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Blood pressure and survival of a Chromosome 7 congenic strain bred from Dahl rats

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Abstract. 11β-hydroxylase (*Cyp11b1*) mutations were previously linked to altered steroid biosynthesis and blood pressure in Dahl salt-resistant (R) and Dahl salt-sensitive (S) rats. In the present work, interval mapping identified a putative blood pressure quantitative trait locus (QTL) near *Cyp11b1* in an F_1(S×R)×S population (LOD = 2.0). Congenic rats (designated S.R-Cyp11b) were constructed by introgressing the R-rat *Cyp11b1* allele into the S strain. S.R-Cyp11b rats had significantly lower blood pressure and heart weight compared with S rats, proving the existence of a blood pressure QTL on Chromosome (Chr) 7 despite the fact that QTL linkage analysis of blood pressure never achieved stringent statistical criteria for significance. To test the effects of the introgressed region on blood pressure and survival, S.R.-Cyp11b and S rats were maintained on a 4% NaCl diet until they died or became moribund. Analysis of variance (ANOVA) indicated significant strain differences in blood pressure and days survived ($P < 0.0001$ for both) as well as gender differences in days survived ($P = 0.0003$). Kaplan-Meier survival analysis also found significant strain ($P < 0.0001$) and gender ($P = 0.007$) differences in days survived. However, when the effects of blood pressure were removed, significant strain differences in survival essentially disappeared. This suggests that the increased survival of S.R-Cyp11b rats was largely due to their decreased blood pressure and thus strongly corroborates the existence of a blood pressure QTL on Chr 7 near or at *Cyp11b1*.

Introduction

Essential hypertension is a complex, multifactorial disorder resulting from the interaction of multiple genetic and environmental factors. While several environmental factors contributing to blood pressure elevation have been identified (for example, excessive dietary NaCl intake), the lack of homogeneous populations makes studying genetic effects difficult in humans. This can be circumvented, in part, by studying rat models of hypertension, such as the inbred strains (Rapp and Dene 1985) of the Dahl salt-sensitive and salt-resistant rats (Dahl et al. 1962). In the Dahl salt-sensitive (S) rat, supplemental dietary NaCl increases blood pressure, whereas in the Dahl salt-resistant (R) rat, this diet has little or no effect on blood pressure (Dahl et al. 1962; Rapp and Dene 1985).

A series of biochemical and genetic studies of steroidogenesis in S and R rat strains suggested that strain differences in the adrenal synthesis of 18-hydroxy-11-deoxycorticosterone (18-OH-DOC) stemming from differences in the 11β-hydroxylase (*Cyp11b1*) gene may contribute to the observed strain differences in blood pressure in the context of an excessive dietary NaCl intake (Rapp and Dahl 1971, 1972a, 1972b, 1976). We have recently shown that in segregating populations derived from inbred S and R rats, *Cyp11b1* polymorphisms cosegregated with both the capacity to synthesize 18-hydroxy-deoxycorticosterone (18-H-DOC) and with blood pressure (Cicila et al. 1993). The R rat carried a *Cyp11b1* allele that: (i) differed from those of 12 other inbred rat strains, (ii) was associated with a uniquely reduced capacity to synthesize 18-OH-DOC, and (iii) encoded five amino acid substitutions in the 11β-hydroxylase protein (Cicila et al. 1993; Matsukawa et al. 1993). The effects of the R-rat *Cyp11b1* allele on blood pressure were highly dependent upon the genetic background in which they were expressed; markedly greater blood pressure differences were observed in the permissive F_1(S×R)×S population compared with the F_2(S×R) or F_1(S×R)×R populations, where the rats had on average lower proportions of background S-rat alleles. *Cyp11b1*, which is on rat Chr 7, was the first gene in which coding sequence mutations were linked to (i) blood pressure and (ii) an altered protein activity that could logically affect blood pressure in an animal model of genetic hypertension.

Our previous results (Cicila et al. 1993) did not provide an estimate of the location of the quantitative trait locus (QTL) associated with (and presumed to be) *Cyp11b1*, nor did linkage results in that study reach the stringent statistical guidelines subsequently suggested to establish linkage (Lander and Kruglyak 1995). Interval mapping of rat Chr 7 in a large, permissive F_1(S×R)×S population was performed to identify the most probable location of the blood pressure QTL. We also developed a congenic strain in which the R-rat *Cyp11b1* locus and flanking chromosomal segments were introgressed into the permissive S-rat genetic background so that the effects of the putative QTL could be observed. Furthermore, we sought to determine the effect of this Chr 7 blood pressure QTL on other relevant phenotypes, heart weight, and survival of rats in the context of an excessive dietary intake of NaCl.

Materials and methods

Genetic crosses. The inbred Dahl salt-hypertension sensitive (SS/Jr) and salt-hypertension resistant (SR/Jr) rat strains were developed (Rapp and Dene 1985) from outbred stock originally obtained from Dahl (Dahl et al. 1962) and will be referred to by their generic designations of S (sensitive) and R (resistant). S and R rats used in breeding the genetic crosses and development of congenic strains were from the colony at the Medical College of Ohio. Two backcross F_1(S×R)×S populations were obtained by crossing F_1 females with S males. One of these populations was used in previous genetic analyses (Cicila et al. 1993, 1994; Rapp and Dene 1990; Rapp et al. 1990). The two populations were combined (n = 150 rats) for the purpose of interval mapping of the blood pressure QTL on Chr 7. A strong linear relationship between heart weight and body weight allowed us

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to remove the influence of body weight from heart weight. The formula for this regression line was heart weight = 383.3 + 2.86 (body weight) and the correlation coefficient (r) was 0.75. In the QTL analysis for the two populations combined, blood pressure was normalized for population and gender differences and adjusted heart weight was normalized for gender differences.

F_1(S×R)×S populations were weaned at 30 days of age and at 35 days of age were placed on a high salt diet (8% NaCl, Teklad diet 82050, Harlan Teklad, Madison, Wis) with access to water ad libitum. Blood pressure was measured by the tail-cuff microphonic method (Friedman and Freed 1949) with the rats under light ether anesthesia as was previously described in detail in our early linkage studies (Rapp et al. 1990). Rats were killed with an overdose of pentobarbital.

Congenic strain breeding The R-rat region of Chr 7 containing the *Cyp11b1* and *Cyp11b2* genes was introgressed into the S-rat genetic background, creating the S.R-Cyp11b congenic strain. In the development of the S.R-Cyp11b congenic strain, polymorphic markers for either of the tightly linked genes, *Cyp11b1* or *Cyp11b2* (Cicila et al. 1993), were used. Because of the tight linkage of these two genes, we will refer to them collectively hereafter as the *Cyp11b* locus. The breeding paradigm was as follows: F_1 rats obtained by crossing S and R were back-crossed to S. A rat heterozygous for the *Cyp11b* locus was selected and again backcrossed to S. This procedure was repeated for a total of eight backcrosses. After the eighth backcross, rats heterozygous for the *Cyp11b* allele were bred, and progeny homozygous for the R-rat *Cyp11b* locus were selected to establish the S.R-Cyp11b congenic strain. The congenic rat strain was subsequently maintained by brother-sister mating.

Testing for blood pressure salt-sensitivity. Blood pressures of the congenic rats were compared with the blood pressure of the parental S strain. Strains to be compared in an experiment were bred at the same time, pups were weaned at 30 days of age, and identified by a numbered skin clip (National Band and Tag Co., Newport, Ky.) placed at the back of the neck. Rats were housed four/cage (two S and two congenic) with access to water ad libitum and fed various test diets for variable lengths of time. Blood pressures were measured for three sessions by the tail-cuff method (Bunag and Butterfield 1982) in conscious, restrained rats with equipment made by IITC, Inc. (Woodland Hills, CA). Rats were warmed to 28°C for measurement of blood pressure, and at least three consistent blood pressure readings were obtained at a given daily session, with the average pressure used as that session's reading. The mean of all the sessions' blood pressure readings was used as the blood pressure of each rat for statistical analysis.

Experiment 1: Male S (n = 21) S.R-Cyp11b rats (n = 16) were maintained on a 1% NaCl (diet 8640, Harland Teklad) diet until 60 days old and then fed a low salt diet (0.2% NaCl, diet 7034, Harlan Teklad) for the next 40 days. Tail-cuff blood pressures were then measured, and the rats were killed by an overdose of pentobarbital and body weight and heart weight measured.

Experiment 2: Male S (n = 20) and S.R-Cyp11b rats (n = 20) were maintained on a low salt (0.2% NaCl) diet until 37 days old and then fed a 2% NaCl diet (diet 94217, Harlan Teklad). Tail-cuff blood pressures were measured after 24 days and 42 days on the 2% NaCl diet.

Experiment 3: Male and female S (n = 22 male, 24 female) and S.R-Cyp11b (n = 11 male, 12 female) rats were maintained on a low salt (0.2% NaCl) diet until 37 days old and then fed a 4% NaCl diet (diet 83033, Harlan Teklad). Tail-cuff blood pressures were measured at 24 days on the 4% NaCl diet, and the diet was continued until the rats died or became obviously terminally ill (for example, strokes), in which case they were euthanized with pentobarbital. Rats were examined twice daily for signs of distress. All animal protocols were approved by the Institutional Animal Care and Use Committee of the Medical College of Ohio.

Genotyping. DNA for genotyping segregating rat populations and S.R-Cyp11b was extracted from frozen liver (Blin and Stafford 1976). DNA for genotyping rats during the development of the congenic strains was extracted from tail biopsy material with the QIAamp Tissue Kit (Qiagen, Chatsworth, Calif.). PCR amplification was performed in a 20-μl reaction containing 50 mmol/L KCl, 10 mmol/L Tris-HCl pH 9.0, 0.1% Triton X-100, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L each dNTP, approximately 40 ng genomic DNA, 10 pmol of each oligonucleotide primer, and 0.75 units *Taq* polymerase, with a PTC-100-96AgV thermocycler (MJ Research, Watertown, Mass.) with the following cycle profile: 94°C for 3.5 min, followed by 35–40 cycles of 94°C for 45 s, 53–65°C (depending on the primer set) for 45 s, and 1.5 min, at 72°C, and a final incubation for 5 min, at 72°C. PCR products were electrophoretically size-fractionated on 4–5% high-resolution agarose gels (Metaphor; FMC BioProducts, Rockport, Me.) or on denaturing polyacrylamide gels. One primer in the reaction was end-labeled with polynucleotide kinase and γ-^{32}P-ATP when PCR products were resolved on polyacrylamide gels and reaction products were detected by autoradiography.

The *Cyp11b* locus was genotyped as described above (Cicila et al. 1993). Primer sets for the PCR-amplification of the *Inha* (Serikawa et al. 1992), *Ptprc* (R52, Serikawa et al. 1992), *Per* (R159, Serikawa et al. 1992), *Cyp11b* (*D7Wox19*), *D3Wox3,* and *D7Wox4* (*RCA24.09*) loci were obtained from GenoSys Biotechnologies (The Woodlands, Tx.). *D3Wox3, D7Wox19,* and *D7Wox4* primer sequences are available on the internet at ftp://ftp.well.ox.ac.uk/pub/genetics/ratmap. Primer sets for the PCR amplification of the *D3Mgh6, D7Mgh4, D7Mgh5, D7Mgh6, Bzrp* (*D7Mgh12*), *D7Mit11,* and *D7Mit13, D13Mit3* loci (Jacob et al. 1995) were obtained from Research Genetics (Huntsville, Ala.). The *Edn3, Ren,* and *Cyp2d2* (*Cyp2d*) loci were PCR-amplified with previously described primer sets (Deng et al. 1994; Rapp et al. 1994; Du et al. 1995, respectively).

The PCR product of *D7Mit3* was sequenced, and new primers 5′-TCC ATC ATC ATT TCC TCT CC-3′ and 5′-AAA TGG CAT GAT AGC ACC TT-3′ were designed with the PrimerSelect program (DNASTAR Inc., Madison, Wis.). This new primer set PCR-amplified a 201-bp fragment from S-rat genomic DNA, distinguishing S-rat and R-rat alleles (S > R), and was designated as *D7Mco1.* An additional locus, *D7Uia1,* was identified from a rat library enriched for tri- and tetranucleotide repeats as described (Sunden et al. 1996) and found to map between the *Cyp11b* and *D7Mit3* loci in an F_2(S×Lewis) population (data not shown). The *D7Uia1* locus was genotyped with the following primer set, 5′-TTC CTG ATA TTG TTC CTG CTG-3′ and 5′-ATG AGG AAT AAA GGA TGA TGG A-3′. These primers amplified a 298-bp fragment from the genomic DNA of a Sprague-Dawley rat and distinguished S-rat and R-rat alleles (S > R) for the *D7Uia1* locus.

Linkage and statistical analysis. Linkage maps and QTL localization were done with the MAPMAKER/EXP and MAPMAKER/QTL programs (Lander and Botstein 1989; Lander et al. 1987; Lincoln et al. 1992a, 1992b; Paterson et al. 1988) obtained from Eric Lander (Whitehead Institute, Cambridge, Mass.) Potential errors in typing, that is, loci involved in double-recombination events were retyped to confirm or correct the results. Threshold values for "suggestive" and "significant" linkage of a locus to a quantitative trail are as defined by Lander and Kruglyak (1995). "Suggestive" and "significant" linkage thresholds correspond to the expectation of one false positive per genome scan and a 0.05 probability of a false positive in a genome scan. Analysis of variance (ANOVA) and survival statistics, respectively, were calculated with the SuperANOVA and StatView 4.5 programs (Abacus Concepts, Mountain View, Calif.) Survival analysis was performed with the Kaplan-Meier method with the logrank (Mantel-Cox) test used to evaluate the equality of the survival functions for the groups.

Results

Linkage analysis of blood pressure and heart weight. A rat Chr 7 map was developed with 150 male and female S×F_1(S×R) rats (Fig. 1a). Polymorphic markers for both of the tightly linked genes, *Cyp11b1* and *Cyp11b2* (Cicila et al. 1993), were used to construct this map. Because of the tight linkage of these two genes (no crossovers were detected), we will refer to them collectively hereafter as the *Cyp11b* locus.

A one-way analysis of variance (ANOVA) indicated that only one locus, *D7Uia1* (P = 0.002), met the recently proposed, stringent criteria (Lander and Kruglyak 1995) for "suggestive" linkage to blood pressure in this population, although the *Cyp11b* locus was close (P = 0.007). One-way ANOVA indicated that the *Cyp11b, D7Uia1,* and *D7Mco1* loci all showed "suggestive" linkage to adjusted heart weight (P = 0.002, 0.001, and 0.002, respectively). Rats genotyped as heterozygous for these loci had lower blood pressures and adjusted heart weights than rats homozygous for S-rat alleles at the same locus (Table 1).

Interval mapping for blood pressure and adjusted heart weight on Chr 7 with the MAPMAKER/QTL program located maximal

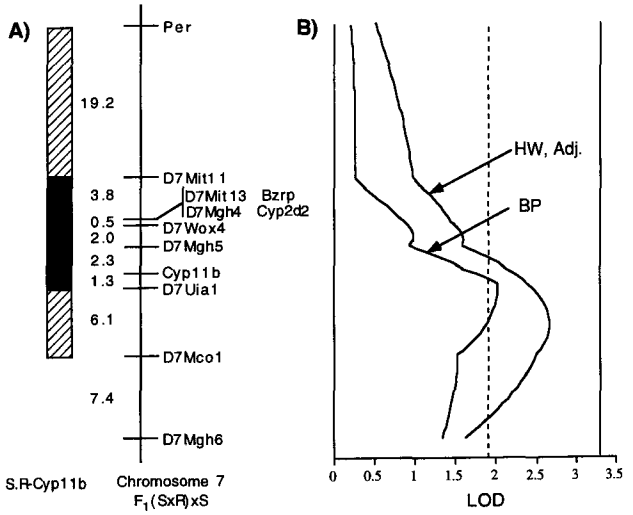

Fig. 1. Chr 7 map showing congenic segment and locations of blood pressure and adjusted heart weight quantitative trait loci (QTL). **(A)** A map of Chr 7 with an F_1(S×R)×S population of 150 rats was drawn with the MAPMAKER/EXP computer program, with the distances between loci expressed in centiMorgans (cM) and corrected using the Kosambi function (Kosambi 1944). The known extent of the R-rat derived portion of chromosome carried by the S.R-Cyp11b strain is designated by the filled portion of the bar alongside F_1(S×R)×S chromosome map, with intervals containing the recombinant endpoints of the R-rat-derived chromosome portion designated by the hatched portions of the bar. **(B)** LOD plots of blood pressure (BP) and adjusted heart weights (HW, Adj.) for rat Chr 7 were drawn with the MAPMAKER/QTL computer program for the F_1(S×R)×S population raised on an 8% NaCl diet. LOD threshold values for "suggestive" (1.9, - - - - - - - -) and "significant" (3.3, _____) linkage are shown.

Table 1. Systolic blood pressure and adjusted heart weight by genotype at selected markers on Chr 7 for the F_1(S×R)×S population.

	Blood pressure (mm Hg)		Adjusted heart weight (mg)	
Locus	SS	SR	SS	SR
D7Mit11	185.0 ± 3.2 (74)	180.3 ± 2.8 (75)	1156 ± 14	1114 ± 12
Cyp2d2	186.9 ± 3.2 (75)	178.3 ± 2.8 (75)	1159 ± 14	1110 ± 12
D7Mgh5	187.2 ± 3.1 (71)	178.4 ± 2.8 (79)	1161 ± 14	1110 ± 12
Cyp11b	188.8 ± 3.3 (69)	177.3 ± 2.6 (81)	1166 ± 15	1107 ± 12
D7Uia1	189.5 ± 3.3 (69)	176.7 ± 2.6 (81)	1168 ± 15	1106 ± 12
D7Mco1	187.1 ± 3.1 (68)	177.1 ± 2.8 (78)	1163 ± 14	1105 ± 12
D7Mgh6	187.6 ± 2.9 (77)	177.3 ± 3.0 (73)	1159 ± 14	1108 ± 12

Systolic blood pressure (BP, mean ± SEM, in mm Hg) and adjusted heart weight (mean ± SEM) for each genotype are given. Adjusted heart weight values were calculated by correcting heart weight values for differences due to blood pressure (see text). Numbers in parentheses are the number of rats in each group, and are shown only in the blood pressure column. S = S-rat allele, R = R-rat allele.

LOD scores for both quantitative traits between *D7Uia1* and *D7Mco1*. These Chr 7 sites of maximal LOD score showed "suggestive" linkage to both blood pressure (LOD = 2.0; Fig. 1b) and adjusted heart weight (LOD = 2.7; Fig. 1b). Thresholds for "suggestive" linkage were as defined by Lander and Kruglyak (1995). The increased adjusted heart weight observed was interpreted to be a consequence of the increased blood pressure, and both measurements are assumed to reflect the same QTL.

The S.R-Cyp11b congenic rat carries the blood pressure QTL. The R-rat region of Chr 7 containing *Cyp11b1* was introgressed into the S-rat genetic background, creating the S.R-Cyp11b congenic strain. *Cyp11b* was the only locus used in the selection of this congenic strain because it was the only marker available when this work began. S.R-Cyp11b rats were subsequently genotyped with the same polymorphic markers used for the F_1(S×R)×S population, providing an estimate of at least 9.9 cM, and as much as 35.2 cM, for the extent of the R-derived portion of rat Chr 7 carried (Fig. 1a).

Table 2. Comparison of the Chromosome 7 Congenic S.R-Cyp11b Strain with the S Strain on Different NaCl Intakes.

Experiment 1:		1% NaCl for 60 days, 0.2% NaCl for 40 days; Males		
Strain	N	Blood Pressure (mm Hg)	Heart Weight (HW, mg)	Body Weight (BW, g)
S	21	244.0 ± 6.2	1284 ± 24	350.0 ± 7.3
S.R-Cyp11b	16	200.3 ± 6.0	1110 ± 17	336.7 ± 6.7
One-way ANOVA P		<0.0001	<0.0001	0.198

Experiment 2:		2% NaCl, Males	
		Blood Pressure (mm Hg)	
Strain	N	(24 days[a])	(42 days[a])
S	20	199.3 ± 7.5	231.4 ± 7.2
S.R-Cyp11b	20	178.0 ± 4.3	198.1 ± 5.7
One-way ANOVA P		0.019	0.0008

[a]Rats were started on a 2% NaCl diet at 37 days of age, and blood pressures were measured after 24 and 42 days on this diet.

Male S and S.R-Cyp11b rats were fed diets with differing amounts of dietary NaCl (1% to 8% NaCl) to determine whether a blood pressure QTL was "trapped" in the congenic strain, and to examine the effects of this QTL on blood pressure under differing degrees of salt challenge. No difference in survival time was observed between the parental and congenic strains fed on 8% NaCl diet, with rats perishing before blood pressure measurement could be completed (data not shown). Because the 8% NaCl diet was so toxic to both S and S.R-Cyp11b rats, diets with lower amounts of dietary NaCl were chosen to evaluate the effect of the Chr 7 QTL on blood pressure between these strains.

Table 2 shows the results of two experiments comparing male S and S.R-Cyp11b rats maintained on diets containing normal or moderately increased (2%) levels of NaCl. In Experiment 1, S.R-Cyp11b rats had significantly lower blood pressure ($P < 0.0001$) and heart weight ($P < 0.0001$) than male S rats on the normal salt diet (Table 2). Decreased heart weight corroborated the observed blood pressure differences. No significant difference in body weight was observed between these two strains. Experiment 2 (Table 2) confirmed that a Chr 7 blood pressure QTL was trapped in the S.R-Cyp11b congenic rats, as they had significantly lower blood pressures than S rats at both 24 days ($P = 0.019$) and 42 days ($P = 0.0008$) on the 2% NaCl diet.

To determine whether the S.R-Cyp11b congenic rat retained any residual R-rat alleles in its background at loci known to be linked to blood pressure on other chromosomes segregating in the S × R comparison, the congenic was genotyped at: endothelin-3 (Cicila et al. 1994; Deng et al. 1994), *D3Mgh6* and *D3Wox3* on Chr (Cicila and Rapp, unpublished); inhibin (α subunit) on Chr 9 (Lathrop and Rapp, unpublished), and renin, protein tyrosine phosphatase (receptor-type) c polypeptide, and *D13Mit3* on Chr 13 (Rapp et al. 1989; Zhang et al. 1997). These markers were all homozygous for the S-rat allele.

Decreased blood pressure and increased survival of the S.R-Cyp11b congenic rat. Experiment 3 was designed to study the effects of the Chr 7 QTL in the context of a higher dietary NaCl intake. In this experiment, male and female S and S.R-Cyp11b rats were fed a 4% NaCl diet, and blood pressure was measured after 24 days on the high salt diet (Table 3). S.R-Cyp11b rats had

Table 3. Strain and Gender Differences in Blood Pressure and Survival Between S.R-Cyp11b and S Rats in the Context of a 4% NaCl Diet.

	N	Strain	Blood pressure (mm Hg)	Survival (days)	Survival, adjusted (days)
Male	22	S	264.0 ± 5.5	39.8 ± 2.7	53.4 ± 5.4
	11	S.R-Cyp11b	200.9 ± 8.2	112.4 ± 24.5	57.1 ± 7.1
Female	24	S	250.1 ± 4.9	33.6 ± 1.3	38.4 ± 1.0
	12	S.R-Cyp11b	215.5 ± 8.5	52.7 ± 6.0	44.6 ± 2.7
Two-Way ANOVA		Strain	<0.0001	<0.0001	0.287
		Gender	0.958	0.0003	0.004
		Strain × Gender	0.036	0.003	0.789

S.R-Cyp11b and S rats were maintained on a low salt (0.2% NaCl) diet to 37 days of age and then fed a 4% NaCl diet until they either died or became moribund. Systolic blood pressure was measured after 24 days on the high salt diet. Adjusted survival values were calculated by correcting the days survived values for differences due to blood pressure (see text).

markedly lower blood pressure ($P < 0.0001$) than S rats (Table 3). While gender differences in blood pressure were not observed, a statistically significant ($P = 0.036$) interaction between strain and gender was found (Table 3).

Analysis using the non-parametric Kaplan-Meier method identified a highly significant overall difference ($P < 0.0001$) in the survival functions of male and female S and S.R-Cyp11b rats in the context of this 4% NaCl intake. Further Kaplan-Meier analysis showed significant survival function differences between: male S and S.R-Cyp11b rats ($P < 0.0001$; Fig. 2a); between female S and S.R-Cyp11b S rats ($P < 0.0001$; Fig. 2b); and between male and female rats of either strain ($P = 0.007$). Strain and gender differences were also obvious by a two-way ANOVA as shown in Table 3. A minor increase (6.2 days) in mean survival time was observed for male S compared with female S rats fed a 4% NaCl diet (Table 3). These gender differences in survival for S rats were minor compared to the large increase (59.7 days) in mean survival time observed for male S.R-Cyp11b rats compared with female S.R-Cyp11b under the same conditions, leading of course to a significant interaction between strain and gender in survival (Table 3).

A hyperbolic relationship between blood pressure taken at 24 days on a 4% NaCl diet and subsequent survival on a continuing 4% NaCl diet was observed for all rats used in Experiment 3 (Fig. 3a). Little difference in survival was seen for rats with blood pressures >220 mm Hg, whereas rats having lower blood pressures survived much longer on the 4% NaCl diet (Fig. 3a). The nonlinear relationship between blood pressure and survival makes it awkward to use blood pressure as a co-variate to remove blood pressure effects on survival. However, the hyperbolic relationship shown in Fig. 3a can be transformed into a linear function by plotting the reciprocal of days survived versus blood pressure (Sokal and Rohlf 1969), as shown in Fig. 3b. The correlation between blood pressure and 1/(days survived) was $r = 0.70$ (Fig. 3b). 1/(days survived) was adjusted (Ostle 1963) for variation in blood pressure by use of the linear relationship between them, and these adjusted values were re-transformed back to days for further analysis by taking the reciprocal of the adjusted 1/(days survived). ANOVA results for days survived adjusted for blood pressure effects are summarized in Table 3. Although the two-way (strain and gender) ANOVA indicated that significant gender effects remained ($P = 0.004$) after survival values on the high salt diet were adjusted to account for blood pressure differences, no significant strain effects nor strain-gender interactions were observed (Table 3).

Kaplan-Meier analysis showed a markedly reduced, though still statistically significant difference in the survival functions calculated with adjusted survival values for all four gender-strain groups (that is, male and female S and S.R-Cyp11b rats; $P = 0.025$), suggesting that increased survival of S.R-Cyp11b congenic rats was largely due to their lower blood pressures in response to a high salt diet (Fig. 2, compare Panels a and b with c and d). Further Kaplan-Meier analysis showed significant differences in the survival functions calculated with adjusted survival values between female S.R-Cyp11b and S rats ($P = 0.012$) and between male and female rats of either strain ($P < 0.0001$). However, significant differences in the survival functions calculated with adjusted survival values were not observed for comparisons of male S.R-Cyp11b and S rats ($P = 0.475$).

Discussion

Comparison of the linkage analysis and congenic strain results. Our interest in the *Cyp11b* locus comes originally from biochemi-

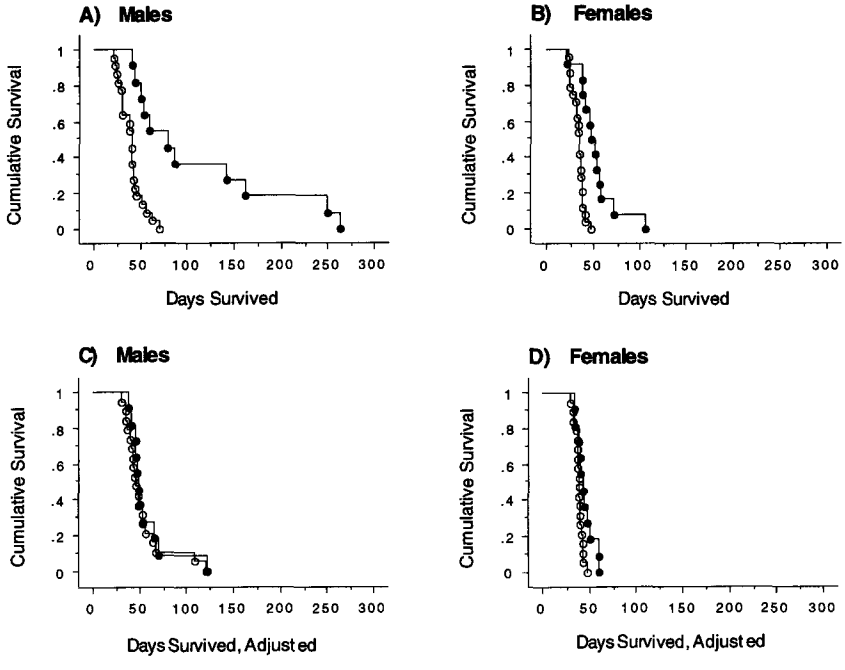

Fig. 2. Cumulative survival curves for S and S.R-Cyp11b rats on a 4% NaCl diet. Thirty-seven-day-old S.R-Cyp11b (●) and S (○) rats were maintained on a 4% NaCl diet until they either died or became moribund. Days survived values were also adjusted for differences due to blood pressure as described in Results. Cumulative survival curves with days survived as the time variable are shown for male (Panel **A**) and female (Panel **B**) rats. Cumulative survival curves with adjusted days survived as the time variable are shown for male (Panel **C**) and female (Panel **D**) rats.

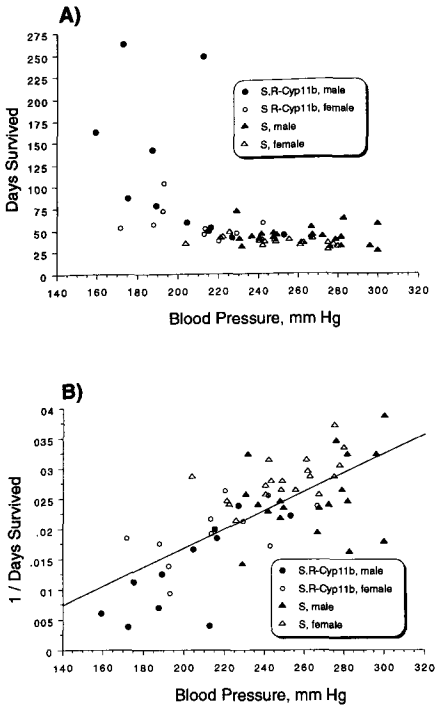

Fig. 3. Survival curves for S and S.R-Cyp11b rats on a 4% NaCl diet. Thirty-seven-day-old rats were placed on a 4% NaCl diet, and systolic blood pressures were measured after 24 days on the high-salt diet. The 4% NaCl diet was continued until the rats died or became moribund. Blood pressure was plotted against either days survived (Panel **A**) or the reciprocal of days survived (Panel **B**) for each rat in Experiment 3, with the gender and strain of each rat designated according to the key in the figure. The formula for the regression line in Panel B was 1 / (days survived) = −0.015 + 0.0001566 (blood pressure) and the correlation coefficient (r) was 0.7.

cal studies of adrenal steroidogenesis (Rapp and Dahl 1971, 1972a, 1972b), which showed marked strain differences due to a mutation in *Cyp11b1* (Rapp and Dahl 1976). Our early (Rapp and Dahl 1972a) and more recent (Cicila et al. 1993) studies showed cosegregation of the *Cyp11b* locus with blood pressure, although the significance levels of this genetic linkage never reached the threshold levels suggested by Lander and Kruglyak (1995) for whole genome scans. The reason for this and the value of stringent guidelines merit discussion.

Recent linkage analyses indicate that there are important genetic background effects on the ability to detect possible linkage of *Cyp11b1* (Cicila et al. 1993) and other candidate genes (Cicila et al. 1994; Rapp et al. 1989, 1990) to blood pressure in genetic studies using S and R rats. In general, the greater the proportion of background genes from S rats in the segregating population, the greater the observed effect of the candidate gene (Cicila et al. 1993, 1994; Rapp et al. 1990). Thus, in several cases genetic linkage to blood pressure has been detectable in the usual segregating populations (fed an 8% NaCl diet), progressing in the order of least useful to most useful, F_1(S×R)×R, F_2(S×R), and F_1(S×R)×S, as the proportion of S-rat-derived genes increases from 25%, 50%, and 75%, respectively, in these populations. This situation implies epistatic interactions and is the reason for selecting the large F_1(S×R)×S population for linkage analysis in the present work.

Construction of the S.R-Cyp11b congenic strain in which the R-rat *Cyp11b* allele is placed on the S background increases the genetic background (exclusive of the chromosomal segments flanking *Cyp11b*) on average to 99.6% S genes after 8 backcrosses. Thus, it is not surprising to find in the case of the *Cyp11b* locus (known to be sensitive to the genetic background) that the S.R-Cyp11b congenic strain shows marked and highly significant effects on blood pressure, much greater than might be predicted from the previous linkage analysis on less permissive genetic backgrounds. It is noted, however, that our previous cosegregation studies of the *Cyp11b* locus in populations derived from S and R rats (Cicila et al. 1993; Rapp and Dahl 1972a) were done with an 8% NaCl diet to maximize the blood pressure response on these less permissive genetic backgrounds. In contrast, diets with lower salt content (4% NaCl or lower) were necessary to compare the parental S strain with the congenic S.R-Cyp11b strain, because an 8% NaCl diet resulted in fulminant hypertension and rapid death, defeating comparisons of chronic differences in blood pressure.

Because the S.R-Cyp11b congenic strain has a markedly lower blood pressure than S rats, the fact that cosegregation analysis for the *Cyp11b* locus never reached the stringent statistical criteria to declare linkage to blood pressure is moot. In our view, although such criteria (Lander and Kruglyak 1995) represent useful guidelines, they are not necessarily the only criteria on which to base a decision to pursue or drop a given line of inquiry (Elston 1997; Witte et al. 1996). The evidence for epistasis (Cicila et al. 1993), the compelling "biological sense" of the steroidogenic changes as a cause of blood pressure differences on a high salt diet (Rapp and Dahl 1972b), and the existence of potentially interesting amino acid substitutions in the R-rat *Cyp11b1* gene (Cicila et al. 1993; Matsukawa et al. 1993) were sufficient to warrant construction of the S.R-Cyp11b congenic strain despite the modest data supporting linkage. Clearly, epistasis can obstruct or defeat attempts to detect QTL by linkage analysis in segregating populations (Frankel and Schork 1996).

11β-hydroxylase as a candidate gene for the Chr 7 QTL. We believe the present data on the S.R-Cyp11b strain is definitive in proving the existence of a Chr 7 blood pressure QTL with differing alleles in Dahl S and R rats. But we do not consider the present S.R-Cyp11b congenic strain to prove that *Cyp11b1* is the gene responsible for the blood pressure differences associated with the Chr 7 QTL. Clearly the next step is to reduce the flanking R-rat chromosome-derived segments around the *Cyp11b* locus to the minimum feasible to reduce the chance that the observed blood pressure differences are really due to an unknown locus near *Cyp11b1* rather than the *Cyp11b1* gene itself (Rapp and Deng 1995).

Aldosterone synthase (*Cyp11b2*), a tightly linked gene with significant sequence homology with *Cyp11b1* (Cicila et al. 1993; Imai et al. 1990; Matsukawa et al. 1990; Mornet et al. 1989), is also a potential candidate gene for the rat Chr 7 QTL. *Cyp11b2* has amino acid substitutions in the R-rat allele, compared with that carried by S rats (Cover et al. 1995), which result in an increased apparent V_{max} and decreased apparent K_m for the R-rat Cyp11b2 enzyme when expressed in heterologous cells, compared with the form of this enzyme found in S rats (Cover et al. 1995). Because of the increased capacity of the R-rat form of aldosterone synthase to synthesize the potent mineralocorticoid, aldosterone, *Cyp11b2* makes little biological sense as a candidate gene for the lower blood pressure associated with the R-rat allele of the Chr 7 QTL.

Survival analysis and blood pressure. In addition to the effect on blood pressure and heart weight, the S.R-Cyp11b strain also showed significantly increased survival on a 4% NaCl diet compared with S. This increased survival was largely attributed to the lower blood pressure observed for the S.R-Cyp11b congenic strain. This relationship between blood pressure and survival is instructive. In Fig. 3b it is clear that in the context of this experiment, blood pressure at 24 days of 4% NaCl feeding was highly predictive of subsequent survival on a continuing 4% NaCl diet.

This is not surprising given knowledge of the biology of hypertension and the beneficial effects of therapeutically lowering blood pressure. It is worth considering, however, that these blood pressure data were collected by the tail-cuff method on conscious, restrained rats warmed to 28°C. Clearly such blood pressure data were obtained from the rat under a condition of some transient stress, and thus the validity of the data as reflecting the chronic blood pressure status of the animal can be questioned. In spite of these theoretical concerns, tail-cuff (stressed) blood pressure measurement clearly predicts pathological events and death, and is, therefore, a biologically relevant measure.

Gender effects on survival and blood pressure. The magnitude and the direction of differences in survival and blood pressure observed for male S.R-Cyp11b congenic rats, compared with female S.R-Cyp11b rats and with S rats in the context of an elevated dietary intake of NaCl, was surprising (Fig. 2, Table 3). Clearly, the increased survival of male S.R-Cyp11b congenic rats was the major source of the significant strain and gender effects as well as the strain-gender interaction observed in this study. We suppose that this strain-gender interaction must stem from an interaction of the Chr 7 QTL (presumably *Cyp11b1*) with the hormonal differences between male and female rats in the context of the S-rat genetic background.

In summary, we confirmed the existence of a Chr 7 QTL for blood pressure and adjusted heart weight by trapping the QTL in a congenic strain. Male S.R-Cyp11b congenic rats showed a marked gender-specific and strain-specific increase in survival in the context of an elevated dietary NaCl intake that was largely a consequence of their decreased blood pressure response to salt.

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