A Chinese familial growth hormone deficiency with a deletion of 7.1 kb of DNA

You Ai He, Shi Shu Chen, Ya Xin Wang, Xing Yu Lin, De Fen Wang

Abstract
Using restriction endonuclease analysis and a human growth hormone cDNA probe, we have found a Chinese family with a human growth hormone gene deletion. Two affected sibs are homozygous for a deletion of approximately 7.1 kb of DNA, which contains the normal human growth hormone gene. The patients' parents and grandmothers are heterozygous for the deleted gene. Their grandfathers are normal and homozygous for the hGH–N gene. All of them have normal stature.

Human growth hormone (hGH) is a protein containing 191 amino acid residues, which is released from the anterior pituitary. It is essential for a normal rate of postnatal growth. Hypopituitarism will result in dwarfism in childhood.

The human growth hormone gene cluster is located on chromosome 17, which contains two genes encoding growth hormone and three genes encoding human chorionic somatomammotrophin (hCS). The order and arrangement of these genes have been defined as 5' hGH–N (normal)–hCS–L (like)–hCS–A–hGH–V (variant)–hCS–B 3'. hGH and hCS have 92% homology between their mRNA coding sequences, so all five genes can be hybridised to a hGH cDNA probe.

In 1981, Phillips et al. first described a Swiss family with growth hormone deficiency resulting from deletion of the hGH–N gene. Recently, families from different ethnic groups have been reported. Using Southern blotting, we examined DNA fragments after digestion with restriction endonucleases and hybridisation with an hGH cDNA probe. We report a family with two affected girls who are homozygous for a deletion of the hGH–N gene. This is the first case of familial isolated growth hormone deficiency type IA with hGH–N gene deletion reported in the Chinese population.

Materials and methods
SUBJECTS
A family with eight members was studied. Two female sibs have isolated growth hormone deficiency as shown in the table. They have the typical facial appearance of IGHD IA as described by Illig et al., namely small maxillae and mandibles, resulting in the appearance of a prominent forehead with a depressed nasal bridge. Their psychomotor development is normal. The family pedigree, showing that their parents are consanguineous, is illustrated in fig 1. The parents and grandparents have normal height.

Materials
Proteinase K and RNase A were purchased from E Merk, restriction enzymes from Boehringer Mannheim, Nytran from Schleicher and Schuell, and P–dCTP (3000 Ci/mmol) from Amersham or New England Nuclear. All chemical reagents are analytical grade.

DNA preparation
High molecular weight DNA was prepared from peripheral blood leucocytes according to standard procedures. Briefly, leucocytes were harvested from 5 ml heparinised whole blood with dextran, lysed with buffer containing SDS, and digested with proteinase K. DNA was extracted with phenol and chloroform. The alcohol precipitated crude DNA was digested with RNase and proteinase K again and re-extracted with phenol, chloroform, and subjected to alcohol precipitation (E 260 nm/E 280 nm of the DNA = 1.6–1.8). The DNA was more than 50 kb as checked by agarose gel electrophoresis.

hGH probe preparation
The recombinant plasmid chGH 800/pBR 322 con-
**Height, weight, and hGH levels of the patients.**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Sex</th>
<th>At birth</th>
<th>Present</th>
<th>Serum hGH (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Weight (g)</td>
<td>Height (cm)</td>
<td>Serum hGH (ng/ml)</td>
</tr>
<tr>
<td>I</td>
<td>F</td>
<td>3400</td>
<td>'Normal'</td>
<td>11½</td>
</tr>
<tr>
<td>II</td>
<td>F</td>
<td>2900</td>
<td>'Normal'</td>
<td>8.2</td>
</tr>
</tbody>
</table>

| I | n | II | n | III | n | IV | n | V | n | VI | n | VII | n |

**Figure 1** Pedigree of the children with IGHD.

Results

On the autoradiograph of genomic DNA from normal subjects digested with *BamHI* and hybridised with \(^{32}P\)-hGH cDNA, six bands, namely 8.3, 6.7, 5.3, 3.8, 3.0, and 1.2 kb, were shown (figs 2 and 3). The first four bands corresponded to hCS-L (human chorionic somatomammotrophin-like gene), hCS-B, hCS-A (human chorionic somatomammotrophin B, A gene), and hGh-N (normal human growth hormone gene), respectively. The 3.0 and 1.2 kb fragments corresponded to hGH–V (variant human growth hormone gene). The autoradiograph patterns of *BamHI* fragments showed that the 3.8 kb fragments containing the hGH–N gene were missing in the DNA from the two affected sibs. Their parents and grandparents containing nearly full length cDNA to human growth hormone was a kind gift from Dr Goodman (Chairman of Department of Molecular Biology, Massachusetts General Hospital, Boston, MA, USA). The 800 bp hGH cDNA insert was isolated from pBR 322 DNA by *HindIII* digestion, followed by agarose gel electrophoresis and electroelution, and recovered with Elude-tip. The probe was radiolabelled with \(^{32}P\)-dCTP to a specific activity of 2.5 to \(7 \times 10^8\) cpm/µg by a random primed DNA labelling method.

**SOUTHERN BLOT ASSAY**

Aliquots (15 µg) of DNA prepared from members of the family and normal subjects were digested with restriction endonuclease *BamHI* or *HindIII* at 12 U/µg DNA for 16 hours. Digested DNA and λ DNA *HindIII* fragments as size markers were subjected to electrophoresis on 0.8% agarose gel. After electrophoresis the procedures recommended by Schleicher and Schuell were followed, that is, the separated DNA fragments in the gel were transferred to nylon filters and hybridised with the hGH probe. After washing, the filters were autoradiographed with x ray film at \(-70^\circ C\).

**Figure 2** Autoradiograph patterns of DNA digested with *BamHI* and hybridised with \(^{32}P\)-hGH cDNA probe from members of the family and control (C).
had normal patterns of BamHI fragments. However, the relative radioactivity of the 3.8 kb bands in the DNA from the parents and grandmothers was lower than that found in the control, while the patterns and intensity of the BamHI bands in the DNA from the grandfathers were identical to those seen in the normal control (fig 3).

Digestion with HindIII yields information about the size of the deletion and permits the detection of heterozygosity. In the autoradiograph of the control DNA HindIII fragments, three bands of 25, 21.3, and 14.8 kb were shown (fig 4), which corresponded to hGH-N+hCS-L, hGH-V+hCS-B, and hCS-A, respectively. The DNA from the affected sibs lacked the 25 kb fragment, but the 21.3 and 14.8 kb fragments were still retained. A new band of about 17.9 kb was generated in the patients' DNA, which might be the result of a deletion of 7.1 kb from the normal 25 kb fragment. The deleted DNA sequence contains the hGH–N gene encoding human growth hormone. The DNA from the affected sibs' parents and grandmothers had both the 25 kb fragment and the 17.9 kb fragment. The results indicated that they were heterozygotes, with a normal allele containing the hGH–N gene and an abnormal one without it.

Therefore, the patients have inherited an abnormal chromosome 17 from each of their heterozygous parents. They are homozygous for a deletion of approximately 7.1 kb of DNA, which contains the hGH–N gene. The HindIII patterns of DNA from the grandfathers were identical to normal ones. This suggests that the grandfathers are normal homozygotes (fig 4). The genotypes and phenotypes of the family members are compatible with an autosomal recessive mode of inheritance.

We also found that the hybridisation patterns of DNA from normal chorionic villi were identical to those from normal peripheral blood leucocytes (figs 5 and 6).

**Discussion**

There are three modes of inheritance in isolated growth hormone deficiency (IGHD), namely auto-
mosomal recessive (type I), autosomal dominant (type II), and X linked recessive (type III). Phillips et al. studied the genomic DNA structure from patients defined as IGHD type IA by Illig et al. using Southern blotting. They found that the patients were homozygous for a deletion of 6.7 kb, which included the gene encoding for normal hGH. Our Chinese patients had an average weight and normal length at birth, but they showed extreme growth retardation during infancy and childhood. The older sister is now 11½ years old and the younger one is 8 years 2 months and their height is the same as that of normal 1 or 2 year olds. Our patients also had the typical facial appearance characteristic of IGHD type IA. The results of Southern blotting showed that the two affected girls had a deletion of the hGH–N gene, but other genes of the growth hormone gene cluster on chromosome 17 are still retained. Although the patients have not received hGH treatment yet, they have many characteristics of IGHD IA and may be considered as IGHD type IA.

So far, 11 families have been reported to have IGHD type IA associated with a deletion of the hGH–N gene. Consanguinity was present in nine of these, including our Chinese family.

There are differences in the location and the extent of the deletion in different IGHD IA families reported. For example, the patients reported from the Swiss, Argentinian, Austrian, and Japanese families were homozygous for a DNA deletion of 6.7 kb,4,7 patients from Iraqi, Yemenite, and Iranian families for 7.5 kb,8 and Italian patients for 7.6 kb.9 Our patients were homozygous for a deletion of about 7.1 kb. A recent paper described a double deletion in the human GH gene cluster and a total of about 40 kb DNA, containing the hGH–N, hCS–A, hCS–B, and hGH–V genes, was absent.10 These data suggest that the breakpoints in the GH gene cluster are different in the patients reported and the molecular mechanisms of hGH–N gene deletion are also different. It is an interesting problem for further study.

Another intriguing phenomenon is that IGHD IA patients from quite different origins may possess the same length of DNA deletion. For example, the Swiss, Argentinian, Austrian, and Japanese patients all had a deletion of 6.7 kb DNA containing hGH–N, while the Italian, Iraqi, Yemenite, and Iranian patients all had a deletion of 7.5 kb or 7.6 kb. This implies that there may be some common sequences in specific sites in the intergenic region on both sides of the hGH–N gene on the abnormal chromosome 17, and these sequences possibly are not present in the DNA of the normal hGH/hCS gene cluster. Mutation in these specific sites may make them accessible to some special proteins, like recombinases, or an unknown mechanism, which results in a deletion of the same length of DNA. Therefore, analysis of the sequences around the breakpoints will help us to understand the molecular basis of hGH–N gene deletion.

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