Brief Genetics Report

Gene Encoding the Catalytic Subunit p110β of Human Phosphatidylinositol 3-Kinase

Cloning, Genomic Structure, and Screening for Variants in Patients With Type 2 Diabetes

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Phosphatidylinositol (PI) 3-kinase is a key signaling molecule in insulin-stimulated glucose transport. Therefore, we investigated the catalytic subunit p110β of human PI 3-kinase as a candidate gene for type 2 diabetes. Human p110β gene was cloned from the placental genomic library. All 22 exons, intronic regions flanking the exons and 1.5 kb of the proximal/5′ region of the p110β gene, were screened for variants by single-strand conformation polymorphism analysis in 79 Finnish patients with type 2 diabetes . Allele frequencies of the variants were also determined in 77 nondiabetic control subjects. No variants were found in exons in diabetic patients. However, we identified two nucleotide polymorphisms in the proximal/5′ region of the p110β gene and a variation in the number of 2-bp repeat sequence $(TA)_n$ in intron 4. The allele frequencies did not differ between diabetic and control subjects. Our results may indicate that the catalytic subunit p110β of PI 3-kinase plays such a fundamental role in the insulin-signaling pathway that structural variants are not likely to exist in that gene. The importance of the polymorphisms in the proximal/5′ region of the p110β gene for insulin signaling remains to be determined. *Diabetes* 49:1740–1743, 2000

Type 2 diabetes is characterized by decreased insulin secretion by the pancreatic β-cells and insulin resistance, particularly in skeletal muscle and adipose tissue. Typical findings in insulin resistance are impaired glucose transport, uptake, and utilization (1). The genetic background of type 2 diabetes is still poorly understood. Phosphatidylinositol (PI) 3-kinase is a key signaling enzyme in the insulin-stimulated GLUT4 translocation and glucose transport (2). It is a heterodimeric enzyme consisting of an 85-kDa regulatory subunit (p85) and a 110-kDa catalytic subunit (p110) (3). Four isoforms of p110 (α, β, δ, and γ) have been identified (4–7). Activation of PI 3-kinase in response to insulin increases GLUT4 translocation to plasma membrane and a subsequent stimulation of glucose transport (8). In addition, constitutively active p110 is able to induce GLUT4 translocation to the plasma membrane (9). Because PI 3-kinase plays a crucial role in the insulin-signaling pathway, it is a promising candidate gene for insulin resistance and type 2 diabetes.

The catalytic subunit p110β has not yet been screened for variants in subjects with type 2 diabetes. Therefore, we determined the exon-intron structure of the gene encoding the catalytic subunit p110β of human PI 3-kinase and screened the exons, intronic regions flanking the exons and 1.5 kb of the proximal/5′ region of this gene, for possible variants in subjects with type 2 diabetes.

The first screening of the human placental genomic library was performed using probe B, which represented the central part (1,502–1,901 bp) of the p110β cDNA (Table 1). The screening yielded two positive phage clones that contained six exons each (exons 12–17). These exons corresponded to base pairs 1,771–2,504 of the p110β cDNA. Subsequently, probes A and C were used in screening. They originated from the 5′ (1–1,217 bp) and 3′ (2,505–3,213 bp) ends of the p110β cDNA, respectively. Twelve positive phage clones were obtained. The first six (exons 1–6) and last five exons (exons 18–22) were determined using probes A and C. These exons represented base pairs 1–1,050 and 2,505–3,213 of the p110β cDNA. Hybridization with probe D yielded one positive phage clone that contained four exons (exons 8–11) corresponding to base pairs 1,303–1,770 of the p110β cDNA. One missing exon (exon 7) was obtained by hybridizing the library filters with probe E. Exon 7 represented base pairs 1,051–1,302 of the p110β cDNA.

Human PI 3-kinase p110β gene contains 22 exons that are 51–252 bp in length (GenBank accession numbers AJ297549–AJ297560). The size and position of the exons in the p110β cDNA are summarized in Table 2. Nucleotides of

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PCR, polymerase chain reaction; PI, phosphatidylinositol; RT, reverse transcriptase; SSCP, single-strand conformation polymorphism.

TABLE 1
Sequence of the primers used for generation of hybridization probes for the screening of the human genomic library

Probe	Upper primer (5′→ 3′)	Lower primer (5′→ 3′)	Size of amplified fragment (bp)
A	ATGTGCTTCAGTTTCATAATG	GCATAAACAGCAAAACATAATC	1,217
B	AACCTTATTATTACCCTCCC	TCTTCGTCACTCATCTGTC	400
C	GATGTTGCCTTATGGCTG	TTAAGATCTGTAGTCTTTCC	708
E	TTCATGTCAGGGCTGGTC	TTCCAGCTTTCCTGATGG	246

the exons are numbered so that number 1 is the first nucleotide of the first potential ATG initiation codon according to Hu et al. (5). The data derived from the sequence indicate that each splice junction conforms to the consensus sequence for 5′ donor and 3′ acceptor sites with conservation of the 5′ GT and 3′ AG. The lengths of introns 1, 4, 5, 12, 15, 16, 18, 19, 20, and 21 were determined by sequencing, and the length of intron 9 was determined by gel electrophoresis. The total lengths of introns 2, 3, 6, 7, 8, 10, 11, 13, 14, and 17 are unknown. A diagram of the exon-intron structure of the p110β gene is shown in Fig. 1.

The exons, intronic regions flanking the exons and 1.5 kb of the proximal/5′ region of the gene encoding the catalytic subunit p110β of human PI 3-kinase, were amplified using polymerase chain reaction (PCR) and were screened by single-strand conformation polymorphism (SSCP) analysis. No variants were found in exons of this gene in 79 subjects with type 2 diabetes. However, we identified two nucleotide polymorphisms in the proximal/5′ region of the gene encoding the catalytic subunit p110β of human PI 3-kinase. Polymorphism T→C located 359 bp upstream from the start codon (–359 bp) (allele frequencies of 0.47 and 0.39 in diabetic and control subjects, respectively, NS) and polymorphism A→G at –303 bp (allele frequencies of 0.05 and 0.09 in diabetic and control subjects, NS). In addition, we found a 2-bp repeat sequence $(TA)_n$ in intron 4 (44 nucleotides downstream from exon 4). The number of this repeat sequence varied between 10 and 13 in diabetic and nondiabetic subjects (allele frequencies: 0.97 vs. 0.95 for $[TA]_{10}$, 0.03 vs. 0.03 for $[TA]_{11}$, and 0.01 vs. 0.02 for $[TA]_{13}$). The allele frequencies did not differ between diabetic and control subjects.

Two possibilities have to be considered to explain the lack of variants in the catalytic subunit p110β of human PI 3-kinase. First, sensitivity of SSCP is ~80%; therefore, we could have missed some of the variants. However, our method has been validated against known mutations of the lipoprotein lipase gene (10). Furthermore, we have successfully used our method in the screening of variants in, for example, the insulin receptor substrate-1 (11) and hexokinase II (12). Therefore, we believe that we have not missed a significant number of variants of this gene. Second, and more likely, the p110β subunit of PI 3-kinase could play such an important role in the insulin-signaling pathway that structural variants are not likely to exist in this gene. In addition to insulin-stimulated GLUT4 translocation and glucose transport, PI 3-kinase participates in the regulation of other metabolic effects of insulin, such as glycogen synthesis, antilipolysis, protein synthesis, and inhibition of gluconeogenesis (13). The importance of p110β in signal transduction pathways is further supported by the observation that it is the only characterized isoform which the lipid kinase can be activated through both p85 and G proteins (5,14).

In conclusion, we determined the exon-intron structure of the gene encoding the catalytic subunit p110β of human PI 3-kinase, but we could not demonstrate any variants in the coding sequence. However, we identified two nucleotide polymorphisms in the proximal/5′ region of the p110β gene and a variation in the number of 2-bp repeat sequence $(TA)_n$ in intron 4. The allele frequencies did not differ between diabetic and control subjects. Our results could imply a fundamental role for the p110β subunit in cellular functions and insulin signaling. The importance of the polymorphisms in the proximal/5′ region of the p110β gene for insulin signaling remains to be determined.

RESEARCH DESIGN AND METHODS

Cloning of the gene encoding the catalytic subunit p110β of human PI 3-kinase. The p110β gene was cloned from human placental genomic library (Stratagene, La Jolla, CA). The following five fragments of human p110β cDNA (5) were used as probes to screen the library: probe A, a 1,217-bp fragment (1 to 1217 with respect to the first potential ATG initiation codon according to Hu et al. [5]); probe B, a 400-bp fragment (1,502–1,901); probe C, a 709-bp fragment (2,505–3,213); probe D, a 722-bp fragment (1,088–1,810), and probe E, a 246-bp fragment (1,052–1,297). Probes A, B and C were prepared by reverse transcriptase (RT)-PCR. RT reactions were performed using human skeletal muscle total RNA as a template and random hexamers (probes A and C) or a lower primer (probe B) as primers. PCR was performed from the RT reaction using primers listed in Table 2. Probes D and E were prepared from plasmid p0101 (containing 960–2,160 bp of human p110β cDNA) by *Sac*I/*Sma*I restriction enzyme digestion and ^{32}P-labeled PCR, respectively. The primers are listed in Table 1. The genomic library was plated and transferred to a nylon membrane (Hypond-N; Amersham Pharmacia Biotech, Uppsala, Sweden), as described

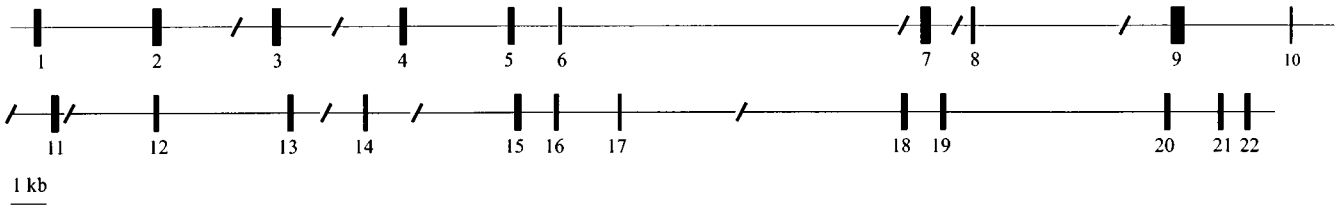

FIG. 1. Structure of the gene encoding the catalytic subunit p110β of human PI 3-kinase. The 22 exons are represented by filled boxes and introns by a thin line. The breaks in the introns 2, 3, 6, 7, 8, 10, 11, 13, 14, and 17 indicate that the total length of the intron is unknown.

TABLE 2
The exon-intron structure of the gene encoding the catalytic subunit p110β of human PI 3-kinase

Exon	Position in cDNA*	Length (bp)	Sequence at the exon-intron junction: Splice acceptor†		Splice donor†	
1	1–171	171			AAGCAG	gtatgt
2	172–397	226	tcatag	ATGTTA	GAAAAG	gtaaat
3	398–621	224	gtttag	GTCTGC	TGCCAG	gtagga
4	622–801	180	cttcag	GACGTG	TTCCAG	gtatgt
5	802–972	171	ctatag	TATATC	ATTTCT	gtaagt
6	973–1,050	78	ttacag	CATGTT	GTAAAA	gtgagt
7	1,051–1,302	252	ttacag	GTTCAT	AAAGTG	gtaatg
8	1,303–1,399	97	caacag	CATTAT	TTCCTG	gtaaga
9	1,400–1,530	131	ttttag	ATGAAC	GATAAG	gtaagc
10	1,531–1,581	51	aaacag	ATTATT	GTGTCA	gtaagt
11	1,582–1,770	189	ttttag	AGTCGA	GCTCAG	gtaaca
12	1,771–1,892	122	aaacag	CTTCAG	GATGAG	gtatct
13	1,893–2,036	144	tttcag	TGATGA	TCTTAG	gtaagt
14	2,037–2,136	100	ctgcag	GTCAGA	AAGCAG	gtatca
15	2,137–2,315	179	tttcag	GTTGAA	ACTCTA	gtaagt
16	2,316–2,425	110	tttcag	TGTTGA	GTGATG	gtaagt
17	2,426–2,504	79	ccttag	ATTTAC	TCTTCG	gtgaga
18	2,505–2,672	168	ttccag	GATGTT	CTCTGG	gttagt
19	2,673–2,796	124	gttcag	GGATGA	GGCCAG	gtgagc
20	2,797–2,942	146	ttccag	CTCTTC	TGGCCG	gtgagt
21	2,943–3,075	133	ccaaag	GTTCCG	CTTAAG	gtataa
22	3,076–3,213	138	ttttag	GACTCT		

*Nucleotides are numbered so that number 1 is the first nucleotide of the first potential ATG initiation codon, according to Hu et al. (5); †the exon sequence is in uppercase, and the intron sequence is in lowercase letters.

by Quertermous (15). The membranes were prehybridized for 2–4 h and hybridized with denaturated α-^{32}P dCTP random primed probe (Ready-To-Go DNA labeling beads; Amersham Pharmacia Biotech) (probes A, B, C, and D) or PCR-labeled probe E for 18 h. The membranes were washed and autoradiographed (16). Positive λ phage clones were purified by two rounds of plating (17) and hybridization steps and were used to make λ phage liquid culture as described (18). Phage DNA was prepared using a Qiagen Lambda Kit (Qiagen, Hilden, Germany).

DNA sequence analysis and determination of genomic structure of the gene encoding the catalytic subunit p110β of human PI 3-kinase. After the digestion of positive phage clones, Southern blot analysis was performed using probes A, B, C, D, and E. The positive fragments were subcloned into pBluescript II SK (+/–) (Stratagene) and then sequenced (ALFexpress, Amersham Pharmacia Biotech). Phage DNA was also directly used as a template in PCR amplification reactions, and the sequence was determined using cycle sequencing (Thermo Sequenase fluorescent-labeled primer cycle sequencing kit with 7-deaza-dGTP; Amersham Pharmacia Biotech). The exon-intron junctions were determined by comparing the genomic sequence with the human p110β cDNA sequence using the Genetics Computer Group software package of the University of Wisconsin.

Subjects. The screening for variants in the gene encoding the catalytic subunit p110β of PI 3-kinase was performed in 79 subjects with type 2 diabetes (39 men and 40 women, aged 63 ± 1 years, BMI 30.0 ± 0.6 kg/m^2, fasting glucose level 9.6 ± 0.3 mmol/l, and fasting insulin level 137.1 ± 10.4 pmol/l). These subjects were randomly selected from our previous population-based study (19), and all subjects had at least one first-degree relative who had type 2 diabetes. The control group comprised 77 healthy men (aged 54 ± 1 years, BMI 26.4 ± 0.4 kg/m^2, fasting glucose level 5.5 ± 0.06 mmol/l, and fasting insulin level 55.8 ± 4.1 pmol/l) who did not have any chronic diseases, hypertension, and abnormality in an oral glucose tolerance test and were not receiving any continuous drug treatment.

SSCP analysis. The exons, intronic regions flanking the exons and 1.5 kb of the proximal/5′ region of the gene encoding the catalytic subunit p110β of human PI 3-kinase, were amplified using PCR. If necessary, the products were digested with restriction enzymes to obtain fragments <250 bp. PCR primers and restriction enzymes are available from the authors. SSCP analysis was performed essentially as described by Orita et al. (20). A PCR reaction was performed in a 6 µl (α-^{33}P) or 10 µl (α-^{32}P) volume containing 100 ng genomic DNA, primers (0.5 µmol/l), 0.136 or 0.25 U DNA polymerase (DynaZyme; Finnzymes, Finland), 0.25 µCi α-^{33}P dCTP or 1 µCi α-^{32}P, dNTP (α-^{32}P: 100 µmol/l dNTP; α-^{33}P: 94 µmol/l d(A,T,G)TP and 75 µmol/l dCTP), and $MgCl_2$ (0.5–1.5 mmol/l). PCR conditions consisted of the following: denaturation at 94°C for 4 min followed by 30–35 cycles of denaturation at 94°C for 30 s, annealing at 51–61°C for 30 s, and extension at 72°C for 30–45 s with a final extension at 72°C for 4 min. PCR fragments from exons 2–4, 6, 7, 9–12, 16, 22, and the proximal/5′ region of the gene encoding the catalytic subunit p110β of human PI 3-kinase were digested with the restriction enzymes. After the enzyme digestion, PCR products were first diluted fourfold with 0.1% SDS (10 mmol/l EDTA) and then (1:1) with loading dye mix (95% formamide, 20 mmol/l EDTA, 0.05% bromphenolblue, and 0.05% xylene cyanol). After denaturation at 98°C for 3 min, the samples were cooled on ice, and 2–3 µl of each sample was loaded onto a 6% nondenaturating polyacrylamide gel. Samples were run at 29 and 38°C. After the electrophoresis, the gel was dried and exposed to an autoradiography film (BioMax; Kodak, Rochester, NY).

Direct sequencing. Genomic DNA from individuals with a variant pattern in SSCP analysis was used as a template in the amplification reaction as previously described (total volume 50 µl, containing 25 pmol of each primer, and 1.25 U Dynazyme DNA polymerase). Amplified fragments were purified by electrophoresis on a 2% low–melting point agarose gel and were sequenced using the Thermo Sequenase radiolabeled terminator cycle sequencing kit (U.S. Biochemicals, Cleveland, OH). Alternatively, the fragments were sequenced using the Perkin Elmer's ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Foster City, CA) on an ABI PRISM Model 310 Genetic Analyzer.

Determination of allele frequencies for the variable lengths of TA repeat sequence in intron 4. The fragment containing the TA repeat sequence was amplified using PCR in a 20-µl volume containing reagents previously described in SSCP analysis. We used the following primers (5′ to 3′): PEX4F (forward, Cy5-labelled) for ACTAATTCAGTTCCAGGTATGT and PEX4R (reverse) for TATTCCAAATGTTCCAGTTGTGG (product size 127 bp). The fluorescently labeled PCR products were electrophoretically separated on an automated laser fluorescence DNA sequencer (Amersham Pharmacia Biotech) using ReproGel Long Read (Amersham Pharmacia Biotech). Two internal size standards were included in each line. The alleles were identified by an ALFwin Fragment Analyzer 1.00 (Amersham Pharmacia Biotech).

Statistical analysis. Clinical characteristics of the study groups were determined using SPSS/Win programs (SPSS, Chicago). All data are represented as means ± SE. Allele frequencies between the study groups were compared with the χ^2 test using the StatXact-3 program (Cytel Software, Cambridge, MA).

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