Assignment of the gene encoding renin binding protein \((\text{Renbp})\) to rat chromosome Xq37 by in situ hybridization and radiation hybrid mapping

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Rationale and significance

The renin-angiotensin system (RAS) plays a crucial role in the regulation of blood pressure (Laragh, 1995). Within the RAS, the physiological relevance of renin binding protein (RnBP) has been controversial for several years (Schmitz et al., 2000). Originally, RnBP was identified as a protein in the kidney that was capable of binding renin in renal homogenates giving rise to a complex designated high molecular weight renin (Takahashi et al., 1983). It was subsequently proposed that RnBP might act as an endogenous cellular renin inhibitor. Recent studies demonstrated that human RnBP is the enzyme N-acetyl-D-glucosamine (GlcNAc) 2-epimerase (Takahashi et al., 1999). Moreover, gene-targeting experiments in the mouse excluded a specific interaction between RnBP and renin in the kidney or in the circulating RAS of the mouse (Schmitz et al., 2000). So far, the gene encoding RnBP has been mapped on chromosome X in different mammals: Xq28 in human (van den Ouweland et al., 1994), X 29.53 cM in mouse (http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=19703) and X in canine (Stoy et al., 1998). As mapping data for the rat homolog \(\text{Renbp}\) is still lacking, it is unclear whether this gene is located in blood pressure quantitative trait loci (QTL) termed \(BP/SP\)-2 and \(SS-X\) on rat chromosome X. \(BP/SP\)-2 has been identified in an intercross between the stroke prone spontaneously hypertensive rat (SHRSP) and the normotensive Wistar-Kyoto (WKY) rat (Hilbert et al., 1991) and \(SS-X\) has been characterized in the Sabra hypertension-prone rat (Yagil et al., 1999). We therefore set out to test the chromosome location of \(\text{Renbp}\) by performing chromosomal mapping analysis in the rat using fluorescence in situ hybridization (FISH) and radiation hybrid (RH) mapping.

Materials and methods

Fluorescence in situ hybridization

A 1092-bp rat \(\text{Renbp}\) cDNA fragment spanning a region between position 140 and 1232 of the reported mRNA sequence (Inoue et al., 1991; GenBank accession no. D10233) was generated by reverse transcription PCR as reported (Kreutz et al., 1997) using the following oligonucleotides:

\[
\begin{align*}
5' & - \text{CCTTGGCCGCGATGGGCAGGTATATG-3} \\
3' & - \text{GGGGCGGGCCC-} \text{AAGGCGTTGGAGTAG-3} 
\end{align*}
\]

The FISH experiments were done as previously described (Laes et al., 1998).

Radiation hybrid mapping

50 ng of genomic DNA from the rat-hamster radiation hybrid panel (Research Genetics, http://www.resgen.com) were amplified with a set of primers specific for a segment of the rat \(\text{Renbp}\) gene in exon 5. PCR was performed on all 106 clones of the RH-panel (Steen et al., 1999; Watanabe et al., 1999) and analyzed by gel electrophoresis. The data vector obtained for \(\text{Renbp}\) was: 00001 00100 00000 00000 00101 00000 00000 00000 00001
firmed by sequencing.

cific band at the expected size of 111 bp of DXWox3 (Fig. 2). Placement for confirmed the telomeric localization of mosomal placement on the X chromosome by RH mapping firmed by somatic cell hybrid analysis (data not shown). Chro-

mosomal placement on chromosome X at Xq37 (Fig. 1). This result was con-

firmed by RH mapping with the vector from exon 5 and an additional vector obtained by PCR amplification of a sequence from intron 9 (data not shown) revealed linkage to both RNO1 (maximum lod score 9.0) and RNOX (maximum lod score 9.4). FISH analysis, however, showed that Renbp is located on chromosome X at Xq37 (Fig. 1). This result was con-

firmed by somatic cell hybrid analysis (data not shown). Chromosomal placement on the X chromosome by RH mapping confirmed the telomeric localization of Renbp close to marker DXWox3 (Fig. 2). Placement for Renbp was next to DXWox3, 26.4 cR away from the closest marker D19Mit1 (D19Mit1 represents a marker previously localized on rat chromosome 19, but reassigned to chromosome X (Bihoreau et al., 1997) on one side and 28.4 cR away from flanking marker DXRat22 on the other side. Construction of a comparative map integrating the previously reported confidence intervals for the placement of the blood pressure QTL BP/SP-2 between markers DXMgh5 and DXMit4 (SHRSP × WKY intercross; Hilbert et al., 1991) and SS-X between markers DXRat4 and DXMgh10 (Sabra rat model, Yagil et al., 1999) revealed that Renbp falls outside the BP QTL regions on chromosome X (Fig. 2).

References


Proof of authenticity: Amplification with the primers generated a spe-

PCR conditions: 94°C 15 s, 60°C 60 s, 72°C 60 s (30 cycles)

Results

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References


