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Familial Hypothyroidism Caused by a Nonsense Mutation in the Thyroid-stimulating Hormone β-Subunit Gene

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Summary

Hereditary hypothyroidism caused by thyroid-stimulating hormone (TSH) deficiency is a rare autosomal recessive disease. Affected individuals show symptoms of severe mental and growth retardation that can be prevented by early administration of exogenous thyroid hormone. In this paper, we describe two related Greek families with three children affected by congenital TSH-deficient hypothyroidism. Sequence analysis of the TSH β-subunit gene (TSHB) showed that the mutation responsible for the hypothyroidism in these families is a nonsense mutation in exon 2. This mutation is a G-to-T transversion at nucleotide 94 that destroys the only *TaqI* site in the TSHB-coding region and gives rise to a novel 8.5-kb *TaqI* fragment. Restriction analysis showed that the three affected children are homozygous for the 8.5-kb allele and that the four parents and two unaffected children are heterozygous. This mutation gives rise to a truncated peptide which includes only the first 11 of 118 amino acids of the mature TSHB peptide.

Introduction

Hereditary thyroid-stimulating hormone (TSH) deficiency is a rare autosomal recessive disease (Miyai et al. 1971); Labbe et al. 1984; Hayashizaki et al. 1989). The severe mental and growth retardation resulting from TSH deficiency can be prevented if treatment with thyroxin is begun within the first 3 mo of life. TSH is a member of the glycoprotein hormone family, which also includes follicle-stimulating hormone (FSH), luteinizing hormone (LH), and chorionic gonadotropin (CG). The glycoprotein hormones are heterodimeric molecules consisting of a common α-subunit and specific β-subunit. The TSH β-subunit gene (TSHB) on the proximal short arm of chromosome 1 (Dracopoli et al. 1986) has been shown to be the site of a point mutation that is responsible for the TSH deficiency in an inbred Japanese family (Hayashizaki et al. 1989). In the present paper, we describe a different mutation that gives rise to hereditary hypothyroidism in two related Greek families with three affected children.

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Material and Methods

Subjects

Blood samples were collected from nine members of two nuclear families with hereditary TSH-deficient hypothyroidism (fig. 1). The families were both from Crete, and the ancestors of the mother and father of both families originated from the same village. The paternal great-grandfathers of the affected children were brothers. Although consanguinity on the maternal side of the families is evident, it is not admitted by the families.

Individual II-1 (family A), a male, was examined at the age of 3.5 mo. Neonatal screening for hypothyroidism by TSH analysis was normal. He was born after an uncomplicated pregnancy and normal delivery with a birth weight of 4,450 g and length of 54 cm. No unusual jaundice was noted. On physical examination, he looked puffy, had a slightly enlarged tongue, depressed nasal bridge, large anterior fontanel, hoarse cry, and omphalocele. His external genitalia were nor-

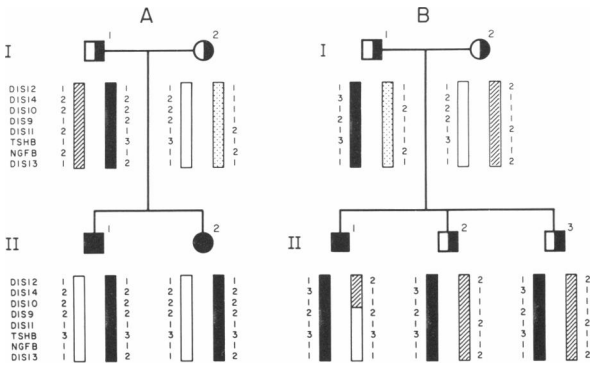

Figure 1 Pedigree of two related nuclear families from Crete with hereditary TSH-deficient hypothyroidism. The paternal great grandfathers of the affected children were brothers. The relationship of the mothers and their relationship to the common paternal ancestor are not known. The inheritance of genotypes at TSHB and at seven closely linked loci are shown. □ = Clinically normal male; male; ○ = clinically normal female; ■ = affected male; ● = affected female; ◨ = heterozygous male gene carrier; ◑ = heterozygous female gene carrier.

mal, the body length was only 59 cm (third percentile), weight was 6,300 g, and head circumference was 40.2 cm. Serum thyroxine (T4) was 0.2 μg/dl at the time of examination. He was started on Na-I-thyroxine replacement therapy and now, at age 12 mo, is developmentally normal and his body length is at the 60th percentile.

Individual II-2 (family A), a female, was examined at the age of 5 mo because she ate little, slept long hours, and had low activity and a hoarse cry. She was delivered by forceps after induced labor because pregnancy was 1 wk overdue. The birth weight was 4,200 g, length was 53 cm, and head circumference was 36 cm. No unusual jaundice was reported. The level of T4 was 0.1 μg/dl at the time of examination. Thyroid scanning with I^{131} showed no visualization of the thyroid gland. Thyroid uptake was only 8% and did not rise after the administration of bovine TSH. The serum TSH values did not change appreciably after treatment with exogenous TRH. She is now 9 years old, takes Na-I-thyroxine replacement therapy, and is growing normally (50th percentile). Her school progress is reported as very good.

Individual II-1 (family B), a male, was first examined at 9 mo because of developmental motor retardation. He was born, after an uneventful pregnancy, with a birth weight of 3,950 g. There was no unusual neonatal jaundice. On physical examination, he had a puffy face, pale yellowish dry skin, a large anterior fontanel, depressed nasal bridge, and slightly enlarged tongue. He had no teeth, and the body length of 66.5 cm was below the third percentile. The head circumference was 44 cm, and weight was 7,700 g. The bone age was less than 3 mo. External genitalia were normal. The level of T4 was 0.9 μg/dl at the time of examination. Thyroid scanning with I^{131} showed no visualization of the thyroid gland. TSH levels did not respond to exogeneous thyrotropin-releasing hormone (TRH) in tests carried out 2 years later, after T4 therapy had been discontinued. He is now 10 years old, growing normally (25th percentile), and is taking Na-I-thyroxine replacement therapy. His school progress is reported as average.

All four parents and the two healthy siblings showed clinically normal levels of T4 (8.2, 9.0, 10.8, 9.4, 9.3, and 10.6 μg/dl for family A members I-1 and I-2 and family B members I-1, I-2, II-2, and II-3, respectively). The levels of triiodothyroxine (T3), prolactin (PRL), FSH, and LH were clinically normal in all family members (data not shown). Serum TSH, T3, T4, LH, FSH, and PRL were determined using commercial radioimmunoassay (RIA) kits or by a commercial immunodiagnostic for serum TSH. The TRH test was carried out by the administration of 200 μg synthetic TRH intravenously. The TSH stimulation test was performed by the daily intramuscular administration of 10 IU of bovine TSH for 3 consecutive days.

Cell Lines

Epstein-Barr virus–transformed lymphoblastoid cell lines were established by standard techniques (Gusella and Anderson 1984) from all nine members of the families with TSH-deficient hypothyroidism (fig. 1). These lymphoblastoid cell lines were grown in Iscoves modified Delbuccos's medium (IMDM) supplemented with 10% inactivated FCS.

DNA Extraction and Southern Blot Hybridization

DNA extraction, restriction-enzyme analysis of lymphoblastoid DNA samples, agarose gel electrophoresis, and Southern transfer to Zetabind membrane (AMF Cuno) were done according to methods described elsewhere (Dracopoli et al. 1987). Hybridization probes were prepared from insert DNA, excised from plasmid vectors, and labeled by random priming (Feinberg and Vogelstein 1984). Hybridization, high-stringency washing, and exposure of filters were done as described elsewhere (Dracopoli et al. 1987). The DNA probes for RFLPs at TSHB, NGFB, and six anonymous loci (D1S9–D1S14) from proximal chromosome 1p have been described elsewhere (Breakefield et al. 1984; Dracopoli et al. 1988*a*, 1988*b*; O'Connell et al. 1989).

Amplification of Genomic DNA

Genomic DNA from an affected child (II-1, family A), a clinically normal parent (I-2, family B), and an unrelated normal control were amplified in vitro using polymerase chain reaction (PCR) (Saiki et al. 1985, 1988). The primer for the coding strand was a 20 mer (5′-CAGCTGTACATATTTCCACC-3′) corresponding to the sequence just 5′ of the *Eco*RV site near TSHB exon 2 (nucleotides 1–20; GENBANK:HUMTSH1) (fig. 2). The primer for the noncoding strand was a 23 mer (5′-CCTACCAGATAAGACTTCTGAGG-3′) corresponding to the sequence close to the 3′ end of TSHB exon 2 (nucleotides 250–272; GENBANK: HUMTSH2) (fig. 2). The primers were selected to have a relatively high GC content and to have at least two G's or C's at the 3′ end of the sequence.

A total of 1 μg of genomic DNA in a 100-μl reaction volume was denatured (180 s at 94°C) and subjected to 30 amplification cycles (each cycle consisting of 120 s annealing at 57°C, 180 s extension at 72°C, and 60 s denaturation at 94°C). The amplified DNA was run on a 1.2% agarose gel and stained with ethidium bromide.

Sequencing

The TSHB exons 2 and 3 from an affected child (II-1, family A) homozygous for the 8.5-kb allele was amplified by PCR and digested with *Eco*RI and *Eco*RV. The 0.8-kb *Eco*RI-*Eco*RV fragment (fig. 2) was subcloned into pBluescript SKII+ (Stratagene) and sequenced by the dideoxy chain-termination method using a modified T7 DNA polymerase, Sequenase (United States Biochemical Corp.) (Tabor and Richardson 1987).

Results

Clinical Analyses

The three affected children are characterized by the early onset of hypothyroidism with low TSH levels that did not rise after the administration of exogenous TRH. These three children all showed isolated TSH deficiency. The levels of the other anterior pituitary hormones (PRL, LH, and FSH) were normal. The affected children all had very low levels of T4 (mean = 0.33 μg/dl), while the four parents and two unaffected siblings had clinically normal levels of T4 (mean = 9.55 μg/dl).

Sequence Analysis

The amplified DNA from the homozygous affected child (II-1, family A) was digested with *Eco*RI and *Eco*RV and cloned into pBluescript SKII+. The 440 nucleotides beginning at the *Eco*RV site, including the complete TSHB exon 2, were determined by sequencing both strands (fig. 3). Comparison with the normal TSHB sequence (Hayashizaki et al. 1985; Guidon et al. 1988; Wondisford et al. 1988) showed only a single nucleotide difference, at nucleotide 94. This mutation was a G-to-T transversion, which destroyed a *Taq*I site (5′-TCGA-3′). This nonsense mutation converts the 12th codon in the mature peptide for glutamic acid (GAA) into a termination codon (TAA). This mutation will result in the production of a prematurely truncated TSHB polypeptide, with a complete signal peptide of 20 amino acids and with only 11 of 118 amino acids of the mature peptide.

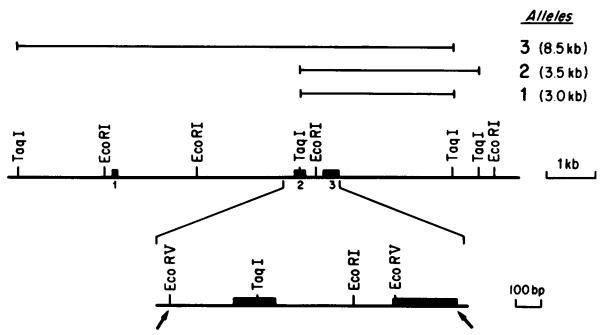

Figure 2 Restriction map of the human TSHB gene, showing location of the three exons (Giudon et al. 1988; Wondisford et al. 1988). The only *Taq*I site within the TSHB coding sequence is located in exon 2. The three *Taq*I fragments generated by polymorphisms at this locus are indicated above the map. The locations of primers used for PCR amplification of this region are indicated by arrows.

PCR Analysis

PCR analysis was used to confirm that the *Taq*I site located in TSHB exon 2 is involved in the generation of the novel 8.5-kb allele. Genomic DNA from an unrelated normal control, from individual I-2 from family B (heterozygous for the 8.5-kb allele), and from individual II-1 from family A (homozygous for the 8.5-kb allele) was amplified. The amplified DNA from all samples was 1.2 kb. Digestion of the amplified DNA with *Taq*I showed two fragments (0.8 kb and 0.4 kb) in the normal control, three fragments in I-2 (1.2 kb, 0.8 kb and 0.4 kb), and one fragment (1.2 kb) in II-1 (data not shown). Consequently, this *Taq*I site is absent from both TSHB alleles in the homozygous affected child and is absent from one of the alleles in the heterozygous parent.

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                .         .         .         .         .         .
-250  GATATCCTAAGGGTTTGGAAGTGGGATCAGGGGGTTCCTAGATTTCTGAGTTAGCCCCTT   -191
                .         .         .         .         .         .
-190  AACACCAGTTGTAATTTCAGTTGACCTTTTTTGGACTTTATCTTTCTGGTGTCTTCCTTG   -131
                .         .         .         .         .         .
-130  ACCAAATGGTAGAATTATAAGCATGATCATATGCATTGGGATGGTACTGAAGTTTGGTTA    -71
                .         .         .         .         .         .
 -70  TACTTTTTCTTGGTTTCTTTGCCCTTTCTGATTTTAACAAATAGGTTCTTTAATTTTATC    -11
                .         .         .         .         .         .
 -10  TTTGATTTAGCATGACTGCTCTCTTTCTGATGTCCATGCTTTTTGGCCTTGCATGTGGGC     49
                M   T   A   L   F   L   M   S   M   L   F   G   L   A   C   G   Q
                .         .         .         .         .         .
                                                        G
  50  AAGCGATGTCTTTTTGTATTCCAACTGAGTATACAATGCACATCTAAAGGAGAGAGTGTG     109
        A   M   S   F   C   I   P   T   E   Y   T   M   H   I   *
                .         .         .         .         .         .
 110  CTTATTGCCTAACCATCAACACCACCATCTGTGCTGGATATTGTATGACACGGGTATGTA     169
                .         .
 170  GTTCATGTCACTTCTTTTGG   189
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Figure 3 Nucleotide and deduced amino acid sequence of TSHB exon 2 from a child affected with TSH-deficient hypothyroidism. Sequence data presented are from the 5′-*Eco*RV site (−250), to the normal end of exon 2 (162) and into intron 2. Comparison with the normal TSHB sequence (Hayashizaki et al. 1985; Guidon et al. 1988; Wondisford et al. 1988) shows only a single nucleotide difference. This mutation is a G-to-T transversion at nucleotide 94, which results in the destruction of the *Taq*I recognition site (*5′ . . TCGA . . 3′*). This nonsense mutation converts the 11th amino acid of the mature peptide from glutamic acid (GAA) to a premature stop codon (TAA).

TaqI Polymorphism at TSHB

Screening of human genomic DNA with the TSHB p3.6 probe usually reveals a two-allele *Taq*I polymorphism with fragment sizes of 3.0 kb and 3.5 kb (Dracopoli et al. 1988*a*). The gene frequencies of the alleles in the Centre d'Etude du Polymorphisme Humain (CEPH) panel are .95 and .05 for the 3.0-kb and 3.5-kb alleles, respectively (Dracopoli et al. 1988*a*, 1988*b*). The novel 8.5-kb allele detected in the Greek families with hereditary TSH deficiency (fig. 4) has not been detected in previous analyses of 425 genomic DNA samples derived from 49 families, including the 40 families of the CEPH reference panel.

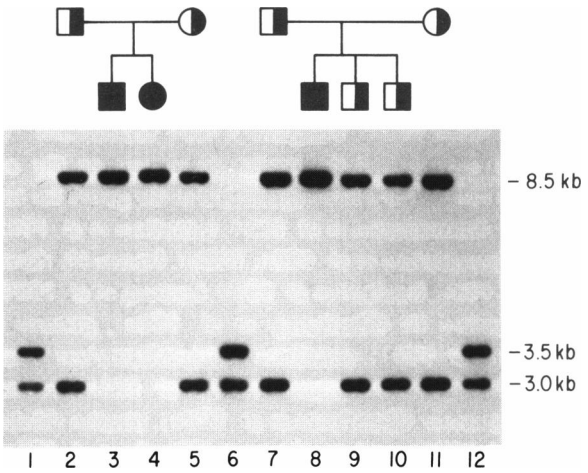

Figure 4 Southern blot of *Taq*I-digested genomic DNA from nine members of two families with hereditary TSH-deficient hypothyroidism and from heterozygous controls (lanes 1, 6, and 12), hybridized to the TSHB p3.6 clone. Samples from family A (lanes 2–5) and family B (lanes 7–11) are loaded in the same order as seen in the pedigree in fig. 1.

Discussion

Hereditary TSH deficiency is likely to result from mutations either in the genes coding for the components of the TSH dimer or in genes controlling its expression. The α-subunit gene is an unlikely candidate to contain the TSH deficiency mutation, since the other anterior pituitary hormones (LH and FSH) are expressed at normal levels in these patients. The recent report of a mutation at the TSHB locus in a Japanese family with hereditary TSH deficiency (Hayashizaki et al. 1989) suggests that the same locus is likely to be involved in the development of hereditary TSH deficiency in the two related Greek families.

The human TSHB gene consists of a 5′ untranslated exon and two coding exons (fig. 2) (Guidon et al. 1988; Wondisford et al. 1988). Analysis of the deduced amino acid sequence (Hayashizaki et al. 1985; Guidon et al. 1988; Wondisford et al. 1988) shows a 20-amino-acid signal peptide and a 118-amino-acid mature TSHB peptide. The mutation resulting in hereditary TSH deficiency in the Greek families is a G-to-T transversion at nucleotide 94 in exon 2. This mutation changes the codon for the 12th amino acid of the mature peptide to a premature stop codon. The mutation destroys the only *Taq*I site (5′-TCGA-3′) in the TSHB coding sequence and generates a unique 8.5-kb allele which is only detected in members of these families (fig. 4). Unlike the mutations detected at *Taq*I sites in many human genes, the TSHB mutation does not result from a transition at the CpG sequence. The *Taq*I recognition site includes a CpG dimer sequence which is a hot spot for mutation in mammalian DNA (Barker et al. 1984). A high frequency of mutations occurs at this CpG sequence because of the failure to repair the transitions resulting from the spontaneous deamination of the 5-methylcytosine (Bird 1980). Frequent C-to-T transitions—or G-to-A transitions if the mutation occurs on the antisense strand—have been identified in the genes for factor VIII (Antonarakis et al. 1985; Gitschier et al. 1985, 1986; Youssoufian et al. 1986, 1988), Factor IX (Bentley et al. 1986; Koeberl et al. 1989; Ludwig et al. 1989), antithrombin III (Duchange et al. 1986), protein C (Romeo et al. 1987), ornithine transcarbamylase (Hata et al. 1989), α1-antitrypsin (Kidd et al. 1983), adenosine deaminase (Bonthron et al. 1985), insulin (Shibasaki et al. 1985), and prealbumin (Maeda et al. 1986).

The mutation detected in the Greek families with hereditary TSH deficiency is different from the mutation described by Hayashizaki et al. (1989) in the Japanese family originally described by Miyai et al. (1971). Two affected members of the Japanese family have a point mutation at nucleotide 145, which is also located in exon 2. This mutation is a G-to-A transition which results in the replacement of Gly (GGA) with Arg (AGA) at the 29th amino acid of the mature TSHB peptide. This mutation occurs in a region of the TSHB polypeptide which is conserved in all mammalian glycoprotein hormones. Mutations in this region result in a conformationally altered β-subunit which cannot associate with the glycoprotein hormone α-subunit to form a functional TSH dimer (Hayashizaki et al. 1989).

In conclusion, hereditary TSH-deficient hypothyroidism is a rare autosomal recessive disease caused by at least two different point mutations in exon 2 of the TSHB gene. The development of direct DNA tests permits the rapid identification of affected fetuses and gene carriers. Consequently, at-risk pregnancies can be screened by prenatal diagnosis as well as by thyroid hormone determination immediately after birth. The identification of affected fetuses or newborns will permit prompt initiation of thyroxin-replacement therapy and the prevention of mental subnormality resulting from TSH deficiency.

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