Impact of rs361072 in the Phosphoinositide 3-Kinase p110β Gene on Whole-Body Glucose Metabolism and Subunit Protein Expression in Skeletal Muscle

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OBJECTIVE—Phosphoinositide 3-kinase (PI3K) is a major effector in insulin signaling. rs361072, located in the promoter of the gene (*PIK3CB*) for the p110β subunit, has previously been found to be associated with homeostasis model assessment for insulin resistance (HOMA-IR) in obese subjects. The aim was to investigate the influence of rs361072 on in vivo glucose metabolism, skeletal muscle PI3K subunit protein levels, and type 2 diabetes.

RESEARCH DESIGN AND METHODS—The functional role of rs361072 was studied in 196 Danish healthy adult twins. Peripheral and hepatic insulin sensitivity was assessed by a euglycemic-hyperinsulinemic clamp. Basal and insulin-stimulated biopsies were taken from the vastus lateralis muscle, and tissue p110β and p85α proteins were measured by Western blotting. The genetic association with type 2 diabetes and quantitative metabolic traits was investigated in 9,316 Danes with glucose tolerance ranging from normal to overt type 2 diabetes.

RESULTS—While hepatic insulin resistance was similar in the fasting state, carriers of the minor G allele had lower hepatic glucose output (per-allele effect: −16%, $P_{add} = 0.004$) during high physiological insulin infusion. rs361072 did not associate with insulin-stimulated peripheral glucose disposal despite a decreased muscle p85α:p110β protein ratio ($P_{add} = 0.03$) in G allele carriers. No association with HOMA-IR or type 2 diabetes (odds ratio 1.07, $P = 0.5$) was identified, and obesity did not interact with rs361072 on these traits.

CONCLUSIONS—Our study suggests that the minor G allele of *PIK3CB* rs361072 associates with decreased muscle p85α:p110β ratio and lower hepatic glucose production at high plasma insulin levels. However, no impact on type 2 diabetes prevalence was found. ***Diabetes*** **59:1108–1112, 2010**

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The phosphoinositide 3-kinase (PI3K) is a key effector in the insulin signaling pathway, mediating, among others, GLUT4 translocation to the cell membrane in skeletal muscle and suppressing hepatic glucose production (HGP). The enzyme consists of regulatory p85 and catalytic p110 subunits existing in different isoforms. In cultured fibroblasts, the quantity of p85 exceeds p110 (1). Free p85 is thought to compete with the p85:p110 complex for phosphotyrosine sites on insulin receptor substrate (IRS)-1. Furthermore, p85 may activate phosphatase and tensin homolog, thereby attenuating insulin signaling (2). The balance between p85 and p110 subunits therefore seems to be critical for signaling through the PI3K pathway (1,3). Hence, gene variants altering transcriptional activity or protein function may affect insulin sensitivity. We have previously shown that an amino acid substitution (Met326Ile) in the p85α subunit was associated with reduced glucose disappearance in homozygous healthy Caucasians (4). rs361072 is a single-nucleotide polymorphism (SNP) located at position −359 in the promoter of *PIK3CB*, the gene encoding the p110β subunit. This promoter region contains a motif for the GATA family of transcription factors. Accordingly, the minor G variant associates with enhanced transcriptional activity, as evidenced by increased p110β mRNA and protein levels in leukocytes (5). In Caucasians, the G allele is common, with a frequency of 48%. Recent studies involving 3,366 French children showed that G allele carriers were protected from obesity-related insulin resistance measured as homeostasis model assessment for insulin resistance (HOMA-IR) (5,6). The effect was smaller in 1,139 obese French adults (7), and in 295 normal-weight Finnish adults, rs361072 did not associate with insulin sensitivity (8).

The aims of the present study were to investigate the functional role of *PIK3CB* rs361072 in metabolically well-characterized Danish adult twins and to examine the association of rs361072 with type 2 diabetes and quantitative metabolic traits in a population-based sample of adult Danes. In addition, we aimed to investigate the potential interaction of the p85α (*PIK3R1*) Met326Ile variant with rs361072 on insulin sensitivity.

RESEARCH DESIGN AND METHODS

In vivo metabolism and heritability were investigated in 196 monozygotic (MZ group; $n = 108$) and same-sex dizygotic (DZ group; $n = 88$) Danish twins without known type 2 diabetes, previously described in detail (9). Subjects were identified having normal glucose tolerance (NGT; $n = 172$), impaired glucose tolerance (IGT; $n = 21$), and screen-detected type 2 diabetes ($n = 3$),

TABLE 1
Characteristics of twin subjects stratified according to *PIK3CB* rs361072 genotypes

PIK3CB rs361072	AA	GA	GG	P_{add}
Genotype frequency (%)	30.4	45.6	24.1	—
n (male/female)	58 (23/35)	87 (42/45)	46 (30/16)	—
Age (years)	44.4 ± 17.4	42.6 ± 16.9	41.2 ± 16.8	—
BMI (kg/m^2)	24.5 ± 3.7	25.6 ± 4.1	24.8 ± 3.7	0.6
Fat percentage	24.8 ± 8.1	25.7 ± 9.3	22.9 ± 7.7	0.3
Fasting insulin (pmol/l)	33.2 ± 17.7	36.6 ± 20.4	34.7 ± 17.6	0.9
30-min insulin (pmol/l)	287.7 ± 129.0	347.4 ± 203.2	334.1 ± 169.9	0.2
120-min insulin (pmol/l)	192.4 ± 193.9	177.2 ± 106.8	154.8 ± 91.0	0.5
Fasting glucose (mmol/l)	5.4 ± 0.8	5.4 ± 0.6	5.3 ± 0.5	0.6
30-min glucose (mmol/l)	8.5 ± 2.0	8.6 ± 1.7	8.4 ± 1.3	0.9
120-min glucose (mmol/l)	6.7 ± 2.4	6.4 ± 1.3	6.3 ± 1.3	0.4
R_d clamp (mg · kg fat-free mass^{-1} · min^{-1})	11.8 ± 3.4	10.5 ± 3.3	10.5 ± 3.5	0.2
HGP clamp (mg · kg fat-free mass^{-1} · min^{-1})	1.7 ± 0.6	1.5 ± 0.5	1.4 ± 0.6	0.004
HGP basal (mg · kg fat-free mass^{-1} · min^{-1})	3.1 ± 0.4	3.1 ± 0.5	2.9 ± 0.4	0.3
Hepatic insulin resistance index	104.3 ± 57.8	112.9 ± 60.0	101.4 ± 54.0	0.7
D_i	$1.7 \pm 1.1 \times 10^{-7}$	$1.8 \pm 1.0 \times 10^{-7}$	$1.7 \pm 1.5 \times 10^{-7}$	0.9

Data are means ± SD. Association of *PIK3CB* rs361072 with quantitative traits is shown in 191 Danish twins. All P_{add} values are adjusted for sex, age, and twin-pair and zygosity status. P_{add} values for plasma levels of glucose and insulin and for metabolic rates and indexes are additionally adjusted for body fat percentage.

according to World Health Organization (WHO) criteria (10). Type 2 diabetes–related quantitative traits were investigated in 5,750 subjects from the Danish population-based Inter99 cohort (11), including individuals with NGT (n = 4,275), impaired fasting glycemia (n = 476), IGT (n = 650), and screen-detected type 2 diabetes (n = 235), according to WHO criteria. Subjects with known type 2 diabetes (n = 114) were excluded from analyses of quantitative traits. The genetic association with type 2 diabetes was assessed in a case-control material including all unrelated type 2 diabetes case and healthy control individuals from the Inter99 sample (case subjects: n = 313, control subjects: n = 4,275), confirmed type 2 diabetic subjects from the ADDITION (Anglo-Danish-Dutch Study of Intensive Treatment in People with Screen-Detected Diabetes in Primary Care) Denmark screening study samples (case subjects: n = 1,577) (12), and type 2 diabetic patients and glucose-tolerant individuals recruited from the outpatient clinic at Steno Diabetes Center (case subjects: n = 1,494, control subjects: n = 495). All study participants had provided informed written consent, and the study was approved by the regional ethical committees and conducted in accordance with the principles of the Helsinki Declaration II.

Clinical examination. All subjects underwent measures of height and weight for calculation of BMI, and a WHO-defined and standardized oral glucose tolerance test (OGTT) was conducted in subjects without known type 2 diabetes. In addition, the twins underwent a dual-energy X-ray absorptiometry scan with measurement of total body fat percentage, a 2-h (40 mU/m^2 per min) euglycemic-hyperinsulinemic clamp, and an intravenous glucose tolerance test (IVGTT) (9). Biopsies were excised from the vastus lateralis muscle (n = 184) during the basal and insulin-stimulated states (13). Plasma glucose and serum insulin were measured as previously described (9). The hepatic insulin resistance index was calculated as basal HGP × fasting serum insulin, and HOMA-IR as fasting serum insulin (pmol/l) × fasting plasma glucose (mmol/l)/22.5. OGTT-derived indexes for acute insulin response (BIGTT-AIR) and insulin sensitivity (BIGTT-S_i) were calculated as described previously (14). 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 IVGTT. β-Cell function was evaluated as the disposition index (D_i) for peripheral insulin action calculated from IVGTT and clamp data (9) or as BIGTT-AIR × BIGTT-S_i.

Genotyping. Genotyping of *PIK3CB* rs361072 and *PIK3R1* rs3730089 was performed by KASPar SNP Genotyping (KBiosciences, Hoddesdon, U.K.), with success rates of 95.7 and 96.8%, respectively. Discordance was 0.34% for rs361072 and 0.84% for rs3730089, as judged from regenotyping of 893 random duplicate samples. Both variants obeyed the Hardy-Weinberg equilibrium ($P > 0.3$).

Muscle protein expression. p85α and p110β contents were determined by Western blotting described in the online appendix (available at http://diabetes.diabetesjournals.org/cgi/content/full/db09-1359/DC1).

Statistical methods. Statistical analyses were performed using R version 2.7.2 (available at http://www.r-project.org) and SAS version 9.1 (SAS Institute, Cary, NC). Heritability (h^2), giving the proportion of the total variation of a trait attributable to genetic variation, was calculated from the intraclass correlations of MZ and DZ twins ($h^2 = 2[r_{MZ} - r_{DZ}]$) (15). Linear models, assuming an additive allele effect, were used to test for associations of genotypes with quantitative traits. Adjustment for twin zygosity and pair status was performed using the SAS PROC MIXED. If not otherwise stated, P values (P_{add}) are adjusted for sex, age, and body fat percentage (BMI in singletons). Interaction analyses compared a two-SNP additive model with a model including additive SNP interaction. Statistical significance was defined as $P < 0.05$. Results are presented as means ± SD, and odds ratios are reported with 95% CI.

RESULTS

Association of *PIK3CB* rs361072 with in vivo metabolism and type 2 diabetes. No differences were identified in BMI, body fat percentage, fasting serum insulin, and fasting plasma glucose among the *PIK3CB* rs361072 genotype groups (Tables 1 and 2). In the Inter99 sample, rs361072 was associated with 30-min post-OGTT plasma glucose with subjects carrying the GG alleles having the lowest value, whereas 2-h plasma glucose was similar. In the twins, the G allele was associated with enhanced insulin suppression of HGP but not with basal HGP or hepatic insulin resistance index. In addition, rs361072 was not associated with insulin-stimulated glucose disposal (R_d) in the twins or indexes of insulin sensitivity in the Inter99 sample. Further stratification of the Inter99 population in lean (BMI <25 kg/m^2), overweight (25 kg/m^2 ≤ BMI ≤ 30 kg/m^2), and obese (BMI >30 kg/m^2) subjects did not change the result ($P_{add} > 0.6$), and no interaction of BMI with rs361072 on HOMA-IR ($P = 0.9$) or BIGTT-S_i ($P = 0.5$) was evident. No association was found between rs361072 and D_i. In the case-control study, rs361072 was not associated with type 2 diabetes (Table 3).

Interaction of *PIK3R1* rs3730089 with *PIK3CB* rs361072. *PIK3R1* rs3730089 did not associate with any glucose metabolic trait in the twin sample, whereas the minor A allele was associated with improved HOMA-IR and BIGTT-S_i in Inter99 subjects (online appendix Tables 1 and 2). rs3730089 did not interact with rs361072 on HOMA-IR ($P = 0.7$) or BIGTT-S_i ($P = 0.3$).

Association of *PIK3CB* rs361072 with muscle PI3K protein. The intraclass correlation coefficients for insulin-stimulated p85α were $r_{MZ} = 0.70$ and $r_{DZ} = 0.57$, giving a

TABLE 2
Characteristics of Inter99 subjects stratified according to *PIK3CB* rs361072 genotypes

PIK3CB rs361072	AA	GA	GG	P_{add}
Genotype frequency (%)	28.6	50.0	21.4	—
n (male/female)	1,613 (824/789)	2,816 (1,400/1,416)	1,206 (584/622)	—
Age (years)	46.3 ± 8.0	46.1 ± 7.8	45.9 ± 7.7	—
BMI (kg/m^2)	26.2 ± 4.5	26.2 ± 4.6	26.1 ± 4.5	0.7
Fasting insulin (pmol/l)	41.0 ± 26.4	42.4 ± 28.7	41.8 ± 28.1	0.3
30-min insulin (pmol/l)	292.0 ± 178.0	287.6 ± 176.8	296.0 ± 203.5	0.9
120-min insulin (pmol/l)	213.9 ± 213.5	219.2 ± 212.0	214.0 ± 208.0	0.4
Fasting glucose (mmol/l)	5.6 ± 0.9	5.5 ± 0.8	5.5 ± 0.7	0.2
30-min glucose (mmol/l)	8.8 ± 1.9	8.7 ± 1.9	8.6 ± 1.8	0.001
120-min glucose (mmol/l)	6.2 ± 2.2	6.2 ± 2.1	6.2 ± 2.0	0.6
HOMA-IR	10.4 ± 7.7	10.7 ± 8.4	10.4 ± 7.6	0.5
BIGTT-S_i	9.2 ± 4.0	9.2 ± 4.1	9.3 ± 4.2	0.4
BIGTT-AIR	1,805.4 ± 934.9	1,851.7 ± 1,124.2	1,872.5 ± 1,072.3	0.1
D_i	15,090.3 ± 7,024.1	15,413.6 ± 7,363.0	15,785.8 ± 7,195.3	0.1

Data are means ± SD. Association of *PIK3CB* rs361072 with quantitative traits in 5,635 Danish subjects. All P_{add} values are adjusted for sex and age. P_{add} values for plasma levels of glucose and insulin, HOMA-IR, BIGTT-S_i, and BIGTT-AIR are additionally adjusted for BMI.

heritability of $h^2 = 0.25$. The similar values for insulin-stimulated p110β were $r_{MZ} = 0.44$ and $r_{DZ} = 0.28$, $h^2 = 0.33$. rs361072 did not associate with p110β protein levels, but carriers of the G allele had decreased p85α and a lower p85α:p110β protein ratio at insulin-stimulated conditions (Table 4). *PIK3R1* rs3730089 did not associate with p85α and p110β protein levels or their ratio (online appendix Table 3). Insulin-stimulated p85α ($P = 0.4$), p110β ($P = 0.8$), or p85α:p110β ($P = 0.4$) were not associated with R_d.

DISCUSSION

Applying the euglycemic-hyperinsulinemic clamp, we demonstrate that the minor G allele of *PIK3CB* rs361072 associates with enhanced suppression of HGP and decreased p85α:p110β protein ratio in skeletal muscle during insulin infusion. However, in the large-scale case-control study, we were unable to demonstrate any association of the G allele with type 2 diabetes.

Previous studies (5–7) have shown that the G allele of rs361072 associates with improved HOMA-IR in obese subjects but not with insulin-stimulated glucose disposal in normal-weight adults (8). Therefore, a role of rs361072 in the development of type 2 diabetes in only obese individuals has been proposed (5). Our present data, however, do not suggest obesity to determine genotype penetrance in adults. The discrepancy between studies using surrogate versus clamp measures of insulin sensitivity may also be explained by the fact that HOMA-IR is influenced predominantly by hepatic insulin resistance (16). Therefore, we examined the association of rs361072 with hepatic insulin sensitivity measured by a euglycemic-hyperinsulinemic clamp at physiological plasma insulin levels. The G allele was associated with a decrease in HGP of 16% during insulin infusion, independent of age and obesity, whereas no influence of rs361072 was seen on hepatic insulin resistance index, indicating a negligible role of genotype at fasting conditions. Given a more pronounced effect of rs361072 at high plasma insulin levels, it could explain the lack of association with HOMA-IR in our study of 5,635 middle-aged Danes. However, the G allele was associated with lower 30-min post-OGTT plasma glucose. At this time point, plasma insulin peaks, and the glucose- and insulin-mediated suppression of HGP has been shown to reach its maximum (17). Therefore, an improved suppression of HGP in G allele carriers might contribute to the decrease in 30-min plasma glucose. Our data do not suggest a role of rs361072 in the regulation of peripheral insulin sensitivity.

Among the currently >20 known type 2 diabetes susceptibility genes, most affect β-cell function, whereas few, including *IRS1*, associate predominantly with insulin resistance (18). The genetic contribution to insulin resistance may be masked by the influence of environmental factors or by the ability of healthy β-cells to compensate (19). As reported previously (7), *PIK3CB* rs361072 was not associated with type 2 diabetes, even in obese subjects,

TABLE 3
Association of *PIK3CB* rs361072 with type 2 diabetes

SNP	Allele (minor/major)	Minor allele frequency (%)	Genotype	Genotype distribution: NGT	Genotype distribution: Type 2 diabetes	Odds ratio (95% CI)
PIK3CB rs361072	(G/A)	47	AA	1,353 (28%)	940 (28%)	1.07 (0.96–1.09); $P = 0.5$
			GA	2,375 (50%)	1,673 (49%)	
			GG	1,042 (22%)	771 (23%)	
Individuals with BMI >30 kg/m^2						
PIK3CB rs361072	(G/A)	47	AA	163 (29%)	484 (28%)	1.11 (0.94–1.31); $P = 0.2$
			GA	283 (50%)	843 (49%)	
			GG	120 (21%)	385 (22%)	

Odds ratios are shown with 95% CI for all subjects and for the subgroup of obese subjects. The association study involved 3,384 type 2 diabetic patients and 4,770 glucose-tolerant control participants. *P* values are adjusted for sex, age, and BMI.

TABLE 4
p85α and p110β muscle proteins in twins stratified according to *PIK3CB* rs361072 genotypes

PIK3CB rs361072	AA	GA	GG	P_{add}
Basal p85α (AU)	762.7 ± 194.9	686.3 ± 226.0	670.2 ± 201.9	0.1
Basal p110β (AU)	488.0 ± 176.1	470.0 ± 152.8	473.8 ± 128.6	0.2
Basal p85α:p110β (AU)	1.74 ± 0.69	1.59 ± 0.73	1.48 ± 0.59	0.06
Insulin p85α (AU)	801.1 ± 260.4	692.3 ± 223.9	624.1 ± 184.0	0.02
Insulin p110β (AU)	510.7 ± 200.2	495.3 ± 196.1	489.7 ± 161.6	0.8
Insulin p85α:p110β (AU)	1.80 ± 0.80	1.60 ± 0.80	1.37 ± 0.51	0.03

Data are means ± SD. Skeletal muscle protein quantities are shown in arbitrary units (AU) for 184 Danish twins. P_{add} values are adjusted for sex, age, body fat percentage, and twin-pair and zygosity status.

despite its association with HGP. This could be explained by the fact that the suppressing effect of the G allele on HGP is evident at high physiological plasma insulin levels only and that the variant does not affect insulin secretion.

Heritability estimates demonstrated a genetic contribution to variance of 25–33% for p85α and p110β protein levels, suggesting a major role of environmental factors in their regulation in skeletal muscle. Accordingly, we did not find an association of rs361072 with p110β muscle protein. Thus, an additional GATA binding site, created by the G variant, may not be sufficient to alter gene expression to a degree that is measurable at the protein level. Surprisingly, subjects homozygous for the A allele had an increase of p85α and a higher p85α:p110β ratio during insulin infusion than GG subjects. Several studies have shown that p110 and p85 are coregulated, and p85 levels may change more dynamically than those of p110 (20,21). Therefore, the p110β variant could possibly play a role in the regulation of p85α expression. A high p85:p110 ratio has been shown to associate with insulin resistance (22), and p85 has been found upregulated in patients with type 2 diabetes (23). We failed to demonstrate an association between muscle p85α:p110β ratio and insulin-stimulated glucose disposal; it is speculated, however, that the improved hepatic insulin sensitivity in G allele carriers might relate to the hepatic p85α:p110β ratio.

Our previous in vitro study of the p85α Met326Ile substitution (rs3730089) showed that the minor A allele was associated with significantly decreased p85α expression in yeast, whereas the decrease was modest in brown preadipocytes (24). Theoretically, a slight reduction of p85α in A allele carriers could explain the improved insulin sensitivity found in the Inter99 sample. However, in skeletal muscle, p85α levels were similar among the genotypes, and as shown previously (25), the variant was not associated with peripheral insulin sensitivity as measured by a euglycemic-hyperinsulinemic clamp. Even though both rs361072 and rs3730089 showed some association with insulin sensitivity, they did not interact.

In conclusion, we demonstrate that the minor G allele of the common *PIK3CB* rs361072 associates with improved hepatic, but not peripheral, insulin sensitivity or type 2 diabetes. The variant does not influence p110β muscle protein expression but may indirectly play a role in the regulation of p85α subunit expression.

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