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Gastric Inhibitory Polypeptide: Structure and Chromosomal Localization of the Human Gene

(ヒト・GIP遺伝子の構造、遺伝子マッピング)
Gastric Inhibitory Polypeptide: Structure and Chromosomal Localization of the Human Gene

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Gastric inhibitory polypeptide (GIP) is a 42-amino-acid hormone which may have a role in the regulation of insulin secretion. The characterization of cDNA clones encoding this hormone indicates that it is derived by proteolytic processing of a 153-amino-acid precursor. The human gene coding for the human GIP precursor spans approximately 10 kilobase pairs and consists of six exons. Similar to genes encoding other members of the glucagon superfamily, each exon appears to encode a distinct region of the GIP precursor or its mRNA. The promoter region of the human GIP gene contains potential binding sites for a number of transcriptional factors including Sp1, AP-1, and AP-2. The human GIP gene has been assigned to chromosome 17q21.3-q22. (Molecular Endocrinology 3: 1014-1021, 1989)

RESULTS

Cloning and Sequencing of the GIP Gene

Eight of approximately one million phage from two different human genomic libraries hybridized with the human GIP cDNA, and based on preliminary restriction analysis, three of these clones (hGIP-1, -3, and -7) were selected for detailed analysis. The gene spans...
approximately 10 kilobase pairs (kb) and has six exons separated by five introns (Fig. 1 and 2). Exon 1 includes the 5'-untranslated region of the mRNA. The signal peptide and a small portion of the amino-terminal peptide are encoded by exon 2. Exon 3 encodes most of the mature GIP peptide. Exons 4 and 5 encode the carboxy-terminal peptide. Exon 6 contains the 3'-untranslated region of the mRNA. The nucleotide sequences of the coding and 5'-untranslated regions are identical to the sequence of cDNA (7), and there is one nucleotide difference in the sequence of the 3'-untranslated region of the cDNA and gene (Fig. 2).

The Promoter Region of the GIP Gene

To define sequences which may be important for expression of the gene, we also sequenced 5'-flanking region of the GIP gene. The transcriptional initiation site was determined by primer extension analysis using human duodenal RNA (Fig. 3). Two major extension products were observed; one corresponding to the 5'-end of the cDNA sequence and another located 3 bases upstream. One minor band was also evident among these two bands. A number of possible regulatory elements were identified in the 5'-flanking region, including a TATA box (Fig. 1) and a binding site for Sp1 (Fig. 2) (9). A sequence homologous with the enhancer core sequence, OγTGG(T)(G) (10), occurs in the region around residue -138. Regions closely resembling the consensus sequences of AP-1 (TGA/-TCA) (11, 12) and AP-2 (C(C/C)(CC) (13) target elements are present at residues -344 and -368, respectively. These factors are involved in the regulation of gene expression by protein kinases A and C (11-13). In addition, there are similarities to a CAMP response element (consensus TGACGTCA) (14, 15) at residues -736, -394, and -306. The role of these putative regulatory elements on regulation of GIP expression remains to be determined.

Sequence of the 3'-Flanking Region of the GIP Gene

The nucleotide sequence of the 3'-flanking region of the GIP gene contains a GT-rich region which may be involved in transcription termination/polyadenylation (Fig. 2) (16).

Fig. 2. Partial Nucleotide Sequence of the Human GIP Gene

The nucleotide sequences of all exons, exon-intron boundaries, and 5'- and 3'-flanking regions are shown. Numbering is from the proposed cap site and the introns are not numbered. Arrows indicate the beginning of exons and the three possible poly(A) addition sites. The larger letters represent exons of the gene. The first and last 10 nucleotides of each intron are shown and the approximate length of each intron is also indicated. Amino acids indicated in italic print are those of the mature 42-amino-acid GIP peptide. Asterisks denote the 3'-end of our previously published cDNA sequence (7). One base substitution relative to the cDNA sequence is dotted under the sequence (at nucleotide residue 589). The TATA box, CCAAT box (8), and Sp1 binding sequence are boxed. Sequence homologous with the consensus sequences of the enhancer core element, AP-1 and AP-2 target elements, and CAMP response element (CRE) are underlined. Putative poly(A) addition signals are double underlined. The GT cluster found in the 3'-flanking region is shown by a wavy line.

Tissue Distribution of GIP mRNA

The tissue distribution of GIP mRNA was examined by RNA blotting (Fig. 4). The 800-base GIP transcript was detected only in RNA prepared from the duodenum. No hybridizing signals were detected in the gallbladder, pancreas, liver, descending colon, antrum, and cardia of the stomach, or esophagus. These results are consistent with immunocytochemical localization of GIP (17).
readily distinguishable from the single mouse DNA fragment of 1.7 kb (Fig. 5). Hybridization to DNA from a panel of 35 somatic cell hybrids indicated that the human DNA fragments were present only in those hybrids which retained chromosome 17 (Table 1). The probe also hybridized to two cell hybrids, TSL-2 and NSL-5 (Fig. 5), derived from human cells with translocations of chromosome 17 (17qter→17p13::3p21→3pter and 17qter→17p11.2::9q12→9qter, respectively), localizing the gene to the p11.2→qter region of chromosome 17. in situ hybridization to prometaphase chromosomes (Fig. 6) confirms the localization to the long arm of chromosome 17, and indicates that the GIP gene is in the region 17q21.3→q22.

DISCUSSION

The exon-intron organization of the human GIP gene is very similar to those of other members of the glucagon superfamily (Fig. 7) (9). Each of the various domains of the precursors of glucagon (18), VIP (19), GFRF (20), and GIP is encoded by a unique exon. The size of each of these genes also is similar (\(10 \text{ kb}\) (18–20)). This comparison suggests that the ancestral gene of the family consisted of four exons which encoded the 5' untranslated region of the mRNA, the signal peptide, the hormone, and the 3' untranslated region of the mRNA, and that the glucagon superfamily arose by amplification of this basic motif. Subsequent amplification of the exon encoding the hormone domain may have generated the multiple glucagon-like and VIP-like peptides observed in the glucagon and VIP precursors (3).

The human GIP gene was mapped to chromosome 17q21.3→q22. Thus, each of the glucagon superfamily genes is localized on a different chromosome: glucagon, chromosome 2p16→q27 (21, 22); VIP, chromosome 6q16→q22 (23, 24); and GIP, chromosome 2q9 (20, 25). Interestingly, the genes for two other gastrointestinal peptides, gastrin (26) and pancreatic polypeptide (27, 28), have also been localized to chro-

![Image](image_url)

Fig. 4. RNA Blot Analysis

Total RNAs were prepared from the following human tissues: lane 1, gallbladder; lane 2, pancreas; lane 3, liver; lane 4, descending colon; lane 5, duodenum; lane 6, antrum; lane 7, cardia of the stomach; and lane 8, esophagus. Positions of 28 S and 18 S ribosomal RNA are indicated.

![Image](image_url)

Fig. 5. Hybridization of the Human GIP cDNA to BamHI-Digested Human-Mouse Cell Hybrid DNAs

Lane 1, ICL-15053F; lane 2, TSL-2; lane 3, NSL-5; lane 4, LM/Tx (mouse control); and lane 5, FBS1 (human control).

![Image](image_url)

Fig. 6. Ideogram of Human Chromosome 17 Showing Silver Grain Distribution after Hybridization with the GIP cDNA

One hundred metaphase chromosomes were examined and 22% (34/154) of the grains were on chromosome 17. Seventy percent of the grains are 17q21.3→q22.

No other human chromosome demonstrated a grain distribution above background.

![Image](image_url)

Table 1. Segregation of the GIP Gene with Human Chromosomes in BamHI-Digested Human-Mouse Cell Hybrid DNA

<table>
<thead>
<tr>
<th>Human Chromosomes</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concordant no. of Hybrids</td>
<td>16</td>
<td>21</td>
<td>17</td>
<td>20</td>
<td>18</td>
<td>21</td>
<td>22</td>
<td>13</td>
</tr>
<tr>
<td>Discordant no. of Hybrids</td>
<td>15</td>
<td>17</td>
<td>14</td>
<td>17</td>
<td>12</td>
<td>12</td>
<td>19</td>
<td>9</td>
</tr>
<tr>
<td>Concordancy (% Discordancy)</td>
<td>50</td>
<td>38</td>
<td>50</td>
<td>43</td>
<td>49</td>
<td>49</td>
<td>35</td>
<td>34</td>
</tr>
</tbody>
</table>

The scores were tabulated by the presence or absence of human DNA fragments in the different somatic cell hybrids. Concordant hybrids are those with at least one specific chromosome, or the reverse. Discordant hybrids are those with no specific chromosome of, or the reverse. Discordancy indicates the degree of discordant segregation for a marker and a chromosome. 0% discordancy is the basis for chromosomal assignment.
HUMAN FIG. MATERIALS Two different human genomic libraries, a Charon 4A library and a GIP library, were used. The GIP library was prepared from human placenta using cDNA derived from a 532-base pair region of the glucagon gene. The filters were washed twice with 0.1 x SSC; 0.1% NaDodSO4, and then incubated at 42°C for 2 hours. The primer-extended products were denatured at 95°C for 1 minute and then at 65°C for 1 minute. The consensus sequence of the signal peptide is indicated by a single box. The +2 reverse transcriptase (Takara, Kyoto, Japan) was used to synthesize the antisense RNA. This research was supported in part by Grant-in-Aid for Scientific Research 63440042 from the Ministry of Education, Science, and Culture (to H.J.), and by Uehara Memorial Foundation, and grant-in-aid from the Mochida Memorial Foundation for Medical and Pharmaceutical Research (to K.K.).


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