Association Testing of Novel Type 2 Diabetes Risk Alleles in the *JAZF1*, *CDC123/CAMK1D*, *TSPAN8*, *THADA*, *ADAMTS9*, and *NOTCH2* Loci With Insulin Release, Insulin Sensitivity, and Obesity in a Population-Based Sample of 4,516 Glucose-Tolerant Middle-Aged Danes

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OBJECTIVE—We evaluated the impact on diabetes-related intermediary traits of common novel type 2 diabetes–associated variants in the *JAZF1* (rs864745), *CDC123/CAMK1D* (rs12779790), *TSPAN8* (rs7961581), *THADA* (rs7578597), AD *AMTS9* (rs4607103), and *NOTCH2* (rs10923931) loci, which were recently identified by meta-analysis of genome-wide association data.

RESEARCH DESIGN AND METHODS—We genotyped the six variants in 4,516 middle-aged glucose-tolerant individuals of the population-based Inter99 cohort who were all characterized by an oral glucose tolerance test (OGTT).

RESULTS—Homozygous carriers of the minor diabetes risk G-allele of the *CDC123/CAMK1D* rs12779790 showed an 18% decrease in insulinogenic index (95% CI 10–27%; $P = 4 \times 10^{-5}$), an 18% decrease in corrected insulin response (CIR) (8.1–29%; $P = 4 \times 10^{-4}$), and a 13% decrease in the ratio of area under the serum-insulin and plasma-glucose curves during an OGTT (AUC-insulin/AUC-glucose) (5.8–20%; $P = 4 \times 10^{-4}$). Carriers of the diabetes-associated T-allele of *JAZF1* rs864745 had an allele-dependent 3% decrease in BIGTT-AIR (0.9–4.3%; $P = 0.003$). Furthermore, the diabetes-associated C-allele of *TSPAN8* rs7961581 associated with decreased levels of CIR (4.5% [0.5–8.4]; $P = 0.03$), of AUC-insulin/AUC-glucose ratio (3.9% [1.2–6.7]; $P = 0.005$), and of the insulinogenic index (5.2% [1.9–8.6]; $P = 0.002$). No association with traits of insulin release or insulin action was observed for the *THADA*, *ADAMTS9*, or *NOTCH2* variants.

CONCLUSIONS—If replicated, our data suggest that type 2 diabetes at-risk alleles in the *JAZF1*, *CDC123/CAMK1D*, and *TSPAN8* loci associate with various OGTT-based surrogate measures of insulin release, emphasizing the contribution of abnormal pancreatic β-cell function in the pathogenesis of type 2 diabetes. ***Diabetes* 57:2534–2540, 2008**

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Recent discoveries using genome-wide association (GWA) studies have led to progression in the understanding of the molecular genetic background of type 2 diabetes, dramatically increasing the number of common validated type 2 diabetes loci with modest impact on relative diabetes risk (1–5). The Diabetes Genetics Replication and Meta-analysis (DIAGRAM) consortium recently reported the outcome of a meta-analysis of data from three GWA studies. Six additional type 2 diabetes loci reaching genome-wide significance levels were identified in the *JAZF1*, *CDC123/CAMK1D*, *TSPAN8*, *THADA*, *ADAMTS9*, and *NOTCH2* loci; all were modestly affecting disease risk with odds ratios between 1.09 and 1.15 (6).

As for most other findings obtained from GWA studies, little is known about the function of the putative regional candidate genes thought to be affected by the at-risk variants. Recent studies have, however, shown that many validated type 2 diabetes risk variants confer an impaired pancreatic β-cell function, which seems to be the case for risk alleles in the *CDKAL1*, *SLC30A8*, *HHEX/IDE*, *CDKN2A/2B*, *IGF2BP2*, *TCF7L2*, and *KCNJ11* loci (2,7–9). Indeed, only the *PPARG* Pro12Ala variant has so far displayed a diabetogenic potential through affecting peripheral insulin sensitivity (10) and variants in *FTO* by increasing fat accumulation (11). Of the six novel type 2 diabetes loci (6), the biological function of *NOTCH2* points to an impact on pancreatic β-cell function because of its critical role in fetal pancreatic development (12), yet little or no prior implication in the pathogenesis of type 2 diabetes or diabetes-related phenotypes can be claimed for genes in the *JAZF1*, *CDC123/CAMK1D*, *TSPAN8*, *THADA*, or *ADAMTS9* regions.

Given the sparse knowledge of the biological functions of the six novel type 2 diabetes–associated variants, we have characterized the influence of these variants on quantitative surrogate measures of oral glucose-stimulated insulin release, insulin sensitivity, and body fat accumulation in a population-based study of glucose-tolerant middle-aged Danes who all had undertaken an oral glucose tolerance test (OGTT).

RESEARCH DESIGN AND METHODS

Studies of quantitative metabolic traits were performed in the Inter99 cohort, which is a population-based, randomized, nonpharmacological intervention

TABLE 1
Unadjusted quantitative metabolic traits in the population-based Inter99 cohort including 4,377 middle-aged subjects with normal glucose tolerance stratified according to genotype of *JAZF1* rs864745

	11 (CC)	12 (CT)	22 (TT)	$P_{ADDITIVE}$	$P_{22 + 12 \text{ VS. } 11}$	$P_{22 \text{ VS. } 12 + 11}$
n (men/women)	996 (453/543)	2,238 (1,056/1,182)	1,143 (513/630)			
Age (years)	45.5 ± 8	45.2 ± 7.8	45.1 ± 7.7			
BMI (kg/m^2)	25.7 ± 4.3	25.6 ± 4.1	25.2 ± 3.9	0.02	0.2	0.008
Waist (cm)	84 ± 13	84 ± 12	83 ± 12	0.04	0.2	0.03
Fasting serum insulin (pmol/l)	33 (23–48)	32 (23–46)	31 (22–44)	0.2	0.1	0.5
Serum insulin at 30 min (pmol/l)	246 (180–359)	250 (182–347)	235 (168–341)	0.3	0.6	0.2
Serum insulin at 120 min (pmol/l)	142 (92–219)	141 (87–212)	134 (87–209)	0.7	0.6	0.8
Fasting plasma glucose (mmol/l)	5.3 (5.0–5.6)	5.3 (5.1–5.6)	5.3 (5.1–5.6)	0.08	0.2	0.1
Plasma glucose at 30 min (mmol/l)	8.2 (7.2–9.1)	8.1 (7.2–9.2)	8.2 (7.2–9.2)	0.7	0.8	0.7
Plasma glucose at 120 min (mmol/l)	5.7 (4.9–6.4)	5.6 (4.7–6.4)	5.6 (4.8–6.3)	0.9	0.6	0.4
ISI	0.13 (0.09–0.18)	0.13 (0.09–0.19)	0.14 (0.09–0.19)	0.3	0.2	0.7
BIGTT-S_I	10.2 ± 3.8	10.3 ± 3.6	10.5 ± 3.7	0.06	0.3	0.06
AUC-insulin/AUC-glucose	28.0 (20.8–38.3)	27.6 (20.8–37.8)	26.5 (19.3–36.9)	0.2	0.5	0.2
CIR	760 (477–1,220)	749 (487–1,210)	747 (462–1,150)	0.4	0.5	0.4
Insulinogenic index	26.1 (18.1–39.1)	26.3 (18.5–38)	25.5 (17–37)	0.4	0.8	0.3
BIGTT-AIR	1,700 (1,370–2,150)	1,690 (1,350–2,120)	1,610 (1,320–2,060)	0.003	0.03	0.007

Data are medians (25% to 75% range) or means ± SD (BMI, waist, and BIGTT-S_I). Values of BMI, plasma glucose, serum insulin, and derived indices were logarithmically transformed before statistical analysis. Calculated *P* values were adjusted for age (BIGTT-S_I and BIGTT-AIR), age and sex (BMI and waist), or age, sex, and BMI (all other traits), assuming an additive, dominant, or recessive model. Indices of insulin release and insulin sensitivity were calculated as described in research design and methods. 1, type 2 diabetes–protective allele; 2, diabetes-associated allele.

study of 6,784 middle-aged subjects for the prevention of ischemic heart disease, conducted at the Research Centre for Prevention and Health in Glostrup, Copenhagen (ClinicalTrials.gov ID-no: NCT00289237) (13). An OGTT was performed in all participants with measurements of plasma glucose and serum insulin at fasting and at 30 and 120 min, and 6,083 subjects with available DNA were subsequently classified as individuals with normal glucose tolerance (NGT) (n = 4,516), impaired fasting glycemia (n = 503), impaired glucose tolerance (n = 692), screen-detected and treatment-naïve type 2 diabetes (n = 253), or previously diagnosed type 2 diabetes (n = 119). In the analysis of quantitative diabetes-related phenotypes, we included 4,516 subjects with NGT (2,101 men/2,415 women, age 45.2 ± 7.9 years and BMI 25.5 ± 4.1 kg/m^2 [mean ± SD]). Type 2 diabetes was diagnosed according to World Health Organization 1999 criteria.

Informed written consent was obtained from all participants. The study was conducted in accordance with the Declaration of Helsinki II and was approved by the local ethics committee of Copenhagen.

Biochemical and anthropometrical measures. Height and weight were measured in light indoor clothing and without shoes. Waist circumference was measured in the upright position midway between the iliac crest and the lower costal margin. Blood samples were drawn after a 12-h overnight fast. Plasma glucose was analyzed by a glucose oxidase method (Granutest; Merck, Darmstadt, Germany). Serum insulin [excluding des (31,32)] and intact proinsulin) was measured using the AutoDELFIA insulin kit (Perkin-Elmer, Wallac, Turku, Finland).

Indexes of insulin release and insulin sensitivity. Oral glucose-stimulated insulin release was reported as the insulinogenic index, the corrected insulin response (CIR), the ratio of the area under the curve (AUC) of insulin to the AUC of glucose during the OGTT (AUC-insulin/AUC-glucose), and the BIGTT-acute insulin response (AIR) index. The insulinogenic index was calculated as follows: (serum insulin$_{30\ min}$ − serum insulin$_{0\ min}$ [pmol/l])/plasma glucose$_{30\ min}$ [mmol/l]. CIR was calculated as follows: 100 × serum insulin$_{30\ min}$/[plasma glucose$_{30\ min}$ × (plasma glucose$_{30\ min}$ − 3.89)] (14). Indexes of insulin sensitivity were reported as the insulin sensitivity index (ISI), calculated as the reciprocal of homeostasis model assessment of insulin resistance {22.5/[plasma glucose$_{0\ min}$ (mmol/l) × serum insulin$_{0\ min}$ (pmol/l)]} (15), and as the OGTT-derived BIGTT-S_I. The BIGTT indexes apply information on sex and BMI combined with plasma glucose and serum insulin during an OGTT to provide indexes for AIR and S_I that are highly correlated with indexes obtained during an intravenous glucose tolerance test and were calculated as reported (16). To construct OGTT-based disposition indexes, we multiplied the CIR index with ISI, since these measures are not intrinsically interdependent. Furthermore, we multiplied the BIGTT-S_I index with the BIGTT-AIR index.

Genotyping. The six gene variants (rs864745, rs12779790, rs7961581, rs7578597, rs4607103, and rs10923931) were genotyped by TaqMan allelic discrimination (KBiosciences, Hoddesdon, U.K). All genotyping success rates were above 96% and all mismatch rates were below 1% in 1,090 duplicate samples. The distributions of genotypes for all variants were in the Hardy-Weinberg equilibrium (all $P > 0.05$).

Statistical analysis. A general linear statistical methodology was used to test quantitative traits in relation to genotype, applying additive, dominant, and recessive models while adjusting for the effect of age (BIGTT-S_I and BIGTT-AIR), age and sex (BMI and waist), or age, sex, and BMI (all other traits). BMI and all values of plasma glucose and serum insulin and derived indexes of insulin release and insulin sensitivity were logarithmically transformed before analysis. In the main text, parameter estimates (95% CI) of associated quantitative traits are given, while data in tables are unadjusted medians or means. The multivariate method, Hotelling's T^2, was applied to test the simultaneous effect of genotype on insulin release and insulin sensitivity. A *P* value of <0.05 was considered significant. All analyses were performed using RGui, version 2.6.1 (http://www.r-project.org).

Estimation of statistical power. Statistical power for the quantitative traits was estimated using simulations. We assumed an additive genetic model for both the simulation of the data and for testing the data using a linear model. We used the empirical variance of the observed traits to simulate phenotypes from a normal distribution so that variance across genotypes is drawn from the estimated variance. Because we also include adjustment factors in our analysis, we estimated the variance from the residuals of a linear model containing the adjustment factors. Thus, we assume that the genotype and the adjustment factors are independent. The power was estimated using 5,000 simulations and a significance threshold of 0.05. Based on the allele frequencies of the six examined gene variants and a sample size of 4,516 subjects, we estimated the effect sizes per allele of quantitative traits for which we had 80 and 90% statistical power, respectively, to detect an association. Depending on allele frequency (range 9.5–48.0%) and assuming an additive model, we had 80% power to detect an allele-dependent difference of 0.8–1.4% in BMI, 2.2–3.8% in BIGTT-AIR, 3.2–5.4% in insulinogenic index, and 3.0–5.0% in ISI. Similarly, we had 90% statistical power to detect a 1.0–1.7% change per allele in BMI, 2.6–4.3% in BIGTT-AIR, 3.7–6.2% in insulinogenic index, and 3.4–5.9% in ISI, respectively.

TABLE 2
Unadjusted quantitative metabolic traits in the population-based Inter99 cohort including 4,395 middle-aged subjects with normal glucose tolerance stratified according to genotype of *CDC123/CAMK1D* rs12779790

	11 (AA)	12 (AG)	22 (GG)	$P_{ADDITIVE}$	$P_{22 + 12 \text{ VS. } 11}$	$P_{22 \text{ VS. } 12 + 11}$
n (men/women)	2,859 (1,324/1,535)	1,365 (620/745)	171 (88/83)			
Age (years)	45.2 ± 7.8	45.3 ± 7.9	45.2 ± 8.1			
BMI (kg/m^2)	25.5 ± 4.1	25.5 ± 4.0	25.8 ± 4.6	0.8	0.9	0.5
Waist (cm)	84 ± 12	84 ± 12	85 ± 12	0.5	0.5	0.6
Fasting serum insulin (pmol/l)	32 (23–46)	32 (23–47)	31 (21–49)	0.7	0.7	0.06
Serum insulin at 30 min (pmol/l)	246 (178–351)	246 (180–347)	217 (159–299)	0.02	0.3	8×10^{-5}
Serum insulin at 120 min (pmol/l)	138 (87–212)	141 (92–216)	139 (80–190)	0.4	0.1	0.2
Fasting plasma glucose (mmol/l)	5.3 (5.0–5.6)	5.4 (5.1–5.6)	5.3 (5.1–5.6)	0.1	0.07	1
Plasma glucose at 30 min (mmol/l)	8.2 (7.2–9.2)	8.2 (7.2–9.1)	8.2 (7.4–9.3)	1	0.9	0.7
Plasma glucose at 120 min (mmol/l)	5.6 (4.7–6.3)	5.7 (4.9–6.4)	5.8 (4.8–6.4)	0.01	0.01	0.3
ISI	0.132 (0.09–0.189)	0.131 (0.09–0.188)	0.131 (0.084–0.201)	0.9	0.6	0.08
BIGTT-S_I	10.4 ± 3.7	10.2 ± 3.7	10.4 ± 3.7	0.4	0.3	0.8
AUC-insulin/AUC-glucose	27.6 (20.5–37.7)	27.2 (20.3–38.1)	25.4 (18.7–31.7)	0.1	0.6	4×10^{-4}
CIR	753 (480–1190)	752 (483–1240)	614 (402–926)	0.07	0.5	4×10^{-4}
Insulinogenic index	26.0 (18.3–38.3)	26.1 (18.2–37.7)	23.1 (15.3–30.4)	0.01	0.2	4×10^{-5}
BIGTT-AIR	1,680 (1,350–2,120)	1,670 (1,350–2,120)	1,620 (1,310–2,040)	0.3	0.5	0.2

Data are median (25% to 75% range) or means ± SD (BMI, waist, and BIGTT-S_I). Values of BMI, plasma glucose, serum insulin, and derived indices were logarithmically transformed before statistical analysis. Calculated *P* values were adjusted for age (BIGTT-S_I and BIGTT-AIR), age and sex (BMI and waist), or age, sex, and BMI (all other traits), assuming an additive, dominant, or recessive model. Indices of insulin release and insulin sensitivity were calculated as described in research design and methods. 1, type 2 diabetes–protective allele; 2, diabetes-associated allele.

RESULTS

We investigated the *JAZF1* rs864745, *CDC123/CAMK1D* rs12779790, *TSPAN8* rs7961581, *THADA* rs7578597, AD *AMTS9* rs4607103, and *NOTCH2* rs10923931 variants for association with type 2 diabetes–related quantitative traits in a population-based sample of 4,516 glucose-tolerant subjects. Assuming an additive genetic model, carriers of the major diabetes-associated T-allele of *JAZF1* rs864745 had a 0.21 kg/m^2 decreased BMI (0.048–0.39 kg/m^2; $P = 0.02$), a 0.47 cm decreased waist circumference (0.03–0.90 cm; $P = 0.04$), and a 2.6% (0.9–4.3%; $P = 0.003$) decreased insulin release per allele as assessed by the BIGTT-AIR index. The variant did not associate with other measures of insulin release (Table 1). Homozygous carriers of the minor diabetes risk G-allele of the *CDC123/CAMK1D* rs12779790 showed a 15% decreased serum insulin at 30 min during an OGTT (7.8–23%, $P = 8 \times 10^{-5}$), an 18% decreased insulinogenic index (10–27%; $P = 4 \times 10^{-5}$), an 18% decreased CIR (8.1–29%; $P = 4 \times 10^{-4}$), and a 13% decreased AUC-insulin/AUC-glucose (5.8–20%; $P = 4 \times 10^{-4}$) (Table 2). When applying a dominant genetic model, the minor diabetes risk C-allele of the *TSPAN8* rs7961581 associated with a modest decrease in serum insulin at 30 min during OGTT (4.9% [1.9–7.9]; $P = 0.001$), a decrease in CIR (4.5% [0.5–8.4]; $P = 0.03$), a decrease in AUC-insulin/AUC-glucose (3.9% [1.2–6.7]; $P = 0.005$), and a decrease in insulinogenic index (5.2% [1.9–8.6]; $P = 0.002$) (Table 3).

The *THADA* rs7578597 did not associate with measures of obesity (BMI: $P = 0.4$), insulin response (insulinogenic index: $P = 0.4$), or insulin sensitivity (BIGTT-S_I: $P = 1$) (Supplementary Table 1 [available in an online appendix at http://dx.doi.org/10.2337/db08-0436]). Similarly, the AD-*AMTS9* rs4607103 and *NOTCH2* rs10923931 variants did not significantly associate with measures of oral glucose-stimulated insulin response (all $P \geq 0.5$), insulin sensitivity ($P \geq 0.1$), or obesity ($P \geq 0.1$) in the Inter99 cohort (Supplementary Tables 2 and 3). Similar results were found when including all 5,964 treatment-naïve individuals from the Inter99 cohort (data not shown).

Because the insulin response to glucose is highly dependent on the level of insulin sensitivity, we constructed two OGTT-based disposition indexes by combining existing indexes of insulin response and insulin sensitivity and tested association with the six genotyped variants. Homozygous carriers of the *CDC123/CAMK1D* diabetes-associated G-allele showed a nominal association with a 13% decrease in a disposition index based on CIR and ISI (1.1–24%; $P = 0.03$). A disposition index based on BIGTT-AIR and BIGTT-S_I did, however, not differ significantly between genotype groups for any of the six variants, although a tendency toward an allele-dependent decrease in minor G-allele carriers of the *CDC123/CAMK1D* variant was observed ($P = 0.05$).

To further evaluate the relationship between insulin release, insulin sensitivity, and genetic predispositions of the type 2 diabetes–associated variants, we applied the multivariate Hotelling's T^2 method to simultaneously test the effect of genotype on a combination of CIR and ISI as well as BIGTT-AIR and BIGTT-S_I (Fig. 1). We demonstrated statistically significant multivariate associations of the *JAZF1* and *CDC123/CAMK1D* variants with the combination of CIR and ISI ($P_{ADDITIVE} = 0.04$ and $P_{RECESSIVE} = 0.002$, respectively). Furthermore, borderline association was observed for the *TSPAN8* variant ($P_{DOMINANT} = 0.09$ and $P_{RECESSIVE} = 0.05$). The multivariate analysis did not show any influence of genotype on the combination of BIGTT-AIR and BIGTT-S_I (data not shown).

TABLE 3
Unadjusted quantitative metabolic traits in the population-based Inter99 cohort including 4,410 middle-aged subjects with normal glucose tolerance stratified according to genotype of *TSPAN8* rs7961581

	11 (TT)	12 (TC)	22 (CC)	$P_{ADDITIVE}$	$P_{22 + 12 \text{ VS. } 11}$	$P_{22 \text{ VS. } 12 + 11}$
n (men/women)	2,404 (1,129/1,275)	1,686 (771/915)	320 (147/173)			
Age (years)	45.3 ± 7.7	45.2 ± 7.9	44.6 ± 8.0			
BMI (kg/m^2)	25.5 ± 4.1	25.5 ± 4.1	25.6 ± 4.3	0.9	0.7	0.7
Waist (cm)	84 ± 12	84 ± 12	84 ± 12	0.9	0.9	0.7
Fasting serum insulin (pmol/l)	32 (23–47)	32 (23–46)	31 (22–44)	0.05	0.2	0.03
Serum insulin at 30 min (pmol/l)	251 (181–352)	238 (175–343)	245 (173–359)	0.003	0.001	0.3
Serum insulin at 120 min (pmol/l)	141 (88–217)	137 (87–202)	140 (90–225)	0.2	0.2	0.6
Fasting plasma glucose (mmol/l)	5.3 (5.1–5.6)	5.3 (5.0–5.6)	5.3 (5.1–5.6)	0.6	0.3	0.7
Plasma glucose at 30 min (mmol/l)	8.1 (7.2–9.1)	8.2 (7.2–9.3)	8.2 (7–9)	0.3	0.7	0.08
Plasma glucose at 120 min (mmol/l)	5.6 (4.8–6.3)	5.6 (4.8–6.4)	5.6 (4.7–6.4)	0.4	0.3	0.9
ISI	0.134 (0.089–0.192)	0.133 (0.092–0.193)	0.136 (0.095–0.211)	0.05	0.2	0.04
BIGTT-S_I	10.3 ± 3.7	10.4 ± 3.6	10.2 ± 3.6	0.4	0.2	0.7
AUC-insulin/AUC-glucose	27.9 (20.4–38.2)	26.6 (20.3–36)	28.2 (20.1–39.0)	0.02	0.005	0.7
CIR	754 (494–1,210)	738 (464–1,130)	741 (469–1,330)	0.1	0.03	0.4
Insulinogenic index	26.7 (18.3–38.6)	25.1 (17.8–36.7)	25.4 (18.3–39.3)	0.01	0.002	1
BIGTT-AIR	1,670 (1,360–2,130)	1,660 (1,330–2,080)	1,720 (1,330–2,140)	0.4	0.2	0.5

Data are medians (25% to 75% range) or means ± SD (BMI, waist, and BIGTT-S_I). Values of BMI, plasma glucose, serum insulin, and derived indices were logarithmically transformed before statistical analysis. Calculated *P* values were adjusted for age (BIGTT-S_I and BIGTT-AIR), age and sex (BMI and waist), or age, sex, and BMI (all other traits), assuming an additive, dominant, or recessive model. Indices of insulin release and insulin sensitivity were calculated as described in research design and methods. 1, type 2 diabetes–protective allele; 2, diabetes-associated allele.

DISCUSSION

We report the association testing of six recently discovered type 2 diabetes risk variants (6) with intermediary diabetes-related phenotypes. Our results, if replicated in independent and statistically well-powered studies, suggest an impairment of pancreatic β-cell function for diabetes risk alleles in or near *JAZF1*, *CDC123/CAMK1D*, and *TSPAN8*, since these variants were associated with various surrogate measures of insulin release during an OGTT. Further support of the role of the *CDC123/CAMK1D* and *TSPAN8* variants in altered pancreatic β-cell function was provided when analyzing an OGTT-based disposition index and for *JAZF1* and *CDC123/CAMK1D* variants when doing multivariate analysis of estimates of insulin sensitivity and insulin release. The observed associations for all three variants are concordant with an impaired oral glucose-stimulated insulin release in subjects carrying the reported type 2 diabetes risk alleles (6).

In the analyses, we primarily focused on glucose-tolerant subjects to avoid the confounding influence of disturbances in glucose homeostasis and to circumvent the risk that associations with especially impaired insulin response were driven by the known association with type 2 diabetes. We did, however, observe similar results when including subjects with impaired fasting glycemia, impaired glucose tolerance, or screen-detected type 2 diabetes.

rs864745 resides in intron 1 of the *JAZF1* (juxtaposed with another zinc finger gene 1) gene, which encodes a transcriptional repressor of the nuclear receptor subfamily 2, group C, member 2 (*NR2C2*) gene (17). NR2C2 (also known as TR4) is a member of the nuclear hormone receptor family and acts as a ligand-activated transcription factor (18). *NR2C2* is widely expressed and *Nr2c2*$^{-/-}$ knockout mice display a phenotype of growth retardation, hypoglycemia, and reduced gluconeogenesis by decreased activation of *PEPCK* (19,20); however, no obvious involvement in pancreatic β-cell function has been demonstrated. Yet, since *JAZF1* is expressed in the pancreas (17), one might speculate that a gain-of-function variant in *JAZF1* may lead to postnatal growth restriction also affecting pancreatic β-cell mass and function.

rs12779790 is located ~90 kb from *CDC123* and ~63.5 kb from *CAMK1D*. *CDC123* (cell division cycle 123 homolog [*S. cerevisiae*]) encodes a protein involved in cell cycle regulation and nutritional control of gene transcription with no known relation to type 2 diabetes pathogenesis (21). Because *CAMK1D* (calcium/calmodulin-dependent protein kinase I delta) regulates granulocyte function (22), it is also possible that a causative variant in this region is related to *CAMK1D* and affects pancreatic β-cell function through increased apoptosis.

Lastly, rs7961581 resides ~110 kb upstream of *TSPAN8* (tetraspanin 8), which encodes a widely expressed cell surface glycoprotein known to form complexes with integrins to regulate cell motility in cancer cell lines (23). Because α6-integrin binding to laminin has been shown to negatively affect pancreatic β-cell mass maintenance (24), it is possible that variation in *TSPAN8* biologically influences pancreatic β-cell function.

In this article, we have performed a thorough evaluation of a range of OGTT-based surrogate estimates of insulin release and insulin sensitivity. The associations of examined gene variants to various measures of pancreatic β-cell function highlight the need for cautious interpretation of outcomes. Variants in the *CDC123/CAMK1D* and *TSPAN8* regions associate with the insulinogenic index, the corrected insulin response, and the ratio of AUC-insulin to AUC-glucose, which are widely used and well-documented

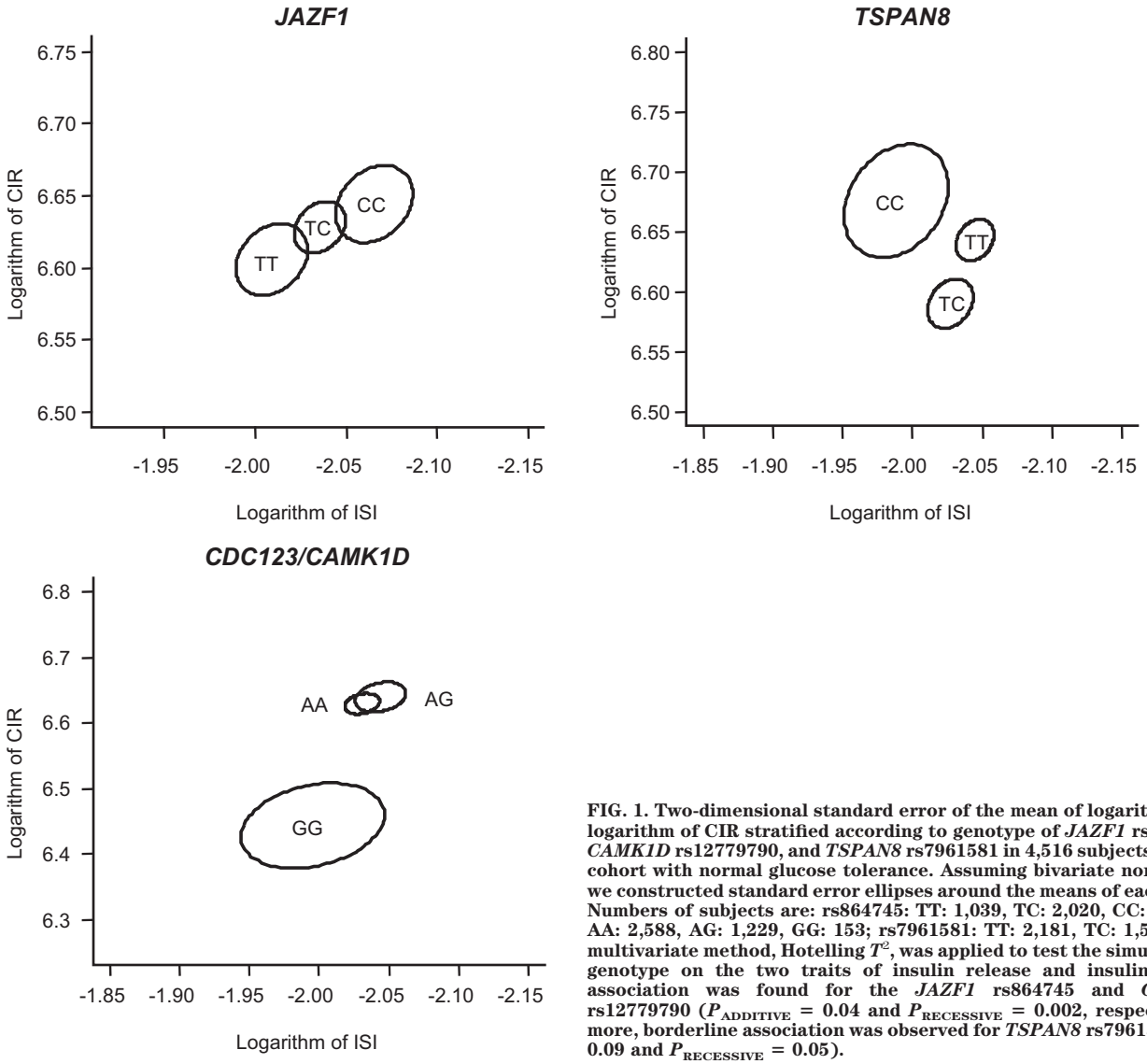

FIG. 1. Two-dimensional standard error of the mean of logarithm of the ISI and logarithm of CIR stratified according to genotype of *JAZF1* rs864745, *CDC123/CAMK1D* rs12779790, and *TSPAN8* rs7961581 in 4,516 subjects from the Inter99 cohort with normal glucose tolerance. Assuming bivariate normal distribution, we constructed standard error ellipses around the means of each genotype level. Numbers of subjects are: rs864745: TT: 1,039, TC: 2,020, CC: 898; rs12779790: AA: 2,588, AG: 1,229, GG: 153; rs7961581: TT: 2,181, TC: 1,518, CC: 289. The multivariate method, Hotelling T^2, was applied to test the simultaneous effect of genotype on the two traits of insulin release and insulin sensitivity, and association was found for the *JAZF1* rs864745 and *CDC123/CAMK1D* rs12779790 ($P_{\text{ADDITIVE}} = 0.04$ and $P_{\text{RECESSIVE}} = 0.002$, respectively). Furthermore, borderline association was observed for *TSPAN8* rs7961581 ($P_{\text{DOMINANT}} = 0.09$ and $P_{\text{RECESSIVE}} = 0.05$).

estimates of insulin release (25,26), yet not with the recently described BIGTT-AIR index (16), and the opposite is true for the *JAZF1* variant. These discrepancies may be caused by different accuracy and/or sensitivity of the applied surrogate indexes or the possibility that the different indexes capture particular and diverse roles of the encoded proteins in specific steps of insulin biosynthesis, insulin secretion, or insulin elimination. However, we cannot exclude that the associations to various measures are caused by statistical type I or II errors. Although we analyzed a range of OGTT-based surrogate indexes of insulin release, we acknowledge that application of more precise measures of insulin release, such as estimates based on an intravenous glucose tolerance test, may have modified the outcome of our analyses.

Type 2 diabetes–associated variants in the *THADA*, *ADAMTS9*, and *NOTCH2* loci did not associate with metabolic traits in the Inter99 cohort. Lack of statistical power is a possible explanation, since these variants confer a modestly increased risk of type 2 diabetes. Based on 95% CIs of effect size estimates, we can with confidence exclude an allele-dependent effect in the current study on BMI, insulinogenic index, BIGTT-AIR, and ISI above 4.5% for *THADA* rs7578597, 3% for *ADAMTS9* rs4607103, and 4% for *NOTCH2* rs10923931. However, we are unable to estimate potential associations below these effect sizes.

We recognize that since no correction for multiple hypothesis testing was applied, the present results are of an explorative nature and call for validation in statistically powered and well-characterized cohorts. If, however, stringent Bonferroni correction for multiple testing (252 tests) was performed, only the associations of the *CDC123/CAMK1D* rs12779790 variant with measures of insulin response (insulinogenic index and serum insulin at 30 min during the OGTT) would remain statistically significant, underlining the need for replication. Based on the effect sizes of the current study, we estimate that ~3,300, 6,100, and 3,900 subjects are needed for future studies to achieve 80% statistical power to replicate associations of *JAZF1* rs864745 with BIGTT-AIR (additive model), *CDC123/CAMK1D* rs12779790 with insulinogenic index (recessive model), and *TSPAN8* rs7961581 with insulinogenic index (dominant model), respectively.

In conclusion, we report data suggesting an impaired pancreatic β-cell function in glucose-tolerant carriers of novel type 2 diabetes risk alleles in the *JAZF1*, *CDC123/CAMK1D*, and *TSPAN8* regions. No associations of common variants in *THADA*, *ADAMTS9*, and *NOTCH2* with

quantitative measures of insulin release or insulin sensitivity could be shown in the cohort of middle-aged people.

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