Selective Amplification of Exons 3 and 8 of the Human Growth Hormone Receptor (hGHR) Gene Based on Newly Identified Intron Sequences

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Abstract. The gene for human growth hormone receptor (hGHR) consists of at least 10 exons, and the corresponding protein is encoded in exons 2-10 which span at least 87 kbp of chromosome 5. Failure to amplify exons 3 and 8 of the hGHR gene from Japanese subjects with the previously reported primers prompted us to determine intron sequences flanking exon 3 and those flanking exon 8 of the hGHR gene, and novel intron sequences flanking exons 3 and 8 of the hGHR gene were identified. We designed new oligonucleotide primers based on these sequences, and successfully amplified DNA fragments encompassing exon 3 and those encompassing exon 8 of the hGHR gene. Since all of the 50 Japanese and the two Caucasians had the very same intron sequences which were different from the previously reported ones, it is more likely that the previously reported sequences were simply wrong than that there exist polymorphic differences in the intron sequences among different ethnic populations.

Key words: Growth hormone (GH) receptor, Gene, GH insensitivity syndrome, Molecular diagnosis

GROWTH hormone receptor (GHR) mediates the actions of growth hormone (GH) on postnatal growth, and its deficiency results in growth hormone insensitivity syndrome [1]. Human GHR (hGHR) consists of 620 amino acids, and its signal sequence, extracellular domain, transmembrane domain, and intracellular domain are encoded in exon 2, exons 3-7, exon 8, and exons 9-10 of the hGHR gene, respectively, which span at least 87 kbp of chromosome 5 [1, 2]. Until now, several types of mutations, including gene deletions, splicing mutations, frameshift mutations, nonsense mutations and missense mutations, have been identified in the hGHR gene of patients with growth hormone insensitivity syndrome [2–17]. Although it has previously been reported that each exon of the hGHR gene was successfully amplified with primers corresponding to the intron nucleotide sequences flanking each exon [5], we were unable to amplify exons 3 and 8 of the hGHR gene from Japanese subjects with the reported primers. In the present study we analyzed intron sequences flanking exon 3 and those flanking exon 8 of the hGHR gene, and designed new oligonucleotide primers to amplify exons 3 and 8 of the hGHR gene based on these sequences.

Materials and Methods

Genomic DNAs were isolated from peripheral blood mononuclear cells of fifty Japanese and two Caucasians, and the intron nucleotide sequences were determined in two Japanese and two Caucasian subjects.

To identify the intron nucleotide sequences flank-
ing exon 3 of the hGHR gene, DNA fragments containing exon 3 were amplified by the linker PCR cloning method. In brief, genomic DNAs were digested with PstI, and then PstI cassettes (Takara Shuzo Co., Ltd., Otsu, Japan) were ligated to the digested DNAs. Polymerase chain reaction (PCR) amplification was carried out with primers corresponding to the antisense strand of exon 3 of the hGHR gene (GHRe3R, 5'-CTGGATTAACACTTGCA-3') and PstI cassette primers (C1 and C2, Takara Shuzo Co., Ltd., Otsu, Japan), and cycled 35 times using Gene Amp PCR System 9600 (Perkin-Elmer Japan Corporation., Ltd., Urayasu, Japan). Each cycle consisted of a denaturation step (94°C for 1 min), an annealing step (55°C for 1 min), and an elongation step (72°C for 1 min). The amplified products were directly sequenced with a Dye Terminator Cycle Sequencing FS Ready Reaction Kit (Perkin-Elmer Japan Corporation., Ltd., Urayasu, Japan).

DNA fragments spanning from exon 8 to 9 were amplified with a sense primer (GHR8F, 5'-CTAGTCGTAATTCTGAAAGCG-3') and an antisense primer (GHR9R, 5'-TATGACACGGAGTCTTGAGTG-3') corresponding to the 5' flanking intron sequence of exon 8 and the 3' flanking intron sequence of exon 9 of the hGHR gene, respectively, and the PCR products were analyzed by direct sequencing as described above.

The 162-bp DNA fragment containing exon 3 was amplified with a sense primer (GHR3F, 5'-GATGACTAATGGTTTTCTTCTC-3') corresponding to the newly identified 5' flanking intron sequence of exon 3 and an antisense primer (GHR3R, 5'-GCTTTAATTACACTAAACATGG-3') corresponding to the 3' flanking intron sequence of exon 3. The 186-bp DNA fragment containing exon 8 was amplified with the sense primer GHR8F corresponding to the 5' flanking intron sequence of exon 8 and an antisense primer (GHR8R, 5'-TGGAAATCTAACAACCTGATCTAC-3') corresponding to the newly identified 3' flanking intron sequence of exon 8. PCR was performed as described above, and the PCR products were directly sequenced with the Dye Terminator Cycle Sequencing FS Ready Reaction Kit.

The nucleotide sequences of primers are summarized in Table 1.

**Results and Discussion**

A 0.5-kbp DNA fragment containing 5' flanking intron sequence of exon 3 of the hGHR gene was successfully amplified, and analyzed. An identical nucleotide sequence was obtained in all of the four subjects examined. The identified nucleotide sequence is shown in Figure 1A. Deletions of nucleotides in the intron sequences flanking exon 3 were identified by comparison with the previously reported sequences [2].

<p>| Table 1. Sequences of oligonucleotide primers for amplification of the hGHR gene |
|-----------------------------------|----------------------------------|</p>
<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Sequence</th>
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<tr>
<td><strong>Oligonucleotides for linker PCR cloning</strong></td>
<td></td>
</tr>
<tr>
<td>GHRe3R</td>
<td>5'-CTGGATTAACACTTGCA-3'</td>
</tr>
<tr>
<td>C1</td>
<td>5'-GTACATAATTGTGTTAGAAGCG-3'</td>
</tr>
<tr>
<td>C2</td>
<td>5'-CGCGTAATAGAGTCACTTAC-3'</td>
</tr>
<tr>
<td><strong>Oligonucleotides for amplification of DNA fragment spanning exons 8 and 9</strong></td>
<td></td>
</tr>
<tr>
<td>GHR8F</td>
<td>5'-CTAGTCGTAATTCTGAAAGCG-3'</td>
</tr>
<tr>
<td>GHR9R</td>
<td>5'-TATGACACGGAGTCTTCCAGTG-3'</td>
</tr>
<tr>
<td><strong>Oligonucleotides for amplification of exon 3</strong></td>
<td></td>
</tr>
<tr>
<td>GHR3F</td>
<td>5'-GATGACTAATGGTTTTCTTCTC-3'</td>
</tr>
<tr>
<td>GHR3R</td>
<td>5'-GCTTTAATTACACTAAACATGG-3'</td>
</tr>
<tr>
<td><strong>Oligonucleotides for amplification of exon 8</strong></td>
<td></td>
</tr>
<tr>
<td>GHR8F</td>
<td>5'-CTAGTCGTAATTCTGAAAGCG-3'</td>
</tr>
<tr>
<td>GHR8R</td>
<td>5'-TGGAAATCTAAACAACCTGGRAC-3'</td>
</tr>
</tbody>
</table>
ANALYSIS OF THE hGHR GENE

A 4.2-kbp DNA fragment that spans exons 8 and 9 of the hGHR gene was also successfully obtained, and sequenced. An identical nucleotide sequence was obtained in all of the four subjects examined. The identified sequence is shown in Figure 1B.

Nucleotide substitutions and deletions in the intron sequences flanking exon 8 were identified by comparison with the previously reported sequences [2].

We constructed primers for the amplification of exons 3 and 8 based on the newly identified sequences (Table 1). With these primers, DNA fragments of the expected sizes were successfully amplified from genomic DNAs obtained from fifty Japanese subjects (Fig. 2). The sequence analysis showed that these fragments were identical among all the subjects.

Fig. 1. DNA sequences flanking exon 3 (panel A) and those flanking exon 8 (panel B) of the hGHR gene. DNA sequences reported previously and the newly identified sequences are shown in the upper and the lower lane, respectively. Exon sequences are boxed. The mismatched nucleotides are marked with stars, and nucleotide deletions are shown with bars. The underlined DNA sequences indicate positions of the primers for selective amplification of exon 3 and exon 8.

Fig. 2. PCR amplification of exon 3 and exon 8 of the hGHR gene from two Japanese subjects. PCR for exon 3 was carried out with the primer pair GHR3F and GHR3R, and that for exon 8 was performed with the primer pair GHR8F and GHR8R, as described in the Materials and Methods section. PCR products were separated on a 3% agarose gel. Molecular size makers are indicated at the left of the panel.
fragments were derived from the corresponding exons of the hGHR gene (data not shown). These data suggest that all of the fifty Japanese subjects have the same intron sequences as those newly identified in this study.

These observations suggest that there may exist polymorphic differences in the intron sequences of the hGHR gene among different ethnic populations, or that the previously reported sequences [2] might have been simply wrong. We speculate that the latter is more likely since all of the Japanese and the Caucasians had the very same intron sequences, which were different from the previously reported sequences [2], although the possibility that the former is the case cannot be totally excluded because of the limited number of DNA samples available in this study.

Mutations in the hGHR gene have recently been reported in Japanese patients with growth hormone insensitivity syndrome [16, 17], so that other Japanese patients with growth hormone insensitivity syndrome are expected to exist, and the methods to amplify DNA fragments encompassing exon 3 and those encompassing exon 8 should be useful in elucidating the molecular basis of these patients' condition.

In conclusion, we have identified novel intron sequences of the hGHR gene flanking exons 3 and 8 and described methods to analyze exons 3 and 8 of the hGHR gene.

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