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The angiotensinogen gene is located on mouse chromosome 8

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We have recently identified a *cis*-acting genetic lesion affecting angiotensinogen gene expression in testis and salivary gland. Accordingly, the angiotensinogen gene was assigned to mouse chromosome 8 by screening a series of hybrid cell lines for retention of mouse angiotensinogen sequences by genomic Southern analysis. In AKXD recombinant inbred mice, the angiotensinogen gene is 2.4 ± 1.8 centiMorgan from *Rn7S-8*,a 7S RNA gene located on chromosome 8 (Taylor, B.A., personal communication). However, the segregation of salivary and testicular angiotensinogen expression phenotypes into inbred mouse strains was not concordant with the known chromosome 8 proviruses *Emv-2*, *Mtv-21*, *Xmv-12* or *Xmv-26*.

Angiotensinogen; Chromosome mapping; Hybrid cell; (Mouse, inbred strain)

1. INTRODUCTION

Angiotensinogen is the only precursor of the vasoactive peptide angiotensin II, and is therefore an important determinant of blood pressure and electrolyte homeostasis [1]. The liver is the predominant site of production of angiotensinogen, but recent interest has centered on synthesis of angiotensinogen in many other tissues, including brain, kidney and brown adipose tissue where it may participate in local angiotensin-generating systems [2].

We have recently identified a genetic lesion affecting angiotensinogen gene expression in testis and salivary gland of transgenic mice [3]. Subsequently, two angiotensinogen expression phenotypes were defined in inbred mouse strains. Low angiotensinogen mRNA levels were found in testis and salivary gland of BALB/c and A/J mice, whereas high angiotensinogen mRNA levels were present in the same organs from C57BL/6, C3H/HeJ, AKR/J, CBA, DBA/2J, SJL and Swiss mice [3]. This variability of angiotensinogen gene expression among inbred mouse strains was characterized further using CXB recombinant inbred mice [3]. Concordance between the angiotensinogen genotype and expression phenotypes in testis and salivary gland of the CXB mice suggested that the genetic lesion was in *cis*. We subsequently identified an *Eco*RI restriction fragment length polymorphism which cosegregated with the angiotensinogen expression phenotypes into inbred mouse strains [3].

In the present study, we determine the chromosomal location of the mouse angiotensinogen gene. The major reason for undertaking this study was to see if the angiotensinogen gene was linked to a known mutation or retroviral insertion, which may account for the observed variability of angiotensinogen gene expression among different mouse

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Abbreviations: A, AKR/J; B, C57BL/6By; C, BALB/cBy; cM, centiMorgan; D, DBA/2J; Rb, Robertsonian translocation; SSC, standard saline citrate

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strains. We were also interested to see if the angiotensinogen gene was linked to the serine protease inhibitor locus on mouse chromosome 12, because angiotensinogen shares significant protein sequence identity with other serine protease inhibitors [4].

2. MATERIALS AND METHODS

2.1. *DNA*

High molecular weight genomic DNAs from inbred mouse strains BALB/cBy and C57BL/6By; the AKXD recombinant inbred set; and congenics B6.C-*H-19*^{c}/By and B6.C-*H-29*^{c}/By were purchased from Dr Benjamin Taylor, The Jackson Laboratory, Bar Harbor, ME 04609, USA. Microcell hybrids used in this study (table 1) have been described previously [5,6].

2.2. *Genomic Southern analysis*

10 μg of genomic DNA was digested to completion with either *Eco*RI or *Bam*HI, electrophoresed through 0.8% agarose gels and subsequently transferred in 0.4 M NaOH onto Biotrace RP nylon membranes (Gelman Sciences, Ann Arbor, MI). Blots were prehybridized for 2–4 h at 65°C in a solution of 3 × SSC, 1% sodium dodecyl sulphate, 0.4% non-fat milk powder and 0.5 μg/ml freshly denatured herring sperm DNA. Blots were then hybridized overnight with a 397 bp *Stu*I–*Bam*HI fragment from exon 2 of the mouse angiotensinogen gene [7,8] which was labeled with [α-^{32}P]dATP and [α-^{32}P]dCTP using the random primer method [9]. Posthybridization washes were taken to a stringency of 0.5 × SSC at 65°C. Blots were autoradiographed using Kodak XAR5 film at −70°C with a single intensifying screen.

3. RESULTS AND DISCUSSION

3.1. *Strain distribution patterns for angiotensinogen in recombinant inbred mice*

The previously determined strain distribution pattern for the angiotensinogen gene in CXB recombinant inbred strains D to K is BCBCBBB [3]. Inspection of all chromosomal assignments for the CXB recombinant inbred set (kindly provided by Dr B.A. Taylor) showed that this angiotensinogen genotype was identical to that determined for the mouse skeletal muscle α actin gene on chromosome 3 [10,11], but also *RP-10* on chromosome 7 [12]. However, there is a 5% probability of a false-positive linkage using the CXB recombinant inbred set alone [13], because this set contains only 7 strains.

In order to resolve this ambiguity, we used a *Bam*HI allelic polymorphism recognized by the mouse angiotensinogen exon 2 probe [8] to genotype the large AKXD recombinant inbred set. The

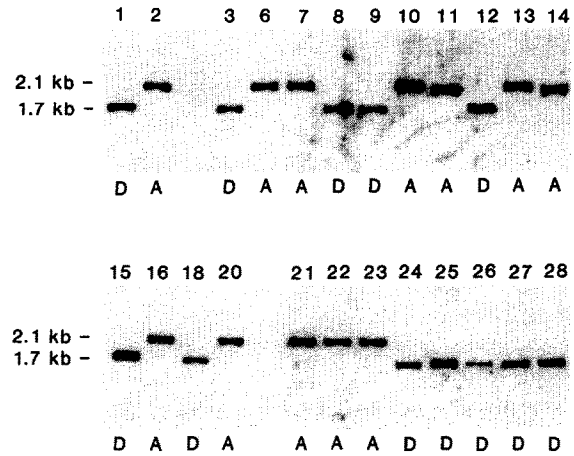

Fig.1. Southern blot of *Bam*HI-digested genomic DNA showing the strain distribution pattern for angiotensinogen in AKXD recombinant inbred mice.

strain distribution pattern for the angiotensinogen gene in AKXD recombinant inbred mice (fig.1) shows no linkage to markers *Pim-1* [14] or *Crya-1* [15] on chromosome 17. Unfortunately, the AKXD set contains no published markers on chromosome 3, 10, 14 or 16 [16]. Accordingly, the chromosomal localization of the angiotensinogen gene was determined using microcell hybrids.

3.2. *Chromosomal localization of the angiotensinogen gene using hybrid cell lines*

Based on the recombinant inbred analysis, we initially analysed microcell hybrids containing mouse chromosomes 3 or 17 carried as Robertsonian translocations onto chromosome 8 [5,6]. Microcell hybrids containing mouse chromosome 8 alone were intended to serve as negative controls, as were the parental rat and hamster cell lines (table 1). Fig.2 demonstrates that microcell hybrids bearing either mouse chromosome 8 alone, Rb(8.17) or Rb(3.8) all contain a 4.5 kb *Eco*RI fragment specific for exon 2 of the mouse angiotensinogen gene. Taken together, these results show that the angiotensinogen gene is located on chromosome 8. This conclusion is reinforced by the fact that the monochromosomal microcell hybrids 8D-1 and F(8)D were independently derived by positive selection for APRT activity carried on chromosome 8 [5,6]. Furthermore, the mouse angiotensinogen gene segregated condordantly with mouse chromosome 8 when the primary F(8)D and F(8)E cell lines

Table 1

Chromosomal localization of the mouse angiotensinogen gene using microcell hybrids

Cell line	No. of passages	Species	Mouse chromosome	APRT activity	Mouse angiotensinogen gene	Ref.
17T-1	13	hamster/mouse	Rb(8.17)	+	+	5
8D-1	11	hamster/mouse	8	+	+	5
DR.31	–	hamster	none	–	–	5
F(3.8)8-6	20	rat/mouse	Rb(3.8)	+	+	6
F(8)D	13	rat/mouse	8	+	+	6
FB(8)D	9	rat/mouse	none	–	–	6
F(8)E	20	rat/mouse	8, 9	+	+	6
FB(8)E	10	rat/mouse	none	–	–	6
Fado-2	–	rat	none	–	–	6

FB(8)D and FB(8)E were derived by backselection for the APRT$^-$ phenotype in medium containing 2,6 diaminopurine [6]

were backselected for the APRT$^-$ phenotype in medium containing 2,6 diaminopurine (fig.3A and table 1). This chromosomal localization for angiotensinogen excludes linkage to the serine protease inhibitor locus on chromosome 12 [17].

3.3. *Linkage of the angiotensinogen gene*

When the CXB recombinant inbred set was used to specifically examine markers on chromosome 8, the strain distribution pattern of the ecotropic murine leukaemia virus *Emv-2* [18,19] showed only one mismatch to that determined for angiotensinogen. The angiotensinogen gene is linked to two unpublished markers on chromosome 8 in AKXD recombinant inbred mice. The strain distribution pattern for angiotensinogen (fig.1) showed only 2 mismatches out of 24 with *Rn7S-8*, a 7S RNA gene (B.A. Taylor, personal communication), and 5 mismatches out of 24 with *Mpmv-21*, a non-ecotropic murine retrovirus (W.N. Frankel, J.P. Stoye, B.A. Taylor and J.M. Coffin (1989), in preparation). Using established formulae [20] we estimate that the angiotensinogen gene is 2.4 ± 1.8 cM from *Rn7S-8*, and 7.5 ± 4.4 cM from *Mpmv-21*. A definitive determination of the order

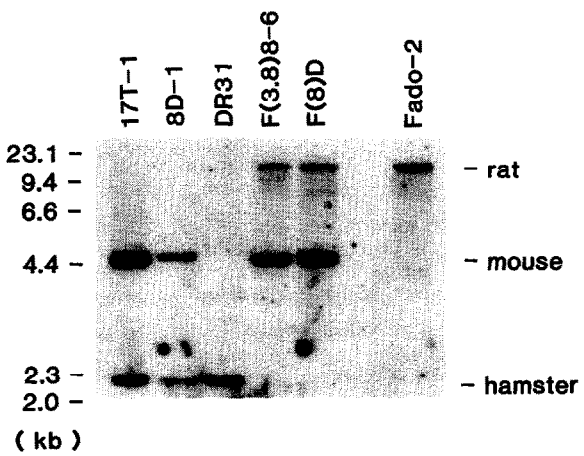

Fig.2. Southern blot of *Eco*RI-digested DNA from microcell hybrids described in table 1, showing segregation of mouse angiotensinogen sequences with chromosome 8. Angiotensinogen bands derived from parental rat and hamster cell lines are also present.

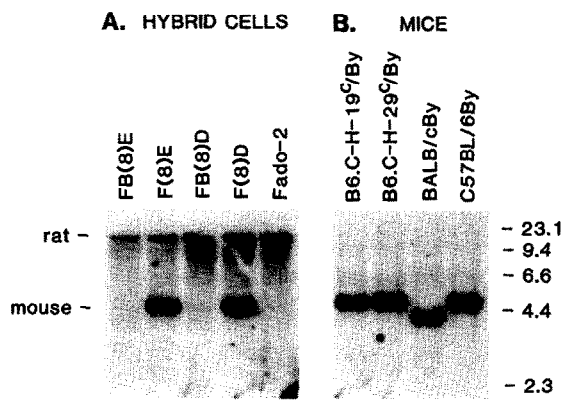

Fig.3. Southern blot of *Eco*RI-digested genomic DNA. (A) Segregation of angiotensinogen sequences after back selection for the APRT$^-$ phenotype in cell lines FB(8)E and FB(8)D. (B) Lack of segregation of the BALB/c angiotensinogen allele with BALB/c chromosome 8 histocompatibility loci *H-19*^{c} and *H-29*^{c} into congenics B6.C-*H-19*^{c} and B6.C-*H-29*^{c}.

of these genes on chromosome 8 requires analysis of a linkage cross. The BALB/c angiotensinogen gene did not segregate with the BALB/c chromosome 8 histocompatibility markers *H-19*^{c} and *H-29*^{c} into congenic strains B6.C-*H-19*^{c}/By and B6.C-*H-29*^{c}/By (fig.3B).

Finally, the segregation of salivary gland and testicular angiotensinogen expression phenotypes into inbred mouse strains [3] was not concordant with known chromosome 8 retroviral insertions *Emv-2* [18], *Mtv-21* [21], *Xmv-12* or *Xmv-26* [16]. We conclude that the nature of the *cis*-acting genetic lesion affecting tissue-specific expression of the angiotensinogen gene will be best defined using molecular cloning techniques.

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NOTE ADDED IN PROOF

Mori et al. [(1989) Cytogenet. Cell Genet. 50, 42–45] have recently assigned the rat angiotensinogen gene chromosome 19 by in situ hybridization analysis of G-banded Wistar-Kyoto rat chromosomes.