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BRIEF REPORT — POLYMORPHISM REPORT

Kazuhiro Tsukamoto · Satoshi Inoue · Takayuki Hosoi
Hajime Orimo · Mitsuru Emi

Isolation and radiation hybrid mapping of dinucleotide repeat polymorphism at the human estrogen receptor β locus

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Abstract A gene for a second type of human estrogen receptor, the estrogen receptor β (ESRβ), was recently identified. We isolated a polymorphic dinucleotide CA repeat marker from a genomic clone containing the human estrogen receptor β gene. High heterozygosity (0.93) makes this polymorphism a useful marker in the genetic study of disorders affecting female endocrine systems; calcium metabolism; and breast, uterine, and ovarian cancers.

Key words Estrogen receptor · CA repeat · Calcium metabolism · Breast cancer · Ovarian cancer

Introduction

A second type of estrogen receptor gene, the estrogen receptor β gene, recently isolated by Mosselman et al. (1996), had 96% conserved amino acid residues in the DNA binding domain and 58% conserved residues in the ligand-binding domain when compared with the estrogen receptor. To understand the relationship between genetic variations at the *ESR*β locus and disorders affecting endocrine systems; calcium metabolism; and breast, uterine, and ovarian cancers (Nakamura 1996; Yanase 1997), we isolated and characterized a dinucleotide repeat polymorphism at this locus.

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

S. Inoue · T. Hosoi · H. Orimo
Department of Geriatrics, Faculty of Medicine, University of Tokyo, Tokyo, Japan

Source and isolation of CA repeat sequence

A human genomic clone containing the *ESR*β 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 the CA repeat was identified by Southern blotting of PAC DNA digested by *Hae*III, *Sau*3A, or *Rsa*I with a (GT)20 probe and was subcloned and sequenced. An autoradiogram of the CA repeat sequence is shown in Fig. 1B. PCR primers were designed to flank this new repeat sequence for polymorphism analysis.

PCR primers

Forward (ERB, 4F) 5′ -GGT AAA CCA TGG TCT GTA CC- 3′
Reverse (ERB, 5R) 5′ -AAC AAA ATG TTG AAT GAG TGG G- 3′

PCR conditions

PCR was performed with 20 ng genomic DNA, 10 mM TrisHCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% of gelatin, 200 μM dNTPs, 2.5 pmol of a [^{32}P] end-labeled forward primer and a nonlabeled reverse primer, and 0.25 units of *Taq* polymerase in a volume of 10 μl. Cycle conditions were 94°C for 4 min, then 30 cycles of 94°C for 30 s, 65°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.

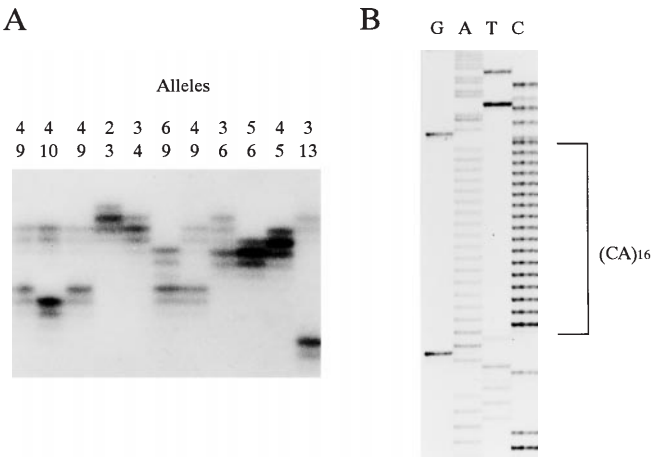

Fig. 1 **A** Autoradiogram showing a polymorphic CA repeat at the estrogen receptor β (*ESR*β) locus in 11 unrelated individuals. **B** Nucleotide sequence of the CA repeat at the *ESR*β locus and the flanking regions

Table 1 Size and frequency of the alleles of the CA repeat polymorphism in the estrogen receptor β (*ESR*β) locus

Allele	Size (bp)	Frequency
A1	194	0.01
A2	192	0.01
A3	190	0.01
A4	188	0.02
A5	186	0.06
A6	184	0.07
A7	182	0.06
A8	180	0.06
A9	178	0.08
A10	176	0.07
A11	174	0.03
A12	172	0.04
A13	170	0.13
A14	168	0.13
A15	166	0.18
A16	164	0.06
A17	162	0.01
A18	160	0.01

Polymorphism and allele frequency

Eighteen alleles were detected in 192 chromosomes of unrelated Japanese individuals. A representative autoradiogram of the CA repeat polymorphism is shown in Fig. 1A. The observed heterozygosity was 0.93. The size and frequency of the 18 alleles are shown in Table 1.

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

Chromosomal localization. The human *ESR*β gene has been assigned to human chromosome 14q (Mosselman et al. 1996).

Radiation hybrid mapping. The newly isolated CA repeat at the *ESR*β locus was mapped to 14q using the G3 RH mapping panel of 83 hybrid cell lines of the Stanford Human Genome Center (SHGC) (Boehnke et al. 1991), by linkage to a marker SHGC-11003 with a logarithm of differences (LOD) score of >100.

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