Promoter Polymorphisms −359T/C and −303A/G of the Catalytic Subunit p110β Gene of Human Phosphatidylinositol 3-Kinase Are Not Associated With Insulin Secretion or Insulin Sensitivity in Finnish Subjects

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OBJECTIVE — Phosphatidylinositol (PI) 3-kinase activity is required for insulin-stimulated translocation of GLUT4 transporters and glucose uptake and utilization. Therefore, genes encoding the subunits of PI 3-kinase are promising candidate genes for insulin resistance and type 2 diabetes. We recently cloned the catalytic subunit p110β gene of human PI 3-kinase and reported two nucleotide polymorphisms, −359T/C and −303A/G, in the promoter region of this gene. In this study, we determined the effects of these polymorphisms on insulin secretion and insulin sensitivity.

RESEARCH DESIGN AND METHODS — We studied two separate groups of Finnish nondiabetic subjects. Insulin secretion was evaluated by intravenous glucose tolerance test and insulin sensitivity by hyperinsulinemic-euglycemic clamp.

RESULTS — Our results showed that the −359T/C and −303A/G polymorphisms did not have a significant effect on fasting plasma insulin levels, insulin secretion, or insulin sensitivity.

CONCLUSIONS — It is unlikely that the promoter polymorphisms −359T/C and −303A/G of the catalytic subunit p110β gene of human PI 3-kinase have a major impact on insulin secretion, insulin sensitivity, or the risk of type 2 diabetes in Finnish subjects.

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Type 2 diabetes is a continuously expanding medical problem in Western countries. The pathogenesis of type 2 diabetes is characterized by defects in insulin action, insulin secretion, or both (1). One of the potential candidate genes for insulin resistance and type 2 diabetes is phosphatidylinositol (PI) 3-kinase, a heterodimeric protein consisting of an 85-kDa regulatory subunit (p85) and a 110-kDa catalytic subunit (p110) (2). So far, two ubiquitously expressed isoforms of p110, p110α (3), and p110β (4), have been characterized. PI 3-kinase is known to participate in the signal transduction pathways of the metabolic and mitogenic effects of insulin and other hormones and growth factors (5,6).

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Abbreviations: IVGTT, intravenous glucose tolerance test; OGTT, oral glucose tolerance test; PI, phosphatidylinositol; S_G, glucose effectiveness; S_I, insulin sensitivity index; WBGU, whole-body glucose uptake.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

PI 3-kinase mediates insulin-stimulated GLUT4 translocation and glucose transport, according to several studies. First, wortmannin and LY294002, inhibitors of PI 3-kinase, block insulin-stimulated glucose transport and GLUT4 translocation to the plasma membrane (5). Second, microinjections of dominant negative mutants of p85, which block the activation of p110, inhibit insulin-stimulated GLUT4 translocation (7,8). Third, overexpression of wild-type or constitutively active forms of p110α (9–11) is sufficient to induce the translocation of GLUT4 to the plasma membrane. Fourth, adenovirus-mediated overexpression of p110β increases GLUT4 translocation and glucose uptake in response to insulin stimulation (12).

Hansen et al. (13) have identified the Met326Ile substitution in the regulatory subunit p85α of PI 3-kinase, and Baier et al. (14) reported that this variant was associated with a low prevalence of type 2 diabetes and improved acute insulin response in Pima women. However, these results were not confirmed in Japanese type 2 diabetic subjects (15). We recently cloned the catalytic subunit p110β gene of human PI 3-kinase and reported two nucleotide polymorphisms, −359T/C and −303A/G, in the 5′ flanking region of this gene (16). Allelic frequencies of the two variants did not differ between diabetic and healthy Finnish subjects (16). This is the first study aiming to investigate the effects of these polymorphisms on insulin secretion and insulin sensitivity in two separate groups of Finnish nondiabetic subjects.

RESEARCH DESIGN AND METHODS

Subjects. The first study group (group I) included 295 normoglycemic subjects

(150 men and 145 women, age 44 ± 1 years, BMI 25.6 ± 0.2 kg/m^2) who were randomly selected from our previous population study (17). They had undergone an intravenous glucose tolerance test (IVGTT) to evaluate first-phase insulin secretion and insulin sensitivity. The other study group (group II) consisted of 110 normoglycemic subjects who had participated in our previous studies (18–20) and who had undergone a hyperinsulinemic-euglycemic clamp (82 men and 28 women, age 51 ± 8 years, BMI 26.1 ± 3.6 kg/m^2). Study subjects (groups I and II, n = 405) did not have any chronic diseases, hypertension, or drug treatment that could affect carbohydrate metabolism. In addition, they did not have abnormalities in liver, kidney, or thyroid function (18). All had normal glucose tolerance according to an oral glucose tolerance test (OGTT) by World Health Organization criteria (21).

The study protocol was approved by the ethics committee of the University of Kuopio and was carried out in accordance with the principals of the Declaration of Helsinki.

OGTT and IVGTT. Both study groups underwent a 2-h OGTT after a 12-h overnight fast (75 g glucose). Levels of blood glucose and plasma insulin were assessed in the fasting state and at 60 and 120 min. In addition, subjects in group I (n = 295) participated in an IVGTT to determine first-phase insulin secretion and insulin sensitivity. After a 12-h overnight fast, two blood samples were taken for the determination of fasting blood glucose and plasma insulin. The blood glucose level was rapidly elevated by an intravenous injection of glucose (0.3 g/kg, 10% solution, administration time 90 s), and the samples for the determination of blood glucose and plasma insulin levels were drawn at 4, 6, 8, 10, 19, 22, 29, 37, 67, 90, and 180 min. At 20 min, additional insulin was administered (0.03 units/kg) to increase the accuracy of the modeling analysis. First-phase insulin secretion was determined by calculating the area under the insulin response curve during the first 10 min of the IVGTT. Insulin sensitivity was assessed by the minimal model of glucose disappearance according to Bergman et al. (22). Insulin sensitivity index (S_I) characterizes insulin sensitivity based on glucose disappearance as a function of insulin in plasma. Glucose effectiveness (S_G) illustrates the enhancement of glucose disappearance due to increased blood glucose level (22).

Hyperinsulinemic-euglycemic clamp. Insulin sensitivity in subjects of group II (n = 110) was evaluated with the hyperinsulinemic-euglycemic clamp as previously described in detail (19). Fasting levels of blood glucose and plasma insulin were determined after a 12-h overnight fast. Plasma insulin was rapidly increased to a desired level with a priming dose of insulin (Actrapid 100 IU/ml; Novo Nordisk, Gentofte, Denmark), which was administered during the first 10 min. The insulin level was maintained constant by a continuous insulin infusion (480 pmol · m^{-2} body surface area · min^{-1}). The level of blood glucose was clamped at 5.0 mmol/l for the next 180 min with an intravenous infusion of 20% glucose solution. The infusion rate was determined according to the sequential measurements (at 5-min intervals) of the level of arterialized blood glucose. The rates of whole-body glucose uptake (WBGU) were calculated on the basis of the mean value of glucose infusion during the last 60 min of the clamp to determine insulin sensitivity.

Indirect calorimetry. Gas exchange measurements were performed using a computerized flow-through canopy gas-analyzer (Deltatrac; Datex, Helsinki, Finland) as previously described (23). In this method, the rates of respiratory O_2 intake and CO_2 production were measured and the rate of glucose oxidation was calculated according to the formula by Ferrannini (24). Two 30-min measurements were done, the first after a 12-h fast and the second during the last 30 min of the hyperinsulinemic-euglycemic clamp. The mean value of the last 20 min of the measurements was used in calculations. The rate of nonoxidative glucose disposal during the hyperinsulinemic-euglycemic clamp was calculated by subtracting the rate of glucose oxidation from the rate of WBGU.

Analysis of the promoter polymorphisms −359T/C and −303A/G of the catalytic subunit p110β gene of human PI 3-kinase in the two study groups. Genomic DNA was isolated from peripheral blood leukocytes. The fragment of promoter region of the p110β gene containing both polymorphisms (−359T/C and −303A/G) was amplified using PCR (forward primer PR1F: 5′-CCT GTC AAG TGC TGG TTA ACT A-3′, reverse primer PR1R2: 5′-CAA TCC ATA CCA CCA ACT AAA G-3′); the size of the PCR product was 191 bp. The reaction was performed in a total volume of 50 μl containing 100 ng genomic DNA, primers (0.5 μmol/μl), 1.25 units DNA polymerase (DynaZyme, Finnzymes, Finland), 100 μmol/l dNTP, and 1.5 mmol/l $MgCl_2$. PCR conditions consisted of denaturation at 94°C for 4 min followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 59°C for 30 s, and extension at 72°C for 30 s with a final extension at 72° for 4 min. Amplified fragments of 295 study subjects were analyzed applying the SNaPshot method according to the manufacturer's instructions (ABI PRISM SNaPshot ddNTP Primer Extension Kit; Applied Biosystems, Foster City, CA). The following probes were used to detect the polymorphisms: 5′-AGT TTA TTC AGA TGT CAA ATA T-3′ (−359T/C) and 5′-GTG TAC ATA TGT ATG TGT ATA T-3′ (−303A/G). The samples of 110 study subjects were analyzed by direct sequencing. Amplified fragments were purified using GFX PCR DNA and Gel Band Purification Kit (Amersham Pharmacia Biotech, Piscataway, NJ) and sequenced using Perkin Elmer's ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) on an ABI PRISM model 310 Genetic Analyzer (Applied Biosystems).

Statistical analysis. Statistical analysis was performed with the SPSS/Win program (version 9.0.1; SPSS, Chicago, IL). ANOVA was used in comparing the means between the genotypes. Insulin and triglyceride values were logarithmically transformed before statistical analysis to obtain normal distribution. All data are represented as means ± SD.

RESULTS

Genotype frequencies of the −359T/C and –303A/G polymorphisms followed Hardy-Weinberg equilibrium and were in linkage disequilibrium in both study groups. The allele frequencies of these polymorphisms in groups I and II were as follows: 0.34 vs. 0.40 for –359T/C and 0.07 vs. 0.09 for –303A/G. In both study groups, these polymorphisms did not have a significant effect on BMI, waist-to-hip ratio (Table 1), fasting plasma insulin level, or area under the insulin curve in the 2-h OGTT (data not shown). Similarly, the −359T/C and –303A/G polymorphisms did not have a significant

Table 1—*Sex, age, BMI, waist-to-hip ratio (groups I and II), insulin secretion at 4 min and insulin response (AUC) during the first 10 min of the IVGTT, S_I, and S_G in 295 Finnish normoglycemic subjects (group I), and the rates of WBGU, glucose oxidation, and nonoxidative glucose disposal during the hyperinsulinemic-euglycemic clamp in 110 Finnish normoglycemic subjects (group II) according to the promoter polymorphisms −359T/C and −303A/G of the catalytic subunit p110β gene of human PI 3-kinase*

	−359T/C			−303A/G		
	T/T	T/C	C/C	A/A	A/G	G/G
Group I						
n	130	128	37	256	39	0
Sex (M/F)	67/63	62/66	21/16	128/128	22/17	—
Age (years)	46 ± 12	44 ± 12	40 ± 10	44 ± 12	46 ± 13	—
BMI (kg/m^2)	26.1 ± 4.0	25.2 ± 3.3	25.0 ± 3.6	25.6 ± 3.7	25.3 ± 3.9	—
Waist-to-hip ratio	0.93 ± 0.10	0.90 ± 0.08	0.90 ± 0.09	0.90 ± 0.08	0.91 ± 0.07	—
4 min insulin (pmol/l)	405 ± 292	326 ± 185	385 ± 224	371 ± 240	350 ± 286	—
Insulin AUC (0–10 min) (pmol/l^{-1} · min^{-1})	2841 ± 1947	2333 ± 1316	2714 ± 1510	2625 ± 1614	2470 ± 1939	—
$S_I \cdot 10^{-4}$ (μU · ml^{-1} · min^{-1})	4.0 ± 2.4	4.5 ± 2.4	4.5 ± 2.4	4.3 ± 2.4	4.2 ± 2.3	—
$S_G \cdot 10^2$ (l/min)	2.1 ± 0.8	2.0 ± 0.7	2.3 ± 1.0	2.1 ± 0.8	2.0 ± 0.8	—
Group II						
n	46	41	23	92	16	2
Sex (M/F)	35/11	32/9	15/8	67/25	13/3	2/0
Age (years)	51 ± 8	52 ± 7	48 ± 7	50 ± 8	52 ± 7	56 ± 8
BMI (kg/m^2)	26.1 ± 3.4	26.5 ± 3.3	25.5 ± 4.5	26.1 ± 3.6	26.1 ± 3.9	24.3 ± 1.3
Waist-to-hip ratio	0.93 ± 0.07	0.95 ± 0.07	0.91 ± 0.08	0.93 ± 0.07	0.96 ± 0.08	0.92 ± 0.07
WBGU (μmol · kg^{-1} · min^{-1})	58.5 ± 16.3	58.2 ± 14.8	57.3 ± 12.6	57.9 ± 14.2	57.9 ± 19.4	70.2 ± 3.9
Glucose oxidation	19.1 ± 4.7	19.4 ± 4.3	20.1 ± 5.6	19.1 ± 4.6	19.9 ± 4.9	28.3 ± 4.7
Nonoxidative glucose disposal	39.4 ± 13.9	38.7 ± 13.5	37.2 ± 11.4	38.7 ± 12.3	38.1 ± 18.1	42.0 ± 8.6

Data are means ± SD. None of the comparisons between the genotypes was statistically significant.

effect on insulin secretion or insulin sensitivity evaluated by IVGTT in subjects of group I (Table 1). Also in group II, these polymorphisms did not have a significant effect on the rates of WBGU or the rates of glucose oxidation and nonoxidative glucose disposal (all *P* values >0.1; adjusted for age, sex, and BMI) (Table 1).

CONCLUSIONS

We recently published an article demonstrating the lack of variants in the coding sequence of the catalytic subunit p110β gene of PI 3-kinase in Finnish subjects with type 2 diabetes (16). The present study based on two independent groups of nondiabetic subjects and different methods to evaluate insulin secretion and insulin sensitivity demonstrated that the −359T/C and −303A/G promoter polymorphisms of p110β gene did not have a significant effect on fasting plasma insulin levels, insulin secretion, or insulin sensitivity (Table 1). Therefore, our study implies that the –359T/C and −303A/G polymorphisms are not likely to regulate the p110β gene expression in such a way as to lead to changes in insulin secretion or insulin sensitivity. However, our results do not exclude the possibility of an impact of these variants, or other variants in this gene, on the development of insulin resistance and type 2 diabetes in other populations.

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