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Cloning and primary structure of a human islet isoform of glutamic acid decarboxylase from chromosome 10

(cDNA cloning/chromosomal localization/islet cell antibodies/insulin-dependent diabetes)

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ABSTRACT **Glutamic acid decarboxylase (GAD; glutamate decarboxylase, L-glutamate 1-carboxy-lyase, EC 4.1.1.15), which catalyzes formation of γ-aminobutyric acid from L-glutamic acid, is detectable in different isoforms with distinct electrophoretic and kinetic characteristics. GAD has also been implicated as an autoantigen in the vastly differing autoimmune disease stiff-man syndrome and insulin-dependent diabetes mellitus. Despite the differing GAD isoforms, only one type of GAD cDNA (GAD-1), localized to a syntenic region of chromosome 2, has been isolated from rat, mouse, and cat. Using sequence information from GAD-1 to screen a human pancreatic islet cDNA library, we describe the isolation of an additional GAD cDNA (GAD-2), which was mapped to the short arm of human chromosome 10. Genomic Southern blotting with GAD-2 demonstrated a hybridization pattern different from that detected by GAD-1. GAD-2 recognizes a 5.6-kilobase transcript in both islets and brain, in contrast to GAD-1, which detects a 3.7-kilobase transcript in brain only. The deduced 585-amino acid sequence coded for by GAD-2 shows <65% identity to previously published, highly conserved GAD-1 brain sequences, which show >96% deduced amino acid sequence homology among the three species. The function of this additional islet GAD isoform and its importance as an autoantigen in insulin-dependent diabetes remain to be determined.**

The inhibitory neurotransmitter γ-aminobutyric acid (GABA), derived from L-glutamic acid by glutamic acid decarboxylase (GAD; glutamate decarboxylase, L-glutamate 1-carboxy-lyase, EC 4.1.1.15) is present in brain as well as several tissues outside the central nervous system (for review see ref. 1). In early work, *Escherichia coli* GAD was crystallized (2) allowing identification of its pyridoxal 5′-phosphate (PLP, or vitamin B_6) cofactor binding site, -Xaa-His-Lys(ε-PLP)-Xaa- (3, 4). Multiple forms of GAD (5, 6) with distinct tissue expression (1, 7), subcellular localization (8–10), and developmental expression (11, 12) have since been reported. Biological functions of GAD and GABA extend beyond regulation of neurotransmission to include effects on the immune system as well as modulation of cell proliferation, protein synthesis, and metabolism (for reviews see refs. 1 and 13).

GAD has recently been associated with autoimmune insulin-dependent diabetes mellitus (IDDM) (14) because of the increased incidence of IDDM in patients with stiff-man syndrome, a disease associated with autoantibodies against GAD (15–18). Also, antibodies from diabetic sera, originally characterized by their ability to immunoprecipitate a M_r 64,000 autoantigen from islets of Langerhans (19–22), reacted with GAD isolated from both brain and islets (14).

Studies of GAD expression demonstrate at least two different isoforms associating into dimeric GAD isoenzymes of approximately M_r 120,000 (23). Various groups have described the subunits as being approximately M_r 59,000–63,000 (23–25), M_r 65,000 and 67,000 (9), or 40,000 and 80,000 (26, 27). Additionally, porcine brain was shown to have three GAD isoforms (5), whereas four forms with different hydrophobic and kinetic properties were demonstrated in rat brain (6). Despite such differing isoforms, only one type of highly conserved mammalian GAD cDNA has been cloned from the brain of rat (28), mouse (29), and cat (30). This cDNA, denoted here by GAD-1, is coded for by a gene mapped to a syntenic region on mouse and human chromosome 2 (31, 32). GAD-1 recognizes a 3.7-kilobase (kb) transcript in brain and shows 96% homology in the deduced amino acid sequence among the three species. Recently, a partial human GAD sequence isolated from testis (7) showed the same high homology to the GAD-1 sequences and demonstrated both 2.5-kb and 3.7-kb transcripts in testis. A third isoform, resulting from alternative splicing, is present in embryonic rat brain (12). Finally, another GAD form with little homology to the GAD-1 sequences and coding for a protein of M_r 80,000 has recently been cloned from mouse brain (27).

Pancreatic islets contain large amounts of GABA (33) and GAD (34), but having found the expression of GAD-1 to be undetectable in islets, we set out to identify the specific GAD form expressed there. A human islet cDNA library was prepared and screened with oligonucleotide probes of the consensus GAD-1 sequence in brain. We report here the isolation of a GAD cDNA recognizing a prominent 5.6-kb transcript in both islets and brain. This additional human GAD cDNA (denoted by GAD-2) further differs from GAD-1 in its deduced amino acid sequence and genomic localization to human chromosome 10. GAD-2 may help to elucidate the function of GAD in the pancreatic β cells as well as its pathogenetic role in IDDM.

MATERIALS AND METHODS

Human Islet Isolation, Cell Culture, and Tissue. Islets from human pancreases, obtained after proper consent from organ transplant donors, were isolated by using collagenase digestion and Ficoll gradient centrifugation as described (35, 36). Dog islets were isolated by similar techniques. Both human and dog islets showed intact first- and second-phase insulin secretion after perifusion with glucose (data not shown).

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Abbreviations: GAD, glutamic acid decarboxylase; GABA, γ-aminobutyric acid; PLP, pyridoxal 5′-phosphate; RACE, rapid amplification of cDNA ends; IDDM, insulin-dependent diabetes mellitus.
§The sequence reported in this paper has been deposited in the GenBank data base (accession no. M74826).

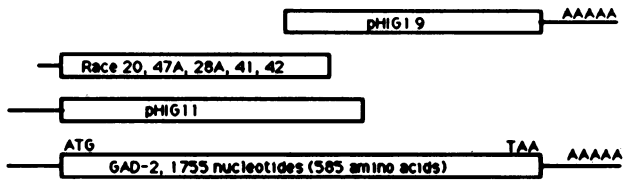

FIG. 1. Cloning strategy of human islet GAD. See the text for an explanation.

Human lymphoblastoid AL-34 cells were cultured under conditions described for RIN-5AH-B cells (37). At the time of mRNA isolation, cells were pelleted, washed in phosphate-buffered saline, and immediately lysed as described below. Human spleen, attached to a pancreas, was obtained with proper consent and was snap frozen for later mRNA isolation. Dog, rat, and monkey brain, as well as dog liver obtained at necropsy, were also snap frozen for later mRNA isolation.

Isolation of mRNA. Two mRNA isolation methods were used. Poly(A)$^+$ RNA from cultured cells and from human islets (for the islet library) was isolated by lysing the cells ($10–15 \times 10^7$ cells) or islets (about 30,000) in 0.2 M Tris·HCl, pH 7.5/0.2 M NaCl/1.5 mM $MgCl_2$/2% SDS/proteinase K at 200 μg/ml (lysis buffer) and homogenizing them by using needles of decreasing caliber, followed by digestion at 45°C for 1–2 hr. The lysis buffer was then adjusted to 0.5 M NaCl, and poly(A)$^+$ RNA was selected by oligo(dT)-cellulose chromatography. After 1 hr, the oligo(dT)-cellulose was washed in 0.5 M NaCl/0.01 M Tris·HCl, pH 7.5 before elution of poly(A)$^+$ RNA with 0.01 M Tris·HCl at pH 7.5 and precipitation at −20°C with 2 volumes of ethanol and 0.1 volume of 3 M NaOAc. In the second method, poly(A)$^+$ RNA was isolated essentially as described (38). Frozen tissue or freshly isolated islets were lysed and homogenized in 8 M guanidine·HCl/25 mM NaOAc and precipitated by 0.6 volume of ethanol at −20°C. The precipitate was reconstituted in guanidine buffer, phenol/chloroform extracted, and reprecipitated before oligo(dT) selection. The concentration and purity of isolated mRNA were determined at A_{260} and A_{280}.

Construction and Screening of the Human Islet cDNA Library. A nonamplified cDNA library was constructed from 2.5 μg of poly(A)$^+$ RNA isolated from human islets by using the Librarian II cDNA library system (Invitrogen, San Diego) according to the manufacturer's instructions. cDNA strands larger than 600 base pairs were ligated into the Librarian II pcDNA II vector and electroporated into ElectroMAX DH10B cells (GIBCO/BRL). After replication to nylon filters, 2×10^6 colonies were screened by hybridization with 20-mer [^{32}P]ATP-labeled oligonucleotide probes (39) representing consensus nucleotide sequences at the 5′ end, center, and 3′ end of GAD-1 (28–30). Six positive clones with insert sizes from 0.7 to 1.4 kb were selected. By rescreening the library with one of these clones, another clone (pHIG1.9) with a 1.9-kb insert was isolated.

Rapid Amplification of cDNA Ends (RACE). To obtain sequence information of the entire coding region of human islet GAD-2, 5′ RACE reactions (40) were performed with

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1    GCACTC GCTGGCGACC TGCTCCAGTC TCCAAAGCCG ATG GCA TCT CCG GGC TCT GGC TTT TGG TCT TTC GGG TCG GAA GAT GGC TCT GGG GAT TCC GAG AAT CCC GGC
                                             Met Ala Ser Pro Gly Ser Gly Phe Trp Ser Phe Gly Ser Glu Asp Gly Ser Gly Asp Ser Glu Asn Pro Gly

109  ACA GCG CGA GCC TGG TGC CAA GTG GCT CAG AAG TTC ACG GGC GGC ATC GGA AAC AAA CTG TGC GCC CTG CTC TAC GGA GAC GCC GAG AAG CCG GCG GAG AGC
     Thr Ala Arg Ala Trp Cys Gln Val Ala Gln Lys Phe Thr Gly Gly Ile Gly Asn Lys Leu Cys Ala Leu Leu Tyr Gly Asp Ala Glu Lys Pro Ala Glu Ser

211  GGC GGG AGC CAA CCC CCG CGG GCC GCC GCC CGG AAG GCC GCC TGC GCC TGC GAC CAG AAG CCC TGC AGC TGC TCC AAA GTG GAT GTC AAC TAC GCG TTT CTC
     Gly Gly Ser Gln Pro Pro Arg Ala Ala Ala Arg Lys Ala Ala Cys Ala Cys Asp Gln Lys Pro Cys Ser Cys Ser Lys Val Asp Val Asn Tyr Ala Phe Leu

313  CAT GCA ACA GAC CTG CTG CCG GCG TGT GAT GGA GAA AGG CCC ACT TTG GCG TTT CTG CAA GAT GTT ATG AAC ATT TTA CTT GAG TAT GTG GTG AAA AGT TTC
     His Ala Thr Asp Leu Leu Pro Ala Cys Asp Gly Glu Arg Pro Thr Leu Ala Phe Leu Gln Asp Val Met Asn Ile Leu Leu Gln Tyr Val Val Lys Ser Phe

415  GAT AGA TCA ACC AAA GTG ATT GAT TTC CAT TAT CCT AAT GAG CTT CTC CAA GAA TAT AAT TGG GAA TTG GCA GAC CAA CCA CAA AAT TTG GAG GAA ATT TTG
     Asp Arg Ser Thr Lys Val Ile Asp Phe His Tyr Pro Asn Glu Leu Leu Gln Glu Tyr Asn Trp Glu Leu Ala Asp Gln Pro Gln Asn Leu Glu Glu Ile Leu

517  ATG CAT TGC CAA ACA ACT CTA AAA TAT GCA ATT AAA ACA GGG CAT CCT AGA TAC TTC AAT CAA CTT TCT ACT GGT TTG GAT ATG GTT GGA TTA GCA GCA GAC
     Met His Cys Gln Thr Thr Leu Lys Tyr Ala Ile Lys Thr Gly His Pro Arg Tyr Phe Asn Gln Leu Ser Thr Gly Leu Asp Met Val Gly Leu Ala Ala Asp

619  TGG CTG ACA TCA ACA GCA AAT ACT AAC ATG TTC ACC TAT GAA ATT GCT CCA GTA TTT GTG CTT TTG GAA TAT GTC ACA CTA AAG AAA ATG AGA GAA ATC ATT
     Trp Leu Thr Ser Thr Ala Asn Thr Asn Met Phe Thr Tyr Glu Ile Ala Pro Val Phe Val Leu Leu Glu Tyr Val Thr Leu Lys Lys Met Arg Glu Ile Ile

721  GGC TGG CCA GGG GGC TCT GGC GAT GGG ATA TTT TCT CCC GGT GGC GCC ATA TCT AAC ATG TAT GCC ATG ATG ATC GCA CGC TTT AAG ATG TTC CCA GAA GTC
     Gly Trp Pro Gly Gly Ser Gly Asp Gly Ile Phe Ser Pro Gly Gly Ala Ile Ser Asn Met Tyr Ala Met Met Ile Ala Arg Phe Lys Met Phe Pro Glu Val

823  AAG GAG AAA GGA ATG GCT GCT CTT CCC AGG CTC ATT GCC TTC ACG TCT GAA CAT AGT CAT TTT TCT CTC AAG AAG GGA GCT GCA GCC TTA GGG ATT GGA ACA
     Lys Glu Lys Gly Met Ala Ala Leu Pro Arg Leu Ile Ala Phe Thr Ser Glu His Ser His Phe Ser Leu Lys Lys Gly Ala Ala Ala Leu Gly Ile Gly Thr

925  GAC AGC GTG ATT CTG ATT AAA TGT GAT GAG AGA GGG AAA ATG ATT CCA TCT GAT CTT GAA AGA AGG ATT CTT GAA GCC AAA CAG AAA GGG TTT GTT CCT TTC
     Asp Ser Val Ile Leu Ile Lys Cys Asp Glu Arg Gly Lys Met Ile Pro Ser Asp Leu Glu Arg Arg Ile Leu Glu Ala Lys Gln Lys Gly Phe Val Pro Phe

1027 CTC GTG AGT GCC ACA GCT GGA ACC ACC GTG TAC GGA GCA TTT GAC CCC CTC TTA GCT GTC GCT GAC ATT TGC AAA AAG TAT AAG ATC TGG ATG CAT GTG GAT
     Leu Val Ser Ala Thr Ala Gly Thr Thr Val Tyr Gly Ala Phe Asp Pro Leu Leu Ala Val Ala Asp Ile Cys Lys Lys Tyr Lys Ile Trp Met His Val Asp

1129 GCA GCT TGG GGT GGG GGA TTA CTG ATG TCC CGA AAA CAC AAG TGG AAA CTG AGT GGC GTG GAG AGG GCC AAC TCT GTG ACG TGG AAT CCA CAC AAG ATG ATG
     Ala Ala Trp Gly Gly Gly Leu Leu Met Ser Arg Lys His Lys Trp Lys Leu Ser Gly Val Glu Arg Ala Asn Ser Val Thr Trp Asn Pro His Lys Met Met

1231 GGA GTC CCT TTG CAG TGC TCT GCT CTC CTG GTT AGA GAA GAG GGA TTG ATG CAG AAT TGC AAC CAA ATG CAT GCC TCC TAC CTC TTT CAG GAA GAT AAA CAT
     Gly Val Pro Leu Gln Cys Ser Ala Leu Leu Val Arg Glu Glu Gly Leu Met Gln Asn Cys Asn Gln Met His Ala Ser Tyr Leu Phe Gln Gln Asp Lys His

1333 TAT GAC CTG TCC TAT GAC ACT GGA GAC AAG GCC TTA CAG TGC GGA CGC CAC GTT GAT GTT TTT AAA CTA TGG CTG ATG TGG AGG GCA AAG GGG ACT ACC GGG
     Tyr Asp Leu Ser Tyr Asp Thr Gly Asp Lys Ala Leu Gln Cys Gly Arg His Val Asp Val Phe Lys Leu Trp Leu Met Trp Arg Ala Lys Gly Thr Thr Gly

1435 TTT GAA GCG CAT GTT GAT AAA TGT TTG GAG TTG GCA GAG TAT TTA TAC AAC ATC ATA AAA AAC CGA GAA GGA TAT GAG ATG GTG TTT GAT GGG AAG CCT CAG
     Phe Glu Ala His Val Asp Lys Cys Leu Glu Leu Ala Glu Tyr Leu Tyr Asn Ile Ile Lys Asn Arg Glu Gly Tyr Glu Met Val Phe Asp Gly Lys Pro Gln

1537 CAC ACA AAT GTC TGC TTC TGG TAC ATT CCT CCA AGC TTG CGT ACT CTG GAA GAC AAT GAA GAG AGA ATG AGT CGC CTC TCG AAG GTG GCT CCA GTG ATT AAA
     His Thr Asn Val Cys Phe Trp Tyr Ile Pro Pro Ser Leu Arg Thr Leu Glu Asp Asn Glu Glu Arg Met Ser Arg Leu Ser Lys Val Ala Pro Val Ile Lys

1639 GCC AGA ATG ATG GAG TAT GGA ACC ACA ATG GTC AGC TAC CAA CCC TTG GGA GAC AAG GTC AAT TTC TTC CGC ATG GTC ATC TCA AAC CCA GCG GCA ACT CAC
     Ala Arg Met Met Glu Tyr Gly Thr Thr Met Val Ser Tyr Gln Pro Leu Gly Asp Lys Val Asn Phe Phe Arg Met Val Ile Ser Asn Pro Ala Ala Thr His

1741 CAA GAC ATT GAC TTC CTG ATT GAA GAA ATA GAA CGC CTT GGA CAA GAT TTA TAA TAA CCTTGCT CACCAAGCTG TTCCACTTCT CTAGAGAACA TGCCCTCAGC
     Gln Asp Ile Asp Phe Leu Ile Glu Glu Ile Glu Arg Leu Gly Gln Asp Leu ••• •••

1845 TAAGCCCCCT ACTGAGAAAC TTCCTTTGAG AATTGTGCGA CTTCACAAAA TGCAAGGTGA ACACCACTTT GTCTCTGAGA ACAGACGTTA CCAATTATGG AGTGTCACCA GCTGCCAAAA
1965 TCGTAGGTGT TGGCTCTGCT GGTCACTGGA GTAGTTGCTA CTCTTCAGAA TATGGACAAA GAAGGCACAG GTGTAAATAT AGTAGCAGGA TGAGGAACCT CAAACTGGGT ATCATTTGCA
2085 CATTACTAAA GACAGAAAAA CGTGCTCTTC TGTTCTCAAA TGCTAAATGC AAACACTGTG TATTTATTAG TTAGGTGTGC CAAACTACCG TTCCCAAATT GGTGTTTCTG AATGACATCA
2205 ACATTCCCCC AACATTACTC AATAAAAACA TAAAATATAC AAACATGTGG CAACCTGTTC TTCCTACCAA ATATAAACTT GTGTATGATC CAAGTATTTT ATCTGTGTTG TCTCTCTAAA
2325 CCCAAATAAA TGTGTAAATG TGGACACAAA AAAAAAAAAA AAAAA   2369
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FIG. 2. Nucleotide sequence and predicted primary structure of human islet GAD-2. The overlapping sequence of pHIG11 and pHIG1.9 is underlined. The PLP-binding site is indicated by a star. The dots indicate stop codons.

oligonucleotide probes representing cloned sequences of GAD-2 on G-tailed islet cDNA.

DNA Sequencing. Double-stranded cDNA was sequenced by using the Sequenase kit as described (version 2.0; United States Biochemical) with synthetic oligonucleotides. The derived nucleotide sequences were analyzed with the sequence analysis software package of the University of Wisconsin Genetics Computer Group (41).

Northern Blot Analysis. Northern blot analyses of 7 μg of poly(A)$^+$ RNA were performed essentially as described (37) with random-primed probes at 42°C in 40% formamide/dextran sulfate buffer. Before reprobing, filters were stripped at 95°C in 2× standard saline citrate/0.1% SDS.

Genomic Southern Hybridization. Approximately 20 μg of genomic DNA isolated from blood of diabetic and control individuals was digested with different restriction enzymes followed by electrophoretic agarose gel separation (39). After Southern blotting, the nylon filters were hybridized with either a GAD-2 or a GAD-1 probe. Before reprobing, the filters were stripped at 95°C in 0.01× standard saline citrate/0.1% SDS.

Genomic Mapping. The human islet pHIG1.9 cDNA was labeled by nick-translation with [^{3}H]thymidine and [^{3}H]dCTP to a specific activity of 2×10^7 cpm/μg. *In situ* hybridization to metaphase chromosomes from a normal male donor was carried out by using the probe at 0.05 ng/μl in the hybridization mixture as described (42). Slides were exposed for 7–14 days, and chromosomes were identified by Q-banding.

RESULTS

Sequence Analysis of Human Islet GAD. The strategy for obtaining the full-length cDNA sequence of human islet GAD (Fig. 1) employed screening the human islet library for cDNA clones, using RACE reactions to obtain additional sequence information of the 5′ end of the cDNA, and finally rescreening the library with RACE products for clones containing the 5′ end of the gene. The sequence of human islet GAD cDNA shown in Fig. 2 is therefore a composite of two overlapping cDNA clones (pHIG11 and pHIG1.9) and of five similar but independent RACE reactions (RACE 20, 47A, 28A, 41, and 42). The pHIG1.9 clone contained the PLP-binding site at nucleotide position 1223, a stop codon at nucleotide position 1793 to give a C-terminal leucine, and a polyadenylylation site (AAATAAA) 17 nucleotides upstream from the poly(A) sequence (Fig. 2). pHIG11 extended 450 base pairs upstream from the predicted N-terminal methionine; only 36 nucleotides of this 5′ untranslated region are shown (Fig. 2) since no other initiator methionine was detected upstream.

Primary and Secondary Structure. Assuming a missing thymidine at nucleotide position 1773 (Val-554) in the published mouse sequence (29), and at position 1790 (Pro-559) in the cat sequence (30), insertion of this resulted in a 96% deduced amino acid sequence homology among GAD-1 sequences of rat (28), mouse (29), and cat (30). In contrast, the GAD-2 translation product shows <65% amino acid sequence similarity to these sequences. The N-terminal region shows the highest diversity between the expected translation products of GAD-1 and GAD-2, whereas the deduced PLP-binding site of all GADs is identical (Fig. 3, asterisk). Of the 15 predicted cysteine residues of the GAD-2 translation product, 11 are preserved within the 13 cysteine residues predicted from GAD-1 (Fig. 3, boxed residues). The human islet GAD-2 codes for 585 amino acids, which is slightly shorter than the predicted sequences from rat, mouse, and cat (594, 594, and 595 amino acids, respectively; Fig. 3).

The Kyte–Doolittle hydropathy plot (43) of the deduced GAD-2 translation product indicates a strikingly similar primary structure to the product of GAD-1 (the latter is represented by the rat sequence in Fig. 3). In addition to a slightly more hydrophobic N terminus of the deduced GAD-2 cDNA, the sequences at amino acid positions 325–355 are more hydrophobic in this isoform, suggesting an overall slightly more hydrophobic protein than that derived from the GAD-1 gene.

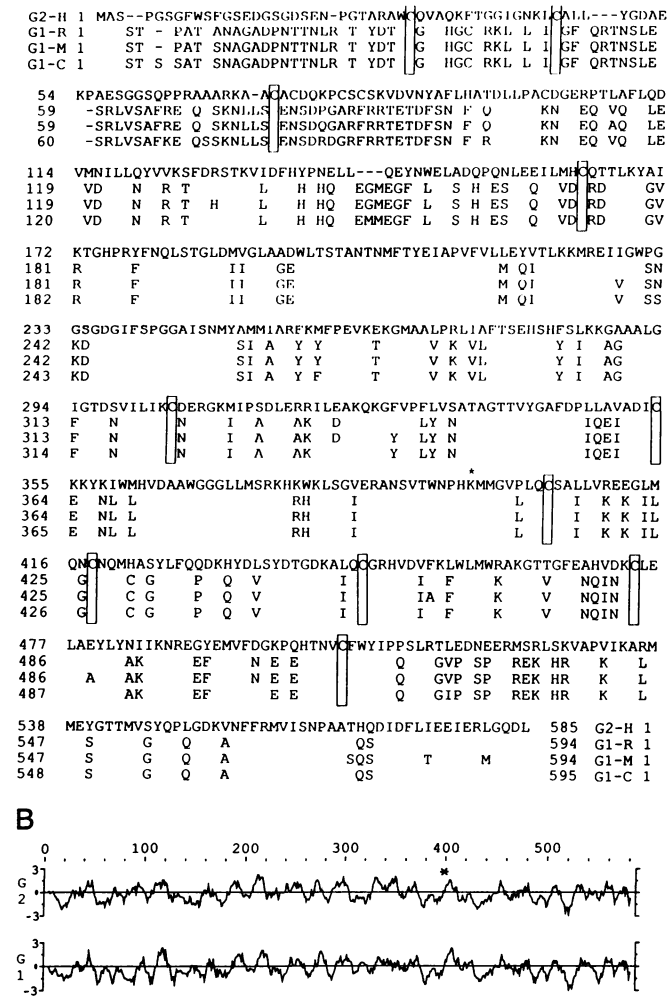


FIG. 3. (*A*) Deduced amino acid sequence of human islet GAD-2 (G2-H) compared to the GAD-1 sequences of rat (G1-R), mouse (G1-M), and cat (G1-C). Amino acid identities are indicated by a blank space, and sequence gaps are indicated by dashes. Conserved cysteine residues are boxed. (*B*) Hydropathy plot of GAD-2 (G2) and GAD-1 (G1). Hydrophobic regions are above the line. The PLP-binding site is indicated by an asterisk.

Tissue Expression. Northern blot analyses of GAD-2 gene expression (Fig. 4*A*) demonstrate a pronounced transcript of ≈5.6 kb in human islets (lane 5) and monkey brain (lane 4). This 5.6-kb transcript is also present, but is less intense, in dog islets (Fig. 4*A*, lane 2), dog brain (Fig. 4*A*, lane 1), and rat brain (Fig. 4*A*, lane 8). Expression of GAD-2 was not detected in dog liver (Fig. 4*A*, lane 3), human spleen (Fig. 4*A*, lane 6), or human lymphoblastoid cells (AL-34; Fig. 4*A*, lane 7). Another transcript of ≈2.5 kb was also detected in the human islets. Reprobing the same blot with a GAD-1 probe (Fig. 4*B*) revealed a 3.7-kb transcript in the brain tissue only (lanes 1, 4, and 8) and not in the islets (lanes 2 and 5). No cross-hybridization to the 5.6-kb transcript was detected.

Genomic Southern Blot Analysis and Chromosomal Localization. DNA prepared from patients with IDDM and from healthy individuals was analyzed by Southern hybridizations. After digestion with seven different enzymes, no restriction fragment length polymorphism was found be-

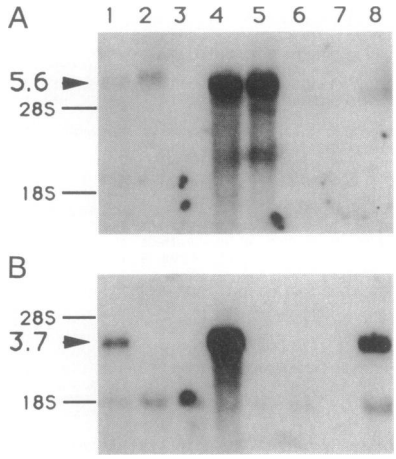


FIG. 4. Northern blot analysis of GAD-2 (*A*) and GAD-1 (*B*) gene expression. Hybridization was done on the same blot. Lanes: 1, dog brain; 2, dog islets; 3, dog liver; 4, monkey brain; 5, human islets; 6, human spleen; 7, human lymphoblastoid cells (AL-34); 8, rat brain.

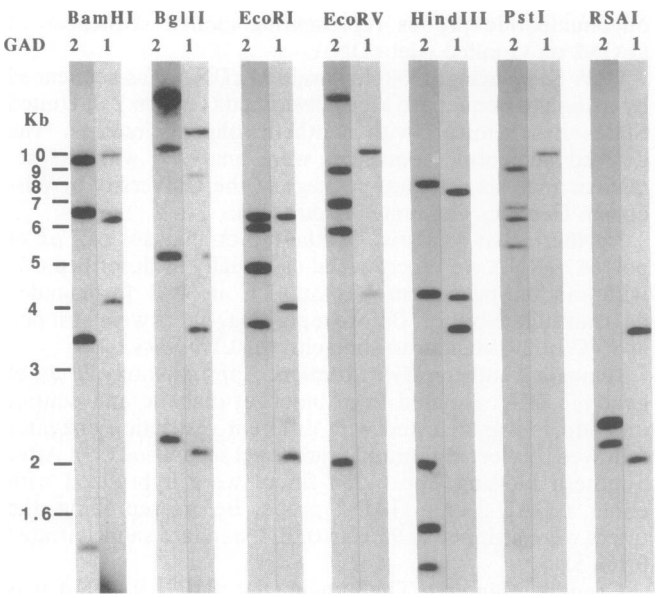


FIG. 5. Southern blot analysis of DNA from one individual with IDDM. The DNA was digested with seven different restriction enzymes as indicated. The same blot was hybridized with islet GAD-2 (lanes labeled 2) and brain GAD-1 (lanes labeled 1), respectively.

tween these individuals (data not shown), but hybridization with the GAD-1 cDNA showed a pattern of bands clearly different from that obtained with the GAD-2 probe (Fig. 5). This suggested that GAD-1 and GAD-2 are different genes.

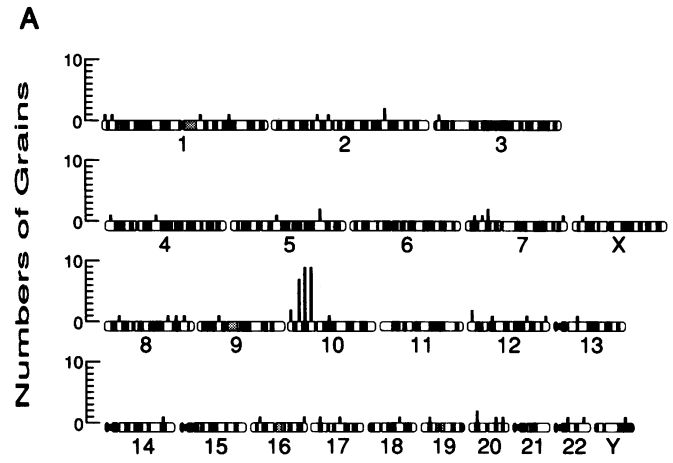
To further explore this possibility, GAD-2 was mapped on the human genome by *in situ* chromosomal hybridization. A total of 55 metaphase cells were examined, and 25 of 73 sites of hybridization (34%) were located on the short arm of chromosome 10 (Fig. 6). The largest numbers of grains were at bands p11.2 and p12 (18 grains); a significant number (7 grains) were at band p13. There was no significant hybridization to other human chromosomes.

DISCUSSION

Despite demonstration of several different GAD isoforms with different tissue expression, function, and kinetics, to our knowledge only one highly conserved mammalian cDNA (GAD-1) isolated from brain has hitherto been described (28–30). Southern analyses have localized its gene to a syntenic region on chromosome 2 of mice and humans (31, 32). Our *in situ* hybridization on mouse metaphase chromosomes with a GAD-1 probe confirms this localization to mouse chromosome 2.

The islets of Langerhans have long been known to contain GABA (33) and GAD (34), and both paracrine and intracellular roles have been proposed (1, 13). The present study identifies an additional human GAD gene, GAD-2, that maps to the p11.2 to p13 region of human chromosome 10 (Fig. 6*B*). The presence of at least two human genes coding for different GAD isoforms is further supported by the different band patterns obtained following Southern hybridization of genomic human DNA with GAD-1 and GAD-2 probes (Fig. 5).

Northern blotting analysis with GAD-2 detected a prominent 5.6-kb transcript in both islet and brain tissue, whereas a GAD-1 probe detected a 3.7-kb transcript in brain only. A similar prominent 3.7-kb transcript and a minor cross-hybridizing 5.6-kb band have previously been demonstrated in mouse brain with a mouse GAD-1 probe (29). In RACE reactions with human islet mRNA, we have identified a sequence with 100% homology to the published partial human testis sequence (7). Since this partial human islet sequence hybridized strongly to a 3.7-kb transcript in brain but was not detected in islets, we speculate that this is the human version of GAD-1 coded for on chromosome 2. Taken together, these findings suggest that human GAD-2 on chromosome 10 is highly expressed in both islets and brain, whereas human GAD-1 on chromosome 2 is primarily expressed in brain. The only cross-hybridizing band with the GAD-2 (except for weak hybridization to the 18S and 28S ribosomal RNA) was an



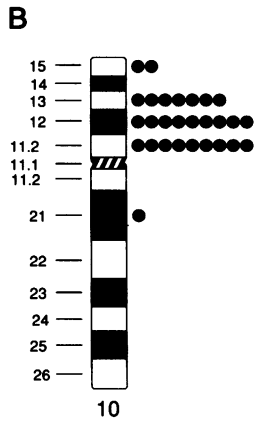


FIG. 6. Regional location of the human islet GAD gene to human chromosome 10. (*A*) Distribution of 73 sites of hybridization on human chromosomes. (*B*) Distribution of autoradiographic grains on human chromosome 10.

≈2.5-kb component in the human islets (Fig. 4, lane 5). A similar-sized GAD transcript was recently reported as the major GAD transcript in human testis (7). The weak 2.5-kb islet transcript remains to be identified and may represent either a spliced product of a GAD transcript or the transcription product of yet another gene.

The discovery of GAD-2 could help to resolve current controversies on the origin of the two isoforms of brain GAD with molecular weights between 59,000 and 67,000 as detected by immunoprecipitation or immunoblotting (9, 10, 23–25). Its cloning should also help elucidate the extent to which these and other products of the GAD genes are generated by alternative splicing. The molecular weights of the proteins predicted from GAD-1 and GAD-2 are about 66,600 and 65,300, respectively. Although this difference *per se* may not completely explain the differing electrophoretic mobilities on SDS gels of the various GAD isoforms, the deduced primary structures show qualitative differences, which may contribute to the variation. Differences were detected primarily at the N terminus and at amino acid positions 325–355.

Although it has been speculated that the diabetes-associated islet M_r 64,000 protein (GAD) might be expressed on the cell surface (19), no conventional leader sequence or specific transmembrane regions are found in either deduced GAD translation product. Further, no C-terminal hydrophobic extension common to phosphatidylinositol membrane-anchored proteins is present. The lack of conventional glycosylation sites (Asn-Xaa-Ser/Thr) is consistent with protein studies failing to show any glycosylation of the human islet M_r 64,000 protein (21, 22). Since we detect only expression of GAD-2 in islets, we speculate that this may code for the diabetes-specific M_r 64,000 protein and be equivalent to the small GAD isoform because of extensive amino acid identity (25), whereas the larger isoform may be the product of GAD-1 and thus primarily expressed in brain tissue.

The differences in protein sequence between the GAD-2 product and the brain isoform derived from GAD-1 should be useful in attempts to determine specific T-cell receptor and autoantibody determinants associated with IDDM. Antibodies to the M_r 64,000 protein seem to be the earliest, most specific markers for IDDM, and the availability of GAD-2 to produce recombinant human islet GAD for diagnostic and therapeutic reagents will be important for studies of the etiology and pathogenesis of IDDM.

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