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Genetics

Molecular basis for familial isolated growth hormone deficiency

(growth hormone loci/gene deletions/DNA)

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ABSTRACT Nuclear DNA from four individuals with familial isolated growth hormone (somatotropin) deficiency (IGHD) type A was studied by restriction endonuclease analysis. By using ^{32}P-labeled human growth hormone (hGH) cDNA sequences as a probe, patterns seen after various digestions indicated that these individuals were homozygous for a deletion of at least 7.5 kilobases (kb) of DNA. This deletion includes the gene that encodes the normal growth hormone but does not include the variant growth hormone gene. Restriction patterns of DNAs from all family members agreed with an autosomal recessive mode of inheritance of the deletion that correlates with the clinical phenotype. Furthermore, independent assortment of the two types of hGH genes suggests that these genes are nonallelic. These findings indicate that, in these families, IGHD type A is caused by deletion of the normal hGH genes and that this disorder can occur in the presence of variant hGH genes.

Human growth hormone (hGH) and human chorionic somatomammotropin (hCS) are closely related polypeptide hormones that have 92% homology between their mRNA coding sequences (1, 2). After digestion with *Eco*RI, the hGH and hCS genes are contained in 2.6- and 2.8-kilobase (kb) genomic DNA fragments, respectively, and another fragment (9.5 kb) also contains homologous sequences (2). These three components of the hGH gene cluster are all located on the long arm of chromosome 17 (3, 4).

Recent data suggest that the hGH and hCS genes arose by duplication, but it is unclear how many copies of each gene exist per genome (1, 3). Although the arrangement of genes in the hGH gene cluster and a regional restriction map have not been established, comparison of different genomic clones containing hGH or hCS genes or both, has shown that there are at least two types of hGH and three types of hCS genes with differing nucleotide sequences. In the case of the hGH genes, one normal hGH (hGH-N) encodes the known protein sequence, whereas the other variant hGH (hGH-V) differs at multiple sites (2, 5, 6).

In most cases of hGH deficiency, the pathogenesis is unknown, but there are single gene disorders that specifically affect GH expression in humans (7). One of these familial disorders, IGHD type A, has an autosomal recessive mode of inheritance, and patients have unusually severe isolated hGH deficiency (8–11). When treated with hGH, these patients develop hGH-antibodies in high titers that inhibit the therapeutic growth effect (9–11). Because these characteristics are compatible with an absence of hGH polypeptide, DNAs from these patients were analyzed to determine if their hGH gene sequences were altered or deleted.

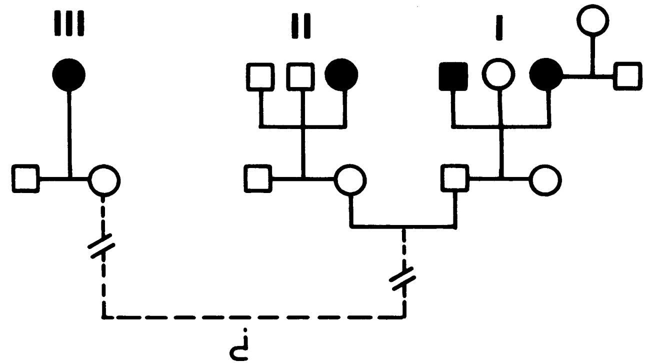

FIG. 1. Pedigrees of families I–III, each of which has one or more individuals affected with IGHD type A (●, ■).

MATERIALS AND METHODS

Clinical Material. We studied DNAs from four individuals with IGHD type A and their relatives (8–11) (Fig. 1). The first two families (I and II), who live in the same town in Switzerland, are thought to be related to family III. Although the pedigree is incomplete, the great-grandfather of the mother in family III had the same surname and was from the same town as the ancestors of the first two families (I and II).

Nuclear DNA Preparation. High molecular weight DNA was prepared as described (12) from peripheral blood (10–20 ml) obtained from controls and individuals shown in Fig. 1.

Genomic DNA Clones. The clones used, which contain either the hGH-N gene (*λhGH-N*) or the hCS gene (*λhCS*) sequences had been characterized after isolation from a library of human genomic DNA (5, 6).

hGH Probe Preparation. The recombinant plasmid chGH 800/pBR322 contains nearly full-length complementary DNA (cDNA) to hGH mRNA (13). The 800-base-pair hGH cDNA insert was isolated from pBR322 DNA by *Hin*dIII digestion, followed by electrophoresis and recovery from agarose gels (14). The purified 800-base-pair DNA fragment was labeled to a specific activity of approximately 10^8 cpm/μg by using [α-^{32}P]dATP and [α-^{32}P]dCTP by "nick translation" with *Escherichia coli* DNA polymerase I (15). Experiments involving recombinant DNA were conducted at P2-EK2 containment in accordance with the National Institutes of Health guidelines.

Restriction Endonuclease Analysis. Aliquots (5 μg) of nuclear DNA prepared from individual family members, controls, and dilutions of the cloned hGH and hCS genes were digested

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Abbreviations: kb, kilobase(s); IGHD, isolated growth hormone deficiency; hCS, human chorionic somatomammotropin; hGH, human growth hormone; -N, normal; -V, variant.

to completion with various restriction endonucleases under conditions recommended by the commercial suppliers (Bethesda Research Laboratories, Rockville, MD). Digested DNAs and appropriate DNA size markers were subjected to electrophoresis in 0.8–1.25% (wt/vol) agarose gels. In some cases, genomic DNA fragments of a specific size were isolated from an agarose gel, digested with a second restriction endonuclease, and fractionated on a second agarose gel (16). The DNA fragments were then transferred to nitrocellulose filters and hybridized with the hGH probe. The filters were washed and autoradiographed (4, 17, 18).

RESULTS

Because the regional restriction map of the hGH gene cluster is unknown, we selected at random six restriction endonucleases to digest DNA samples. The autoradiogram patterns obtained after *Bam*HI, *Bgl* II, *Eco*RI, *Hinc*II, *Hin*dIII, or *Sst* I digestion all had either an absence of or a reduced amount of hybridization for a specific fragment. For example, Fig. 2 compares the autoradiograms of DNA from a control, the mother, and the affected son in family I after different restriction endonuclease digestions. Control DNA (lane 1) had specific fragments that were absent in DNAs from the affected son (lane 3) after digestion with *Sst* I (2.6 kb; Fig. 2*A*), *Eco*RI and *Bgl* II (2.2 kb; Fig. 2*B*), or *Eco*RI and *Bam*HI (2.15 kb, Fig. 2*C*). The amount of hybridization seen for the 2.6-, 2.2-, and 2.15-kb fragments in the DNA of the mother (lane 2) was intermediate between that observed in DNA from the control (lane 1) and the son (lane 3). Finally, the son's DNA after *Eco*RI digestion (Fig. 2*D*) had a decrease in the amount of hybridization of the 2.6-kb fragment as compared to the 2.8-kb fragment. Because after *Eco*RI digestion the hGH genes were contained in the 2.6-kb fragment, this decrease in hybridization suggests that the fragments missing in Fig. 2 *A–C* may contain hGH sequences.

To determine if individuals from all three families had alterations in the restriction patterns of their DNAs, we compared their patterns to those of three controls. The autoradiograms obtained after *Bam*HI digestion are shown in Fig. 3. All four affected individuals lacked the normal 3.8-kb fragment that was seen in control samples (C_{1-3}). These patients did not have any additional hybridizing fragments of abnormal size. In all three

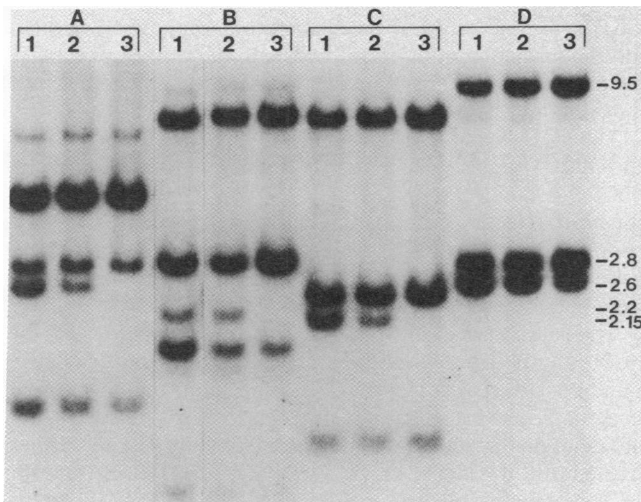

FIG. 2. Autoradiogram patterns of DNAs from a control (lane 1) and from family I mother (lane 2) and son (lane 3) after restriction endonuclease digestion with *Sst* I (*A*), *Eco*RI and *Bgl* II (*B*), *Eco*RI and *Bam*HI (*C*), or *Eco*RI (*D*) and hybridization with the hGH probe. Fragment sizes in kb are on the right.

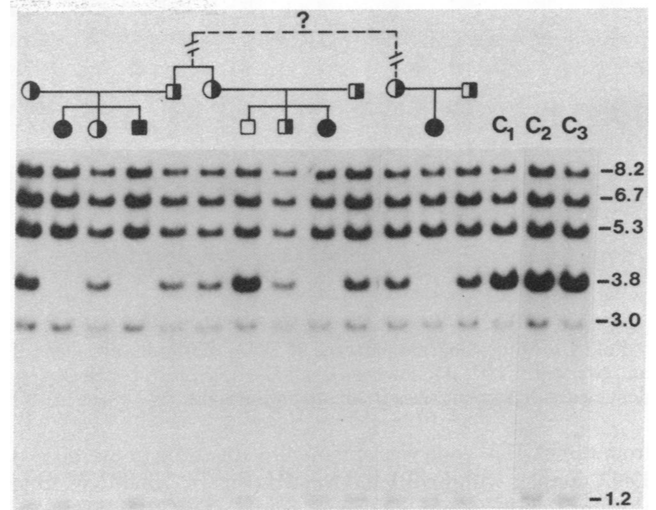

FIG. 3. Autoradiogram patterns of DNAs from families I–III and three controls (C_{1-3}) after digestion with *Bam*HI restriction endonuclease. Fragment sizes in kb are on the right.

families, the parents, who were presumed to be heterozygotes, had a relatively decreased amount of the 3.8-kb fragment compared to controls. Two of the siblings of the affected individuals appeared to be heterozygous for the lack of the 3.8-kb fragment. The third sibling had a normal pattern, suggesting that he inherited a normal chromosome from each of his heterozygous parents.

The absence of specific hybridizing fragments in DNAs from affected individuals after digestion with various endonucleases (Figs. 2 and 3) suggested that these alterations were due to deletion of DNA containing hGH-like sequences. To establish the size of the deletion, DNAs of various family members were digested with *Hin*dIII before blotting. *Hin*dIII was chosen because the pattern seen after digestion with *Eco*RI plus *Hin*dIII was identical to that seen after *Eco*RI alone (Fig. 2). This suggests that the *Hin*dIII recognition sites flank the *Eco*RI sites. The autoradiogram obtained after *Hin*dIII digestion is shown in Fig. 4. The controls ($C_{1,2}$) had hybridizing fragments of 26, 23, and 16 kb. Affected individuals were homozygous for a deletion of approximately 7.5 kb, which changed the size of the 26-kb fragment to 18.5-kb (Fig. 4). Furthermore, the decreased amount of hybridization of the 18.5-kb fragment of heterozygotes as compared to their 26-kb fragment agreed with the loss of hGH-like sequences. The patterns of the parents and two siblings were compatible with heterozygosity for a normal chromosome and a chromosome containing the deletion. The third sibling had a normal pattern, consistent with inheritance of a normal chromosome from each parent.

To prove that this deletion (Figs. 2–4) affects the hGH gene rather than the hCS gene, DNAs from families I and II, as well as λ*hGH-N* and λ*hCS*, were digested with *Eco*RI and *Bam*HI (Fig. 5). These restriction endonucleases were used because the hGH-V gene, but not the hGH-N gene, has a *Bam*HI recognition site in the intron preceeding amino acid 128 (2, 6). The DNAs from all affected individuals lacked the fragment that contains hGH-N gene sequences (Fig. 5, lanes 2, 7, and 9). Again the parents (lanes 1, 5, 6, and 10) and two unaffected sibs (lane 3 and 8) were shown to be heterozygous, whereas the remaining sibling (lane 4) was homozygous for the normal chromosome. The smaller fragments derived from the hGH-V gene (approximately 1.1 kb) were seen in DNAs from all family members.

Further evidence that the remaining hGH sequences are

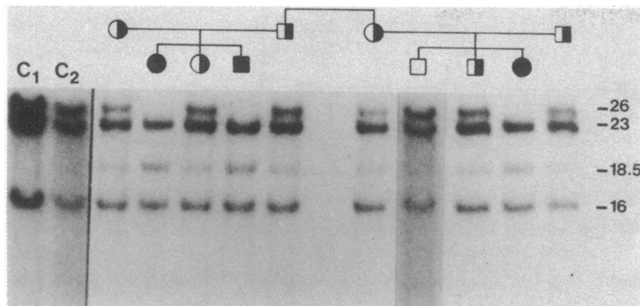

FIG. 4. Autoradiogram patterns of DNAs from families I and II and two controls ($C_{1,2}$) following digestion with *Hin*dIII restriction endonuclease. Fragment sizes in kb are on the right.

from the hGH-V gene was obtained by digestion of the various DNA samples with *Eco*RI and *Hae* III (Fig. 6). Total DNA from the affected son in family I (lane 4) contained hCS-derived fragments. However, the large fragment containing hGH-N gene was absent from his total DNA (lane 4) and was decreased in the total DNA from his mother (lane 3) as compared to the control (lane 2). In contrast, his DNA seemed to contain normal amounts of the fragments that correspond to the hGH-V gene (5). To prove that the 2.6-kb *Eco*RI fragments in this individual (Fig. 2*D*, lane 3) contain hGH-V gene sequences, we isolated fragments of genomic DNA that ranged in size from 2.5 to 2.7 kb after digestion with *Eco*RI (16). After *Hae* III digestion this DNA yielded the pattern shown in Fig. 6, lane 6. Because the sizes of the two hybridizing fragments observed correspond to those expected from the hGH-V gene but not the hGH-N gene, we assume that the remaining hGH sequences in this individual are derived from the variant gene. Furthermore, his mother's total DNA (Fig. 6, lane 3) gave a pattern consistent with a normal complement of hGH-V gene but a reduced number of hGH-N gene sequences as compared to the control (lane 2).

DISCUSSION

Deletion of hGH Genes is the Basis of IGHD Type A. Taken as a whole, the patterns obtained after various restriction endonuclease digestions of DNAs from each of the four affected subjects indicate each is homozygous for a deletion of at least 7.5 kb, which includes the hGH-N sequences (Figs. 2–6). All six parents and two of the three siblings (all of normal stature) are heterozygotes, having one chromosome 17 which yields a normal pattern and one which has the deletion. The remaining normal sibling (also of normal stature) has inherited a normal

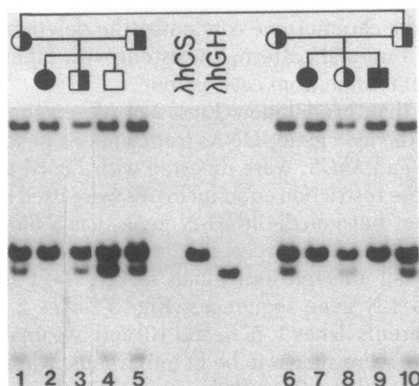

FIG. 5. Autoradiogram patterns of DNAs from families I and II and genomic clones containing either the hCS gene (λ*hCS*) or the normal hGH gene (λ*hGH*) after digestion with *Eco*RI and *Bam*HI restriction endonucleases. For fragment sizes, see Fig. 2 and text.

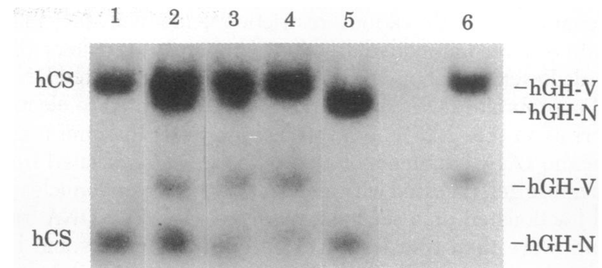

FIG. 6. Autoradiogram patterns of total DNAs from a control (lane 2), the mother (lane 3), and the son (lane 4) in family I and DNA from genomic clones containing either the hCS gene (λ*hCS*; lane 1) or the normal hGH gene (λ*hGH-N*; lane 5) after digestion with *Eco*RI and *Hae* III restriction endonucleases. Lane 6 contains the fragments produced by *Hae* III digestion of the 2.6-kb fragments that were isolated after digestion of the son's total DNA (lane 4) with *Eco*RI. Symbols on both sides of the autoradiogram indicate fragments derived from the hCS, hGH-N, and hGH-V genes (5).

chromosome 17 from each of his heterozygous parents. We were unable with these methods to detect any heterogeneity in the size or type of deletion in these families.

Gene deletions are a well-documented basis for certain human globin gene disorders, including hemoglobin Lepore, hereditary persistence of fetal hemoglobin, and some thalassemias (19–21). In several of these conditions, the deletions arose by unequal crossing-over after mispairing of duplicated or analogous globin genes (22–24). We have not determined if the hGH deletion described could be due to such a mechanism.

Affected Individuals Have hGH-V but no hGH-N Gene Sequences. As shown in Fig. 5 the deletion includes hGH-N gene sequences. In Fig. 6, the sizes of the two hGH-derived fragments correspond to those expected from the hGH-V gene but not the hGH-N gene (2, 5). The smallest hybridizing fragment seen in total DNA from the affected individual (Fig. 6) comes only from the hCS gene because it is absent in the *Hae* III digest of the isolated 2.6-kb fraction of DNA produced by *Eco*RI digestion. This fraction normally contains all hGH (-N and -V) gene sequences (2, 5, 6, 13).

The hGH-V Gene Product is Either Deficient or Poorly Functional in IGHD Type A. The four individuals studied with IGHD type A have deletion of their hGH-N genes but retention of their hGH-V genes. Their phenotype of extremely short stature, the absence of hGH production in immunoassay studies, and the formation of hGH-antibodies in high titers after hGH treatment (8–11) suggest that the product of their hGH-V genes is deficient or poorly functional, or both.

The hGH-N and hGH-V Genes are Nonallelic. The hGH-N and hGH-V genes were found by comparing the sequences of isolated genomic clones (2, 5). These two genes differ in that the hGH-N gene codes for the known growth hormone polypeptide whereas the hGH-V gene, which is a variant rather than a pseudogene, encodes a protein with 14 amino acid differences from mature normal hGH (unpublished data).

The data shown in Figs. 5 and 6 also strongly suggest that the hGH-N and -V genes represent different loci. First, the relative number of hGH-N copies in DNAs from family members varies in agreement with an autosomal recessive mode of inheritance of a deletion that includes hGH-N (Fig. 5). Second, the smallest fragment in this digest (Figs. 5 and 2*C*) is produced from the hGH-V gene by the presence of an additional *Bam*HI site (6). The amount of this fragment and, hence, hGH-V genes appears constant if allowance is made for variations in the amount of total DNA loaded. Fig. 6 also suggests that different numbers of hGH-N genes but a constant number of hGH-V genes may be inherited. By combining these data, the genotypes and segre-

tation patterns of the hGH-V and -N genes become clear (Fig. 5, *Upper*). The children in the family shown on the left are affected (-V/-V), heterozygous (NV/-V), and homozygous normal (NV/NV). These genotypes are compatible with two hGH loci (N and V) but incompatible with N and V alleles at one locus.

Note Added in Proof. We have subsequently found that the 2.2-kb fragment seen in Fig. 2*B* is produced by the presence of a polymorphic *Bgl* II restriction site 3′ to an hCS gene. The next lower band corresponds to a 1.8-kb fragment which is derived from the hGH-N and hGH-V genes. The amount of hybridization seen for the 1.8-kb fragment is less in the son's DNA (lane 3) than in DNA from the control (lane 1).

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