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Genetic mapping of the human pituitary-specific transcriptional factor gene and its analysis in familial panhypopituitary dwarfism

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Abstract We have analyzed the human pituitary-specific transcription factor (Pit-1) gene using PCR amplification of DNA fragments that span intron III and contain portions of exons III and IV. A PCR restriction fragment length polymorphism (PCRFLP) was detected in intron III by *Rsa*I digestion, which was used to assign the human Pit-1 locus to chromosome 3p by linkage analysis of the CEPH panel. Analysis of corresponding Pit-1 segments from six nonrelated probands with familial panhypopituitary dwarfism (FPD) did not reveal any alterations in size and co-segregation of Pit-1, or a tightly linked microsatellite marker (D3S1559), and FPD was excluded in all six kindreds. Our data (1) assign Pit-1 to human chromosome 3p by linkage, (2) provide a PCRFLP and identify a variety of tightly linked markers, for analysis of FPD, and (3) exclude Pit-1 defects as the basis of at least one form of FPD.

Introduction

The human pituitary-specific transcription factor or Pit-1 gene is a member of a family of genes containing homologies to the POU domain whose protein products are involved in development (Bodner et al. 1988; Ingraham et al. 1988). The Pit-1 product is a transcription factor for the growth hormone (GH) and prolactin (PrL) genes, and has been shown to regulate thyroid-stimulating hormone (TSH; Nelson et al. 1988). Expression of Pit-1 occurs during development of the somatotroph, lactotroph and thyrotroph cell types of the anterior pituitary gland, which secrete GH, PrL and TSH, respectively (Karin et al. 1990). Mutations at the Pit-1 locus in the Snell dwarf (*dw*) mouse cause deficiencies of GH, PrL and TSH as well as hypoplasia of the corresponding secretory cells of the anterior pituitary (Camper et al. 1990). Multiple allelic defects of Pit-1 are reported in humans and the locus has been assigned to 3p11 by fluorescence in situ hybridization (FISH; Ohta et al. 1992; Phillips and Cogan 1994). The bovine, murine and rat Pit-1 cDNA sequences have been reported and the Pit-1 locus has been mapped to murine chromosome 16 (Li et al 1990).

In this study, we detected an informative PCR amplification product polymorphism (PCRFLP) located within intron 3 of Pit-1. This PCRFLP was used in linkage analysis of DNAs from the CEPH panel and six nonrelated familial panhypopituitary dwarfism (FPD) kindreds to further determine the localization of the human Pit-1 gene by linkage and its status in FPD.

Materials and methods

This study was approved by the committee for the protection of human subjects and performed in accordance with the ethical standards outlined in the 1964 Helsinki Declaration. All patients gave their informed consent prior to inclusion in this study.

Oligonucleotides for PCR were selected from Pit-1 sequences conserved between bovine, rat, and murine cDNAs. The forward and reverse primers corresponded to nucleotides 351–375 (5′-GGTGGAAGAGC CAATAGACATGGAC-3′) and 460–438 (5′-CCACGTTTGTCTGGGTGTATCCT-3′) from murine Pit-1 cDNA (Li et al 1990). The PCR reactions were denatured for 6 min at 94°C, cycled 31 times (94°C, 1.5 min; 58°C, 2 min; 72°C, 2 min), followed by a 10-min extension at 72°C. The resulting 2145-bp amplification product was comprised mostly of intron III with some 3′ exon III and 5′ exon IV sequences. The PCR product was confirmed to be derived from Pit-1 by comparison of its exonic sequences to those of human Pit-1 cDNA. Aliquots were then double digested with *Rsa*I and *Hha*I and separated by size on a 5% polyacrylamide gel. Gels were stained with ethidium bromide and visualized under UV light.

Results

The CEPH panel of DNAs were genotyped for the PCRFLP (see Fig. 1). The alleles detected by *Rsa*I digestion

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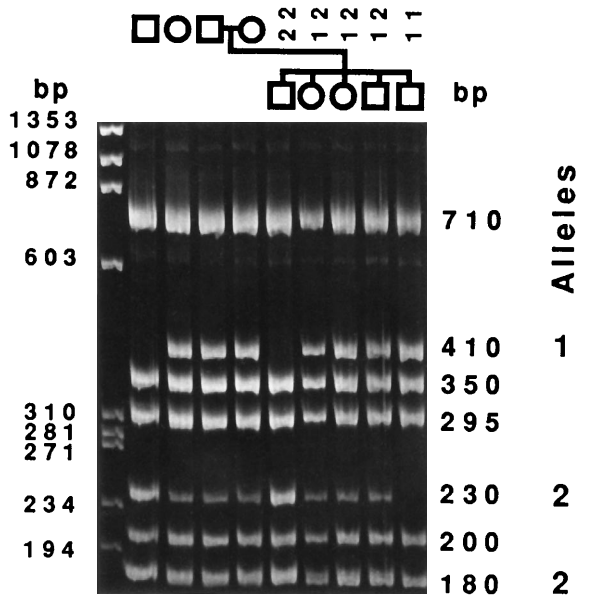

Fig. 1 Ethidium bromide staining patterns of DNA fragments obtained by PCR amplification of DNA from an unaffected family (CEPH panel) and two nonrelated controls after restriction enzyme digestion with *Rsa*I and *Hha*I. Note the three genotypes detected: *11* (no 230-bp band), *22* (no 410-bp band), and *12* (has both the 230- and 410-bp bands at half intensity)

Table 1 Two-point linkage data with Pit-1 locus and previous data

Locus	Θ/lod with Pit-1	Probe
D3S742	0.00/17.8	LIB22-64
D3Z1	0.00/8.4	P3-5
D3S2318	0.00/7.9	UT6384
D3S1559	0.00/3.01	AFM023 × g1
D3S739	0.01/16.1	LIB9-19
D3S4	0.03/16.8	B67
D3S625	0.03/11.9	LIB38-85
D3S15	0.03/9.8	CRI-L325
D3S216	0.05/12.2	LIB32-90
D3S672	0.06/17.8	cCI3-438
D3S1186	0.06/8.1	LIB15-47
D3S1281	0.08/10.7	AFM177 × h
D3S1591	0.09/8.2	AFM292 × g
D3S1616	0.09/8.2	AFM348te
D3S13	0.10/11.6	CRI-R96

were 410 (allele 1) and 230 plus 180 (allele 2) bp in size (see Fig. 1). Additional digestion with *Hha*I was required to remove a PCR fragment, also derived from intron III, that was similar in size to allele 1. Pairwise and multipoint linkage analysis was performed on the CEPH panel results using the Linkage program 5.01 kindly supplied by Dr. Jurg Ott. The resulting two-point lod scores for Pit-1 and 14 linked loci on chromosome 3p and 3q near the centromere are shown in Table 1 (Lathrop et al. 1984). The multipoint map of Pit-1 and linked markers are shown in Fig. 2. This map is estimated to span ~37 cM and is localized to the pericentromeric region of human chromosome 3 according to the Cooperative Human Linkage Center (CHLC) database.

Allele frequencies were determined in 152 chromosomes from 76 unrelated Caucasians (CEPH parents). The frequency for allele 1 was 0.38 and for allele 2 was 0.62 with a heterozygosity of 0.47.

DNA was isolated from six kindreds with multiple members affected by FPD. In the families, deficiencies of GH and TSH were documented, Prl levels were not determined, and additional deficiencies of gonadotrophins (FSH, LH) were noted. Analysis of Pit-1 segments from probands did not reveal any alterations in size, and cosegregation of the Pit-1 locus and FPD phenotype was excluded in all three informative kindreds by the occurrence of multiple, affected recombinants. Segregation studies were performed on the three uninformative families using the tightly linked microsatellite marker D3S1559 ($\theta = 0$, lod = 3.01). The results showed one to three affected recombinants per family demonstrating that the Pit-1 gene also does not segregate with the disease phenotype in these three families.

Discussion

Our results are consistent with the FISH assignment of the Pit-1 locus to human chromosome 3p and further extend the homology between human chromosome 3 and mouse chromosome 16 (Ohto et al 1992; Searle et al. 1994). In addition, the markers described should be useful in detecting co-segregation of Pit-1 alleles with the FPD phenotype and facilitating identification of human Pit-1 alterations that are analogous to the *dw* mutations found in mice.

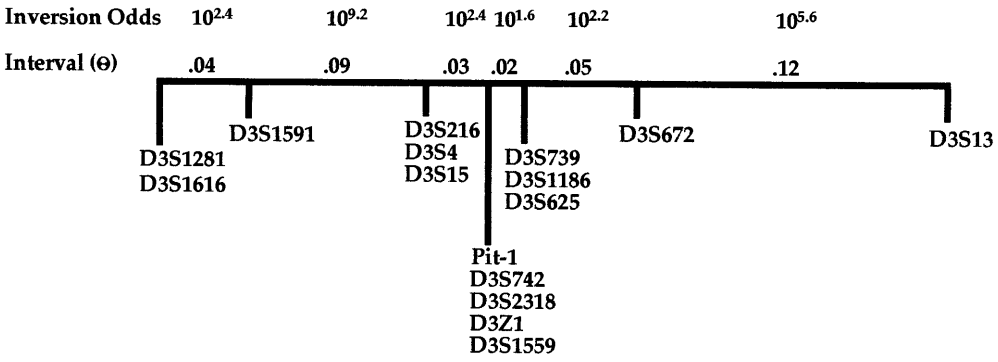

Fig. 2 Multipoint map of Pit-1 and various loci on chromosome 3

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