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Human pancreatic Beta-cell glucokinase: cDNA sequence and localization of the polymorphic gene to chromosome 7, band p 13

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Summary. The glucose phosphorylating enzyme glucokinase plays an important role in the regulation of glucose homeostasis. Studies in rodents indicate that pancreatic Beta cells and hepatocytes express different isoforms of this protein as a consequence of the presence of tissue-specific promoters and exon 1 sequences which are spliced to a shared group of nine exons which encode most of the mRNA and protein. Here, we report the isolation and characterization of cDNA clones encoding human Beta-cell glucokinase. The sequence of human Beta-cell glucokinase shows 97% amino acid identity with that of the cognate rat protein. We also mapped the human glucokinase gene to the short arm of chromosome 7 by analysing its segregation in a panel of reduced human-mouse somatic cell hybrids. In situ hybridization to metaphase chromosomes confirmed the localization of the human glucokinase gene to chromosome 7 and indicated that it was in band p 13. A microsatellite DNA polymorphism that can be typed using the polymerase chain reaction was identified upstream of exon 1a, the Beta-cell specific first exon. The glucokinase cDNA clone and highly informative DNA polymorphism will be useful for examining the role of this gene in the pathogenesis of diabetes mellitus.

Key words: Glucokinase, polymerase chain reaction, microsatellite DNA polymorphism.

Glucokinase (ATP: D-hexose 6-phosphotransferase, EC 2.7.1.1) is expressed by hepatocytes and pancreatic Beta cells and plays a key role in the regulation of glucose homeostasis [1, 2]. In the hepatocyte, the phosphorylation of glucose by glucokinase facilitates the uptake and metabolism of glucose by maintaining a gradient for glucose transport into these cells. In Beta cells, glucokinase is believed to comprise part of the glucose sensing mechanism which regulates insulin secretion. In the rat, glucokinase is encoded by a single-copy gene that has 11 exons [1, 3, 4]. The glucokinase transcripts present in the liver and Beta-cell share sequences encoded by the last nine exons. The sequences of the liver and Beta-cell glucokinase mRNA differ at their 5′-ends because the glucokinase gene has two promoters, one of which functions in the Beta cell and the other which is active in liver. As a consequence, the sequences of the 5′-untranslated regions of the two transcripts differ. In addition, since translation is initiated within the tissue-specific first exon, the sequence of amino acids 1–15 of the liver and Beta-cell isoforms are different. The presence of tissue-specific alternative promoters allows the glucokinase gene to be differentially regulated in these two tissues.

Because of the important role played by glucokinase in the regulation of insulin secretion and the uptake of glucose by the liver, it has been implicated as a candidate gene whose genetic variation or altered regulation could contribute to the development of Type 2 (non-insulin-dependent) diabetes mellitus. Tanizawa et al. [5] have recently described the isolation and characterization of cDNA clones encoding the hepatic isoform of human glucokinase. In this report, we present the sequence of the human Beta-cell isoform. In addition, we have mapped the human glucokinase gene (GCK) to chromosome 7, band p13, and identified a microsatellite DNA polymorphism that will facilitate genetic studies of its role in the development of Type 2 diabetes.

Materials and methods

General methods

Standard methods were carried out as described in Sambrook et al. [6] and as described previously [7]. DNA sequencing was done by the dideoxynucleotide chain-termination procedure after subcloning appropriate DNA fragments into M13mp18 or M13mp19. The sequence was confirmed on both strands.

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GGACACTAAGCCCCACAGCTCAACACAACCAGGAGAGAAAGCGCTGAGGACGCCACCCAAGCGCCCAGCAATGGCCCTGCCTGGAGAACATCCAGGCTCAGTGAGGAAGGGTCCAGAAG  119

GGAATGCTTGCCGACTCGTTGGAGAACAATGAAAAGGAGGAAACTGTGACTGAACCTCAAACCCCAAACCAGCCCGAGGAGAACCACATTCTCCCAGGGACCCAGGGCGGGCCGTGACC  238

                                                                                            1
                                                                                            Met Leu Asp Asp Arg Ala Arg
CCTGCGGCGGAGAAGCCTTGGATATTTCCACTTCAGAAGCCTACTGGGGAAGGCTGAGGGGTCCCAGCTCCCCACGCTGGCTGCTGTGCAG ATG CTG GAC GAC AGA GCC AGG  350

         10 Thr                                  20                                      30                 Ser
Met Glu Ala Ala Lys Lys Glu Lys Val Glu Gln Ile Leu Ala Glu Phe Gln Leu Gln Glu Glu Asp Leu Lys Lys Val Met Arg Arg Met
ATG GAG GCC GCC AAG AAG GAG AAG GTA GAG CAG ATC CTG GCA GAG TTC CAG CTG CAG GAG GAG GAC CTG AAG AAG GTG ATG AGA CGG ATG  440

         40                                      50                                      60
Gln Lys Glu Met Asp Arg Gly Leu Arg Leu Glu Thr His Glu Glu Ala Ser Val Lys Met Leu Pro Thr Tyr Val Arg Ser Thr Pro Glu
CAG AAG GAG ATG GAC CGC GGC CTG AGG CTG GAG ACC CAT GAA GAG GCC AGT GTG AAG ATG CTG CCC ACC TAC GTG CGC TCC ACC CCA GAA  530

         70                                      80                                      90                     Ala
Gly Ser Glu Val Gly Asp Phe Leu Ser Leu Asp Leu Gly Gly Thr Asn Phe Arg Val Met Leu Val Lys Val Gly Glu Gly Glu Glu Gly
GGC TCA GAA GTC GGG GAC TTC CTC TCC CTG GAC CTG GGT GGC ACT AAC TTC AGG GTG ATG CTG GTG AAG GTG GGA GAA GGT GAG GAG GGG  620

        100                                     110                                     120
Gln Trp Ser Val Lys Thr Lys His Gln Met Tyr Ser Ile Pro Glu Asp Ala Met Thr Gly Thr Ala Glu Met Leu Phe Asp Tyr Ile Ser
CAG TGG AGC GTG AAG ACC AAA CAC CAG ATG TAC TCC ATC CCC GAG GAC GCC ATG ACC GGC ACT GCT GAG ATG CTC TTC GAC TAC ATC TCT  710

        130                                     140                                     150
Glu Cys Ile Ser Asp Phe Leu Asp Lys His Gln Met Lys His Lys Lys Leu Pro Leu Gly Phe Thr Phe Ser Phe Pro Val Arg His Glu
GAG TGC ATC TCC GAC TTC CTG GAC AAG CAT CAG ATG AAA CAC AAG AAG CTG CCC CTG GGC TTC ACC TTC TCC TTT CCT GTG AGG CAC GAA  800

    Leu 160                                     170                                     180 Ile
Asp Ile Asp Lys Gly Ile Leu Leu Asn Trp Thr Lys Gly Ile Lys Ala Ser Gly Ala Glu Gly Asn Asn Val Val Gly Leu Leu Arg Asp
GAC ATC GAT AAG GGC ATC CTT CTC AAC TGG ACC AAG GGC ATC AAG GCC TCA GGA GCA GAA GGG AAC AAT GTC GTG GGG CTT CTG CGA GAC  890

        190                                     200                                     210
Ala Ile Lys Arg Arg Gly Asp Phe Glu Met Asp Val Val Ala Met Val Asn Asp Thr Val Ala Thr Met Ile Ser Cys Tyr Tyr Glu Asp
GCT ATC AAA CGG AGA GGG GAC TTT GAA ATG GAT GTG GTG GCA ATG GTG AAT GAC ACG GTG GCC ACG ATG ATC TCC TGC TAC TAC GAA GAC  980

Arg     220                                     230                                     240
His Gln Cys Glu Val Gly Met Ile Val Gly Thr Gly Cys Asn Ala Cys Tyr Met Glu Glu Met Gln Asn Val Glu Leu Val Glu Gly Asp
CAT CAG TGC GAG GTC GGC ATG ATC GTG GGC ACG GGC TGC AAT GCC TGC TAC ATG GAG GAG ATG CAG AAT GTG GAG CTG GTG GAG GGG GAC  1070

        250                                     260                                     270                     Met
Glu Gly Arg Met Cys Val Asn Thr Glu Trp Gly Ala Phe Gly Asp Ser Gly Glu Leu Asp Glu Phe Leu Leu Glu Tyr Asp Arg Leu Val
GAG GGC CGC ATG TGC GTC AAT ACC GAG TGG GGC GCC TTC GGG GAC TCC GGC GAG CTG GAC GAG TTC CTG CTG GAG TAT GAC CGC CTG GTG  1160

        280                                     290     Ile                             300
Asp Glu Ser Ser Ala Asn Pro Gly Gln Gln Leu Tyr Glu Lys Leu Ile Gly Gly Lys Tyr Met Gly Glu Leu Val Arg Leu Val Leu Leu
GAC GAG AGC TCT GCA AAC CCC GGT CAG CAG CTG TAT GAG AAG CTC ATA GGT GGC AAG TAC ATG GGC GAG CTG GTG CGG CTT GTG CTG CTC  1250

Lys     310                                     320                                     330
Arg Leu Val Asp Glu Asn Leu Leu Phe His Gly Glu Ala Ser Glu Gln Leu Arg Thr Arg Gly Ala Phe Glu Thr Arg Phe Val Ser Gln
AGG CTC GTG GAC GAA AAC CTG CTC TTC CAC GGG GAG GCC TCC GAG CAG CTG CGC ACA CGC GGA GCC TTC GAG ACG CGC TTC GTG TCG CAG  1340

        340     Ser                         His 350                                     360 Val Asn
Val Glu Ser Asp Thr Gly Asp Arg Lys Gln Ile Tyr Asn Ile Leu Ser Thr Leu Gly Leu Arg Pro Ser Thr Thr Asp Cys Asp Ile Val
GTG GAG AGC GAC ACG GGC GAC CGC AAG CAG ATC TAC AAC ATC CTG AGC ACG CTG GGG CTG CGA CCC TCG ACC ACC GAC TGC GAC ATC GTG  1430

        370                                     380                                     390
Arg Arg Ala Cys Glu Ser Val Ser Thr Arg Ala Ala His Met Cys Ser Ala Gly Leu Ala Gly Val Ile Asn Arg Met Arg Glu Ser Arg
CGC CGC GCC TGC GAG AGC GTG TCT ACG CGC GCT GCG CAC ATG TGC TCG GCG GGG CTG GCG GGC GTC ATC AAC CGC ATG CGC GAG AGC CGC  1520

        400                                     410                                     420
Ser Glu Asp Val Met Arg Ile Thr Val Gly Val Asp Gly Ser Val Tyr Lys Leu His Pro Ser Phe Lys Glu Arg Phe His Ala Ser Val
AGC GAG GAC GTA ATG CGC ATC ACT GTG GGC GTG GAT GGC TCC GTG TAC AAG CTG CAC CCC AGC TTC AAG GAG CGG TTC CAT GCC AGC GTG  1610

        430         Asn                         440                                     450
Arg Arg Leu Thr Pro Ser Cys Glu Ile Thr Phe Ile Glu Ser Glu Glu Gly Ser Gly Arg Gly Ala Ala Leu Val Ser Ala Val Ala Cys
CGC AGG CTG ACG CCC AGC TGC GAG ATC ACC TTC ATC GAG TCG GAG GAG GGC AGT GGC CGG GGC GCG GCC CTG GTC TCG GCG GTG GCC TGT  1700

        460             Ala 465
Lys Lys Ala Cys Met Leu Gly Gln OP
AAG AAG GCC TGT ATG CTG GGC CAG TGA GAGCAGTGGCCGCAAGCGCAGGGAGGATGCCACAGCCCCACAGCACCCAGGCTCCATGGGGAAGTGCTCCCCACACGTGCTCGC 1811

AGCCTGGCGGGGCAGGAGGCCTGGCCTTGTCAGGACCCAGGCCGCCTGCCATACCGCTGGGGAACAGAGCGGGCCTCTTCCCTCAGTTTTTCGGTGGGACAGCCCCAGGGCCCTAACGGG 1931

GGTGCGGCAGGAGCAGGAACAGAGACTCTGGAAGCCCCCCACCTTTCTCGCTGGAATCAATTTCCCAGAAGGGAGTTGCTCACTCAGGACTTTGATGCATTTCCACACTGTCAGAGCTGT 2051

TGGCCTCGCCTGGGCCCAGGCTCTGGGAAGGGGTGCCCTCTGGATCCTGCTGTGGCCTCACTTCCCTGGGAACTCATCCTGTGTGGGGAGGCAGCTCCAACAGCTTGACCAGACCTAGAC 2171

CTGGGCCAAAAGGGCAGCCAGGGGCTGCTCATCACCCAGTCCTGGCCATTTTCTTGCCTGAGGCTCAAGAGGCCCAGGGAGCAATGGGAGGGGGCTCCATGGAGGAGGTGTCCCAAGCTT 2291

TGAATACCCCCAGAGACCTTTTCTCTCCCATACCATCACTGAGTGGCTTGTGATTCTGGGATGGACCCTCGCAGCAGGTGCAAGAGACAGAGCCCCCAAGCCTCTGCCCCAAGGGGCCCA 2411

CAAAGGGGAGAAGGGCCAGCCCTACATCTTCAGCTCCCATAGCGCTGGCTCAGGAAGAAACCCCAAGCAGCATTCAGCACACCCCAAGGGACAACCCCATCATATGACATGCCACCCTCT 2531

CCATGCCCAACCTAAGATTGTGTGGGTTTTTAATTAAAAATGTTAAAAGTTTTAAAAAAAAAAAAAAAAAAAA                                                2606
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Fig. 1. Composite nucleotide sequence of human Beta-cell glucokinase cDNA and predicted amino acid sequence of the protein. The sequence encoded by the Beta-cell-specific exon 1a is shown in bold-face type. The number of the nucleotide at the end of each line is noted. The sequence was obtained from the following clones: hGK-p5, nucleotides 1-393; hGK3.1, nucleotides 372-831; and hGK12-1, nucleotides 771-2606. The corresponding amino acid residue of rat Beta-cell glucokinase [4, 14] is indicated above that of the human sequence at those sites at which the sequences differ. There are three differences between the cDNA sequence presented here and the sequence of human liver glucokinase [15] in the region in which these two sequences overlap– Codon 107 is ATG (Met) here, in the human glucokinase gene (unpublished) and in the rat glucokinase cDNA sequence [14], and ACG (Thr) in Matsutani et al. [15]; and there are two differences in the 3′-untranslated region (underlined) which are G and C in our sequence and GG and CC, respectively, in Reference 15. We show the sequence of codon 74 as being TTC (Phe) which is also the sequence in Reference 15 and in the gene (unpublished). However, in two independent polymerase chain reaction products that were obtained from this region it was TCC (Ser). We assume that this difference is a consequence of misincorporation by either reverse transcriptase or Taq DNA polymerase. It is unknown if the sequence differences noted above represent polymorphisms in the human glucokinase gene

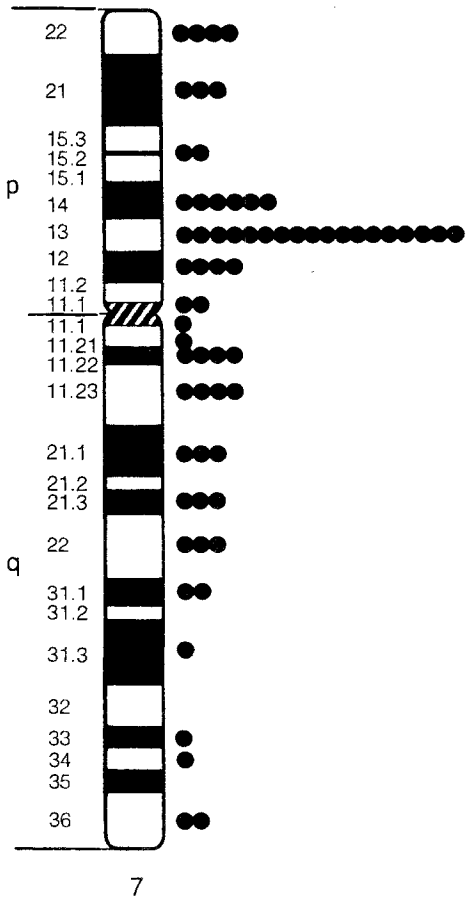

Fig. 2. Ideogram of human chromosome 7 showing silver grain distribution after hybridization with phGK12-1. One hundred metaphase spreads were examined. Of the total number of grains, 27.1% (65 of 240) were on chromosome 7, and of these, 36.9% (24 of 65) were at 7p13→p14. Eighteen percent of metaphase spreads examined showed a silver grain at 7p13→p14

Isolation of human glucokinase cDNA clones

A human liver cDNA library was screened using low-stringency hybridization conditions [8] (hybridization conditions – 5 × SSC, 25% formamide, 2 × Denhardt's solution, 20 mmol/l sodium phosphate buffer, pH 6.5, 0.1% sodium dodecyl sulphate, 100 μg/ml of sonicated and denatured salmon testes DNA, 10% dextran sulphate, and 1×10^6 cpm/ml of probe at 37 °C for 16–20 h; washing conditions – 2 × SSC and 0.1% sodium dodecyl sulphate, for 1 h each at room temperature and then at 40 °C) with a ^{32}P-labelled 3248 base pair (bp) EcoRI fragment of the human hexokinase I cDNA clone, λhHEX-15 [7], which codes for amino acids 92–917 and the 3′-untranslated region of the mRNA. The cDNA clone λhGK12-1 was obtained using this procedure. The remainder of the cDNA was obtained by reverse transcription-polymerase chain reaction (PCR) amplification of human insulinoma mRNA using specific primers [rGK-13 (sense primer) 5′-GTCGAGCAGATCCTGGCAGAG-3′ and ohGK-2r (antisense primer) 5′-TGGTCCAGTTGAGAAG-GAAG-3′; the sequence of rGK-13 was based on the sequence of rat glucokinase mRNA] which gave cDNA clone – hGK3.1, and by the rapid amplification of cDNA ends (RACE) procedure [9] using human insulinoma mRNA and the specific primer (antisense) 5′-CTCTGCCAGGATCTGCTCTAC-3′ which generated a cDNA clone, hGK-p5, containing the 5′-end of human Beta-cell glucokinase mRNA. At least two PCR and RACE products obtained from each amplification were sequenced to control for errors that might be introduced in the amplification by Taq DNA polymerase. A cDNA encoding the human Beta-cell isoform of glucokinase, designated pGEM-hGK20 (vector-pGEM4Z) was generated using the three clones described above.

Gene mapping

The chromosomal location of human GCK was determined by hybridization of ^{32}P-labelled phGK12-1 (the insert of λGK12-1 subcloned into pBR327) to Southern blots of EcoRI-digested DNA from 36 different reduced human-mouse somatic cell hybrid cell lines [10] as described previously [11]. The regional localization of GCK was determined by in situ hybridization of ^{3}H-labelled phGK12-1 to normal human prometaphase chromosomes as described by Nakai et al. [12].

Isolation of the human GCK gene and identification of a microsatellite DNA polymorphism

The human GCK gene was isolated from a genomic library (946203, Stratagene, La Jolla, Calif., USA) by hybridization with the insert from the cDNA clone pGEM-hGK20. Five clones [λhGK-1, -2, -4, -5 and -7] were isolated; these clones contain all the exons of the human GCK gene. The clones containing CA-dinucleotide repeats were identified by hybridization with nick-translated ^{32}P-labelled poly (dA-dC)-poly (dG-dT) (Pharmacia LKB Biotechnology, Piscataway, NJ, USA) [13] (hybridization conditions – 0.5 mol/l sodium phosphate buffer, pH 7.0, 7% sodium dodecyl sulphate, 1% bovine serum albumin, and 1×10^6 cpm/ml of probe at 42 °C for 16–20 h; washing conditions – 1 × SSC and 1% sodium dodecyl sulphate, for 1 h each at room temperature and then at 50 °C). A 3.0 kb Hind III fragment from λhGK-7 was isolated, digested with Sau3A I, and ligated into BamHI-digested M13mp19. The inserts in M13 clones hybridizing with ^{32}P-labelled poly (dA-dC)-poly (dG-dT) were sequenced.

Amplification of the microsatellite DNA polymorphism

Two primers (hGK-CA-1, 5′-AACAGATACGCTTCATCCTG-3′; and hGK-CA-2, 5′-TGTCTGCAACTTACTCTTAC-3′) were used to amplify a 127–143 bp TC and AC repeat-rich region upstream of the Beta-cell specific exon, exon 1a, of human GCK. The PCR was performed using ^{32}P-labelled hGK-CA-1 and unlabelled hGK-CA-2. DNA was initially denatured at 94 °C for 6 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 2 min, extension at 72 °C for 2 min and a final extension step of 10 min. The PCR products were analysed on a 5% denaturing polyacrylamide gel. The PCR reactions were carried out in a volume of 25 μl containing 50 mmol/l KCl, 10 mmol/l Tris-HCl (pH 8.3), 1.5 mmol/l $MgCl_2$, 100 μg/ml gelatin, 200 μmol/l each of dATP, dGTP, dCTP, and dTTP; and 0.1 μg of DNA, 10 pmol of each primer, and 1.5 U of Taq polymerase (Perkin Elmer Cetus, Norwalk, Conn., USA).

Results

Sequence of human Beta-cell glucokinase cDNA and protein

Human glucokinase cDNA clones were isolated from a liver cDNA library by low-stringency cross-hybridization with a human hexokinase I cDNA probe. The sequence of the insert in one of these clones, now termed λhGK12-1, did not correspond to hexokinase I. With the publication

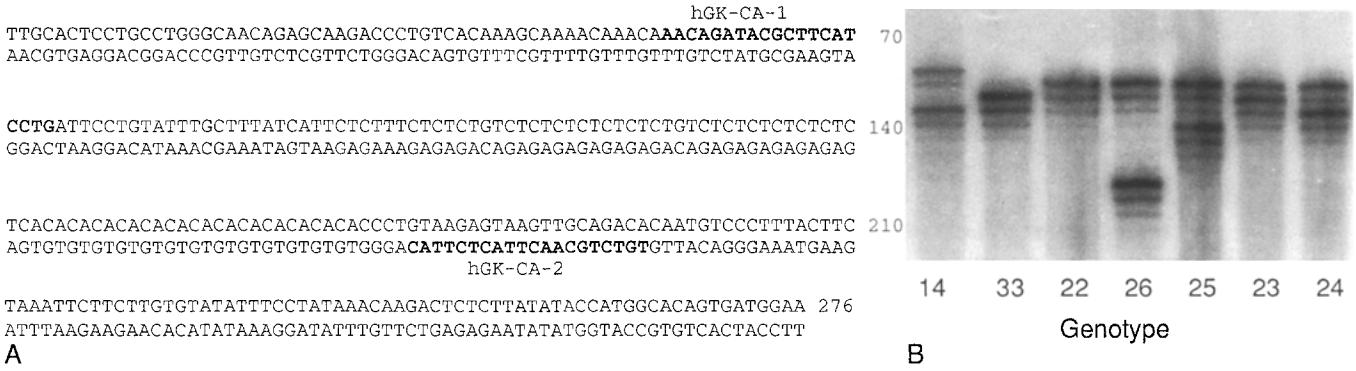

Fig. 3 A, B. Microsatellite DNA polymorphism in the human glucokinase gene. **A** The nucleotide sequence of the polymorphic region upstream of exon 1 a is shown. The sequences of the nucleotide primers used to amplify this region are shown in bold-face type. **B** Polymerase chain reaction amplification of microsatellite DNA polymorphism. The genotypes of the unrelated individuals studied here are shown at the bottom of the figure

of the rat glucokinase cDNA sequence by Andreone et al. [14], it became evident that λhGK12-1 encoded part of human glucokinase. Since subsequent reports indicated that the region of glucokinase mRNA present in this clone was common to transcripts expressed by both liver and Beta cells/insulinomas, it was used as a focus to obtain the sequence for the human Beta-cell isoform. The remainder of the human sequence was obtained with a combination of reverse transcription-PCR and RACE-based approaches using human insulinoma RNA. The composite sequence of the human glucokinase cDNA clones is shown in Figure 1 (the strategy for obtaining this sequence is outlined in Materials and methods and in Fig. 1 legend). This sequence indicates that human Beta-cell glucokinase is 465 amino acids ($M_r = 52166$). There is 97% amino acid identity between human and rat Beta-cell glucokinase. The fifteen differences between human and rat Beta-cell glucokinase are shown in Figure 1. They are scattered throughout the protein and one is in the region encoded by the Beta-cell specific exon 1. Six of the differences (amino acids 159, 181, 276, 292, 308, and 342) represent conservative amino acid replacements.

Localization of the human glucokinase gene

The human glucokinase cDNA probe phGK12-1 hybridized to EcoRI fragments of 11 and 6.1 kb in digests of human DNA which were readily distinguished from a mouse-specific EcoRI fragment of 18 kb. The presence or absence of the human-specific fragments were scored in a somatic cell hybrid panel comprised of 36 different cell lines. The human specific fragments were only present in those cell lines that retained human chromosome 7 (data not shown) indicating that GCK was on this chromosome. The human GCK gene was also present in two hybrid cell lines with no intact chromosome 7, but with chromosome 7 translocations: hybrid JSRL-17S2 with a 7/9 translocation [7pter→7q22::9p24→pter]; and XOL-21 [ISO7p: 7pter→7centromere] localizing the gene to the short arm of chromosome 7, pter→centromere.

The assignment of GCK to chromosome 7 and a more precise regional localization was obtained by in situ hybridization of ^{3}H-labelled phGK12-1 to normal prometaphase chromosomes. The distribution of silver grains over chromosome 7 (Fig. 2) indicated that GCK was in bands p13→p14, with the most likely location being in band p13.

Characterization of a microsatellite DNA polymorphism

A region upstream of exon 1 a, the Beta-cell-specific exon of human GCK hybridized with a ^{32}P-labelled poly (dA-dC)-poly (dG-dT) probe. The sequence of this region (Fig. 3 A) revealed two adjacent regions of repeating dinucleotide repeats; $(TC)_n$ and $(CA)_n$. Oligonucleotide primers flanking this region were selected and used to amplify this region in a series of unrelated subjects. This region was polymorphic (Fig. 3 B). Six alleles were noted in a group of 56 unrelated individuals of different races and their frequencies appear to vary among these racial groups (Table 1). This DNA polymorphism showed codominant inheritance in five informative families.

Discussion

We have determined the sequence of cDNAs encoding the Beta-cell isoform of human glucokinase. Human Beta-cell glucokinase is a protein of 465 amino acids that shares 97% amino acid identity with the corresponding rat protein. However, in contrast to rat Beta-cell and liver glucokinases which are both comprised of 465 amino acids [3, 4], the human Beta-cell and liver isoforms are 465 and 466 amino acids, respectively. This difference in size is a consequence of the differences in the sizes of the segments encoded by the Beta-cell and liver-specific first exons which are 15 and 16 amino acids, respectively. Thus, the sequences of the NH_2-terminal domains of the human Beta-cell and liver proteins differ in length and in size. The functional consequences of these differences are unknown.

We have physically mapped the human GCK gene to chromosome 7 in band p13 and described a microsatellite DNA polymorphism which will be useful for genetic studies. While this manuscript was in preparation, Matsu-

Table 1. Characterization of microsatellite DNA polymorphism in the human glucokinase gene

Allele frequencies		Racial group		
Allele	Size (base pairs)	Caucasian ($n = 29$)	Asian ($n = 14$)	African-American ($n = 13$)
1	143	–	–	0.04
2	141	0.32	0.25	0.46
3	139	0.62	0.57	0.23
4	137	0.02	0.18	0.27
5	135	0.02	–	–
6	127	0.02	–	–
Heterozygosity		0.51	0.58	0.66
PIC value		0.43	0.52	0.60

PIC, polymorphic information content

tani et al. [15] reported the mapping of human GCK to chromosome 7p by linkage analysis. Our results are in complete agreement with those of these authors and, in addition, provide a precise physical localization for GCK on the short arm of chromosome 7 within band p13. Matsutani et al. [15] also described a $(CA)_n$ repeat polymorphism in GCK. This microsatellite DNA polymorphism is different from the one that we have reported here. These two microsatellite DNA polymorphisms can be combined to make GCK more informative for linkage studies.

Froguel et al. [16] have recently reported studies showing close linkage of DNA polymorphisms in the glucokinase locus with early-onset Type 2 diabetes. The sequences of human Beta-cell glucokinase mRNA and protein described above will facilitate studies of the role of this candidate diabetes-susceptibility gene in the development of Type 2 diabetes mellitus.

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