BRIEF REPORT — POLYMORPHISM REPORT

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Isolation of a polymorphic CA repeat sequence at the human progesterone receptor (PGR) locus

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Abstract We isolated a polymorphic dinucleotide (CA) repeat sequence from a genomic clone containing the human progesterone receptor (PGR) gene. This polymorphism will be a useful marker in the genetic study of disorders affecting female endocrine systems, such as progesterone resistance and breast, uterine, and ovarian cancers.

Key words Progesterone receptor · CA repeat · Progesterone resistance · Pseudocorpus luteum insufficiency · Female endocrine system

Introduction

Progesterone prepares the endometrium for blastocyst implantation and allows maintenance of pregnancy. Failure of the endometrium to respond to progesterone via the progesterone receptor (PGR) leads to impaired blastocyst implantation and infertility (Chrousos et al. 1986). A disorder of progesterone resistance, pseudocorpus luteum insufficiency, has been reported by Keller et al. (1979) and other researchers. Progesterone has a protective effect against the development of breast cancer, and the presence of progesterone receptors in human breast cancer is associated with hormone dependence and prolonged survival. In addition, several PGR mRNA variants have been found in some breast cancer tissues (Leygue et al. 1996). Human cDNA for progesterone receptor encodes a polypeptide of 933 amino acids with a molecular weight of 98,868 Da (Misrahi et al. 1987). To understand the relationship between genetic variations at the *PGR* locus, and disorders affecting female

endocrine systems, such as progesterone resistance and breast, uterine, and ovarian cancers (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 *PGR* 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 (GT)₂₀ probe, 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

The PCR primers used were: Forward (PGR,7F) 5' TAG TTA ACT AGC TAA ATT CCA A 3', and reverse (PGR,7R) 5' TTA CTT ACC TTC ACG AAT GAA 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 MgCl₂, 0.01 % of gelatin, 200 µM dNTPs, 2.5 pmol of a [³²P] end-labeled forward primer and a non-labeled 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 PCR 9600 System (Perkin Elmer Cetus, Palo Alto, 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

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Fig. 1 **A** Autoradiogram showing a polymorphic CA repeat at the *PGR* locus in seven unrelated individuals. **B** Nucleotide sequence of the CA repeat and flanking regions at the *progesterone receptor* (*PGR*) locus

were transferred to filter papers, dried at 80°C, and autoradiographed. Sizes of alleles were determined by comparison with a sequencing ladder of a control plasmid.

Polymorphism and allele frequency

Ten alleles were detected in 192 chromosomes of unrelated Japanese individuals. A representative autoradiogram of the CA repeat polymorphism is shown in Fig. 1A. Observed heterozygosity was 0.66. The size and frequency of the ten alleles are shown in Table 1.

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

Chromosomal localization. The human *PGR* gene was assigned to human chromosome 11q22 (Mattei et al. 1988).

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Table 1 Size and frequency of the ten alleles of the CA repeat polymorphism in the progesterone receptor (*PGR*) locus

Allele	Size(bp)	Frequency
A1	174	0.02
A2	172	0.01
A3	170	0.05
A4	168	0.01
A5	166	0.02
A6	164	0.03
A7	162	0.32
A8	160	0.49
A9	158	0.01
A10	154	0.04

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