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The relaxin gene is located on chromosome 19 in the mouse

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Relaxin is a polypeptide hormone that exerts a variety of physiological effects during pregnancy. To investigate the possibility that known genetic mutations affecting aspects of reproductive physiology result in alterations in the structure or production of relaxin, we have determined the chromosomal location of the mouse gene. The finding of a *Bam*HI restriction fragment length polymorphism in AKR mice enabled us to use recombinant inbred strains to make an assignment to chromosome 19. This was confirmed by Southern analysis of DNA from microcell hybrids.

Relaxin; *Bam*HI RFLP; Chromosome mapping; Mouse, inbred strain

1. INTRODUCTION

Relaxin is a mammalian peptide hormone which is produced predominantly by the corpus luteum during pregnancy and acts on the smooth muscle and connective tissue of the reproductive tract. A number of biological actions, including cervical ripening, promoting growth of interpublic ligaments, and inhibiting uterine contractility, have been elucidated using tissues from a variety of species [1], however the importance of these and other possible roles for relaxin remains unclear.

One approach to demonstrating an essential physiological role is to examine animals in which relaxin has a reduced bioactivity or is present at substantially lowered levels. In the long term this could be done by targetting the relaxin gene using homologous recombination. As an initial step, however, we have determined the chromosomal location of the mouse relaxin gene to find out whether it shows concordance with any mutations known to affect reproduction.

2. MATERIALS AND METHODS

2.1. *DNA*

Genomic DNA was prepared from the tails [2] of random-bred Swiss and inbred mouse strains A/J, C57BL/6By, BALB/cBy purchased from ARC, and C3H/HeJ purchased from the Department of Pathology, University of Melbourne, Australia.

Genomic DNA from the inbred mouse strains SWR/J, DBA/2J, C57L/J, C57BL/6J, C3H/HeJ, AKR/J, SJL, C57BL/10J, BALB/cJ, and the AKXD and AKXL recombinant inbred (RI) sets, was purchased from Dr B.A. Taylor, The Jackson Laboratory, Bar Harbor, Maine 04609, USA.

Microcell hybrid and parental cell lines used in this study (Table III) have been described previously [3–5].

2.2. *Genomic Southern analysis*

10 or 20 μg of genomic DNA was digested to completion with *Bam*HI (Amersham), electrophoresed on 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 55°C in a solution of 3× SCC, 1% SDS, 0.04% non-fat milk powder and 0.5 μg/ml freshly denatured herring sperm DNA (1× SSC contains 0.15 M NaCl and 0.015 M sodium citrate). Blots were then hybridized overnight with a 230 bp fragment from the mouse relaxin A chain (Evans et al., manuscript submitted), labeled with [α^{32}P]dATP plus dCTP (each 2000 Ci/mmol, Bresatec, Adelaide) using the random primer method [6]. Post-hybridization washes contained 1% SDS, and were in 0.5 × SSC at 65°C. Blots were autoradiographed using Kodak XAR5 film at −70°C with a single intensifying screen.

10 μg of genomic DNA from DBA/2J and C57BL/6J or C57BL/6By was digested with the following restriction enzymes: *Alu*I, *Bam*HI, *Bgl*II, *Eco*RI, *Hin*dIII, *Hin*fI, *Nco*I, *Pst*I, *Sac*I and *Xho*I (Amersham); *Hpa*II (New England Biolabs); *Ava*I, *Hae*III, *Hha*I and *Pvu*II (Pharmacia). Digests were done according to the enzyme manufacturer's instructions. Southern analysis was done as above.

2.3. *Linkage analysis in recombinant inbred mice*

Computer printouts of all known strain distribution patterns for AKXD and AKXL recombinant inbred mice were kindly provided by Dr B.A. Taylor, The Jackson Laboratory, Bar Harbor, ME 04609. Recombination frequencies (r) were calculated by using the formula: $r = R/(4-6R)$, where R is the proportion of discordant strains in a recombinant inbred set [7]. The 95% confidence intervals (Table II) for linkage analysis with recombinant inbred mice are those tabulated by Silver [8].

3. RESULTS

3.1. *Analysis of mouse genomic DNA*

We had shown previously that the mouse genome contains a single relaxin gene (Evans et al., manuscript

†We regret that Dr. W.M. Clouston is deceased. Bill was a generous person and a fine scientist, and will be warmly remembered by all who worked with him.

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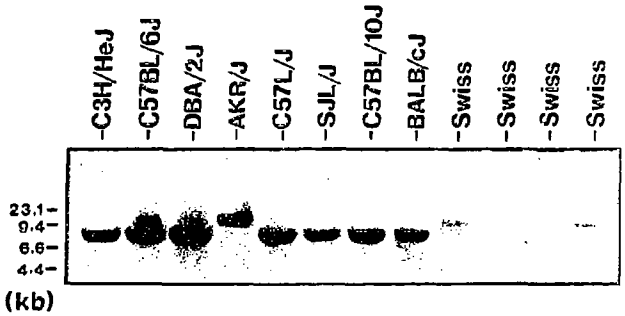

Fig. 1. Southern blot showing segregation of a *Bam*HI RFLP for relaxin in different mouse strains. The blot was washed in 0.5 × SSC at 65°C, and exposed to X-ray film for 10 days. Size markers are λcI857 DNA digested with *Hin*dIII.

submitted). The results of our current search for restriction fragment length polymorphisms (RFPL's) confirm this finding. Genomic DNAs from DBA/2J and C57BL/6J or C57BL/6By were analysed using a panel of 15 different restriction enzymes (see section 2), however no RFLP could be detected in these strains. In a second series of experiments, genomic DNAs from 8 different inbred strains and 4 random-bred Swiss mice were digested to completion with *Bam*HI and analysed as above. We found that both Swiss mice and the AKR/J inbred strain give a high molecular weight band (10.5 kb), whereas all other strains examined give a band of 7.8 kb (Fig. 1).

3.2. *Strain distribution patterns (SDP) in recombinant inbred mice*

Recombinant inbred mouse strains were used to determine the chromosomal location of the mouse relaxin gene. The BXD RI set contains 26 strains [9] but could not be used in this study as no RFLP could be detected in the parental inbred strains (C57BL/6J and DBA/2J).

The *Bam*HI allelic polymorphism recognised by the mouse relaxin A chain probe was used to genotype the AKXD and AKXL RI sets [9]. The higher molecular weight band in the AKR/J strain was designated A and the lower bands in the DBA/2J and C57L/J were designated D and L respectively (Fig. 1). The SDPs for the mouse relaxin gene (*Rln*) in the AKXD (24 strains) and AKXL (18 strains) RI sets are listed in Table I.

Table I

Strain distribution patterns for the mouse relaxin gene in AKXD and AKXL recombinant inbred (RI) mice

RI strain	Series No.	Progenitor DNA pattern
AKXD	6, 7, 9, 10, 11, 18, 21, 23, 24, 27	A
	1, 2, 3, 8, 12, 13, 14, 15, 16, 20, 22, 25, 26, 28	D
AKXL	5, 7, 9, 12, 21, 29, 37	A
	6, 8, 13, 14, 16, 17, 19, 24, 25, 28, 38	L

Table II

Linkage of the mouse relaxin gene to chromosome 19 markers in recombinant inbred mice

Locus	Discordant RI strains/ total analysed	Percent recombination [7] (cM)	95% confidence limits [8] (cM)
P450-2C	2/18	3.3	0.4–18.1
Ah-2	3/18	5.6	0.95–27.3
Ly-1	11/42	10.8	4.4–28.5
Ly-10	11/41	11.2	4.5–30.2

The SDP for *Rln* in the AKXD RI set shows 5/24 mismatches to the mouse lymphocyte alloantigens *Ly-1* and *Ly-10* [9]. The SDP for *Rln* in the AKXL RI set shows 6/18 mismatches to *Ly-1* (B.A. Taylor, personal communication), 6/17 mismatches to *Ly-10* [9], 3/18 mismatches to *Ah-2* (B.A. Taylor, personal communication), the locus controlling constitutive aryl hydrocarbon hydroxylase [10], and 2/18 mismatches to the murine hepatic cytochrome *Cyp2c* locus (formerly *P450-2C*; [11]) on chromosome 19.

Using established formulae [7] we estimate that the relaxin gene is 3.3 centiMorgans (cM) from *Cyp2c*, 5.6 cM from *Ah-2*, 10.8 cM from *Ly-1*, and 11.2 cM from *Ly-10*, all on chromosome 19. The 95% confidence limits [8] for these map distances are shown in Table II.

3.3. *Chromosomal localization using hybrid cell lines*

On the basis of the RI analysis and known homology with human chromosome 9, microcell hybrids containing mouse chromosomes 2, 4 and 19 were analysed. The parental hamster (DR.31) and parental rat (Fado-2) cell lines were used as negative controls (Table III). Figure

Table III

Chromosomal localization of the mouse relaxin gene using microcell hybrids

Cell line	No. of passages	Species	Mouse chromosome	Mouse relaxin gene	Reference
ABm-28	6	hamster/ mouse	2,4,17	–	[4]
DR.31	–	hamster	none	–	[3]
F(2,8)D	11	rat/mouse	Rb(2.8)	–	[4]
F(8)E	20	rat/mouse	8,9	–	[5]
F(8,19)E	5	rat/mouse	Rb(8.19)	+	*a*
FF4-3a	10	rat/mouse	9,10,13,14,15, 18, X	–	[5],*b*
Fado-2	–	rat	none	–	[5]

(*a*) F(8,19)E was constructed and characterized as described in [3]. Of 24 metaphase preparations scored, 22 were found to have Rb(8.19). No other mouse chromosomes were observed.

(*b*) FF4-3a at passage 5 contained mouse chromosome 19 [5] in only 40% of cells. The population of FF4-3a used in this study was scored as chromosome 19-negative because at passage 11 none of the cells retained mouse chromosome 19. 15% representation of a chromosome is required for detection by Southern analysis.

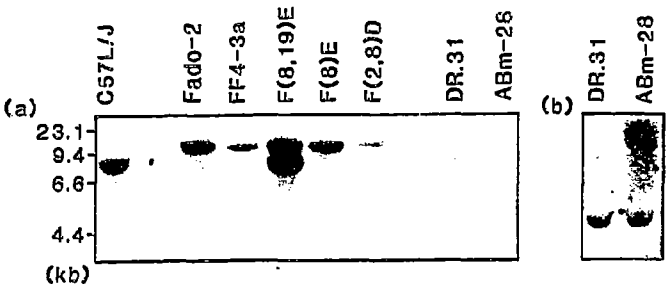

Fig. 2. Southern blot of *Bam*HI digested genomic DNA from microcell hybrids. The hybrid lines are described in Table III. (a) Mouse relaxin hybridization is associated with the presence of chromosome 19. The left track shows control mouse DNA from strain C57L/J. Higher molecular weight bands in the central tracks are due to cross-hybridization of the mouse probe with the rat relaxin gene of parental cells. (b) To demonstrate that the DNA from hybrids DR.31 and ABm-28 had been transferred properly, this portion of the blot was reprobed with a mouse angiotensinogen exon 2 fragment [19] which cross-hybridized with the hamster DNA. Both blots were washed in 0.5 × SSC at 65°C, and exposed to X-ray film for 5 days.

2 demonstrates that the microcell hybrid F(8,19)E bearing mouse chromosome 19, carried as Robertsonian translocation onto mouse chromosome 8, contains a 7.8 kb *Bam*HI fragment specific for the mouse relaxin gene. Relaxin was not detected in F(2,8)D or F(8)E microcell hybrid DNA which contains mouse chromosome 8. These results confirm localization of the relaxin gene to mouse chromosome 19.

4. DISCUSSION

We have shown that the mouse relaxin gene (*Rln*) is linked to the *Cyp2c*, *Ly-1* and *Ly-10* genes on chromosome 19. There are no known mutations in this region which affect reproductive processes, however this finding is of interest in clarifying data on the parturition defect in mice with the Hertwig's anemia (*an/an*) phenotype. The mouse chromosomal location is also consistent with previous data on human relaxin genes [12].

The human genome contains two non-allelic relaxin genes (*RLN1* and *RLN2*) localized to the 9 pter-9q12 region of chromosome 9. Human chromosome 9 shows homology with mouse chromosomes 2, 4 and 19 [13]. The only locus previously shown to display homology between human chromosome 9 and mouse chromosome 19 is the aldehyde dehydrogenase isozyme gene *ALDH1* (corresponding to mouse *Ahd-2*), which has been mapped to the 9q21 band of the human chromosome [14]. In the mouse, *Ahd-2* is localized in close proximity (approximately 1 cM) to *Ly-10* on chromosome 19 [15,16], indicating linkage to *Rln*. Our results thus demonstrate a second homologous locus on human chromosome 9 and mouse chromosome 19.

There are no known mutations at loci on either mouse chromosome 19 or human chromosome 9 which affect aspects of fertility, pregnancy or parturition. The location of mouse *Rln* on chromosome 19 is relevant, however, to a suggestion made by Taney and coworkers [17] concerning the parturition defect in *an/an* mice. These mice suffer macrocytic anemia (Hertwig's anemia), due to an X-ray induced recessive mutation on chromosome 4 [18]. Homozygous affected animals which survive to maturity also show an inability to deliver their offspring, due at least in part to inadequate lengthening of the interpubic ligament. This length is 1.11±0.09 mm in day 18 pregnant animals, compared to 2.28±0.32 mm in matched normal controls [17]. Since relaxin is known to be a major stimulus to changes in the interpublic ligament, it was thought that *an/an* mice may show a defect in this hormone. However, no significant difference in the levels of immunoreactive relaxin between the *an/an* and control mice was observed. One possible explanation of these results was that *an/an* mice produce an aberrant relaxin which retains immunoreactivity but has lowered biological activity. Our findings render this explanation very unlikely. Although the *an* mutation itself results in a stem cell defect, there may be other abnormalities which are closely linked on chromosome 4, possibly due to radiation-induced deletion [18]. If one of these was the production of relaxin with an altered sequence, then the structural gene for this hormone would also have to be located on chromosome 4, whereas we have shown that the relaxin gene is on chromosome 19. Thus the alternative explanation of an end organ defect [17] affecting the ability of relaxin to promote lengthening of the interpublic ligament seems much more likely.

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