J Hum Genet (1998) 43:280–282

BRIEF REPORT — POLYMORPHISM REPORT

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Isolation and radiation hybrid mapping of a dinucleotide repeat polymorphism at the human calcium-sensing receptor (CASR) locus

Received: June 2, 1998 / Accepted: June 24, 1998

Abstract Calcium-sensing receptor (CASR) in parathyroid gland regulates calcium homeostasis by sensing decreases in extracellular calcium levels and effecting an increase in secretion of parathyroid hormone. A polymorphic dinucleotide (CA) sequence was isolated from a genomic clone containing the human *CASR* gene and was mapped to 3q13.3–q21. This polymorphism will be useful in the genetic study of disorders affecting calcium metabolism, such as hypercalcemia, hypocalcemia, osteoporosis, hyperparathyroidism, and hypoparathyroidism.

Keywords Calcium-sensing receptor parathyroid gland · Calcium metabolism dinucleotide repeat

Introduction

Calcium-sensing receptor (CASR) is a member of a superfamily of G-protein-coupled cell surface receptors expressed in parathyroid gland and kidney. Parathyroid cells respond to decreases in extracellular calcium levels by means of this receptor, which ultimately effects an increase in parathyroid hormone secretion. Brown et al. (1993) identified a cDNA encoding a 120-Kda receptor containing a large extracellular domain and seven membrane-spanning regions, characteristic of G protein-coupled receptors, by expression cloning.

Defects in the *CASR* gene have been implicated in several genetic disorders with disturbed parathyroid calcium homeostasis, such as familial hypocalciuric hypercalcemia (Pollak et al. 1993), autosomal dominant hypocalcemia (Pollak et al. 1994), neonatal severe hyperparathyroidism (Pollak et al. 1993), and autosomal dominant hypoparathyroidism (Baron et al. 1996). To understand the relationship between genetic variations at the *CASR* locus and various disorders affecting calcium metabolism (Nakamura 1996; Yanase 1997), we isolated and characterized a dinucleotide repeat polymorphism at this locus.

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Source and Isolation of CA repeat sequence

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

PCR primers

The PCR primers used were:
Forward (CASR, 5F) 5′ TAA TGT TGC TAT GAA CAT TTT ATG 3′
Reverse (CASR, 5R) 5′ GAA CTT GGG GTC CTC CAT AG3′

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 deoxyribonucleotide triphosphate (dNTP)s, 2.5 pmol of a [^{32}P] end-labeled forward primer and a nonlabeled 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

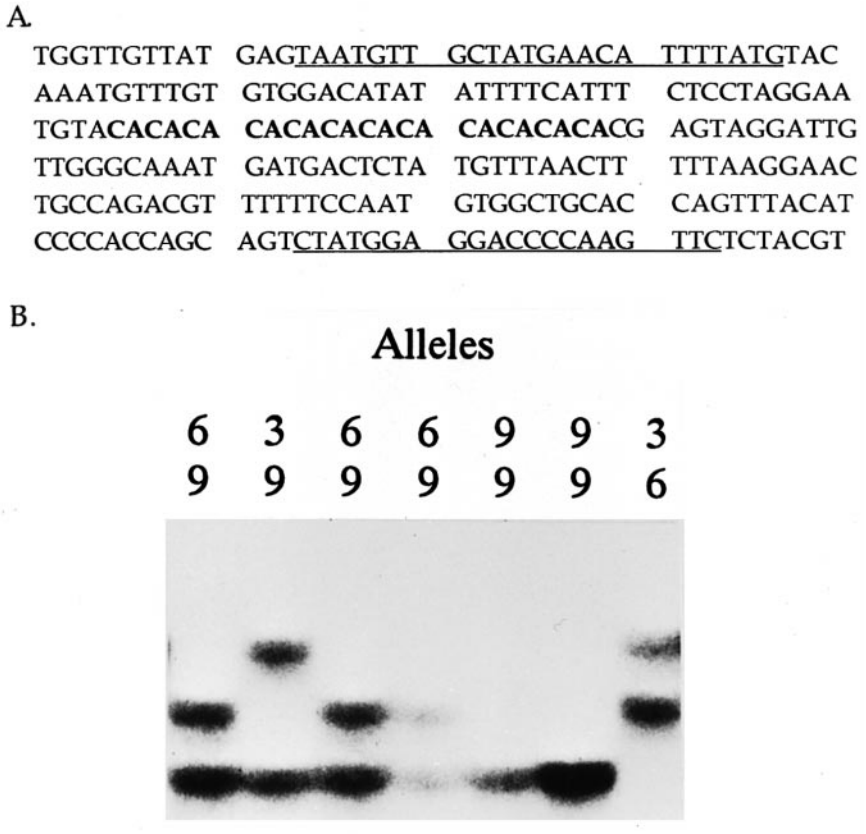

Fig. 1 **A** Nucleotide sequence of the CA repeat and the flanking region at the *calcium-sensing receptor* (*CASR*) locus. Sequences used for forward and reverse primers are *underlined*. CA repeats are shown in *bold*. **B** Autoradiogram showing a polymorphic CA repeat at the *CASR* locus in seven unrelated individuals

72°C in a Gene Amp PCR9600 System (Perkin Elmer Cetus, Foster City, CA, 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 volts for 2–4 h. The gels were transferred to filter papers, dried at 80°C, and autoradiographed. The sizes of alleles were determined by comparison with the sequencing ladder of a control plasmid.

Polymorphism and allele frequency

Ten alleles (Table 1) were detected in 192 chromosomes of unrelated Japanese individuals. Representative autoradiogram of the CA repeat polymorphism is shown in Fig. 1. Observed heterozygosity was 0.55.

Mendelian inheritance

Codominant inheritance was observed in two two-generation families.

Chromosomal localization

The human *CASR* gene was assigned to human chromosome 3q13.3–q21 (Janicic et al. 1995).

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

Allele	Size (bp)	Frequency
A1	232	0.01
A2	230	0.02
A3	228	0.32
A4	226	0.01
A5	224	0.02
A6	222	0.02
A7	220	0.01
A8	218	0.01
A9	216	0.57
A10	214	0.01

Radiation hybrid mapping

The newly isolated CA repeat at the *CASR* locus was mapped to chromosome 3, using the G3 RH mapping panel of 83 hybrid cell lines of the Stanford Human Genome Center (Boehnke et al. 1991), by linkage to a marker SHGC-7096 with a Logarithm of the ODds (LOD) score of more than 6.0.

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.

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