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N J Samani, ... , J Sassard, M Lathrop

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Research Article

The role of the kidney in initiating hypertension has been much debated. Here we demonstrate that a recently identified gene of yet unknown function, termed SA, which is differentially expressed in the kidney of the spontaneously hypertensive rat, cosegregates with an increase in blood pressure in F2 rats derived from a cross of the spontaneously hypertensive rat with normotensive Wistar-Kyoto rats, accounting for 28 and 21% of the genetic variability in systolic and diastolic blood pressures, respectively. Further, the genotype at this locus appears to determine the level of expression of the gene in the kidney. The findings provide strong evidence for a primary genetic involvement of the kidney in hypertension.

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A Gene Differentially Expressed in the Kidney of the Spontaneously Hypertensive Rat Cosegregates with Increased Blood Pressure

Nilesh J. Samani,* David Lodwick,* Madeleine Vincent,† Christopher Dubay,‡ Michael A. Kaiser,* Martin P. Kelly,* Ming Lo,‡ Janet Harris,* Jean Sassard,‡ Mark Lathrop,‡ and John D. Swales*

*Department of Medicine, University of Leicester, Leicester LE2 7LX, United Kingdom, ‡URA Centre National de la Recherche Scientifique 1483, Département de Physiologie et de la Pharmacologie Clinique, Faculté de Pharmacie, 69373, Lyon, France; and †Institut National de la Santé et de la Recherche Médicale U. 358, Centre d'Étude du Polymorphisme Humain, 75010, Paris, France

Abstract

The role of the kidney in initiating hypertension has been much debated. Here we demonstrate that a recently identified gene of yet unknown function, termed S_A , which is differentially expressed in the kidney of the spontaneously hypertensive rat, cosegregates with an increase in blood pressure in F_2 rats derived from a cross of the spontaneously hypertensive rat with normotensive Wistar-Kyoto rats, accounting for 28 and 21% of the genetic variability in systolic and diastolic blood pressures, respectively. Further, the genotype at this locus appears to determine the level of expression of the gene in the kidney. The findings provide strong evidence for a primary genetic involvement of the kidney in hypertension. (*J. Clin. Invest.* 1993; 92:1099–1103.) Key Words: hypertension • genetics • kidney • spontaneously hypertensive rat • gene expression

Introduction

The mapping of genetic loci underlying simple mendelian traits has now become readily achievable. For polygenic traits, especially those with additional environmental determinants such as hypertension (1) and atheroma (2), major difficulties still remain. However, the feasibility of mapping genetic loci directly involved in blood pressure regulation has recently been demonstrated in a number of rodent models of genetic hypertension (3–9) where a limited number of genes control the hypertensive phenotype (10). Nevertheless, in most cases the mechanisms by which the linked loci influence blood pressure remain to be determined, and no intermediate phenotypes have as yet been identified. Recently, a gene, designated S_A , demonstrating increased expression in the kidney of the spontaneously hypertensive rat (SHR)¹ compared with its normotensive control, the Wistar-Kyoto (WKY) rat, has been identified by differential hybridization (11). The kidney plays a central

role in blood pressure regulation (12–14), and there is evidence from transplantation studies of its involvement in both genetic hypertension in rats (15–17) and in human essential hypertension (13, 14). We therefore undertook a study to investigate a possible role for the S_A gene in the etiology of hypertension in the SHR. In addition to carrying out cosegregation analysis of alleles at the locus with blood pressure in F_2 rats derived from a cross of SHR and WKY rats, we also determined the influence of the genotype at the locus on S_A mRNA levels in the kidney.

Methods

Generation of F_2 rats. 3 SHR male rats and 3 SHR female rats were reciprocally mated with the same number of WKY rats (all animals obtained from the breeding stock of Charles River Laboratories, Margate, UK) to obtain F_1 hybrids. F_1 animals from each half of the cross were randomly mated to generate 233 male F_2 rats. All animals were housed under controlled conditions (temperature $21 \pm 1^\circ\text{C}$, humidity $60 \pm 10\%$, 12-h day/night cycle), fed standard rat chow containing 0.25% Na and 0.66% K (Rat & Mouse No. 3 Breeding Diet; Special Diet Services Ltd., Witham, Essex, UK), and given free access to tap water.

Blood pressure measurements. Indirect systolic blood pressure in tail artery was measured at 12, 16, and 20 wk of age after prewarming of the animals to 32°C . A 7/16-in tubular cuff (Linton Instrumentation, Diss, UK) was used to occlude the artery and a pneumatic sensor and pulse transducer (Physiograph; Narco Bio Systems Inc., Houston, TX) connected to an oscilloscope to detect the pulsations on release of the cuff. Direct blood pressure measurements were obtained at 25 ± 1 wk of age using a modification of our computerized technique (18). Under anesthesia, a catheter was inserted via the femoral artery into the lower abdominal aorta, and the rat was placed into an individual recording cage and allowed to recover for 24 h. The arterial catheter was connected to a blood pressure transducer (Statham P23ID; Gould Inc., Cleveland, OH) via a rotating swivel that allowed the animals to be unrestrained. Calibration of the instrument was verified before each measurement. Blood pressure measurements were recorded beat by beat for 2 consecutive h between 1000 and 1700. The data were processed off-line following the method of Gustin et al. (19).

Genotyping. DNA typing was carried out for a S_A polymorphism at the S_A locus (20). DNA was prepared from tail fragments as described (21) and Southern blot analysis carried out using standard protocols (22). Probe was a 1.6-kb S_A cDNA subcloned from a fragment isolated by reverse transcription and PCR amplification (23) of 6-wk-old SHR kidney total RNA using primers based on the published sequence (11) (5'-TGGCTTCTCTCCATTAAG (nucleotides 7–26); 3'-TGTTGTCCATTCCTTCTCC (nucleotides 1904–1923)). Authenticity of the amplified fragment was confirmed by restriction mapping and sequencing of the ends of the clone (22).

RNA analysis. Total kidney RNA was prepared by LiCl/urea precipitation (24) and 60- μg aliquots analyzed using standard Northern blot techniques (22). The probes used were the S_A cDNA fragment

Address correspondence to Dr. N. J. Samani, Department of Medicine, University of Leicester, Clinical Sciences Building, Leicester Royal Infirmary, P. O. Box 65, Leicester LE2 7LX, United Kingdom.

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1. Abbreviations used in this paper: LOD, likelihood of the odds; MT1PA, metallothionein-1 pseudogene a; SHR, spontaneously hypertensive rat; WKY, Wistar-Kyoto rat.

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described above and an HGPRT cDNA (25). Genotypes of the F₂ rats used in this analysis were ascertained as described above.

Statistical analysis. Statistical analysis was carried out using MINITAB (Release 7) (Minitab Inc., University Park, PA) and the LINKAGE program (26).

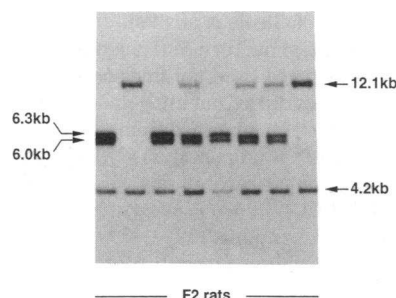
Results

S_A polymorphism cosegregates with blood pressure. 233 male F₂ rats were generated from the SHR × WKY cross. All animals had indirect tail cuff blood pressures measured at 12, 16, and 20 wk of age. In addition, 193 randomly chosen animals had direct blood pressures measured at 25 ± 1 wk of age. 16 male SHR and WKY rats were also studied in a similar fashion.

Fig. 1 illustrates the various S_A genotypes observed in the F₂ rats, and Table I shows the direct blood pressures at 25 wk of age of F₂ rats, according to the S_A genotypes. As can be seen, the S_A locus had a highly significant effect on both systolic and diastolic blood pressures. The effect of the SHR allele was co-dominant and the locus accounted for 11 and 10% respectively of the total variance of systolic and diastolic blood pressures in the F₂ population.

The total variances of systolic and diastolic blood pressures in F₂ rats are the sums of their respective genetic and environmental variances. The degrees of genetic determination (DGD = ratio of genetic to total variance) of systolic and diastolic blood pressures in the F₂ rats were calculated as described by Rapp (10) using the variances of the respective parental blood pressures as estimates of the environmental variances. The values calculated for DGD (±SE) were 39 ± 15% for systolic blood pressure and 46 ± 12% for diastolic blood pressure, therefore suggesting that the locus accounts for up to 28% of the genetic variance of systolic blood pressure and 21% of the genetic variance of diastolic blood pressure in the F₂ population.

Longitudinal effects on blood pressure. The blood pressure of the F₂ rats rose progressively from 12 to 20 wk of age (12 wk: 144.00 ± 0.79; 16 wk: 151.85 ± 0.86; 20 wk: 158.98 ± 0.99) with highly significant correlations between blood pressures at the different ages (data not shown). In addition, blood pressures at 25 wk correlated significantly with the indirect blood pressures at all ages with, as expected, increasing correlation from 12 to 20 wk ($r = 0.378, 0.526, \text{ and } 0.574$ for mean arterial pressure at 25 wk and 12-, 16-, and 20-wk BPs, respectively; $P < 0.0001$ in all cases). The effect of the S_A locus on the longitudinally measured indirect blood pressures is shown in Fig. 2. The effect appears to be age dependent, only becoming significant at 20 wk of age. While this finding needs to be interpreted with some caution because of the indirect method of measuring blood



allele (S genotype) while the presence of two bands at 6.0/6.3 kb (e.g., tracks 1 and 3) homozygosity for the WKY allele (W genotype).

Figure 1. Southern blot showing the F₂ genotypes at the S_A locus identified by StuI. Results of the typing of eight individual rats are shown. In addition to the constant band (4.2 kb), a single band at 12.1 kb (e.g., tracks 2 and 8) indicates homozygosity for the SHR

Table I. Linkage of S_A Gene to Blood Pressure Phenotypes

Genotype	No	SBP	DBP
W	49	179.0 ± 1.8	115.3 ± 1.3
H	100	184.1 ± 1.4	120.1 ± 1.1
S	44	192.6 ± 2.0	125.0 ± 1.4
F value (ANOVA)		11.7	10.3
P value (ANOVA)		<0.0001	<0.0001
LOD score statistic* (at 0% recombination)		4.5	3.8

Linkage of S_A gene to blood pressure phenotypes in F₂ rats derived from an SHR × WKY cross. W, homozygous for WKY allele; H, heterozygous; S, homozygous for SHR allele; SBP, systolic blood pressure; DBP, diastolic blood pressure.

* Base 10 logarithm of the likelihood ratio statistic for the test of linkage.

pressure, it emphasizes the importance of considering age as a factor when investigating the effect of a gene on blood pressure.

Chromosomal location. The chromosomal location of the rat S_A gene was established by linkage analysis with other polymorphic markers typed in the F₂ rats whose chromosomal locations had previously been established (27). Significant linkage was found between S_A and a microsatellite marker R231 of the rat metallothionein-1 pseudogene (MT1PA) located on chromosome 1 (27) with a maximum likelihood of the odds (LOD) score of 44.8 at 7.3% recombination. This marker was also associated with increased blood pressure, but the linkage was much weaker than that found with the S_A locus (Table II). Two other informative markers located elsewhere on rat chromosome 1 (27) showed no linkage with either S_A or MT1PA or with blood pressure (Table II).

S_A genotype and kidney expression. The S_A gene shows very limited tissue distribution of expression, with most marked expression in the kidney and some expression in the liver and brain (11, and unpublished data of our own). In the SHR kidney it also shows age-related changes in expression, with low levels before 4 wk of age and then a dramatic (> 10-fold) rise that is maintained up to at least 16 wk of age (11). This pattern parallels closely the rise in blood pressure in the SHR (28). In the WKY kidney, expression remains constant with age and after 4 wk is < 10% of that in the SHR kidney. To determine whether the level of expression in the kidney was directly controlled by the S_A locus, we examined S_A mRNA levels in kidneys of 15 F₂ rats with the various S_A genotypes. As shown in Fig. 3 the S_A locus seems itself to be the main determinant of the level of S_A gene expression in the kidney with the SHR allele again, as in the case of blood pressure, acting in a codominant fashion.

Heterogeneity of S_A transcripts. Some heterogeneity was seen between SHR and WKY in the size of the kidney S_A gene transcripts detected by Northern blotting (Fig. 3). This has also been previously reported (11). To investigate the possibility that this heterogeneity was due to differences in the coding region, PCR amplification of SHR and WKY kidney total RNA was carried out using primers flanking the coding region. In both cases a single fragment of the size predicted from the published sequence (11) was detected (data not shown). This indicates, first, that at least one of the mRNA species detected

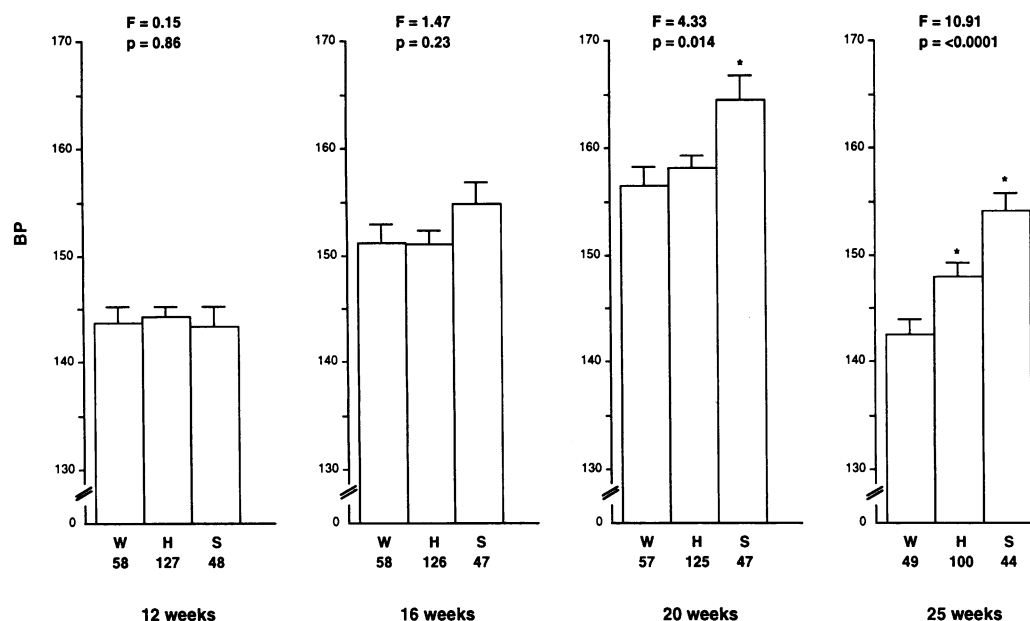


Figure 2. Age-dependent effects of the S_A gene on blood pressure in F_2 rats from SHR \times WKY cross. See Methods for experimental details. W, homozygous for WKY allele; H, heterozygous; S, homozygous for SHR allele. The numbers below each label indicate the number of animals in that group. In addition to the indirect blood pressures at 12, 16, and 20 wk, the direct mean arterial blood pressures at 25 wk are also shown. F and P values are from ANOVA. *Significant difference ($P < 0.01$) from other groups by pairwise comparisons using Scheffe's test.

by Northern blotting in the WKY is an S_A gene transcript, and second, that the heterogeneity is not due to a difference between the coding regions of the SHR and WKY transcripts. The heterogeneity may therefore be due to differences in the noncoding regions, or one of the transcripts may be the product of a separate but related gene. The absence of a similar transcript in the SHR (Fig. 3) and Southern blot analysis of SHR and WKY DNA (data not shown) however argues against the presence of a related gene.

Discussion

Hypertension is a common disorder (prevalence $\sim 15\%$) of largely unknown cause. It is the main risk factor for stroke and significantly influences the risk of ischemic heart disease, heart failure, kidney failure, and peripheral vascular disease (1). Family studies have demonstrated that hypertension is a multifactorial trait with a strong genetic component (1, 10, 29). The identification of the major genetic determinants of hypertension would enable both preventive and therapeutic maneuvers

to be targeted more precisely and thus improve the management of the condition.

Studies of the genetics of human hypertension are complicated by the late onset of hypertension, the small sizes of human pedigrees, and the impact of environmental effects, in addition to the likely heterogeneity of the disease in human populations (1, 10, 29). Nevertheless, the locus that causes a rare form of inherited hypertension (glucocorticoid-remedial hypertension) has recently been identified (30) and affected sibship analysis has implicated the angiotensinogen locus in essential hypertension (31).

Inbred rodent models of genetic hypertension, with their higher heritability of blood pressure and uniform genetic causes within each strain and cross, provide an attractive alternative to identifying genes that regulate blood pressure (10). Using molecular approaches, several loci that influence blood pressure have recently been identified in a number of such strains (3–9). In most cases however, the mechanisms (so-called intermediary phenotypes) through which blood pressure is affected remain unclear.

Table II. Linkage of Other Chromosome 1 Markers to Blood Pressure Phenotypes

	Locus					
	MT1PA		KAL		PKC	
	SBP	DBP	SBP	DBP	SBP	DBP
W	181.7 \pm 2.2	117.8 \pm 1.6	183.0 \pm 2.1	119.2 \pm 1.6	182.7 \pm 2.1	118.2 \pm 1.5
H	182.5 \pm 1.4	119.1 \pm 1.2	184.5 \pm 1.5	120.2 \pm 1.1	184.2 \pm 1.5	119.6 \pm 1.2
S	191.3 \pm 1.9	124.2 \pm 1.4	187.6 \pm 2.4	122.2 \pm 1.8	186.1 \pm 2.3	122.0 \pm 1.7
F value	7.2	4.6	1.0	0.8	0.5	1.2
P	0.001	0.011	NS	NS	NS	NS
LOD score	3.0	1.8				

Linkage of other rat chromosome 1 markers to blood pressure phenotypes in F_2 rats. 15 loci located on rat chromosome 1 (27) were screened for microsatellite polymorphism between SHR and WKY DNA as previously described (27). Three were found to be polymorphic (MT1PA, renal kallikrein [KAL], protein kinase C type 1 [PKC]). F_2 rats were then typed for these loci. The table shows the blood pressures of the F_2 rats according to the genotypes at each locus. Abbreviations as per Table I.

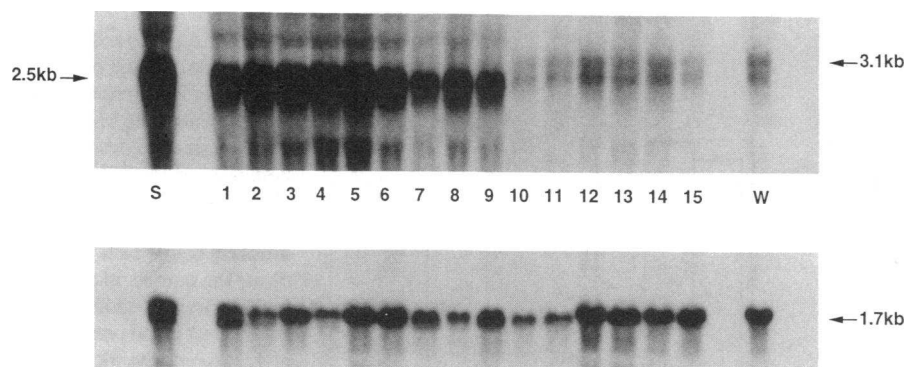


Figure 3. Effect of S_A genotype on kidney S_A mRNA levels at 6 wk of age. A Northern blot of kidney RNA was prepared from 6-wk-old SHR (S), WKY (W), and 15 F_2 rats, probed for S_A mRNA (top) and then reprobed for HGPRT mRNA (bottom). F_2 rats 1–6 had S genotype, 7–9 had H genotype, and 10–15 had W genotype. Despite some variation in the levels of the control HGPRT mRNA, the data demonstrate the marked effect of the S_A genotype on kidney S_A mRNA levels (2.5-kb band) at 6 wk of age. An additional 3.1-kb species was also detected in kidneys from animals with the W genotype (see text for discussion).

In this study, we demonstrate that a polymorphism in a gene that shows increased expression in the kidney of the SHR strongly cosegregates with raised blood pressure. While we cannot definitively exclude the possibility that a closely linked locus is responsible for the effect, the data strongly point to the S_A gene itself. Additional support for a direct involvement of S_A is provided by the observations (11) that its expression is modulated by antihypertensive treatment (with captopril) in the SHR and that, at least in some strains, it is also affected by dietary salt intake, a factor well known to influence blood pressure. Cosegregation of the locus with blood pressure has also recently been reported in other smaller crosses involving the SHR (20, 32, 33). Located on chromosome 1, the S_A gene is not linked to any of the other loci that have been reported to cosegregate with blood pressure in previous crosses involving the SHR or the closely related stroke-prone SHR (4–9). In previous studies of this type, linkage of specific loci to blood pressure phenotypes has been found to be cross-dependent (3, 4, 9), and whether the S_A locus plays a role in the elevated blood pressure of other strains of genetically hypertensive rats (e.g., Dahl, Milan) remains to be determined.

We further show that the S_A genotype also influences the level of S_A mRNA in the kidney. It is important to note that the effect of S_A on its kidney expression was manifested by 6 wk of age, while the effect on blood pressure was not significant till beyond 16 wk of age. This implies that the difference seen in kidney S_A expression is not secondary to a change in systemic blood pressure. We have therefore identified a single gene locus that affects blood pressure and also appears to directly influence its expression in a key cardiovascular tissue. Lower levels of expression of S_A also occur in the liver and brain (11, 32, and our unpublished data). The level of S_A mRNA is also higher in the SHR liver but lower in SHR brain compared with WKY (11, 32, and our unpublished data). While an effect of the S_A locus on blood pressure through expression in these sites cannot at present be ruled out, especially as antihypertensive treatment (with captopril) has been reported to have a more pronounced effect on liver than kidney S_A mRNA levels (11), our findings together with the limited tissue distribution of expression of the gene and its temporal pattern in the SHR (11) suggest that the effect of the S_A gene locus on blood pressure is mediated via an effect on S_A gene expression in the kidney.

The findings particularly bear upon previous observations made in experiments involving transplantation of SHR and WKY kidneys (16, 17). These studies have shown that the tendency to hypertension follows the SHR kidney and that this is not due to secondary changes in the SHR kidney as a conse-

quence of increased blood pressure. Our findings offer a potential genetic mechanism for these physiological observations. The relevance of our findings to human hypertension remains to be investigated although it is notable that the tendency to hypertension in human kidney recipients has also been shown to be related to the blood pressure status of the donor (13, 14).

The function of the S_A gene product is unknown. The nucleotide sequence of the SHR S_A cDNA suggests a putative protein comprising 546 amino acids (11). The primary sequence has little homology to any other known protein sequence. The S_A protein therefore likely represents a hitherto unidentified molecule involved in blood pressure homeostasis. The disparate temporal effects of the S_A genotype on kidney expression and blood pressure suggest that if the effect of S_A gene on blood pressure is mediated through its renal expression, then the mechanism involved is slow and/or indirect. In this context it may be relevant that an increased renal vascular resistance in young F_2 rats derived from an SHR \times WKY cross has been shown also to cosegregate with hypertension (34). At present, however, the localization of the S_A gene product in the kidney is not known. Determining this and elucidating its mechanism of action may not only provide clues to the etiology of human essential hypertension but also offer novel therapeutic options.

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