending on the stimulus used and allele frequency of the SNPs. All data are given as estimated mean (95% CI) unless otherwise stated. After correction for multiple hypothesis, testing results were regarded significant at $P \leq 0.008$ (six tests). Apart from the meta-analysis, SPSS version 16.0 software (SPSS, Chicago, Illinois) was used for all statistical analyses.

RESULTS

As previously shown second-phase insulin secretion measured with the hyperglycemic clamp was only slightly reduced in the subjects with IGT ($P > 0.1$), whereas all

other measures of glucose-stimulated insulin release and ISI were significantly lower (all $P < 0.0001$; Table 1) (28). Genotype distributions for each of the tested gene variants are given in Table 2. Genotype distributions were comparable with other Caucasian populations.

First, no associations were found with insulin sensitivity with the sole exception of *THADA*, where we noted a significantly lower ISI ($P = 6.9 \times 10^{-3}$) in carriers of the T risk allele. Five loci, however, significantly affected β-cell function. These associations are shown in Table 2 and will be briefly summarized below. Throughout, reported *P* values represent the values obtained for the full model that includes the genotype of interest and age, sex, BMI, glucose tolerance status, family relatedness, and insulin sensitivity (where appropriate) as covariates. A model without BMI yielded essentially the same results (data not shown). A meta-analysis of the results in the three separate study samples instead of the analysis of the pooled data yielded virtually identical results (data not shown).

CDC123/CAMK1D. The rs12779790 variant in the *CDC123/CAMK1D* locus was not significantly associated with first-phase GSIS; however, we do note a significantly decreased second-phase GSIS in carriers of the at-risk genotype (Table 2; $P = 4.9 \times 10^{-3}$). The response to GLP-1, arginine stimulation, and insulin sensitivity were not significantly different, although we do note a trend toward a reduced response to arginine (−32%; $P = 0.015$).

THADA. Because the protective C/C genotype of the rs7578597 SNP is only present in three subjects, we pooled the CC and CT genotype groups. The TT risk genotype was not significantly associated with first-phase GSIS ($P = 0.77$), but all other measures of β-cell function were reduced (11–37%), although not always statistically significant: second-phase insulin response ($P = 0.019$), DI ($P = 0.039$), GLP-1 ($P = 1.6 \times 10^{-3}$), and arginine-stimulated insulin response (2.3×10^{-4}; Table 2). As stated above we also noted a significantly lower ISI ($P = 6.9 \times 10^{-3}$) in carriers of the at-risk genotype.

ADAMTS9. Analysis of rs4607103 in *ADAMTS9* provided evidence for an effect on first-phase GSIS. Carriers of the type 2 diabetes risk genotype CC showed, paradoxically, a 40% increased first-phase GSIS than the nonrisk TT reference genotype ($P = 5.9 \times 10^{-3}$). This effect was similar in direction in both NGT and IGT subjects (Table 3). Furthermore, the risk allele carriers also showed a higher DI ($P = 2.6 \times 10^{-3}$). Second-phase GSIS, the response to GLP-1 or arginine, and ISI were not significantly affected by the *ADAMTS9* genotype.

BCL11A. Carriers of the rs10490072 TT risk genotype of the *BCL11A* locus had on average a 16% lower first-phase GSIS ($P = 3.1 \times 10^{-3}$). The DI was also lower, although not statistically significant ($P = 0.010$). Other measures of β-cell function and ISI were not significantly different (Table 2).

MTNR1B. The risk allele for *MTNR1B* was significantly associated with a decreased DI ($P = 1.5 \times 10^{-3}$) but not other measures of glucose-stimulated insulin secretion. Although not statistically significant, there were increased responses to GLP-1 (30%; $P = 0.026$) and arginine stimulation (19%; $P = 0.037$) in carriers of the risk allele for rs10830963.

Other novel type 2 diabetes loci. Gene variants in the *JAZF1*, *TSPAN8/LGR5*, *DCD*, *NOTCH2/ADAM30*, and *VEGFA* loci were not significantly associated with any of the β-cell measures or insulin sensitivity (Table 2).

DISCUSSION

The DIAGRAM consortium and others recently showed that *JAZF1*, *CDC123/CAMK1D*, *TSPAN8/LGR5*, *THADA*, *ADAMTS9*, *NOTCH2/ADAMS30*, *HNF1B*, *WFS1*, *MTNR1B*, and possibly also *DCD*, *VEGFA*, and *BCL11A* should be added to the list of confirmed type 2 diabetes loci (8,14–19). In this study we have shown that gene variants in five of these loci are associated with measures of β-cell function obtained during hyperglycemic clamps, either in response to glucose alone and/or in combination with other β-cell secretagogues during hyperglycemia. In contrast to our previous work, which showed that most other known loci primarily affect first-phase GSIS (6,7,34), the current set of loci also affected various other aspects of β-cell function.

***CDC123/CAMK1D*, rs12779790.** Previously, Grarup et al. (9) reported that the G risk allele of rs12779790 *CDC123/CAMK1D* was associated with a lower insulinogenic index, corrected insulin response, and area under the insulin/glucose curve during OGTTs. They also noted a lower DI in carriers of the G allele. The β-cell defect was confirmed in a study of subjects of Asian Indian descent (10). Three other studies in Caucasians failed to replicate the observation made by Grarup et al. However, in all three studies a similar, though not significant, trend toward lower β-cell function could be observed (11–13). These results are in line with our observation of a lower insulin response to glucose stimulation. We also noted a trend toward a reduced insulin response after arginine stimulation (−32%; $P = 0.015$). Arginine stimulation during hyperglycemia is a measure of (near) maximal insulin secretion and has been suggested as a proxy for β-cell mass. Given the putative role of *CAMK1D* in granulocyte function, it seems plausible that this gene variant affects β-cell function by causing reduced β-cell mass due to enhanced apoptosis (35). Further research, however, is needed to verify this hypothesis.

***THADA*, rs7578597.** We have shown that homozygous carriers of the risk allele have lower levels of various measures of β-cell function. This was not previously reported in any of the OGTT-based studies, although Stancakova et al. showed some evidence for a reduced early phase insulin response ($P = 0.045$) (13). *THADA*, encoding thyroid adenoma–associated protein, has been suggested to be involved in the death receptor pathway and apoptosis (36). Given the fact that the gene variant is associated with reduced response to arginine stimulation during the clamp, this could imply that those subjects with the rs7578597 (T1187A) gene variant in *THADA* have a reduced β-cell mass due to increased apoptosis. Again, further studies are needed to confirm our hypothesis of increased apoptosis and lower β-cell mass as the underlying disease mechanism. The *THADA* variant was the only variant associated with insulin sensitivity; this, however, was not corroborated by any of the other studies and may thus be a false-positive association.

***ADAMTS9*, rs4607103.** Remarkably, we noted a significantly increased first-phase GSIS and DI in carriers of the risk allele. The observed increased β-cell function was present in all separate samples and in NGT and IGT subjects when analyzed separately, arguing against a chance finding. Also Lyssenko et al. (11) reported an increased DI during follow-up in carriers of the risk genotype. The other studies, however, did not report any changes in β-cell function or insulin sensitivity

TABLE 2
Insulin response according to genotype

Gene	*n*	First-phase insulin response (pmol/l)	Second-phase insulin response (pmol/l)	ISI (μmol · min^{-1} · kg^{-1} · pmol^{-1} · l^{-1})	DI (μmol · min^{-1} · kg^{-1})	*n* (GLP-1, Arg)	GLP-1–stimulated insulin release (pmol/l)*	Arginine-stimulated insulin release (pmol/l)*
JAZF1, rs864745								
C/C	73	727 (652–812)	262 (236–292)	0.141 (0.122–0.162)	107 (95–121)	26	1,034 (799–1,337)	1,728 (1,495–1,998)
C/**T**	161	723 (672–778)	239 (223–255)	0.155 (0.142–0.170)	111 (103–120)	48	1,374 (1,122–1,683)	1,992 (1,727–2,297)
T/T	100	759 (686–841)	263 (243–286)	0.160 (0.145–0.177)	124 (111–139)	49	1,200 (951–1,514)	2,233 (1,969–2,532)
P		0.54	0.80	0.15	0.07		0.63	0.018
CDC123/ CAMK1D, rs12779790								
A/A	212	755 (704–810)	260 (245–275)	0.155 (0.143–0.168)	117 (109–127)	74	1,318 (1,094–1,588)	2,181 (1,979–2,403)
A/**G**	110	713 (656–774)	238 (220–258)	0.153 (0.138–0.169)	112 (101–123)	48	1,106 (881–1,389)	1,817 (1,588–2,078)
G/G	12	617 (478–797)	200 (176–228)	0.146 (0.108–0.198)	94 (71–125)	1	1,142 (913–1,428)	1,486 (1,322–1,671)
P		0.10	0.0049	0.68	0.16		0.24	0.015
TSPAN8/LGR5, rs7961581								
T/T	159	738 (687–793)	253 (237–270)	0.149 (0.135–0.164)	113 (103–123)	47	1,253 (1,028–1,529)	2,094 (1,860–2,357)
T/**C**	141	724 (668–784)	247 (229–265)	0.158 (0.142–0.175)	113 (105–123)	65	1,222 (994–1,503)	2,024 (1,797–2,280)
C/C	34	738 (613–889)	254 (219–295)	0.160 (0.135–0.190)	118 (97–142)	11	1,148 (796–1,657)	1,710 (1,362–2,146)
P		0.88	0.84	0.34	0.72		0.73	0.24
THADA, rs7578597								
C/C	3	905 (484–1694)	365 (317–421)	0.125 (0.067–0.230)	121 (80–182)	0	NA	NA
C/**T**	72	739 (662–825)	271 (247–296)	0.180 (0.160–0.204)	127 (113–142)	25	1,783 (1,352–2,352)	2,605 (2,236–3,035)
T/T	261	732 (689–778)	244 (232–257)	0.147 (0.137–0.158)	110 (103–118)	98	1,120 (970–1,292)	1,897 (1,744–2,064)
P		0.77†	0.019†	0.0069†	0.039†		0.0016†	0.00023†
ADAMTS9, rs4607103								
T/T	20	549 (467–646)	206 (172–246)	0.136 (0.106–0.175)	83 (69–99)	7	777 (597–1,011)	1,632 (1,335–1,994)
T/**C**	119	725 (668–787)	256 (238–274)	0.152 (0.137–0.169)	111 (101–123)	47	1,291 (1,028–1,621)	1,990 (1,753–2,260)
C/C	187	767 (714–824)	252 (237–268)	0.157 (0.145–0.171)	121 (112–130)	69	1,244 (1,032–1,498)	2,094 (1,866–2,350)
P		0.0059	0.26	0.32	0.0026		0.38	0.18
NOTCH2/ADAM30, rs2641348								
A/A	253	736 (692–782)	248 (234–262)	0.152 (0.141–0.163)	114 (107–121)	94	1,226 (1,045–1,438)	2,035 (1,858–2,228)
A/**G**	73	746 (661–841)	256 (230–285)	0.154 (0.133–0.179)	113 (97–131)	27	1,228 (896–1,683)	2,036 (1,671–2,482)
G/G	10	654 (502–852)	278 (242–319)	0.189 (0.156–0.229)	121 (96–152)	2	1,323 (1,100–1,593)	1,398 (1,251–1,563)
P		0.76	0.33	0.37	0.89		0.93	0.59
DCD, rs1153188								
T/T	24	811 (670–982)	279 (243–321)	0.169 (0.136–0.210)	128 (103–160)	5	1,448 (1,143–1,834)	2,068 (1,467–2,915)
T/**A**	120	726 (675–781)	248 (231–267)	0.154 (0.138–0.171)	113 (103–124)	40	1,018 (757–1,368)	1,976 (1,723–2,268)
A/A	192	732 (678–790)	247 (232–263)	0.152 (0.140–0.165)	113 (104–123)	78	1,336 (1,151–1,551)	2,043 (1,845–2,262)
P		0.55	0.29	0.49	0.48		0.27	0.83
VEGFA, rs9472138								
C/C	176	722 (674–774)	245 (231–260)	0.156 (0.145–0.169)	114 (106–123)	68	1,207 (1,014–1,436)	1,908 (1,715–2,121)
C/**T**	131	765 (704–832)	263 (243–284)	0.153 (0.136–0.172)	117 (106–130)	48	1,278 (989–1,652)	2,203 (1,942–2,498)
T/T	28	695 (578–835)	229 (197–268)	0.141 (0.115–0.174)	101 (83–123)	7	1,096 (556–2,161)	1,922 (1,267–2,917)
P		0.77	0.80	0.44	0.55		0.97	0.35
BCL11A, rs10490072								
C/C	32	810 (703–934)	226 (199–256)	0.169 (0.141–0.201)	132 (111–158)	13	812 (595–1,108)	1,774 (1,553–2,028)
C/**T**	126	799 (738–866)	255 (237–274)	0.145 (0.131–0.161)	120 (110–132)	49	1,266 (978–1,639)	2,073 (1,814–2,369)
T/T	178	685 (637–737)	251 (236–268)	0.157 (0.144–0.171)	107 (99–116)	61	1,311 (1,139–1,508)	2,040 (1,810–2,300)
P		0.0031	0.39	0.92	0.010		0.060	0.41

TABLE 2
Continued

Gene	*n*	First-phase insulin response (pmol/l)	Second-phase insulin response (pmol/l)	ISI (μmol · min^{-1} · kg^{-1} · pmol^{-1} · l^{-1})	DI (μmol · min^{-1} · kg^{-1})	*n* (GLP-1, Arg)	GLP-1–stimulated insulin release (pmol/l)*	Arginine-stimulated insulin release (pmol/l)*
HNF1B, rs757210								
C/C	118	746 (696–799)	255 (237–274)	0.149 (0.134–0.166)	112 (103–122)	51	1,218 (966–1,535)	2,049 (1,792–2,342)
C/**T**	145	737 (672–809)	251 (233–270)	0.154 (0.139–0.170)	116 (105–128)	49	1,265 (1,034–1,546)	2,034 (1,828–2,263)
T/T	71	704 (634–782)	240 (218–263)	0.161 (0.144–0.179)	111 (99–125)	23	1,174 (874–1,577)	1,946 (1,586–2,387)
P		0.38	0.33	0.35	0.99		0.93	0.70
WFS1, rs10010131								
A/A	39	623 (527–737)	258 (217–306)	0.160 (0.128–0.200)	99 (84–117)	11	1,564 (1,155–2,120)	2,311 (1,773–3,011)
A/**G**	176	751 (701–804)	257 (243–272)	0.149 (0.138–0.162)	114 (106–123)	66	1,298 (1,086–1,551)	2,066 (1,854–2,303)
G/G	119	749 (686–818)	238 (221–257)	0.158 (0.143–0.175)	119 (108–131)	46	1,072 (848–1,356)	1,900 (1,663–2,171)
P		0.14	0.21	0.81	0.09		0.058	0.18
MTNR1B, rs10830963								
C/C	187	757 (706–813)	239 (226–253)	0.163 (0.150–0.177)	122 (112–131)	57	1,044 (865–1,259)	1,869 (1,675–2,085)
C/**G**	113	758 (700–821)	270 (248–294)	0.139 (0.123–0.157)	110 (101–120)	49	1,440 (1,142–1,814)	2,157 (1,868–2,490)
G/G	35	561 (487–647)	239 (207–276)	0.158 (0.132–0.190)	90 (77–106)	17	1,360 (1,084–1,705)	2,231 (1,973–2,523)
P		0.010	0.27	0.22	0.0015		0.026	0.037

Data are estimated means (95% CI) unless otherwise indicated. Alleles identified as risk alleles for type 2 diabetes are indicated in bold. All variables were log transformed before analysis. *P* values were computed for additive models using linear generalized estimating equations, which takes into account the family relatedness when computing the standard errors. First- and second-phase GSIS and GLP-1– and arginine-stimulated insulin secretion were adjusted for study center, family relatedness, glucose tolerance status, age, sex, BMI, and ISI. ISI and DI were adjusted for study center, family relatedness, glucose tolerance status, age, sex, and BMI. *Available for 123 subjects from the NTR twin sample. †*P* values are for the recessive model.

(9,10,12,13). Given these counterintuitive results and the unknown function of *ADAMTS9* in type 2 diabetes susceptibility and/or β-cell function, our data warrant further replication and studies into the disease mechanism.

***BCL11A*, rs10490072.** For carriers of the risk allele in *BCL11A* we noted a significant reduction in first-phase GSIS. Only Staiger et al. (12) included *BCL11A* in their analyses, and they did not corroborate our results. *BCL11A*, encoding B-cell CLL/lymphoma 11A, has been implicated in several blood-related phenotypes and acts as a DNA sequence–specific transcriptional repressor, acting on genes like *BCL6*, *COUP-TF*, and *SIRT1* (37). Sirtuins like *SIRT1* have been implicated in several processes directly linked to type 2 diabetes (38), and one may speculate that *BCL11A* gene variants exert their effect via the regulation of *SIRT1* expression.

***MTNR1B*, rs10830963.** Recently, the melatonin receptor 1B gene has been identified as a novel type 2 diabetes and fasting plasma glucose gene (17–19). Also in this study the risk allele was associated with increased fasting plasma glucose levels ($P = 0.004$). Several studies have shown that gene variants in this locus are associated with lower oral and intravenous glucose–stimulated insulin secretion (39). Our results regarding the lower DI seem to corroborate these previous findings. Alhough not formally statistically significant due to the smaller sample size, we surprisingly also noted increased insulin responses toward GLP-1 (30%) and arginine stimulation (19%). This seems to contradict the observed decreased insulin response to oral glucose during OGTT in *MTNR1B* carriers because it is known that the insulin response to oral glucose is in part mediated via the positive effects of incretins like GLP-1 (40). In vitro short-term exposure of β-cells and islets to melatonin results in a decreased insulin response to glucose and GLP-1 (39), but studies using INS-1E cells have also suggested that prolonged exposure to melatonin, in contrast to short-term exposure, results in a potentiation of the response to GLP-1 (41). If replicated our results indicate that carriers of this gene variant may well benefit from treatment with GLP-1 agonists or dipeptidyl peptidase-IV inhibitors.

***WFS1*.** Previously, it has been reported that *WFS1* gene variants are associated with reduced insulin response to oral but not intravenous glucose (11,13,20–22). In line with those previous reports we also could not detect an effect of intravenous glucose. Furthermore, Schäfer et al. (22) demonstrated a reduced response to GLP-1 stimulation during hyperglycemic clamps. In this study with similar size and power, we were unable to confirm this observation. Our data do not confirm previously reported β-cell defects in *JAZF1* and *TSPAN8* (9), which is in line with the other reports based on OGTTs (10–13).

One of the main limitations of the current study is the relatively small number of participants. Although this is the largest study applying the gold-standard method for assessing β-cell function, the hyperglycemic clamp, we cannot exclude that we have missed subtle defects associated with the various gene variants, especially given the fact that their effects on type 2 diabetes risk are also small. Furthermore, we have applied a rather lenient correction for multiple hypotheses testing, which means that some of the current findings may be spurious. Our results should therefore be regarded exploratory, and we fully subscribe the need for replication, although such replication is nontrivial because the hyperglycemic clamp methodology

TABLE 3
Insulin response according to genotype in NGT and IGT subjects (genes with significant effects only)

	NGT					IGT				
Gene	*n*	First-phase insulin response (pmol/l)	Second-phase insulin response (pmol/l)	ISI (μmol · min^{-1} · kg^{-1} · $pmol^{-1}$ · l^{-1})	DI (μmol · min^{-1} · kg^{-1})	*n*	First-phase insulin response (pmol/l)	Second-phase insulin response (pmol/l)	ISI (μmol · min^{-1} · kg^{-1} · $pmol^{-1}$ · l^{-1})	DI (μmol · min^{-1} · kg^{-1})
CDC123/ CAMK1D, rs12779790										
C/C	109	888 (812–971)	257 (239–276)	0.202 (0.182–0.225)	178 (160–199)	103	717 (610–843)	249 (216–286)	0.109 (0.090–0.132)	79 (67–93)
C/**T**	66	792 (714–877)	247 (226–269)	0.202 (0.177–0.230)	162 (146–181)	44	715 (584–875)	208 (174–249)	0.102 (0.081–0.128)	77 (61–97)
T/T	5	720 (540–962)	197 (164–237)	0.221 (0.166–0.295)	154 (122–193)	7	564 (363–877)	186 (144–239)	0.096 (0.059–0.157)	60 (36–99)
P		0.034	0.14	0.89	0.13		0.42	0.0028	0.44	0.37
THADA, rs7578597										
C/C	1	1,109 (1,005–1,224)	388 (355–424)	0.280 (0.247–0.318)	264 (240–290)	2	920 (359–2,358)	360 (270–478)	0.070 (0.035–0.138)	76 (46–124)
C/**T**	43	843 (728–976)	272 (244–303)	0.249 (0.215–0.288)	192 (164–224)	29	730 (570–936)	257 (209–316)	0.122 (0.092–0.162)	87 (68–112)
T/T	136	840 (776–910)	243 (228–259)	0.190 (0.173–0.208)	164 (150–180)	125	710 (606–833)	232 (202–266)	0.104 (0.087–0.125)	76 (64–90)
P		0.91*	0.057*	0.0017*	0.073*		0.67*	0.14*	0.32*	0.21*
ADAMTS9, rs4607103										
T/T	12	694 (581–830)	207 (171–251)	0.182 (0.122–0.271)	137 (110–170)	8	487 (340–699)	193 (137–272)	0.093 (0.068–0.127)	52 (37–73)
T/**C**	71	832 (759–912)	245 (224–267)	0.204 (0.180–0.231)	169 (150–191)	54	699 (582–840)	252 (215–296)	0.102 (0.082–0.128)	74 (62–89)
C/C	97	867 (788–955)	259 (241–279)	0.204 (0.182–0.228)	176 (160–194)	94	747 (625–892)	227 (196–263)	0.111 (0.092–0.135)	84 (69–102)
P		0.11	0.054	0.71	0.12		0.051	0.75	0.22	0.012
BCL11A, rs10490072										
C/C	18	976 (828–1,151)	230 (197–269)	0.213 (0.172–0.263)	210 (170–258)	14	740 (553–990)	207 (159–268)	0.127 (0.091–0.177)	84 (60–119)
C/**T**	71	885 (799–979)	261 (241–283)	0.191 (0.168–0.217)	175 (155–197)	55	815 (663–1,000)	231 (196–272)	0.101 (0.082–0.126)	85 (70–104)
T/T	91	785 (719–858)	246 (227–267)	0.211 (0.188–0.236)	161 (146–177)	87	670 (570–787)	238 (205–276)	0.108 (0.088–0.132)	74 (62–88)
P		0.0066	0.96	0.59	0.019		0.10	0.33	0.73	0.19
MTNR1B, rs10830963										
C/C	91	853 (778–936)	243 (226–260)	0.212 (0.189–0.237)	177 (159–196)	96	762 (645–900)	226 (195–263)	0.115 (0.094–0.140)	88 (74–104)
C/**G**	65	882 (802–970)	267 (241–295)	0.188 (0.160–0.221)	172 (154–193)	48	732 (609–879)	260 (218–309)	0.093 (0.075–0.114)	73 (61–87)
G/G	21	696 (593–818)	241 (210–276)	0.228 (0.191–0.272)	157 (135–183)	14	509 (410–633)	223 (175–284)	0.096 (0.066–0.141)	53 (40–70)
P		0.23	0.49	0.83	0.31		0.0067	0.38	0.078	0.00036

Data are estimated means (95% CI) unless otherwise indicated. Alleles identified as risk alleles for type 2 diabetes are indicated in bold. All variables were log transformed before analysis. *P* values were computed for additive models using linear generalized estimating equations, which take into account the family relatedness when computing the standard errors. First- and second-phase GSIS were adjusted for study center, family relatedness, age, sex, BMI, and ISI. ISI and DI were adjusted for study center, family relatedness, glucose tolerance status, age, sex, and BMI. **P* values are for the recessive model.

is demanding for both researchers and participants. However, our current results clearly justify these investments.

A further limitation is the inclusion of a mix of NGT and IGT subjects. It is well known that subjects with IGT often have insulin resistance and/or insufficient β-cell function to maintain normal glucose homeostasis and are thus at high risk to develop type 2 diabetes. One may argue that the observed associations with decreased β-cell function are thus due to the known association with type 2 diabetes and the risk implied by the IGT state. However, our data analyzing NGT and IGT subjects separately showed that the direction of the effects for the gene variants we found to be associated was in general similar in both groups and not mainly driven by the IGT subjects, arguing against this potential bias. Furthermore, we used a random-effects meta-analysis approach to test whether the relationship between the genes and the outcome variables is homogeneous over the three cohorts. Also, this analysis yielded virtually identical results, providing further evidence that our data are not influenced by the inclusion of the IGT subjects. However, although the associations we found are resistant to the above-described analyses and present in both NGT and IGT subjects, we cannot exclude that for other genes/loci this would not be the case.

In conclusion, we found novel associations between gene variants in *THADA*, *ADAMTS9*, and *BCL11A* loci and various aspects of β-cell function. In carriers of the *THADA* variant we observed decreases in both GLP-1– and arginine-induced insulin release hinting at lower β-cell function and/or mass. Carriers of gene variants in *ADAMTS9* and *BCL11A* show alterations in first-phase GSIS, suggesting they may primarily affect processes involved in the rapid recruitment and release of insulin from insulin granules.

In addition to the above-mentioned associations we have confirmed that a gene variant in *CDC123/CAMK1D* is associated with reduced β-cell function, and our data suggest it may do so via a reduced β-cell mass. Furthermore, our data suggest that carriers of the *MTNR1B* risk allele may be more sensitive toward the stimulatory effects of GLP-1, which may offer therapeutic possibilities if confirmed. These findings point to a clear diversity in the impact that these various gene variants may have on (dys)function of pancreatic β-cells and justify the use of the hyperglycemic clamp methodology, especially with additional secretagogues, to resolve the pathogenic mechanisms of these loci.

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