

Studies of Association of Variants Near the *HHEX*, *CDKN2A/B*, and *IGF2BP2* Genes With Type 2 Diabetes and Impaired Insulin Release in 10,705 Danish Subjects

Validation and Extension of Genome-Wide Association Studies

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OBJECTIVE—In the present study, we aimed to validate the type 2 diabetes susceptibility alleles identified in six recent genome-wide association studies in the *HHEX/KIF11/IDE* (rs1111875), *CDKN2A/B* (rs10811661), and *IGF2BP2* (rs4402960) loci, as well as the intergenic rs9300039 variant. Furthermore, we aimed to characterize quantitative metabolic risk phenotypes of the four variants.

RESEARCH DESIGN AND METHODS—The variants were genotyped in the population-based Inter99 cohort ($n = 5,970$), the ADDITION Study ($n = 1,626$), a population-based sample of young healthy subjects ($n = 377$), and in additional type 2 diabetic case ($n = 2,111$) and glucose-tolerant ($n = 521$) subjects. The case-control studies involved a total of 4,089 type 2 diabetic patients and 5,043 glucose-tolerant control subjects.

RESULTS—We validated association of variants near *HHEX/KIF11/IDE*, *CDKN2A/B*, and *IGF2BP2* with type 2 diabetes. Interestingly, in middle-aged people, the rs1111875 C-allele of *HHEX/KIF11/IDE* strongly associated with lower acute insulin response during an oral glucose tolerance test ($P = 6 \times 10^{-7}$). In addition, decreased insulin release following intravenous tolbutamide injection was observed in young healthy subjects ($P = 0.02$). Also, a reduced insulin release was observed for the *CDKN2A/B* rs10811661 T-allele after both oral and intravenous glucose challenges ($P = 0.001$ and $P = 0.009$, respectively).

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BIGTT-AIR, BIGTT-acute insulin response; BIGTT-S, BIGTT-insulin sensitivity index; GWA, genome-wide association; IVGTT, intravenous glucose tolerance test; OGTT, oral glucose tolerance test.

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CONCLUSIONS—We validate that variants in the proximity of the *HHEX/KIF11/IDE*, *CDKN2A/B*, and *IGF2BP2* loci associate with type 2 diabetes. Importantly, variations within the *HHEX/KIF11/IDE* and *CDKN2A/B* loci confer impaired glucose- and tolbutamide-induced insulin release in middle-aged and young healthy subjects, suggesting a role for these variants in the pathogenesis of pancreatic β -cell dysfunction. *Diabetes* 56: 3105–3111, 2007

Type 2 diabetes is a rapidly growing public health problem with a tremendous impact on morbidity and premature mortality worldwide. Although the epidemic nature of the disease may be attributable mainly to environmental factors leading to obesity, genetic factors also predispose to the disease. Progress in finding type 2 diabetes susceptibility genes has been sparse; however, recent genome-wide association (GWA) studies have revolutionized this field of research (1–6). In collaboration with deCODE Genetics (Iceland), we recently reported results of a GWA study showing associations of variants in *SLC30A8* and *CDKAL1* with type 2 diabetes and impaired insulin response (2). Results of four additional GWA studies have presented a list of 10 validated type 2 diabetes loci, including several previously unknown genomic regions (1,3–6). However, for these novel loci, no phenotype besides type 2 diabetes has been demonstrated. We hypothesized that given the prior knowledge of the biological significance of the neighboring genes in the *CDKN2A/B* and *HHEX/KIF11/IDE* regions, the diabetes-associated variants confer β -cell dysfunction.

The aim of the present study was to validate association with type 2 diabetes and to establish possible quantitative metabolic risk phenotypes of previously uncharacterized variants in the *HHEX/KIF11/IDE* (rs1111875), *CDKN2A/B* (rs10811661), and *IGF2BP2* (rs4402960) loci. Furthermore, we aimed to evaluate the impact of the intergenic rs9300039 variant for which data from the GWA studies have been inconsistent (1–6).

RESEARCH DESIGN AND METHODS

Details of the study populations are given in online appendix Table 1 (available at <http://dx.doi.org/10.2337/db07-0856>). Participants from the population-based Inter99 cohort (ClinicalTrials.gov ID no. NCT00289237) (7,8) involving 5,970 middle-aged subjects who were characterized by an oral

TABLE 1
Association studies of type 2 diabetes in 4,089 type 2 diabetic case and 5,043 glucose-tolerant control subjects

Variant	Risk allele	Nearest gene	Genotype distribution		Risk-allele frequencies		Unadjusted*		Adjusted†		
			Glucose tolerant n (%)	Type 2 diabetes n (%)	Glucose tolerant % (95% CI)	Type 2 diabetes % (95% CI)	Allele frequency model OR (95% CI)	Additive model OR (95% CI)	Dominant model OR (95% CI)	Recessive model OR (95% CI)	
rs1111875	C	HHEX	TT	602 (16)	58.5	60.2	1.07 (1.01–1.14)	1.13 (1.03–1.23)	1.28 (1.08–1.50)	1.11 (0.98–1.26)	
			CT	2,279 (47)	1,864 (48)	(57.4–59.5)	(59.1–61.3)	$P_{\text{freq}} = 0.03$	$P_{\text{add}} = 0.008$	$P_{\text{dom}} = 0.004$	$P_{\text{rec}} = 0.1$
			CC	1,694 (35)	1,389 (36)						
rs10811661	T	CDKN2B	CC	135 (2.7)	83.1	86.4	1.26 (1.16–1.38)	1.30 (1.16–1.47)	1.35 (0.90–2.02)	1.36 (1.19–1.55)	
			CT	1,365 (28)	933 (24)	(82.3–83.8)	(85.6–87.1)	$P_{\text{freq}} = 4 \times 10^{-8}$	$P_{\text{add}} = 1 \times 10^{-5}$	$P_{\text{dom}} = 0.2$	$P_{\text{rec}} = 8 \times 10^{-6}$
			TT	3,410 (70)	2,884 (74)						
rs4402960	T	IGF2BP2	GG	2,389 (49)	30.2	32.2	1.10 (1.03–1.18)	1.10 (1.00–1.21)	1.17 (1.04–1.32)	1.02 (0.83–1.25)	
			GT	2,018 (42)	1,660 (43)	(29.3–31.2)	(31.1–33.2)	$P_{\text{freq}} = 0.004$	$P_{\text{add}} = 0.04$	$P_{\text{dom}} = 0.01$	$P_{\text{rec}} = 0.9$
			TT	455 (9.4)	403 (11)						
rs9300039	C		AA	43 (0.9)	91.0	90.5	0.93 (0.84–1.03)	0.89 (0.77–1.03)	1.11 (0.60–2.07)	0.87 (0.74–1.02)	
			AC	777 (16)	650 (17)	(90.4–91.6)	(89.8–91.2)	$P_{\text{freq}} = 0.2$	$P_{\text{add}} = 0.1$	$P_{\text{dom}} = 0.7$	$P_{\text{rec}} = 0.08$
			CC	4,030 (83)	3,127 (82)						

Data are n (%), risk-allele frequencies in % (95% CI), or OR (95% CI). Patients having type 2 diabetes were recruited at the Steno Diabetes Center (n = 2,111), from the population-based Inter99 cohort (7,8) (n = 352), and from the ADDITION Study (10) (n = 1,626). Glucose-tolerant subjects were recruited from population-based studies at the Steno Diabetes Center (n = 521) and the Inter99 cohort (n = 4,522). *Differences in allele frequencies (P_{freq}) not adjusted for age, sex, and BMI were calculated using Fisher's exact test. †P values compare genotype distributions between type 2 diabetic case and glucose-tolerant control subjects applying an additive (P_{add}), dominant (P_{dom}), or recessive (P_{rec}) logistic regression model, while adjusting for age, sex, and BMI. The risk alleles were determined as previously reported (1–6).

glucose tolerance test (OGTT) as subjects with normal glucose tolerance (n = 4,522), impaired fasting glycemia (n = 503), or impaired glucose tolerance (n = 693), as well as patients with screen-detected and treatment-naïve type 2 diabetes (n = 252), were investigated for associations between genotype and quantitative metabolic traits. Patients with treated type 2 diabetes (n = 100) were not included. Further studies of quantitative metabolic traits were performed in 377 subjects from a population-based sample of young healthy Danish Caucasians recruited at the Research Centre for Prevention and Health (9).

The case-control studies of type 2 diabetes included all unrelated type 2 diabetic case subjects and all glucose-tolerant control subjects from the Inter99 cohort (n = 352 and 4,522 for case and control subjects, respectively) (7,8) and the ADDITION Study (ClinicalTrials.gov ID no. NCT00237548) (n = 1,626 case subjects) (10), as well as samples recruited from the outpatient clinic at Steno Diabetes Center (n = 2,111 and 521 for case and control subjects, respectively). All control subjects had normal fasting glycemia and were glucose tolerant following an OGTT. Diabetes, impaired fasting glycemia, and impaired glucose tolerance were defined in accordance with World Health Organization 1999 criteria (11).

All participants were of Danish nationality, and informed written consent was obtained from all subjects before participation. The studies were approved by the ethics committees of Copenhagen and Aarhus and were in accordance with the principles of the Declaration of Helsinki II.

Biochemical and anthropometric measures. The plasma-glucose and serum-insulin concentrations were measured as described in the online appendix. **Genotyping.** Genotyping of the *HHEX/KIF11/IDE* rs1111875, *CDKN2A/B* rs10811661, *IGF2BP2* rs4402960, and intergenic rs9300039 variants was performed using TaqMan allelic discrimination (KBioscience, Herts, U.K.). Genotype data were obtained in more than 97% of the DNA samples, with a genotype error rate of less than 0.5% for all variants estimated from 1,464 duplicate samples. All genotype groups were in Hardy-Weinberg equilibrium. **Statistical analysis.** Logistic regression analyses and Fisher's exact test were applied to test for differences in genotype distribution or allele frequencies. A general linear model was applied to test anthropometrical and biochemical variables for differences between genotype groups. Heterogeneity in the case-control material was evaluated by comparing allele frequencies within case or control subjects ascertained from different cohorts separately. These analyses were performed using the χ^2 test. To correct for multiple testing, we used a permutation procedure for all of the performed tests in which the genotypes of each variant in each cohort were permuted together in each iteration. The best P value from each model and trait at each iteration was saved and used as an empirical null distribution to evaluate significance (12). The statistical analyses were performed using RGui version 2.4.0 (available at <http://www.r-project.org>). Permutation testing of each individual quantitative trait was performed to obtain an empirical P value. For each trait, 100,000 permutations were done using Blossom version 2005.11.23 (available at <http://www.fort.usgs.gov/Products/Software/Blossom/>). A P value <0.05 was considered significant.

RESULTS

Potential association of the *HHEX/KIF11/IDE* rs1111875, *CDKN2A/B* rs10811661, *IGF2BP2* rs4402960, and intergenic rs9300039 variants with susceptibility of type 2 diabetes was evaluated in case-control studies involving 4,089 type 2 diabetic patients and 5,043 glucose-tolerant control subjects (Table 1). The *CDKN2A/B* rs10811661 T-allele was highly associated with type 2 diabetes with an odds ratio (OR) of 1.30 per risk allele (95% CI 1.16–1.47) ($P_{\text{add}} = 1 \times 10^{-5}$). In addition, the *HHEX/KIF11/IDE* rs1111875 C-allele was associated with a higher risk of having type 2 diabetes with an OR of 1.13 per allele (1.03–1.23) ($P_{\text{add}} = 0.008$). An association of the *IGF2BP2* rs4402960 variant with type 2 diabetes was observed for a dominant genetic model (OR 1.17 [95% CI 1.04–1.32]) ($P_{\text{dom}} = 0.01$) (Table 1). Since subjects included in the case-control study were ascertained from several different study populations, we evaluated possible heterogeneity by comparing allele frequencies of the four variants in type 2 diabetic case or control subjects from the different subgroups. No evidence of heterogeneity was observed.

Furthermore, the four variants were investigated for influence on quantitative metabolic traits in the popula-

TABLE 2
Quantitative metabolic traits in the population-based Inter99 cohort including 5,970 middle-aged subjects with normal glucose tolerance ($n = 4,522$), impaired fasting glycaemia ($n = 503$), impaired glucose tolerance ($n = 693$), or screen-detected and treatment-naïve type 2 diabetes ($n = 252$) stratified according to genotype

	rs1111875 (HHEX/KIF11/IDE)				rs4402960 (GFP2BP2)							
	TT	TC	CC	P_{add}	P_{dom}	P_{rec}	GG	GT	TT	P_{add}	P_{dom}	P_{rec}
n	1,028	2,675	2,017				2,773	2,432	541			
Men/women (n)	519/509	1,318/1,357	1,001/1,016				1,368/1,405	1,230/1,202	261/280			
Age (years)	46.2 ± 7.7	46.2 ± 7.9	46 ± 8.0				46.1 ± 7.9	46.1 ± 8.0	46.4 ± 7.7			
BMI (kg/m ²)	26.2 ± 4.5	26.3 ± 4.6	26.1 ± 4.5	0.5	0.7	0.2	26.1 ± 4.6	26.2 ± 4.5	26.3 ± 4.6	0.4	0.4	0.5
Fasting serum insulin (pmol/l)	$34 (24-51)$	$35 (24-52)$	$34 (24-50)$	0.7	0.6	0.9	$34 (23-50)$	$35 (24-52)$	$35 (24-52)$	0.4	0.6	0.3
Serum insulin at 30 min (pmol/l)	$260 (190-380)$	$251 (178-363)$	$233 (167-331)$	$3 \times 10^{-11*}$	$1 \times 10^{-6*}$	$2 \times 10^{-9*}$	$248 (176-358)$	$243 (178-348)$	$242 (176-358)$	0.1	0.08	0.7
Fasting plasma glucose (mmol/l)	5.5 ± 0.7	5.6 ± 0.9	5.5 ± 0.7	0.2	0.4	0.2	$5.4 (96-252)$	5.5 ± 0.7	5.5 ± 0.7	0.3	0.3	0.4
Plasma glucose at 30 min (mmol/l)	8.6 ± 1.9	8.7 ± 1.9	8.7 ± 1.9	0.3	0.1	0.8	8.7 ± 1.8	8.7 ± 1.8	8.7 ± 1.8	0.5	0.5	0.8
Plasma glucose at 120 min (mmol/l) × HOMA-IR (mmol/l × pmol/l)	6.1 ± 2.0	6.2 ± 2.1	6.2 ± 2.2	0.3	0.4	0.3	6.1 ± 2.1	6.3 ± 2.2	6.3 ± 2	0.04	0.02	0.6
BiGTT-S _i	$8.2 (5.6-12.4)$	$8.5 (5.7-13.1)$	$8.2 (5.7-12.9)$	0.6	0.4	0.9	$8.2 (5.5-12.7)$	$8.4 (5.7-13.1)$	$8.7 (5.7-13.1)$	0.5	0.6	0.5
Insulinogenic index _{Insulin}	$9.1 (6.4-12.3)$	$9.0 (6.2-11.9)$	$9.4 (6.4-12.1)$	0.1	0.8	0.05	$9.2 (6.5-12.2)$	$9.1 (6.3-11.4)$	$9.3 (5.8-12.2)$	0.3	0.2	0.8
BiGTT-AIR	$26.9 (18.3-40.5)$	$24.9 (16.9-36.5)$	$22.5 (15.5-34.1)$	$1 \times 10^{-13*}$	$2 \times 10^{-8*}$	$1 \times 10^{-10*}$	$24.8 (16.9-36.4)$	$24.1 (16.4-36.3)$	$24.0 (17.2-37.1)$	0.1	0.06	0.7
	$1,680 (1,330-2,140)$	$1,650 (1,300-2,080)$	$1,580 (1,250-2,040)$	$6 \times 10^{-7*}$	$1 \times 10^{-4*}$	$2 \times 10^{-5*}$	$1,620 (1,280-2,070)$	$1,630 (1,290-2,090)$	$1,630 (1,280-2,130)$	0.8	0.8	0.7
	rs10811661 (CDKN2A/B)											
	rs9300039											
	CC	CT	TT	P_{add}	P_{dom}	P_{rec}	AA	AC	CC	P_{add}	P_{dom}	P_{rec}
n	154	1,608	4,036				48	939	4,742			
Men/women (n)	78/76	813/795	1,992/2,044				25/23	456/483	2,358/2,384			
Age (years)	46.4 ± 8.1	46.3 ± 7.9	46.1 ± 7.8				47.4 ± 7.1	45.9 ± 8.0	46.1 ± 7.9			
BMI (kg/m ²)	26.2 ± 4.1	26.2 ± 4.6	26.2 ± 4.5	0.8	0.9	0.8	26 ± 4.5	26.1 ± 4.5	26.2 ± 4.6	0.4	0.7	0.4
Fasting serum insulin (pmol/l)	$35 (25-47)$	$35 (24-53)$	$34 (24-51)$	0.7	1	0.7	$30 (23-47)$	$35 (24-52)$	$34 (24-51)$	0.4	0.6	0.3
Serum insulin at 30 min (pmol/l)	$244 (175-352)$	$254 (177-365)$	$242 (174-348)$	0.005	0.3	0.004*	$258 (174-378)$	$247 (178-354)$	$244 (175-353)$	0.6	0.7	0.6
Serum insulin at 120 min (pmol/l)	$171 (103-248)$	$161 (101-258)$	$155 (93-254)$	0.06	0.8	0.04	$151 (96-251)$	$155 (93-261)$	$157 (97-254)$	0.9	0.8	0.9
Fasting plasma glucose (mmol/l)	5.5 ± 0.6	5.5 ± 0.9	5.5 ± 0.8	0.03	0.4	0.03	5.6 ± 1.3	5.5 ± 0.7	5.5 ± 0.8	0.8	0.8	0.8
Plasma glucose at 30 min (mmol/l)	8.8 ± 1.8	8.7 ± 1.9	8.7 ± 1.9	0.3	0.3	0.2	9.1 ± 2.7	8.7 ± 2	8.7 ± 1.9	1	0.5	0.9
Plasma glucose at 120 min (mmol/l) × HOMA-IR (mmol/l × pmol/l)	6.0 ± 1.4	6.2 ± 2.2	6.2 ± 2.1	0.4	0.4	0.5	6.2 ± 2.7	6.2 ± 2.1	6.2 ± 2.1	0.4	0.6	0.4
BiGTT-S _i	$8.4 (5.8-11.8)$	$8.3 (5.6-13.2)$	$8.3 (5.7-12.9)$	1	0.8	1	$7.2 (5.0-13.2)$	$8.6 (5.5-13.2)$	$8.3 (5.7-12.7)$	0.4	0.7	0.3
Insulinogenic index _{Insulin}	$9.7 (6.7-12.0)$	$9.3 (6.4-12.1)$	$9.1 (6.3-12.1)$	0.8	0.4	0.9	$9.0 (6.7-13.4)$	$9.2 (6.4-11.9)$	$9.2 (6.4-12.1)$	0.8	0.5	0.9
BiGTT-AIR	$24.9 (17.3-37.5)$	$25.1 (17.2-37.7)$	$24.0 (16.5-35.7)$	0.003*	0.4	0.002*	$25.1 (17.8-38.8)$	$25.1 (17.5-36.3)$	$24.4 (16.7-36.4)$	0.5	0.6	0.6
	$1,620 (1,320-1,970)$	$1,660 (1,310-2,150)$	$1,610 (1,270-2,060)$	0.007	0.8	0.001*	$1,610 (1,300-2,080)$	$1,650 (1,290-2,070)$	$1,620 (1,280-2,080)$	0.6	1	0.6

Data are median (interquartile range) or means ± SD (age, BMI, and values of plasma glucose, serum insulin, homeostasis model assessment of insulin resistance (HOMA-IR), insulinogenic index_{Insulin} and BiGTT-AIR were logarithmically transformed before statistical analysis. Calculated P values were adjusted for age, sex, and BMI, where appropriate, assuming an additive (P_{add}), dominant (P_{dom}), or recessive (P_{rec}) model. Homeostasis model assessment of insulin resistance was calculated as fasting plasma glucose (mmol/l) multiplied by fasting serum insulin (pmol/l) and divided by 22.5. Insulinogenic index_{Insulin} was calculated as (serum insulin at 30 min [pmol/l] - fasting serum insulin [pmol/l])/plasma glucose at 30 min (mmol/l). BiGTT-S_i and BiGTT-AIR uses information on sex and BMI combined with analysis of plasma glucose and serum insulin levels at the time points 0, 30, and 120 min to provide indexes for S_i and AIR that highly correlate with indexes obtained during an IVGTT and were calculated as described elsewhere (13). *Statistically significant at the 5% level after correcting of multiple testing of traits and models within this cohort and variant using a permutation procedure.

TABLE 3
Quantitative metabolic traits in a population-based sample of 377 young healthy subjects stratified according to genotype

	rs1111875 (HHEX/KIF11/IDE)				rs4402960 (IGF2BP2)			
	TT	TC	CC	P_{add} P_{dom} P_{rec}	GT	TT	P_{add} P_{dom} P_{rec}	
<i>n</i> (men/women)	61 (29/32)	117 (56/61)	191 (97/94)		156 (77/79)	27 (13/14)		
Age (years)	25.9 ± 3.3	25.0 ± 3.5	25.1 ± 3.7		25.5 ± 3.4	25.5 ± 3.1		
BMI (kg/m ²)	23.7 ± 4.5	23.4 ± 3.6	23.76 ± 3.47	0.5 1 0.4	23.5 ± 3.8	24.0 ± 4.7	0.9 0.9 0.6	
Acute serum insulin response								
0–8 min (pmol/l × min)	2,290 (1,540–3,220)	1,860 (1,220–2,590)	1,930 (1,160–2,930)	0.08 0.04 0.4	1,710 (1,180–2,500)	1,890 (1,060–2,950)	0.02 0.004 0.9	
Δ Plasma glucose 2 min	–0.44 (–0.67 to –0.22)	–0.56 (–0.89 to –0.33)	–0.56 (–0.89 to –0.33)	0.007 0.005 0.09	–0.56 (–0.89 to –0.33)	–0.44 (–1.00 to –0.33)	1 0.8 0.5	
Δ Serum insulin 2 min	219 (123–348)	178 (98–319)	182 (91–295)	0.02 0.09 0.05	176 (76–285)	126 (74–369)	0.03 0.02 0.4	
Insulin sensitivity index								
$[10^{-5} \times (\text{min} \times \text{pmol/l})^{-1}]$	11.6 (8.5–19.9)	13.6 (9.1–19.5)	12.7 (9.1–20.0)	0.4 0.4 0.6	13.2 (9.1–20.9)	10.0 (5.8–17.9)	0.7 0.8 0.2	
Birth weight (g)	3,390 ± 578	3,370 ± 579	3,350 ± 552	0.7 0.7 0.8	3,390 ± 520	3,290 ± 580	0.8 1 0.4	
Disposition index	2.45 (1.76–3.96)	2.65 (1.65–3.72)	2.65 (1.73–4.01)	0.6 0.5 0.8	2.45 (1.60–3.37)	2.03 (0.99–3.83)	0.009 0.01 0.1	
rs10811661 (CDKN2A/B)								
	CC	CT	TT	P_{add} P_{dom} P_{rec}	AC	CC	P_{add} P_{dom} P_{rec}	
<i>n</i> (men/women)	11 (5/6)	92 (42/50)	262 (132/130)		52 (26/26)	304 (152/152)		
Age (years)	25.9 ± 3.1	24.6 ± 3.9	25.4 ± 3.4		25.4 ± 3.6	25.2 ± 3.5		
BMI (kg/m ²)	22.7 ± 3.2	23.6 ± 4.0	23.7 ± 3.7	0.8 0.4 1	23.6 ± 3.6	23.6 ± 3.7	0.6 1 0.6	
Acute serum insulin response								
0–8 min (pmol/l × min)	1,770 (1,140–2,470)	2,160 (1,430–3,080)	1,890 (1,170–2,790)	0.02 0.8 0.009	2,000 (1,230–2,720)	1,970 (1,230–2,890)	0.4 0.4 0.5	
Δ Plasma glucose 2 min	–0.33 (–0.67 to –0.28)	–0.56 (–0.89 to –0.33)	–0.56 (–0.89 to –0.33)	0.9 0.7 1	–0.44 (–0.89 to –0.33)	–0.56 (–0.78 to –0.33)	0.9 0.6 1	
Δ Serum insulin 2 min	150 (117–308)	235 (126–351)	177 (97–303)	0.2 0.8 0.1	191 (110–303)	192 (98–336)	0.6 0.8 0.6	
Insulin sensitivity index								
$[10^{-5} \times (\text{min} \times \text{pmol/l})^{-1}]$	19.1 (12.8–22.0)	11.8 (8.5–17.1)	13.6 (8.9–20.1)	0.2 0.5 0.07	11.7 (8.8–16.9)	13.3 (8.9–20.1)	0.03 1 0.02	
Birth weight (g)	3,570 ± 582	3,200 ± 533	3,410 ± 570	0.1 0.2 0.02	3,270 ± 647	3,380 ± 559	0.2 0.8 0.2	
Disposition index	3.25 (2.52–4.01)	2.59 (1.58–3.84)	2.65 (1.63–4.00)	0.4 0.4 0.5	2.41 (1.44–3.40)	2.72 (1.73–3.95)	0.04 0.5 0.04	

Data are median (interquartile range) or means ± SD (age, BMI, and birth weight). Values of serum insulin, insulin sensitivity index, and disposition index were transformed using cube root before statistical analysis. Calculated *P* values were adjusted for age, sex, and BMI (where appropriate) assuming an additive (P_{add}), dominant (P_{dom}), or recessive (P_{rec}) model. Acute serum insulin response 0–8 min, Δ plasma glucose 2 min post-folbutamide, Aserum insulin 2 min post-folbutamide (pmol/l), insulin sensitivity index, and the disposition index were calculated as described elsewhere (9).

tion-based Inter99 sample involving 5,970 treatment-naïve middle-aged subjects (Table 2). Surrogate measures of insulin resistance and insulin secretion were reported as homeostasis model assessment of insulin resistance and insulinogenic index_{insulin}, as well as assessed by BIGTT-insulin sensitivity index (BIGTT-S_i) and BIGTT-acute insulin response (BIGTT-AIR). The BIGTT indexes are based on information on sex and BMI combined with analysis of plasma glucose and serum insulin levels at time 0, 30, and 120 min during an OGTT to provide indexes for S_i and AIR that highly correlate with indexes obtained during an intravenous glucose tolerance test (IVGTT) (13). At the population level, the *HHEX/KIF11/IDE* rs1111875 C-allele strongly associated with an allele-dependent decrease in acute insulin release as assessed by BIGTT-AIR ($P_{\text{add}} = 6 \times 10^{-7}$), insulinogenic index_{insulin} ($P_{\text{add}} = 1 \times 10^{-13}$), and lower 30-min serum-insulin concentrations during an OGTT ($P_{\text{add}} = 3 \times 10^{-11}$). Similarly, homozygous carriers of the type 2 diabetes risk T-allele of the *CDKN2A/B* rs10811661 variant associated with a decrease in BIGTT-AIR, insulinogenic index_{insulin}, and 30- and 120-min serum-insulin concentrations ($P_{\text{rec}} = 0.001$, $P_{\text{rec}} = 0.002$, $P_{\text{rec}} = 0.004$, and $P_{\text{rec}} = 0.04$, respectively) (Table 2). All analyses of quantitative traits were also performed in a subgroup consisting of middle-aged normal glucose-tolerant subjects from the Inter99 cohort ($n = 4,522$), and similar results were observed (online appendix Table 2).

In a second study of quantitative metabolic traits the variants were further evaluated in a population-based sample of 377 young healthy Caucasians (Table 3). We found that carriers homozygous for the *CDKN2A/B* rs10811661 T-allele had decreased acute insulin release during an IVGTT ($P_{\text{rec}} = 0.009$). Likewise, considering a dominant genetic model, the *IGF2BP2* rs4402960 variant was associated with decreased acute insulin release ($P_{\text{dom}} = 0.004$), as well as lowered insulin release in response to tolbutamide injection ($P_{\text{dom}} = 0.02$). Also, tolbutamide-stimulated insulin release decreased in an allele-dependent manner for carriers of the *HHEX/KIF11/IDE* rs1111875 risk C-allele ($P_{\text{add}} = 0.02$) (Table 3).

To ensure the robustness of the quantitative trait analysis, we obtained an empirical P value by permutation testing of each single trait individually, and consistency was observed.

DISCUSSION

The present study aides in establishing the more precise nature of the metabolic effect of the recently reported common type 2 diabetes susceptibility alleles identified by GWA. We validate association of the *HHEX/KIF11/IDE* rs1111875, *CDKN2A/B* rs10811661, and *IGF2BP2* rs4402960 variants with type 2 diabetes. Furthermore, analyses in two population-based samples of well-characterized middle-aged or young subjects show that variations within the *HHEX/KIF11/IDE* and *CDKN2A/B* loci confer an impairment of glucose-induced insulin release pointing to pancreatic β -cell dysfunctions.

Variants belonging to a 270-kb haplotype block on chromosome 10q, including the *HHEX*, *KIF11*, and *IDE* genes, were associated with type 2 diabetes in all (1,2,4–6) but one (3) of six recent GWA studies. *HHEX* encodes a transcription factor essential for embryonic formation of the ventral pancreas (14). Insulin-degrading enzyme, encoded by *IDE*, degrades amylin, thereby decreasing the pathogenic amyloid deposition in the pancreatic β -cell

(15). Interestingly *Ide*^{-/-} knockout mice display hyperinsulinemia and glucose intolerance (16); however, the insulin-degrading function of IDE points to a possible effect of this enzyme on insulin clearance. Association studies of variants in *IDE* in relation to type 2 diabetes have yielded inconsistent results (17,18). *KIF11* encodes a member of the kinesin-like protein family possibly involved in mitosis and meiosis. In the present study, a glucose- and tolbutamide-induced insulin release dysfunction was evident in carriers of the *HHEX/KIF11/IDE* rs1111875 type 2 diabetes risk allele, yet the results of association with acute insulin release following an intravenous glucose challenge was borderline significant. This discrepancy may be due to a differential influence of specific estimates of insulin release or to the relatively small sample size in the cohort of young healthy subjects. The quantitative phenotype that we observed is compatible with causal variants in both *HHEX* and *IDE*, perhaps influencing the fetal pancreatic development making carriers vulnerable to β -cell stress in adult life.

The rs10811661 variant is located 125 kb upstream of the *CDKN2A/B* genes. In the present study, we find a substantial impact of this variant on type 2 diabetes risk with an OR of 1.30 per risk allele, and given a risk-allele frequency >80%, this variant contributes considerably to the population-attributable risk. In two population-based samples, we find an impaired glucose- and tolbutamide-induced insulin release in risk-allele carriers; both studies pointing to a recessive mode of inheritance. Yet, the study of the association with type 2 diabetes is also consistent with an additive genetic model. The *CDKN2A/B* genes are expressed in adipocytes and pancreatic islets (4). *CDKN2A* encodes p16^{INK4a}—a tumor suppressor influencing pancreatic β -cell proliferation (19,20)—thus making it likely that a causal variant is situated in *CDKN2A*, possibly increasing the susceptibility of type 2 diabetes through a decreased β -cell mass and subsequent decreased insulin release in conditions with a high insulin demand.

The GWA studies also associated a cluster of variants in intron 2 of *IGF2BP2* with type 2 diabetes (3–5). However, two other GWA studies did not detect this association (1,2). We show a decrease in glucose-stimulated insulin release, yet this effect was only observed in a cohort of young healthy subjects exposed to an IVGTT, whereas these findings could not be replicated during an OGTT in the Inter99 cohort. This may be accounted for by differences in the age of the subjects or the glucose tolerance tests performed or by statistical type I or II errors. However, as studies of insulin-like growth factor-binding protein homologs in animal models have shown a potential implication in pancreatic development (21,22), a β -cell dysfunction in risk-allele carriers seems likely.

Interestingly, concerning the *HHEX/KIF11/IDE* rs1111875 and *IGF2BP2* rs4402960 variants, the decrease in insulin release was also evident after intravenous tolbutamide injection. This suggests a pharmacogenetic-modifying effect, implying a decreased efficacy of treatment with sulfonylurea in risk-allele carriers.

In the FUSION Study (3), a variant located in an intergenic region on chromosome 11p associated with type 2 diabetes at a genome-wide statistical significance level, yet other GWA studies obtained no signal from this region (1–6). In the present study, no association of rs9300039 with type 2 diabetes or examined metabolic phenotypes was observed despite the application of statistically well-powered case-control materials.

Given prior knowledge on the biological function of the genes located close to rs111875 (*HHEX/KIF11/IDE*) and rs10811661 (*CDKN2A/B*), we hypothesized that these variants influenced quantitative traits related to β -cell function. Therefore, we argue that the need for correction for multiple testing only applies to other phenotypes. Nevertheless, we have applied a permutation procedure to correct for multiple testing within each cohort and variant taking into account correlation between traits. Based on these results, we observe statistically significant corrected *P* values for measures of insulin release for *HHEX/KIF11/IDE* and *CDKN2A/B* variants in the Inter99 cohort of 5,970 middle-aged subjects; yet in the sample of 377 young healthy subjects, none of the nominally significant findings are significant after correction. This is probably due to a rather small sample size, and applying a stringent correction procedure therefore makes type II errors likely.

In the present report, we evaluated quantitative measures of insulin release and sensitivity in two population-based cohorts. In the cohort of young healthy subjects, all participants were subjected to an IVGTT yielding well-defined estimates of insulin release and sensitivity. In the Inter99 cohort, however, only OGTT data were available; we therefore rely on surrogate estimates. Even though we analyzed both well-known indexes, as well as the more recently defined BIGTT indexes, which show a high correlation to IVGTT data, we recognize that the application of more accurate and biologically relevant measures of insulin release and sensitivity may have changed the results.

In conclusion, we present evidence of decreased glucose- and tolbutamide-elicited insulin responses in the diabetes risk-allele carriers of variants in the *HHEX/KIF11/IDE* and *CDKN2A/B* loci indicating β -cell dysfunctions. The causality of the associated variants has not yet been shown, and future fine-mapping and functional studies are needed to determine the origin of association.

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