J Hum Genet (1998) 43:146–147

BRIEF REPORT — POLYMORPHISM REPORT

Kazuhiro Tsukamoto · Mitsuru Emi

A polymorphic CA repeat sequence at the human calcitonin locus

Received: November 10, 1997 / Accepted: December 5, 1997

Abstract A polymorphic dinucleotide (CA) repeat sequence was isolated from a genomic clone containing the human calcitonin gene at 11p15.2–p15.1. This polymorphism will be a useful marker in the genetic study of disorders affecting calcium metabolism including hyperparathyroidism, hypoparathyroidism, and osteoporosis.

Key words CA repeat, Calcitonin gene · Calcium metabolism · Osteoporosis · Hyperparathyroidism

Introduction

Calcitonin, a calcium-regulating hormone, lowers the calcium levels in serum by inhibiting bone resorption, and is implicated in the pathogenesis of disorders of calcium metabolism (Jacobs et al. 1981; Rosenfeld et al. 1983). In order to understand the relationship between genetic variations at the calcitonin locus and disorders of calcium metabolism including hyperparathyroidism, hypoparathyroidism, and osteoporosis (Alevizaki et al. 1989; Nakamura 1996; Yanase 1997), we isolated and characterized a dinucleotide repeat polymorphism at this locus.

Source and isolation of CA repeat sequence

A human genomic clone containing the calcitonin gene was identified from a P1-derived artificial chromosome (PAC) library by polymerase chain reaction (PCR) 3-dimensional screening using primer sequences derived from the 3′ portion of the gene. A fragment containing a CA repeat was identified by Southern blotting of a PAC DNA digested by *Hae*III, *Sau*3A, or *Rsa*I with the $(GT)_{20}$ probe, subcloned, and sequenced (Fig. 1). PCR primers were designed to flank this repeat sequence for polymorphism analysis.

K. Tsukamoto · M. Emi (✉)
Department of Molecular Biology, Institute of Gerontology, Nippon Medical School, 1-396 Kosugi-cho, Nakahara-ku, Kawasaki 211, Japan
Tel. +81-44-733-5230; Fax +81-44-733-5192
e-mail: memi@nms.ac.jp

PCR primers

Forward (CT, 4F): 5′ -GGA GAC AAA CAG GGA TGA CA- 3′
Reverse (CT, 4R): 5′ -CAG AAA CAT GGT GTG CCA GC- 3′

PCR conditions

PCR was performed in a volume of 10 μl containing 20 ng genomic DNA, 10 mM Tris·HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 200 μM dNTPs, 2.5 pmol of a [^{32}P] end-labeled forward primer and an unlabeled reverse primer, and 0.25 units of *Taq* polymerase. Cycle conditions were 94°C for 4 min, then 30 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s, with a final extension step of 5 min at 72°C, in a Gene Amp PCR9600 System (Perkin Elmer Cetus, Norwalk, CT, USA) (Nakura et al. 1994). PCR products were electrophoresed in 0.3-mm-thick denaturing 6% polyacrylamide gels containing 36% formamide and 8 M urea, at 2000 V for 2–4 h. Gels were transferred to filter papers, dried at 80°C, and autoradiographed. The sizes of alleles were determined by comparison with a sequencing ladder of a control plasmid.

Polymorphism and allele frequency

Six alleles were detected in 192 chromosomes of unrelated Japanese individuals (Fig. 1A). The observed heterozygosity was 0.52. The size and frequency of the six alleles are shown in Table 1.

Mendelian inheritance. Codominant inheritance was observed in two three-generation families.

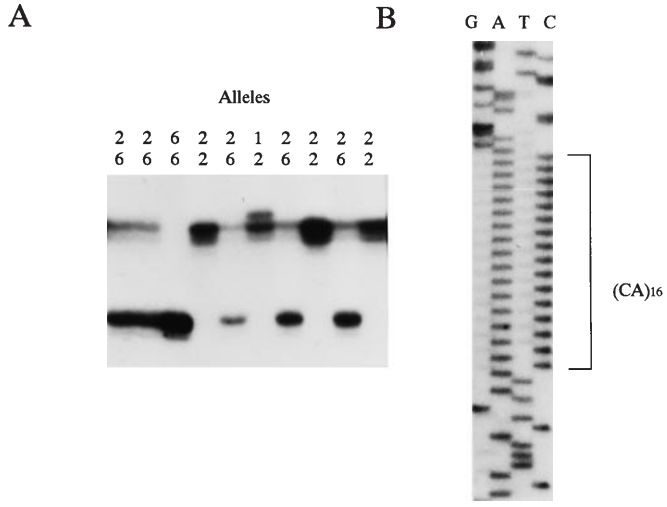

Fig. 1 A Autoradiogram showing a polymorphic CA repeat at the calcitonin locus in 10 unrelated individuals. **B** Nucleotide sequence of the CA repeat and the flanking regions at the calcitonin locus

Table 1 Size and frequency of the alleles of the CA repeat polymorphism at the human calcitonin locus

Allele	Size (bp)	Frequency
A1	126	0.06
A2	124	0.61
A3	122	0.01
A4	114	0.01
A5	112	0.02
A6	110	0.29

Chromosomal localization. The human calcitonin gene was assigned to chromosome 11p15.1–p15.2 by fluorescence in situ hybridization (Hoovers et al. 1993).

Acknowledgments This work was supported by research grants for osteoporosis from the Ministry of Health and Welfare of Japan and the Novartis foundation for gerontological research.

References

Alevizaki M, Stevenson JC, Girgis SI, MacIntyre I, Legon S (1989) Altered calcitonin gene in a young patient with osteoporosis. Br Med J 298: 1215–1216

Hoovers JMN, Redeker E, Speleman F, Hoppener JWM, Bhola S, Bliek J, van Roy N, Leschot NJ, Westerveld A, Mannens M (1993) High-resolution chromosomal localization of the human calcitonin/CGRP/IAPP gene family members. Genomics 15: 525–529

Jacobs J, Goodman RH, Chin WW, Dee PC, Habener JF, Bell NH, Potts JT (1981) Calcitonin messenger RNA encodes multiple polypeptides in a single precursor. Science 213: 457–459

Nakamura Y (1996) Application of DNA markers to clinical genetics. Jpn J Hum Genet 41: 1–14

Nakura J, Miki T, Ye L, Mitsuda N, Ogihara T, Ohta T, Jinno Y, Niikawa N, Takahashi A, Ishini Y (1994) Six dinucleotide repeat polymorphisms on chromosome 7. Jpn J Hum Genet 39: 447–449

Rosenfeld MG, Mermod JJ, Amara SG, Swanson LW, Sawchenko PE, Rivier J, Vale WW, Evans RM (1983) Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing. Nature 304: 129–135

Yanase T (1997) Human genetics: past, present, and future. Jpn J Hum Genet 42: 265–316