

## SHORT COMMUNICATION

# Molecular Characterization of an Intragenic Minisatellite (VNTR) Polymorphism in the Human Parathyroid Hormone-Related Peptide Gene in Chromosome Region 12p12.1–p11.2

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**The human parathyroid hormone-related peptide (hPTHrP) gene in chromosome region 12p12.1–p11.2 plays an important role in mammalian development and specifically in skeletogenesis. We have characterized a VNTR polymorphism in the hPTHrP gene that is located in an intron 100-bp downstream of exon VI that encodes a 3' untranslated region. By PCR analysis eight different alleles were identified in a group of 112 unrelated individuals. All eight alleles were sequenced and the repeat unit was identified as the general sequence [G(TA)<sub>n</sub>C]<sub>N</sub>, where *n* = 4 to 11 and *N* = 3 to 17. This polymorphic sequence-tagged site will be useful for mapping chromosome 12p and will aid in testing for linkage of genetic diseases to the hPTHrP gene. © 1993**

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Parathyroid hormone-related peptide (PTHrP; human gene mapping symbol PTHLH) was initially isolated from tumors associated with hypercalcemia and has been demonstrated to be a causative agent of the hypercalcemia of malignancy. The expression of PTHrP is now appreciated to be widespread in fetal and adult tissues, both normal and malignant. It is thought to normally act in an autocrine/paracrine fashion and have important effects upon cell growth and differentiation (2). A mouse model with a defective PTHrP locus has been created (3). While mice heterozygous for the PTHrP null mutation are fertile and apparently phenotypically normal, mice homozygous for the mutation die in the perinatal period. These animals suffer a multitude of skeletal abnormalities involving both axial and appendicular skeleton. The human (h)PTHrP gene spans more than 15 kb and consists of at least nine exons (see 2 and references therein). Multiple alternative 5' and 3'

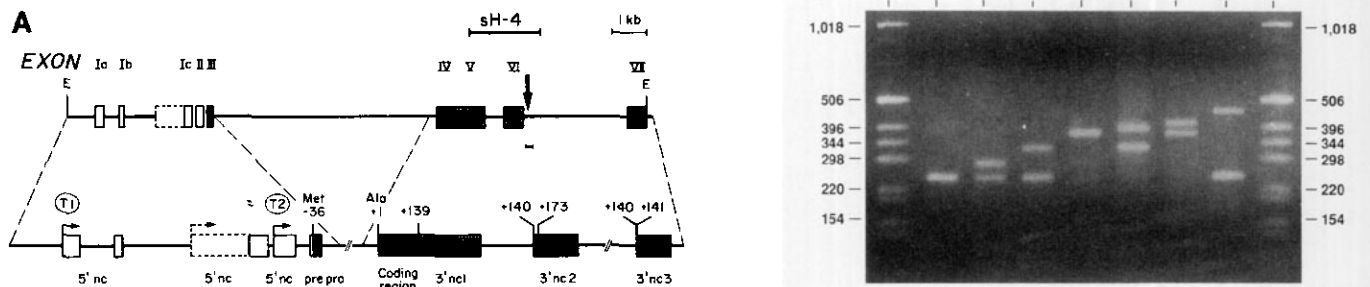
untranslated region exons are present in the gene, which encodes three polypeptide isoforms of 139, 141, and 173 amino acids. The hPTHrP gene has been assigned to chromosome region 12p12.1–11.2 (see 2 and references therein).

Figure 1A shows the polymorphic site in the hPTHrP gene, which was identified in the following manner. The subcloned *Hind*III restriction fragment sH-4 was used as a probe in Southern blot analysis of human genomic DNA to identify a polymorphism (1). Double digests of genomic DNA with *Hind*III and *Bam*HI showed that the polymorphism was located toward the 3' end of the sH-4 sequence (data not shown). DNA sequence analysis of subclone sH-4 was extended from that previously obtained (2) through the putative VNTR region by the di-deoxy chain termination method. Polymerase chain reaction (PCR) primers were designed on either side of the polymorphic site. The forward primer was 5'-GACCTA-GTTCTGATTGTATCCTCTACC-3' and the reverse primer was 5'-GTTCCAGGCGTAAGAATTGACGAG-TG-3'. Five picomoles of each of the primers with 0.5 μg genomic DNA, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.001% (w/v) gelatin, 200 μM each deoxynucleotide triphosphate, and 2.5 units of Amplitaq DNA polymerase (Perkin-Elmer-Cetus) in a total volume of 50 μl was subjected to 27 cycles of PCR consisting of 10 s at 94°C and 95 s at 62°C using a GeneAmp PCR System thermocycler Model 9600 (Perkin-Elmer-Cetus).

Analysis of PCR-amplified products from genomic DNA of 112 unrelated individuals revealed the presence of eight alleles ranging in size from approximately 250 to 460 bp (Fig. 1B). DNA sequences of all eight unique alleles were determined both by direct sequencing of PCR-amplified material and after subcloning into a plasmid vector. The repeat unit itself consists of TA dinucleotide repeats in blocks that are interspersed with a single CG dinucleotide repeat, which therefore imposes a higher order on what would otherwise be a simple dinucleotide repeat of the microsatellite type (Fig. 2). The consensus sequence of the repeat is well conserved, although within several alleles single nucleotide substitutions are ob-

Sequence data from this article have been deposited with the GenBank Data Library under Accession No. L07553.

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**FIG. 1.** (A) The organization of PTHrP gene and the site of the polymorphic region. The gene is located on an *EcoRI*(E) restriction fragment, approximately 15 kb in size. Exons are indicated by boxes designated by a Roman numeral: open boxes, 5' noncoding (nc); solid boxes, coding; hatched boxes, 3' noncoding (nc). T1 and T2 denote upstream and downstream transcription initiation sites, respectively. The vertical arrow indicates the polymorphic region which starts approximately 100 bp downstream of the end of exon VI and sH-4 is the subcloned *HindIII* restriction fragment that was used as a probe in Southern blot analysis of human genomic DNA to initially identify the polymorphism (1). The PCR-amplified region is indicated by the solid bar below the arrow. (B) Agarose gel electrophoresis (1%, 1× TBE, pH 8.0) of allelic products from the hPTHrP VNTR. The size markers and products shown are lanes 1 and 9, the BRL 1-kb DNA ladder; lanes 2–8, hPTHrP VNTR amplified products: 252, 252 and 288, 252 and 332, 378, 332 and 393, 378 and 414, and 252 and 460 bp. An additional allele of 356 bp was also identified (not shown).

served either within a TA repeat block or less frequently within a CG dinucleotide. The smallest allele contains 3 blocks of the sequence and the largest allele 17 blocks.

In five different families the polymorphism was inherited in a Mendelian fashion with codominant autosomal segregation of the hPTHrP VNTR alleles. The frequency distribution of the alleles in 112 unrelated individuals of various ethnic origins was 252 bp (32%), 288 bp (7%), 332 bp (17%), 356 bp (2%), 378 bp (35%), 393 bp (1%), 414 bp (2%) and 460 bp (4%). There were 80 (71%) heterozygotes, which was not significantly different than expected under Hardy–Weinberg equilibrium (83 expected). The PIC was 0.7.

This “higher ordered” VNTR minisatellite does not exhibit the PCR-generated slippage pattern that can

sometimes make genotype assignment problematic with simple dinucleotide repeats. A mouse PTHrP “knockout gene” model has provided evidence implicating PTHrP in mammalian development, with an important role of this peptide in skeletogenesis (3). The polymorphic sequence-tagged site described here should be useful for analysis of linkage of diseases to the hPTHrP gene and for mapping the proximal region of chromosome 12p.

#### ACKNOWLEDGMENTS

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      10      20      30      40      50      60
1  GACCTAGTTC TGATTGTATC CTCTACCTAT CATCTACTCA TTTATATCTA TCTATCTATC
61 TAGGGGGATA TGTTTCTATG AGGCAAAAAG TATATATATA CGTATATATA TATATATATA
121 TACACCTATA TATATATACG TATATATATA TACCTATATA TATCGCTATA TATATATACG
181 TATATATATA TACCTATATA TATACCTATA TATATATACG TATATATATA TACCTATATA
241 TATATACCTA TATATATGAG TTATAAAAAG AGGTATAAAT AACTTTGCAC GGATTTTATT
301 TATTTCAAAT TTGCAATGTT CTTTAAGTAT CTGGAGAATT GATGAAAATT AACACTCGTC
361 AATTCTTACG CCTGGAAC

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**FIG. 2.** Nucleotide sequence of the most common hPTHrP VNTR allele. The repeat unit is of the general sequence G(TA)<sub>n</sub>C, where n = 4 to 11. The PCR primers are shown as arrows. TA repeats are boxed. Nucleotide substitutions deviating from the consensus sequence are indicated by asterisks.