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

Niels Grarup,1 Gitte Andersen,1 Nikolaj T. Kragup,1 Anders Albrechtsen,2 Ole Schmitz,3,4 Torben Jørgensen,5 Knut Borch-Johnsen,1,5,6 Torben Hansen,1 and Oluf Pedersen1,6

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), ADAMTS9 (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 × 10⁻⁵), an 18% decrease in corrected insulin response (CIR) (8.1–29%; P = 4 × 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 × 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 (4.9% [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
Subjects with NGT (2,101 men/2,415 women, age 45.2 ± 7.9 years and BMI 25.5 ± 4.1 kg/m²) were reported as the insulin sensitivity index (ISI), calculated as plasma glucose (mmol/l)/serum insulin (pmol/l) (14). In the analysis of quantitative diabetes-related phenotypes, we included 4,516 cases of the six examined gene variants and a sample size of 4,516 subjects, we had 80% power to detect an allele-dependent difference of 0.8 –1.4% in BMI, 2.6 – 4.3% in BIGTT-AIR, 3.7– 6.2% in insulinogenic index, and 3.4 –5.9% in ISI, respectively.

We used empirical variance of the observed traits to simulate phenotypes with the BIGTT-AIR index with the BIGTT-SI index and BIGTT-AIR). Statistical power. The power was estimated using simulations. We assumed 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% power to detect an allele-dependent difference of 0.5–1.4% 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 1

| n (men/women) | 11 (CC) | 12 (CT) | 22 (TT) | P\_ADDITIVE | P\_22 + 12 vs. 11 | P\_22 vs. 12 + 11 |
|---------------|---------|---------|---------|-------------|------------------|------------------|
| n             | 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/m²)   | 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 (0.09–0.19) | 0.13 (0.09–0.19) | 0.14 (0.09–0.19) | 0.3         | 0.2             | 0.7              |
| BIGTT-SI      | 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-SI). Values of BMI, plasma glucose, serum insulin, and derived indices were logarithmically transformed before statistical analysis. Calculated P values were adjusted for age (BIGTT-SI and BIGTT-AIR), age and sex (BMI and waist), or age, sex, and BMI (all other traits), assuming an additive, dominant, or recessive model.
**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)          | \(P_{\text{ADDITIVE}}\) | \(P_{22 + 12 \text{ vs. } 11}\) | \(P_{22 \text{ vs. } 12 + 11}\) |
|----------------|------------------|------------------|------------------|--------------------------|-------------------------------|-------------------------------|
| \(n\) (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                           | \(8 \times 10^{-5}\)         |
| 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                           | \(4 \times 10^{-4}\)         |
| CIR             | 753 (480–1190)   | 752 (483–1240)   | 614 (402–926)    | 0.07                     | 0.5                           | \(4 \times 10^{-4}\)         |
| Insulinogenic index | 26.0 (18.3–38.3) | 26.1 (18.2–37.7) | 23.1 (15.3–30.4) | 0.01                     | 0.2                           | \(4 \times 10^{-5}\)         |
| 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%; \(P = 4 \times 10^{-5}\)), 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 G-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 (Table 1). 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 (\(P_{\text{ADDITIVE}} = 0.04\) and \(P_{\text{RECESSIVE}} = 0.002\), respectively). Furthermore, borderline association was observed for the TSPAN8 variant (\(P_{\text{DOMINANT}} = 0.09\) and \(P_{\text{RECESSIVE}} = 0.05\)). The multivariate analysis did not show any influence of genotype on the combination of BIGTT-AIR and BIGTT-S\(_I\) (data not shown).
Further support of the role of the TSPAN8 (transmembrane protein with another zinc finger gene 8) surrogate measures of insulin release during an OGTT.

NR2C2 (nuclear receptor subfamily 2, group C, member 2) gene, which encodes a transcriptional repressor of the nuclear hormone 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 CAMKID. 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 CAMKID (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 CAMKID and affects pancreatic β-cell function through increased apoptosis.

Lastly, rs7961581 resides ~110 kb upstream of TSPAN8 (tetrasinpan 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/CAMKID 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

|          | 11 (TT)      | 12 (TC)      | 22 (CC)      | P_{ADDITIVE} | P_{22 + 12 vs. 11} | P_{22 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/m²)    | 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-SI | 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) | 0.02 | 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-SI). Values of BMI, plasma glucose, serum insulin, and derived indices were logarithmically transformed before statistical analysis. Calculated P values were adjusted for age (BIGTT-SI 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/CAMKID, 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/CAMKID and TSPAN8 variants in altered pancreatic β-cell function was provided when analyzing an OGTT-based disposition index and for JAZF1 and CDC123/CAMKID 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 glycaemia, 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 CAMKID. 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 CAMKID (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 CAMKID and affects pancreatic β-cell function through increased apoptosis.

Lastly, rs7961581 resides ~110 kb upstream of TSPAN8 (tetranspanin 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/CAMKID 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.
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 diabetes. VOL. 57, SEPTEMBER 2008
quantitative measures of insulin release or insulin sensitivity could be shown in the cohort of middle-aged people.

ACKNOWLEDGMENTS

The study was supported by grants from the Lundbeck Foundation Centre of Applied Medical Genomics for Personalized Disease Prediction, Prevention and Care (LUCAMP); the Danish Health Research Council, The European Union (EUGENE2, grant no. LSHM-CT-2004-512013), Danish Council for Strategic Research (DanORC, grant no. 2101-06-0005), the Faculty of Health Sciences of Aarhus University, the Danish Clinical Intervention Research Academy, and the Danish Diabetes Association and Nordov Nordisk.

We are thankful to Dr. Mark McCarthy, Dr. Michael Boehnke, Dr. David Altshuler, Dr. Leif Groop, and Dr. Francis Collins from the DIAGRAM consortium for pre-publication information on the identity of novel type 2 diabetes genes and loci. The authors thank A. Forman, I.-L. Wantzin, and M. Stendal for technical assistance and G. Lademann for secretarial support.

We acknowledge all the members of the Inter99 team. The steering committee of the Inter99 study comprises: T. Jørgensen (principal investigator [PI]), K. Borch-Johnsen (co-PI), H. Ibsen, T. Thomsen, C. Pisinger, and C. Glümer. The study was financially supported by The Danish Medical Research Council, The Danish Centre for Health Technology Assessment, Novo Nordisk, Research Foundation Denmark, Ministry of Internal Affairs and Health, The Augustinus Foundation, The Royal Danish Academy of Fine Arts, The Danish Diabetes Association and the Danish Diabetes Federation (co-PI), H. Ibsen, T. Thomsen, C. Pisinger, and C. Glümer.

The study was financially supported by The Danish Medical Research Council, The Danish Centre for Health Technology Assessment, Novo Nordisk, Research Foundation Denmark, Ministry of Internal Affairs and Health, The Augustinus Foundation, The Royal Danish Academy of Fine Arts, The Danish Diabetes Association and the Danish Diabetes Federation (co-PI), H. Ibsen, T. Thomsen, C. Pisinger, and C. Glümer.

The steering committee of the Inter99 study comprises: T. Jørgensen (principal investigator [PI]), K. Borch-Johnsen (co-PI), H. Ibsen, T. Thomsen, C. Pisinger, and C. Glümer. The study was financially supported by The Danish Medical Research Council, The Danish Centre for Health Technology Assessment, Novo Nordisk, Research Foundation Denmark, Ministry of Internal Affairs and Health, The Augustinus Foundation, The Royal Danish Academy of Fine Arts, The Danish Diabetes Association and the Danish Diabetes Federation (co-PI), H. Ibsen, T. Thomsen, C. Pisinger, and C. Glümer.

Jørgensen (principal investigator [PI]), K. Borch-Johnsen (co-PI), H. Ibsen, T. Thomsen, C. Pisinger, and C. Glümer. The study was financially supported by The Danish Medical Research Council, The Danish Centre for Health Technology Assessment, Novo Nordisk, Research Foundation Denmark, Ministry of Internal Affairs and Health, The Augustinus Foundation, The Royal Danish Academy of Fine Arts, The Danish Diabetes Association and the Danish Diabetes Federation (co-PI), H. Ibsen, T. Thomsen, C. Pisinger, and C. Glümer.

The steering committee of the Inter99 study comprises: T. Jørgensen (principal investigator [PI]), K. Borch-Johnsen (co-PI), H. Ibsen, T. Thomsen, C. Pisinger, and C. Glümer. The study was financially supported by The Danish Medical Research Council, The Danish Centre for Health Technology Assessment, Novo Nordisk, Research Foundation Denmark, Ministry of Internal Affairs and Health, The Augustinus Foundation, The Royal Danish Academy of Fine Arts, The Danish Diabetes Association and the Danish Diabetes Federation (co-PI), H. Ibsen, T. Thomsen, C. Pisinger, and C. Glümer.

REFERENCES

1. Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, Boutin P, Vincent D, Belisle A, Hadjadj S, Balkau B, Heude B, Charpentier G, Hudson T, Montpetit A, Pasche-Haak SV, Premkari M, Posner BI, Balding DJ, Meyre D, Polychronakos C, Fugelssjaer J. A genome-wide association study identifies new loci associated with type 2 diabetes and lipid levels. Nat Genet 38:203–208, 2006.

2. Steinthorsdottir V, Thorleifsson G, Reynisdottir I, Benediktsson R, Samani NJ, Snaebjornsson B, Polychronakos C, Froguel P: A genome-wide association study identifies new loci for type 2 diabetes. Nature 445:881–885, 2007.

3. Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, Lango H, Freathy RM, Frayling, T, Mistry RM, Gyllensten UB, Manning AM, Vranizan K, Rayman M, O'Donnell KJ, Prokunina-Olsson L, Sanna S, Amin N, Barrett JC, Lindgren CM, Groffen J, Willer CJ, Sanna MN, Parra MC, Asselbergs FW, Speliotes EK, Hattersley AT, McCarthy MI, Hattersley AT, McCarthy MI: T2DM locus on chromosome 3q27 is associated with BMI and waist circumference. Nat Genet 39:66–70, 2007.

4. Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, Lango H, Freathy RM, Frayling, T, Mistry RM, Gyllensten UB, Manning AM, Vranizan K, Rayman M, O'Donnell KJ, Prokunina-Olsson L, Sanna S, Amin N, Barrett JC, Lindgren CM, Groffen J, Willer CJ, Sanna MN, Parra MC, Asselbergs FW, Speliotes EK, Hattersley AT, McCarthy MI, Hattersley AT, McCarthy MI: T2DM locus on chromosome 3q27 is associated with BMI and waist circumference. Nat Genet 39:66–70, 2007.

5. Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, Lango H, Freathy RM, Frayling, T, Mistry RM, Gyllensten UB, Manning AM, Vranizan K, Rayman M, O'Donnell KJ, Prokunina-Olsson L, Sanna S, Amin N, Barrett JC, Lindgren CM, Groffen J, Willer CJ, Sanna MN, Parra MC, Asselbergs FW, Speliotes EK, Hattersley AT, McCarthy MI, Hattersley AT, McCarthy MI: T2DM locus on chromosome 3q27 is associated with BMI and waist circumference. Nat Genet 39:66–70, 2007.
18. Chang C, da Silva SL, Ideta R, Lee Y, Yeh S, Burbach JPH: Human and rat TR4 orphan receptors specify a subclass of the steroid receptor superfamily. *Proc Natl Acad Sci USA* 91:6040–6044, 1994

19. Collins LL, Lee YF, Heinlein CA, Liu NC, Chen YT, Shyr CR, Meshul CK, Uno H, Platt KA, Chang C: Growth retardation and abnormal maternal behavior in mice lacking testicular orphan nuclear receptor 4. *Proc Natl Acad Sci USA* 101:15058–15063, 2004

20. Liu NC, Lin WJ, Kim E, Collins LL, Lin HY, Yu IC, Sparks JD, Chen LM, Lee YF, Chang C: Loss of TR4 orphan nuclear receptor reduces phosphoenolpyruvate carboxykinase-mediated gluconeogenesis. *Diabetes* 56:2901–2909, 2007

21. Bieganowski P, Shilinski K, Tsichlis PN, Brenner C: Cdc123 and checkpoint forkhead associated with RING proteins control the cell cycle by controlling eIF2gamma abundance. *J Biol Chem* 279:44656–44666, 2004

22. Verploegen S, Ulfman L, van-Deutekom HW, van-Aalst C, Honing H, Lammers JW, Koenderman L, Coffier PJ: Characterization of the role of CaMKI-like kinase (CKLiK) in human granulocyte function. *Blood* 106:1076–1083, 2005

23. Gesierich S, Paret C, Hildebrand D, Weitz J, Zgraggen K, Schmitz-Winnenthal FH, Horejsi V, Yoshie O, Herlyn D, Ashman LK, Zoller M: Colocalization of the tetraspanins, CO-029 and CD151, with integrins in human pancreatic adenocarcinoma: impact on cell motility. *Clin Cancer Res* 11:2840–2852, 2005

24. Kilkenny DM, Rocheleau JV: Fibroblast growth factor receptor-1 signaling in pancreatic islet beta-cells is modulated by the extracellular matrix. *Mol Endocrinol* 22:196–205, 2008

25. Stunzoll M, Mitrakeon A, Pinmenta W, Jenessen T, Yki J, Van Haetton T, Renn W, Gerich J: Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. *Diabetes Care* 23:295–301, 2000

26. Hanson RL, Pratley RE, Bogardus C, Narayan KM, Roumain JM, Imperatore G, Fagot-Campagna A, Pettitt DJ, Bennett PH, Knowler WC: Evaluation of simple indices of insulin sensitivity and insulin secretion for use in epidemiologic studies. *Am J Epidemiol* 151:190–198, 2000