Analysis of human growth hormone gene 5' sequences in isolated growth hormone deficiency patients

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Abstract
Human growth hormone (hGH) gene deletion (6.7 to 7.6 kb) is one of the causes of isolated growth hormone deficiency (IGHD), named IGHD IA. IGHD IA, however, only accounts for about 10% of the total IGHD patients. Most IGHD is caused by unknown mechanisms. Here, hGH gene 5' sequences in three IGHD patients without hGH gene deletion were analysed to see if there was any mutation hindering the expression of the hGH gene.

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IGHD IA is one of the causes of isolated growth hormone deficiency.¹⁻⁴ The human growth hormone gene cluster is located on chromosome 17. Since the GH1 (GH-N), GH2 (GH-V), and hCS genes are very similar,⁵ two primers for PCR amplification can only be designed for sequencing at -60 to -40 and 54 to 71 (fig 1). Stringent conditions were used to perform PCR amplification (100 µl reaction mixtures contained 0.1 µg of genomic DNA, 10 mmol/l Tris-HCl, pH 8.3, 1.5 mmol/l MgCl₂, 50 mmol/l KCl, 0.1% gelatin, 0.05 mmol/l each of dATP, dCTP, dGTP, and dTTP, 0.5 µmol/l of each primer, and 2.5 units of *Taq* polymerase). Amplification was performed for 30 cycles at 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for one minute, using a Perkin Elmer Cetus DNA thermal cycler. The PCR products were purified, combined with M13mp19 phages, and cloned in *E coli* JM101.⁶ The sequences were then analysed by the dideoxy termination method. The sequence data showed that most clones were from the GH1 gene indicating the successful amplification of this gene. The 5' sequences of the GH1 gene in two normal controls and one IGHD patient were exactly the same as the

normal one which has been published^{5,7} (fig 2). In another two IGHD patients, however, there were two types of sequence, one which was similar to the normal sequence with A replaced by G at -6 (figs 1 and 2). This sequence has been reported by DeNoto *et al*⁸ in a normal subject, so it may be a polymorphic site. Another sequence, however, showed four nucleotide differences at position -1, 3, 16, and 25. To make sure that this was not the result of experimental error, we repeated the PCR amplification, cloning, and sequencing and found the same sequence again. The four nucleotide changes in this sequence have not been reported up to now and it was not found in normal Chinese subjects either. Therefore we presume that they are the mutations which may be related to IGHD.

It is known that nucleotides 1 to 62 of the hGH gene represent the 5' untranslated region of mRNA. The mutations at -1 and 3 may affect the initiation of transcription and the addition of the 5' cap. The 5' untranslated sequence is also important for the initiation of translation. Therefore, a mutation in this region may interfere with the protein synthesis of hGH. The existence of two sequences in both patients indicated that they came from two different chromosomes 17 of their parents.

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Figure 1 Human growth hormone gene 5' sequences. (1) Normal hGH gene. (2, and 3) Sequences from IGHD patients without hGH gene deletion. The dashes indicate identity with sequence 1. Primer sites are also indicated.

Figure 2 Autoradiograph of DNA sequencing of a IGHD patient with two hGH gene 5' sequences (1 and 2), compared with that of a normal subject (3). The base changes are indicated by arrows.

The correlation of these mutations with IGHD, however, is still awaiting further studying.

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