Locus for the Inducible, but Not a Constitutive, Nitric Oxide Synthase Cosegregates with Blood Pressure in the Dahl Salt-sensitive Rat

Alan Y. Deng and John P. Rapp
Department of Physiology and Molecular Medicine, Medical College of Ohio, Toledo, Ohio 43699

Abstract

Alleles of the inducible nitric oxide synthase locus (*Nos2*) cosegregated highly significantly ($P < 0.0001$) with blood pressure in an F_2 population [F_2(S × MNS), $n = 171$] derived from a cross of inbred Dahl salt-sensitive (S) rats with Milan normotensive rats (MNS). In contrast, alleles at the constitutive brain nitric oxide synthase locus (*Nos1*) did not cosegregate with blood pressure in several F_2 populations. *Nos2* was mapped on rat chromosome 10. Nine genetic markers, including the angiotensin-converting enzyme (*Ace*) and *Nos2* loci spanning roughly 46 cM on rat chromosome 10, all cosegregated strongly with blood pressure in the F_2(S × MNS) population. *Nos2* showed the highest LOD score of 6.3. *Ace* and *Nos2* are 30 cM apart. In an F_2 population [F_2(S × WKY), $n = 159$] derived from a cross of S rats with Wistar-Kyoto (WKY) rats, *Nos2* alleles did ($P = 0.0070$), but *Ace* alleles did not ($P = 0.91$), cosegregate with blood pressure. We conclude that the *Nos2* locus rather than the *Nos1* locus is a candidate for influencing blood pressure in the S rat. There are probably two separate but linked quantitative trait loci (QTL) for blood pressure on rat chromosome 10, one marked by *Ace* and the other marked by *Nos2*. In F_2(S × MNS) functionally variant alleles at both QTL influence blood pressure, but in F_2(S × WKY) only the QTL marked by *Nos2* is segregating alleles influencing blood pressure. (*J. Clin. Invest.* 1995. 95:2170–2177.) Key words: genetic hypertension • rat chromosome 10 • endothelium-derived relaxing factor • quantitative trait loci • Milan normotensive rat

Introduction

Nitric oxide (NO) (1, 2), possibly along with labile nitroso compounds, is thought to account for the activity of an endothelium-derived relaxing factor originally described by Furchgott and Zawadzki (3). Since NO is unstable, its oxidized products nitrite and nitrate are usually assayed as indicators of NO production (4). Using L-arginine as the substrate, three types of enzymes, known as nitric oxide synthase (NOS),[1] responsible for the production of NO have been purified from humans and the mouse (5–7) and cloned (8–12). Two types of NOS, designated as NOS1 and NOS3, are constitutively expressed and are Ca^{2+} and calmodulin dependent. The remaining type, designated as NOS2, is inducible and is Ca^{2+} and calmodulin independent (13). So far, only NOS1 (8) and NOS2 (14–16) have been identified and cloned in the rat. *NOS1* (17), *NOS2* (18, 19), and *NOS3* (18, 20–22) have been assigned to human chromosomes 12, 17, and 7, respectively. *Nos2* has also been mapped to mouse chromosome 11 (23) and rat chromosome 10 (24). Among its alleged physiological functions, such as aiding in cytotoxicity mediated by macrophages (25–27) and serving as a neurotransmitter (25–28), NO can regulate blood pressure by causing vasodilation (1–3, 25–27, 29).

It is unknown whether genetic variation in the NO synthesizing enzymes is involved in causing inherited hypertension. To address this issue, we utilized a genetic approach using genetically hypertensive inbred Dahl salt-sensitive rats in segregating populations to analyze the NO synthesizing enzymes for cosegregation with blood pressure.

Methods

Animal procedures. Rat strains used and animal procedures were given in detail previously (30–33) and are only summarized briefly here. The inbred Dahl salt-sensitive and inbred Dahl salt-resistant rats were bred in our own colonies and will be designated as S and R, respectively. To generate segregating F_2 populations, S rats were crossed with various contrasting strains and the F_1 offspring were intercrossed to produce F_2. The contrasting strains used were spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY) obtained from Harlan Sprague-Dawley (Indianapolis, IN), Milan normotensive rats (MNS) from Vincent Gattone (University of Kansas), LEW/NCrlBR from Charles River Laboratory (Wilmington, MA), and Brown Norway from Harlan Sprague-Dawley.

F_2 rats were weaned at 30 d of age and fed on a high salt (8% NaCl) diet (TD82050, Teklad, Madison, WI) starting at 37 d of age. Systolic blood pressure was measured by the tail-cuff microphonic manometer method with the rats warmed to 28°C either under light ether anesthesia or in the conscious restrained state (IITC Inc., Woodland Hills, CA). When the highest blood pressures of rats in the population reached over 200 mmHg, the blood pressure of the entire population was measured intensively during a 7–10-d period. At least three consistent blood pressure readings at a given session were acquired for each rat and averaged as that session's reading. Three such separate sessions on different days were conducted for each rat. The blood pressure from three sessions was then averaged and taken as the final blood pressure measurement of that rat. All rats were males, except for those crosses with R rats, in which case both male and female rats were studied. Data from males and females were combined in the present analysis as both sexes gave similar results.

Address correspondence to A. Y. Deng, Ph.D., Department of Physiology and Molecular Medicine, Medical College of Ohio, P.O. Box 10008, Toledo, OH 43699-0008. Phone: 419-381-4026; FAX: 419-381-3124.

Received for publication 2 November 1994 and in revised form 3 January 1995.

1. *Abbreviations used in this paper:* MNS, Milan normotensive rat; NOS, nitric oxide synthase; QTL, quantitative trait locus (loci); R, Dahl resistant rat; S, Dahl salt-sensitive rat; SHR, spontaneously hypertensive rat; SSR, simple-sequence repeats; WKY, Wistar-Kyoto rats.

J. Clin. Invest.

0021-9738/95/05/2170/08 $2.00
Volume 95, May 1995, 2170–2177

Generation of polymorphic markers containing simple-sequence repeats (SSR) for rat NOS genes. A cDNA probe for the rat brain nitric oxide synthase (*Nos1*) was provided by Dr. S. Snyder (Johns Hopkins University, Baltimore, MD), and a cDNA probe for the mouse inducible nitric oxide synthase (*Nos2*) was provided by Dr. C. Nathan (Cornell University, New York).

A cDNA probe for the bovine endothelial nitric oxide synthase (*Nos3*) was generated as follows from a bovine endothelial cell cDNA library constructed in λgt11, which was custommade by Clontech Laboratory Inc. (Palo Alto, CA). A pair of primers flanked by *Eco*RI restriction sites was designed to produce a product of 422 bp by PCR from the bovine endothelial Nos3 gene according to a published sequence (11). The forward primer was 5′GGAATTCGATCAGCAACGCTATCACGA3′ and the reverse primer was 5′GGAATTCTCACCTAAACACCAAAGGGC3′. The PCR product of the expected size was produced, separated on an 1.5% agarose gel, excised, purified using glass milk (Bio. 101, La Jolla, CA), and subcloned into a TA cloning vector (Invitrogen, San Diego, CA). The sequence of the PCR product was confirmed to be the bovine *Nos3* sequence as published (11).

The cDNA probes for *Nos1, Nos2,* and *Nos3* were then used to screen a genomic library (courtesy of Dr. D. Ginn [34]) of the Dahl R rat. Probes were labeled using a kit for random priming (Boehringer Mannheim, Indianapolis, IN) and purified using a Bio-6 column (Bio-Rad, Mellville, NY). Standard procedures for growing phage, screening by filter lift, treatment of DNA on the filters, and hybridization were used (35). The prehybridization and hybridization solutions consisted of 5× SSPE, 1% SDS, 10× Denhart, 0.05 mg/ml of salmon sperm DNA, 50% formamide, pH 7.4. Hybridization took place at 42°C overnight. Washing conditions were 2× SSC, 1% SDS, twice at room temperature 5 min each, followed by 0.2× SSC, 1% SDS, twice at 68°C, 30–60 min each.

Purified phage clones containing the rat *Nos* genes were prepared in a 2× NZCYM liquid medium by the standard method (35). The inserts were digested separately with a series of restriction enzymes that cut the vector only once. The digested fragments were then separated on a 1% agarose gel, transferred to a sheet of Duralon-UV membrane (Stratagene, La Jolla, CA), fixed by UV cross-linking and probed with a mixture of $(CA)_{15}$ and $(CT)_{15}$ oligonucleotides. The oligonucleotides were end labeled with ^{32}P as described previously (30). Prehybridization and hybridization were carried out as described above.

The genomic fragments recognized by the $(CA)_{15}$ and/or $(CT)_{15}$ oligonucleotides were then subcloned into a pT7T3 18U (Pharmacia LKB Biotechnology Inc., Piscataway, NJ). The subcloned fragments containing $(CA)_n$ and/or $(CT)_n$ SSR in or near the gene for *Nos1* and *Nos2* were digested with different enzymes followed by subcloning until the fragment of interest became < 1 kb. These clones were then sequenced. Flanking sequences around $(CA)_n$ and/or $(CT)_n$ repeats were used to design primers for PCR using the Primer Detective Program (Clontech). PCR of the SSR was done as given previously (30) except that labeled primers were used directly without purification. Briefly, the PCR program was run on a Thermocycler (M.J. Research, Watertown, MA). The PCR cycles for rat primers were as follows: 95°C for 5 min; 30 cycles of 94°C for 40 s, 55°C for 40 s, and 72°C for 1.5 min; extension for 5 min at 72°C. The PCR cycles for mouse primers were the same as above except the annealing temperature was set at 50°C rather than 55°C.

To verify that the genomic region containing the SSR was continuous and closely linked being the *Nos* gene in question rather than being a chimera formed by ligating unlinked random genomic fragments during cloning, all the clones positive for both a gene of interest and for containing a SSR were digested with restriction enzymes. Restriction mapping revealed that all such positive clones shared a similar restriction pattern and overlapped one another. Therefore, they were all cloned from the same chromosomal region. SSR PCR primers around SSR for both the rat *Nos1* and *Nos2* genes are given in Table I.

Statistical analysis for genotyping and linkage. To eliminate genotyping errors, all homozygous genotypes were confirmed by retesting. When several loci on a particular chromosome region were genotyped, the MAPMAKER program (36) was used to detect potential genotyping errors. If such possible errors were noted, the rats for the loci in question were genotyped again to either confirm or correct the results from the original genotyping.

A one-way ANOVA comparing blood pressure among segregating genotypes was done using SPSS programs (SPSS, Chicago, IL). Linkage maps and quantitative trait locus (QTL) localization were done with MAPMAKER/QTL programs (36, 37) obtained from Dr. Eric Lander (Whitehead Institute, Cambridge, MA). In QTL analysis of blood pressure in the rat, Jacob et al. (38) suggested that a LOD score of 3 or a probability from ANOVA of $P < 0.001$ should be set as the significance level to reasonably establish the existence of a QTL when many marker loci are screened.

Results

Cosegregation analyses for Nos1 and Nos2. Rat genomic clones for *Nos1* and *Nos2* were obtained by screening the rat genomic library. But no rat genomic clones were obtained using the bovine *Nos3* probe even under lower stringency conditions for both hybridization (37°C) and the final wash (42°C). Our attention was then focused on the rat *Nos1* and *Nos2* loci.

Using the SSR markers we developed (Table I), we genotyped the *Nos1* and *Nos2* loci for cosegregation with blood pressure in F_2 populations derived from crosses of the S rat with various control rats. Alleles of *Nos2* cosegregated significantly with blood pressure in the F_2(S × MNS) population ($P < 0.0001$) and in the F_2(S × WKY) population ($P = 0.007$), but not in F_2(S × R) or a backcross population F_1(S × R) × S (Table II). We tested as many F_2 populations as polymorphisms allow between the S and contrasting alleles for *Nos1*. No association between the alleles of *Nos1* and blood pressure has been detected in any of the four F_2 populations tested (Table III).

The rat *Nos1* gene could not be assigned to a rat chromosome by using rat-mouse somatic hybrids due to a lack of marker polymorphisms between the rat *Nos1* and the mouse *Nos1* genes nor was the rat *Nos1* gene linked to any markers we have genotyped thus far (30–34). Thus, the chromosome localization of *Nos1* in the rat remains to be determined.

Genetic mapping of the Nos2 locus. We previously assigned *Nos2* to rat chromosome 10 in mouse-rat somatic hybrids using a different SSR marker for *Nos2* (24). To further map *Nos2* to a specific region of chromosome 10, we used two approaches to obtain more markers. The first approach was to map rat loci already assigned to chromosome 10 by linkage. Of 21 such rat loci containing SSR (Table I), 8 were polymorphic between the S and MNS rats.

The second approach was to take advantage of a conserved synteny relationship between rat chromosome 10 and mouse chromosome 11 (39, 40). We tested 141 mouse SSR markers for mouse chromosome 11 (purchased from Research Genetics, Huntsville, AL). Eight of them (5.6% of the total mouse markers tested) were polymorphic between S and MNS (Table I). Of these eight mouse markers, six (4.2% of the total mouse markers tested) were usable for genotyping the rat F_2(S × MNS) population as the other two mouse markers failed to yield PCR products for a majority of the rats in the population. Taken all together, 14 markers including *Nos2* were analyzed for cosegregation with blood pressure and for linkage among themselves in the F_2(S × MNS) population. The data for these loci are presented in Table II and Fig. 1. These 14 markers fell

Table I. PCR Primers for Rat Loci and Comparison of PCR Products among Strains

Locus	GenBank accession number	Primers (5′ → 3′)	Expected size	Strain comparison of PCR products
			bp	
Abp	M19993	GCTCACCTCTCCATAAAACC CTGGGGATTTAGCTCAGT	258	S = R = MNS > WKY = SHR = LEW = AS = BN
Aldoc	M63656	ACGGGAGCAAAACACAATGC GCTTTGGAGATCATCAGGTAGG	255	R = WKY = SHR = MNS = LEW = AS = BN > S
Asgr	K02817	GGAAATTCAGGACCAAAAGGGG AGGGAATAGACTTGGAACTGGG	253	S = R = WKY = SHR = MNS = LEW = AS = BN
Clatp	J05210	CTGGAAACAGAGCCCGTTAT CGCAAACGTCACAAGTATTGGC	186	S = WKY = MNS = LEW = BN > R = SHR = AS
*Grm6**	b	CTTTGGTCAGAGTGTCCATG AGTTACATAGTGAGACCCTC	150	WKY = R = MNS = LEW = BN > S = SHR = AS
Il-3	c	CTGCTTAGAGCCTTCACACA AGGAATTCGTCCAGGTTTAC	930	R = S = MNS > WKY > BN
Mhcg	X04267	CATCACATTGGTTGCCATC AATGAAGTCCACTCAGG	261	S = R = MNS = BN > WKY = SHR = LEW = AS
Mhcg	X04267	TTCATCTGGTGGGGACATAACC GGTGGGGGAAGTGATTAAGA	202	WKY = SHR = LEW = AS > S = R = MNS = BN
Ngfr	X61269	GCTTGGCATTTGTAAGGTCC CTGTCTCTGTCTTTCTGTTCCC	297	WKY > S = R = MNS = LEW = BN > SHR > AS
Ngfrf	X05137	CTTAATGTCAGCCCTCG ATGAAGCTCAGCTCTGTC	215	AS = WKY > S = R = SHR = MNS = LEW = BN
Nos1‡	U14522	GGTTGTCTCTACACTCTGGAGC TGCTTTGGCTGCTTCTCTCG	240	BN > WKY = MNS = SHR > S = R = LEW = AS
Nos2‡	U14523	CAGCTCTCACTGATTGACTTGG GTCAGGTTCCCATGTGCTTA	269	BN = S > WKY = MNS = R = SHR = LEW = AS
Nos2‡	U16359	GGACGTTGTTGTGATTTCCTGG AGCAGAGGCAGAGATTGATTGC	301	S = R = WKY = SHR = MNS = LEW = AS = BN
Ppp	M18207	TCCCTTCTGTCTCTCTCCTA ATCAACTTTACCTGCTCCTGCC	257	R = WKY = SHR > S = MNS = LEW = AS
Ppp	M18207	GGAATCCCTGTAGACTATCTCC CTTCAGATCCTGTCTGTG	210	SHR > S = MNS
Syb2	M24105	TGTGTATGGGACTTGCTGGA CTCAGACCCCTAAAACTGTG	187	S = R = WKY = SHR = LEW = BN > AS > MNS

within a region of roughly 81 cM on chromosome 10. The data for cosegregation for *Ace* in F_2(S × MNS) were reported previously (30). The *Ace* and *Nos2* loci are separated by > 30 cM. All markers in the region ranging from D11MIT58 to MIT-R1704 spanning ~ 46 cM cosegregated strongly with blood pressure ($P < 0.0001$) in the F_2(S × MNS) population, with the strongest point at the *Nos2* locus (blood pressure difference = 22.7 mmHg). The LOD score between loci and blood pressure were 4.8 at *Ace* and 6.3 at *Nos2*.

When nine loci and their blood pressure effects were studied in F_2(S × WKY) (Fig. 2), a QTL with a smaller effect on blood pressure was detected near *Nos2* with a maximum LOD score of 2.30. As opposed to the F_2(S × MNS) population, alleles at the *Ace* locus in F_2(S × WKY) did not cosegregate ($P = 0.91$) with blood pressure (Table II, Fig. 2) as reported previously (30).

Statistical analysis of epistatic interaction between different loci. In our previous work (30) an interaction was observed between QTL associated with the guanylyl cyclase A/atrial natriuretic peptide receptor (*Gca*) locus on chromosome 2 and the *Ace* locus on chromosome 10 in the F_2(S × MNS) population. In the present work, no interaction was found between *Nos2* (30 cM from *Ace*) and markers on chromosome 2.

Discussion

A highly significant cosegregation of alleles at the *Nos2* locus ($P < 0.0001$, $F = 15.0$, LOD = 6.4; Table II, Fig. 1) with systolic blood pressure in F_2(S × MNS) provides strong evidence for a possible role of the inducible nitric oxide synthase (NOS2) in genetic hypertension in the S rat. In contrast, a constitutive and Ca^{2+}-dependent nitric oxide synthase (NOS1) may not be involved in genetic hypertension because alleles at the *Nos1* locus did not cosegregate with blood pressure in any of the four F_2 populations tested (Table III).

The results of physiological and pharmacological studies are pertinent to our genetic analysis. In studying both young S and R rats raised on a high-salt (8% NaCl) diet, Chen and

Table I. (Continued)

Locus	GenBank accession number	Primers (5′ → 3′)	Expected size	Strain comparison of PCR products
			bp	
MIT-RR24	a	CTCTTTGGGATGAACCGGTA AATGGGAAGCAACAGCATTC	260	S = R = WKY = MNS = LEW > SHR
MIT-RR92	a	ACCTTTGGTTCCAGGGAATC TTCCTATAAGCTCTCTGACTCACA	190	S = R = WKY = SHR = MNS = LEW = AS = BN
MIT-RR1023	a	AGCCTCACTGATGCTCCTGT CCAAGAGCTACCTGCACTCC	230	WKY = MNS > S = R = SHR = LEW = AS = BN
MITR1704*		Research Genetics	190[§]	MNS > WKY = LEW > S
MITR183*		Research Genetics	200[§]	WKY = MNS > LEW = S = R = SHR = AS = BN
D11MIT4		Research Genetics	280[§]	S = R > WKY = SHR = LEW = AS > MNS = BN
D11MIT12		Research Genetics	220[§]	S = R = WKY = SHR = MNS = LEW = BN
D11MIT15*		Research Genetics	260[§]	MNS > S = R = BN > SHR = LEW = AS
D11MIT28*		Research Genetics	150[§]	R > S > MNS = WKY = SHR = LEW = AS = BN
D11MIT37		Research Genetics	190[§]	WKY = AS = BN > LEW
D11MIT53		Research Genetics	190[§]	SHR > S = R = WKY = MNS = LEW = AS = BN
D11MIT58		Research Genetics	260[§]	BN > WKY > LEW = AS > S = R > MNS
D11MIT60		Research Genetics	100[§]	S = R = WKY = SHR = MNS = LEW = AS = BN
D11MIT84*		Research Genetics	190[§]	BN > WKY > SHR > AS = LEW > MNS > S = R
D11MIT91		Research Genetics	160[§]	S = R = WKY = SHR = MNS = LEW = AS = BN
D11MIT101		Research Genetics	200[§]	WKY > S = R = SHR = AS > MNS = LEW = BN
D11MIT119*		Research Genetics	170[§]	MNS = AS > WKY = SHR = BN > S = R > LEW
D11MIT145		Research Genetics	230[§]	S = R = WKY = SHR = MNS = LEW = AS = BN
D11MIT168		Research Genetics	196[§]	R = S > MNS = WKY = LEW > SHR
D11MIT175*		Research Genetics	118[§]	MNS > S = R = WKY = SHR = LEW = AS = BN
MITR265		Research Genetics	300[§]	BN > S = MNS = R = WKY = LEW = AS
MIT-MHCG		Research Genetics	180[§]	S = MNS
MIT-PPP		Research Genetics	180[§]	S = MNS = R = AS = BN > WKY = SHR
MIT-BAND3		Research Genetics	120[§]	S = R = MNS = LEW = AS = BN > WKY = SHR

* Newly mapped markers in the rat. [‡] Newly developed and/or mapped markers. [§] Estimated size determined on agarose gels. Gene loci are in italics. a–c, primers for markers were the same as given in references 38, 56, and 57, respectively. *Abp*, androgen binding protein; *Aldoc*, aldolase C, primers for PCR and its localization to rat chromosome 10 were reported previously (24); *Asgr*, asialoglycoprotein receptor; *Clatp*, ATP citrate lyase; *Grm6*, glutamate receptor, metabolic subtype 6; *Il-3*, interleukin-3; *Mhcg*, Myosin heavy chain, embryonic skeletal muscle; *Ngfr*, nerve growth factor receptor; *Ngfrf*, nerve growth factor receptor, fast; *Nos1*, nitric oxide synthase, brain; *Nos2*, nitric oxide synthase, inducible; *Ppp*, pancreatic polypeptide; *Syb2*, synaptobrevin 2 (vesicle-associated membrane protein, VAMP-2); MITR1704, MITR183, MITR265, MIT-MHCG, MIT-PPP, MIT-BAND3, D11MIT4, D11MIT12, D11MIT15, D11MIT28, D11MIT37, D11MIT53, D11MIT58, D11MIT60, D11MIT84, D11MIT91, D11MIT101, D11MIT119, D11MIT145, D11MIT168, D11MIT175 are markers purchased from Research Genetics (Huntsville, AL). All the rat loci that are not mapped to rat chromosome 10 in Figs. 1 and 2 are also known to locate on chromosome 10 (40), except *Nos1*. LEW, Lewis rat; AS, albino-surgery rat; BN, Brown Norway rat.

Sanders (41) showed that L-arginine, the substrate for NOS, but not D-arginine, caused a decrease in blood pressure in the S rat but not in the R rat when infused intravenously. N^G-monomethyl-L-arginine, a relatively stereospecific competitive inhibitor of NOS, raised blood pressure more in the R rat than in the S rat. L-Arginine did not lower blood pressure in the S rat fed on a low-salt (0.3% NaCl) diet. Their results suggested that NOS(s) is likely involved in the regulation of blood pressure in the Dahl rats fed on a high-salt diet and the S rat may be defective in raising the activity of NOS compared with the R rat in response to a high-salt diet. The S rat appeared to be unique among hypertensive rat models in using the NO system because intravenous injection of L-arginine had no effect on blood pressure in the SHR (41). Furthermore, a comparison of NO released and hypotensive effects in response to a bolus injection of acetylcholine, a stimulator of NO production, showed that there was no difference between the SHR rat and its regularly used normotensive control WKY rat (42). N^G-monomethyl-L-arginine increased blood pressure in a similar magnitude in both SHR and WKY (42). Studies by Chen and Sanders (43) showed that the L-arginine–induced decrease in blood pressure in the S rat fed on a high-salt diet could be prevented by infusion of dexamethasone, a selective inhibitor for the expression of NOS2, but not of NOS1 and NOS3 (44). Thus, it appeared that NOS2, rather than NOS1 or NOS3, was involved in pathogenesis of hypertension in the S rat.

Several caveats need to be added to the genetic and physiological interpretations. A cosegregation analysis did not, and cannot, prove that the *Nos2* locus, rather than an unknown locus linked to *Nos2*, was the actual QTL for blood pressure

Table II. Cosegregation Analysis of Systolic Blood Pressure (mmHg) with Alleles at Loci on Rat Chromosome 10 in F_2 Populations and One Backcross Population Raised on a High-Salt (8% NaCl) Diet

Population	Locus	Blood pressure and genotype			*P* (one-way ANOVA)
F_2(S × MNS)		MM	MS	SS	
	MIT-RR1023	167.2±2.28 (42)	172.0±2.09 (92)	185.9±4.20 (37)	0.0002
	D11MIT58	166.5±2.09 (48)	172.1±2.16 (84)	187.2±4.18 (38)	< 0.0001
	Ace	166.2±2.11 (47)	172.2±2.13 (85)	186.7±4.10 (39)	< 0.0001
	D11MIT119	165.6±2.27 (50)	172.7±2.30 (81)	186.5±3.56 (40)	< 0.0001
	Nos2	165.0±2.34 (48)	172.3±2.32 (83)	187.7±3.48 (40)	< 0.0001
	Aldoc	164.3±2.28 (51)	173.4±2.24 (83)	188.1±3.56 (37)	< 0.0001
	D11MIT15	163.7±2.23 (46)	174.0±2.24 (86)	185.3±3.62 (39)	< 0.0001
	Syb2	163.0±2.09 (47)	174.6±2.27 (85)	185.4±3.63 (39)	< 0.0001
	D11MIT28	163.0±2.04 (48)	175.4±2.31 (86)	184.2±3.70 (37)	< 0.0001
	MIT-R1704	163.0±2.18 (44)	174.7±2.32 (89)	184.5±3.35 (38)	< 0.0001
	Grm6	164.1±2.70 (39)	174.1±2.20 (94)	183.2±3.39 (38)	0.0003
	D11MIT175	165.4±2.65 (43)	173.4±2.21 (89)	184.1±3.50 (39)	0.0002
	MIT-R183	167.8±2.81 (40)	172.9±2.22 (92)	182.4±3.60 (38)	0.0074
	D11MIT84	170.5±2.83 (39)	171.0±2.18 (90)	182.3±3.50 (41)	0.0087
F_2(S × WKY)		WW	WS	SS	
	Ace	173.1±4.90 (39)	174.8±2.47 (89)	172.9±3.70 (31)	0.91
	MITBAND3	170.6±4.39 (43)	176.0±2.60 (86)	172.9±3.56 (30)	0.48
	Ppp	170.4±4.30 (44)	176.2±2.63 (85)	172.9±3.56 (30)	0.44
	NGFRF	168.2±4.40 (38)	175.1±2.50 (95)	178.2±4.18 (26)	0.22
	D11MIT119	166.8±4.13 (40)	175.0±2.45 (95)	181.8±4.74 (24)	0.050
	Nos2	164.8±3.64 (40)	175.4±2.58 (90)	183.3±4.56 (28)	0.0070
	D11MIT4	164.3±3.91 (36)	175.2±2.51 (96)	182.5±4.46 (27)	0.0099
	Mhcg	164.3±3.70 (36)	175.8±2.59 (93)	179.9±4.40 (30)	0.020
	Grm6	165.8±3.96 (40)	175.7±2.50 (88)	179.7±4.63 (31)	0.039
F_2(S × R)		RR	RS	SS	
	Nos2	164.2±3.53 (33)	162.6±2.60 (53)	167.5±4.64 (26)	0.61
	Aldoc	162.8±3.50 (32)	163.6±2.77 (51)	167.5±4.64 (26)	0.65
F_1(S × R) × S			RS	SS	
	Nos2		166.3±3.32 (34)	163.1±2.92 (36)	0.47
	Aldoc		166.9±4.05 (24)	162.9±3.25 (31)	0.44

Values are means±SEM with number of rats for each genotype in parentheses. Primers and cosegregation data for *Ace* in F_2(S × MNS) and F_2(S × WKY) were also given previously (30). In F_2(S × MNS) and F_2(S × WKY) populations, loci are given in the same order as they are on the linkage maps. For designations of the loci and for designations of rat strains, see the footnote for Table I. L, allele for the LEW strain; M, allele for the MNS strain; R, allele for the R strain; S, allele for the S strain; W, allele for the WKY strain.

on chromosome 10. L-Arginine, also a substrate for arginase in the urea cycle (45), may lower blood pressure of the S rat by a totally unknown mechanism other than being the substrate for NOS. Dexamethasone, although a selective inhibitor for NOS2 (not NOS1 and NOS3), is not a specific inhibitor for NOS2 and can also inhibit the production of the vasodilator, atrial natriuretic peptide (46). Furthermore, dexamethasone can inhibit the induction of arginino-succinate synthase, an enzyme involved in regeneration of arginine from citrulline (47). Also, S rats were compared with R rats in the physiological studies cited. Physiological inferences involving the NO system dependent on an S versus R comparison are probably irrelevant because alleles of *Nos2* did not cosegregate with blood pressure in F_2(S × R) or F_1(S × R) × S rats.

Nevertheless, combining the physiological data relevant to the S rat per se with our genetic evidence, it is within reason to hypothesize that the production of NOS2 in the S rat, as compared with the MNS and/or WKY rat, might be defective in response to the stimulation by a high-sodium chloride (8%) diet. If so, insufficient NO would be generated in tissues involved in regulating blood pressure of the S rat.

Our other previous work at chromosome mapping of blood pressure QTL showed that there are chromosome regions on rat chromosomes 2 (32), 5 (31), and 17 (31), where single blood pressure QTL were identifiable, and that there were two separate and distinguishable blood pressure QTL in a region on rat chromosome 1 (48). Typically, it has been possible to localize the QTL to regions 20–30 cM in size. In contrast, the markers spanning the entire chromosome region of roughly 46 cM between D11MIT58 and MIT-R1704 on rat chromosome 10 (Fig. 1) all cosegregated strongly with blood pressure in F_2(S × MNS) ($P < 0.0001$, $F > 11.8$ throughout this region). One interpretation is that on rat chromosome 10, there might exist two major blood pressure QTL so closely linked that they

Table III. Cosegregation Analysis of Systolic Blood Pressure (mmHg) for Alleles at the Brain Nitric Oxide Synthase Locus (Nos1) in F_2 Populations Raised on a High-Salt (8% NaCl) Diet

Population	Blood pressure and genotype			*P* (one-way ANOVA)
F_2(S × MNS)	MM	MS	SS	
	179.4±3.69	173.0±2.30	171.0±2.84	0.18
	(37)	(86)	(48)	
F_2(S × WKY)	WW	WS	SS	
	168.8±2.68	174.5±2.85	178.2±4.61	0.20
	(44)	(71)	(44)	
F_2(S × SHR)	HH	SH	SS	
	162.7±3.34	173.0±4.32	167.8±5.58	0.27
	(30)	(49)	(29)	
F_2(S × BN)	BB	BS	SS	
	172.6±3.64	175.0±2.89	174.5±6.01	0.89
	(28)	(51)	(17)	

Values are means±SEM with number of rats for each genotype in parentheses. M, allele for MNS rats; S, allele for S rats; W, allele for WKY rats; H, allele for the SHR strain; B, allele for Brown Norway rats. For strain designations, see footnotes for Table I.

F_2 (S x MNS) POPULATION

Chromosome 10 Map	Blood Pressure Difference	One-Way ANOVA F	P
MIT-RR1023	+18.7	9.2	0.0002
9.5			
D11MIT58			
Ace	+20.5	11.8	< 0.0001
27.0			
D11MIT119	+20.9	12.5	< 0.0001
3.1			
Nos 2	+22.7	15.0	< 0.0001
Aldoc			
4.8			
D11MIT15			
Syb2	+22.4	13.7	< 0.0001
D11MIT28			
11.4			
MIT-R1704	+21.5	12.0	< 0.0001
4.1			
Grm6	+19.1	8.5	0.0003
7.5			
D11MIT175	+18.7	8.8	0.0002
5.1			
MIT-R183	+14.6	5.0	0.0074
8.5			
D11MIT84	+11.8	4.9	0.0087

Figure 1. Genetic linkage map for rat chromosome 10 and statistical analysis for cosegregation of marker alleles with blood pressure in F_2(S × MNS) rats fed 8% NaCl diet. The numbers along the vertical line to the left of the map for chromosome 10 denote map units between loci in centiMorgans using the Haldane correction. The orders for the loci in brackets cannot be accurately determined. But the orders of the loci (except among those bracketed) are ≥ 10^6-fold better than the next best order based on the likelihood analysis in the MAPMAKER Program (36, 37). Blood pressure difference at each locus refers to the blood pressure of homozygous SS minus the blood pressure of homozygous MM. For the unresolved loci in brackets, only the statistical data for one representative locus are given.

F_2 (S x WKY) POPULATION

Chromosome 10 Map	Blood Pressure Difference	One-Way ANOVA F	P
Ace	-0.3	0.1	0.91
5.8			
MIT-BAND3	+2.2	0.7	0.48
Ppp			
12.6			
Ngfrf	+10	1.5	0.22
9.5			
D11MIT119	+14.9	3.0	0.050
4.5			
Nos 2	+18.4	5.1	0.0070
7.3			
D11MIT4	+18.2	4.8	0.0099
2.9			
Mhcg	+15.6	4.0	0.020
8.3			
Grm6	+13.8	3.3	0.039

Figure 2. Genetic linkage map for rat chromosome 10 and statistical analysis for cosegregation of marker alleles with blood pressure in F_2(S × WKY) rats fed 8% NaCl diet. The numbers along the vertical line to the left of the map for chromosome 10 denote map units between loci in centiMorgans using the Haldane correction. The orders for the loci in a bracket cannot be accurately determined. But the orders of the loci (except among those bracketed) are ≥ 10^6-fold better than the next best order based on the likelihood analysis in the MAPMAKER Program (36, 37). Blood pressure difference at each locus refers to the blood pressure of homozygous SS minus the blood pressure of homozygous WW. For the unresolved loci in a bracket only the statistical data for one representative locus are given.

become unresolvable using cosegregation analysis in F_2(S × MNS). In this regard, *Ace* (30, 38, 49) and *Nos2*, which are 30 cM apart, are plausible candidate genetic loci for the two respective blood pressure QTL.

The concept that there are two blood pressure QTL on chromosome 10 is strongly supported by the fact that in the F_2(S × WKY) population, alleles of *Nos2* did ($P = 0.007$) but alleles of *Ace* did not ($P = 0.91$) cosegregate with blood pressure (Table II, Fig. 2). This implies that in the F_2(S × WKY) population only one of the two putative QTL on chromosome 10 is functionally variant between S and WKY. This contrasts with the significant cosegregation with blood pressure of alleles for both *Nos2* and *Ace* in the F_2(S × MNS) population, implying that both putative QTL on chromosome 10 are functionally variant between S and MNS. However, a definitive resolution of the two putative QTL on chromosome 10 awaits the construction of rat congenic strains, each of which contains a nonoverlapping chromosome fragment influencing blood pressure from this region.

Studies in some human populations did not find an association of the ACE locus with essential hypertension (50–52), whereas an insertion/deletion polymorphism of ACE showed an association with essential hypertension in another study population (53, 54). Likely explanations were the ACE locus might not be a blood pressure QTL in humans, or the ACE locus failed to cosegregate with blood pressure in the particular human populations tested due to the nature of population dependence in cosegregation analysis (30–33), or the association of the ACE alleles with essential hypertension could be an age-dependent phenomenon (55). However, the positive and strong cosegregation of the *Nos2* locus, ~ 30 cM away from Ace, with blood pressure in the F_2(S × MNS) and F_2(S × WKY) populations, indicates that a blood pressure QTL might be near or identical to *Nos2*. It is, therefore, logical to test the *NOS2* locus for cosegregation with blood pressure in human populations.

Acknowledgments

We thank Dr. H. Dene for helping submit sequences to GenBank, Dr. D. Ginn for an R genomic library, Dr. S. Snyder of Johns Hopkins University for a rat brain *Nos* cDNA probe, and Dr. C. Nathan of Cornell University for a mouse macrophage *Nos* cDNA probe.

This work was supported in part by grants from the National Institutes of Health.

References

1. Palmer, R. M. J., A. G. Fertige, and S. Moncada. 1987. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature (Lond.).* 327:524–526.

2. Ignarro, L. J., G. M. Buga, K. S. Wood, R. E. Byrns, and C. Chaudhuri. 1987. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc. Natl. Acad. Sci. USA.* 84:9265–9269.

3. Furchgott, R. F., and J. V. Zawadzki. 1980. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature (Lond.).* 288:373–376.

4. Marletta, M. A., P. S. Yoon, R. Iyenger, C. D. Leaf, and J. S. Wishnok. 1988. Macrophage oxidation of L-arginine to nitrite and nitrate: nitric oxide is an intermediate. *Biochemistry.* 27:8706–8711.

5. Palmer, R. M. J., D. S. Ashton, and S. Moncada. 1988. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature (Lond.).* 333:664–666.

6. Sakuma, I., D. J. Stuehr, S. S. Gross, C. Nathan, and R. Levi. 1988. Identification of arginine as a precursor of endothelium-derived relaxing factor. *Proc. Natl. Acad. Sci. USA.* 85:8664–8667.

7. Nathan, C. 1992. Nitric oxide as a secretory product of mammalian cells. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 6:3051–3064.

8. Bredt, D. S., P. M. Hwang, C. E. Glatt, C. Lowenstein, R. R. Reed, and S. H. Snyder. 1991. Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase. *Nature (Lond.).* 351:714–718.

9. Xie, Q., H. J. Cho, J. Calaycay, R. A. Mumford, K. M. Swiderek, T. D. Lee, A. Ding, T. Troso, and C. Nathan. 1992. Cloning and characterization of inducible nitric oxide synthase from mouse macrophages. *Science (Wash. DC).* 256:225–228.

10. Lamas, S., P. A. Marsden, G. K. Li, P. Tempst, and T. Michel. 1992. Endothelial nitric oxide synthase: molecular cloning and characterization of a distinct constitutive enzyme isoform. *Proc. Natl. Acad. Sci. USA.* 89:6348–6352.

11. Sessa, W. C., J. K. Harrison, C. M. Barber, D. Zeng, M. E. Durieux, D. D. D'Angelo, K. P. Lynch, and M. J. Peach. 1992. Molecular cloning and expression of a cDNA encoding endothelial cell nitric oxide synthase. *J. Biol. Chem.* 267:15274–15276.

12. Forstermann, U., E. I. Closs, J. S. Pollock, M. Nakane, P. Schwarz, I. Gath, and H. Kleinert. 1994. Nitric oxide synthase isozymes: characterization, purification, molecular cloning, and functions. *Hypertension (Dallas).* 23:1121–1131.

13. Marletta, M. A. 1993. Nitric oxide synthase structure and mechanism. *J. Biol. Chem.* 268:12231–12234.

14. Nunokawa, Y., N. Ishida, and S. Tanaka. 1993. Cloning of inducible nitric oxide synthase in rat vascular smooth muscle cells. *Biochem. Biophys. Res. Commun.* 191:89–94.

15. Wood, E. R., H. J. Berger, P. A. Sherman, and E. G. Lapetina. 1993. Hepatocytes and macrophages express an inducible cytokine inducible nitric oxide synthase gene. *Biochem. Biophys. Res. Commun.* 191:767–774.

16. Adachi, H., S. Iida, S. Oguchi, H. Ohshima, H. Suzuki, K. Nagasaki, H. Kawasaki, T. Sugimura, and H. Esumi. 1993. Molecular cloning of a cDNA encoding an inducible calmodulin-dependent nitric-oxide synthase from rat liver and its expression in COS-1 cells. *Eur. J. Biochem.* 217:37–43.

17. Kishimoto, J., N. Spurr, M. Liao, L. Lizhi, P. Emson, and W. Xu. 1992. Localization of brain nitric oxide synthase (NOS) to human chromosome 12. *Genomics.* 14:802–804.

18. Xu, W., I. G. Charles, S. Moncada, P. Gorman, D. Sheer, L. Liu, and P. Emson. 1994. Mapping of the genes encoding human inducible and endothelial nitric oxide synthase (NOS2 and NOS3) to the pericentric region of chromosome 17 and to chromosome 7, respectively. *Genomics.* 21:419–422.

19. Marsden, P. A., H. H. Q. Heng, C. L. Duff, X. M. Shi, L. C. Tsui, and A. V. Hall. 1994. Localization of the human gene for inducible nitric oxide synthase (NOS2) to chromosome 17q11.2-q12. *Genomics.* 19:183–185.

20. Robinson, L. J., S. Weremowicz, C. C. Morton, and T. Michel. 1994. Isolation and chromosomal localization of the human endothelial nitric oxide synthase (NOS3) gene. *Genomics.* 19:350–357.

21. Naduad, S., A. Bonnardeaux, M. Lathrop, and F. Soubrier. 1994. Gene structure, polymorphism and mapping of the human endothelial nitric oxide synthase gene. *Biochem. Biophys. Res. Commun.* 198:1027–1033.

22. Marsden, P. A., H. H. Q. Heng, R. J. Schereer, A. V. Hall, X. M. Shi, L. C. Tsui, and K. T. Schappert. 1993. Structure and chromosomal localization of the human constitutive endothelial nitric oxide synthase gene. *J. Biol. Chem.* 268:17478–17488.

23. Gerling, I. C., A. E. Karlsen, H. D. Chapman, H. U. Anderson, E. Boel, J. M. Cunningham, J. Nerup, and E. H. Leiter. 1994. The inducible nitric oxide synthase gene, *Nos2,* maps to mouse chromosome 11. *Mamm. Genome.* 5:318–320.

24. Deng, A. Y., L. Gu, J. P. Rapp, C. Szpirer, and J. Szpirer. 1994. Chromosomal assignment of 11 loci in the rat by mouse-rat somatic hybrids and linkage. *Mamm. Genome.* 5:712–716.

25. Moncada, S., R. M. Palmer, and E. A. Higgs. 1991. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.* 43:109–142.

26. Furchgott, R. F., and P. M. Vanhoutte. 1989. Endothelium-derived relaxing and contracting factors. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 3:2007–2018.

27. Ignarro, L. J. 1990. Biosynthesis and metabolism of endothelium-derived nitric oxide. *Annu. Rev. Pharmacol. Toxicol.* 30:535–560.

28. Snyder, S. H. 1992. Nitric oxide: first in a new class of neurotransmitters. *Science (Wash. DC).* 257:494–496.

29. Rees, D. D., R. M. J. Palmer, and S. Moncada. 1989. Role of endothelium-derived nitric oxide in the regulation of blood pressure. *Proc. Natl. Acad. Sci. USA.* 86:3375–3378.

30. Deng, Y., and J. P. Rapp. 1992. Cosegregation of blood pressure with angiotensin converting enzyme and atrial natriuretic peptide receptor genes using Dahl salt-sensitive rats. *Nature Genet.* 1:267–272.

31. Deng, A. Y., H. Dene, M. Pravenec, and J. P. Rapp. 1994. Genetic mapping of two new blood pressure quantitative trait loci in the rat by genotyping endothelin system genes. *J. Clin. Invest.* 93:2701–2709.

32. Deng, A. Y., H. Dene, and J. P. Rapp. 1994. Mapping of a quantitative trait locus for blood pressure on rat chromosome 2. *J. Clin. Invest.* 94:431–436.

33. Rapp, J. P., H. Dene, and A. Y. Deng. 1994. Seven renin alleles in rats and their effect on blood pressure. *J. Hypertens.* 12:119–125.

34. Ginn, D. I., C. A. C. Baptista, K. Y. Alam, A. Y. Deng, H. Dene, H. Le, T. W. Kurtz, and J. P. Rapp. 1994. Genetic analysis of α2-adrenergic receptors and blood pressure using Dahl salt-sensitive rats. *J. Hypertens.* 12:127–135.

35. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

36. Lander, E., P. Green, J. Abrahamson, A. Barlow, M. J. Daly, S. E. Lincoln, and L. Newburg. 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics.* 1:174–181.

37. Lander, E., and D. Bostein. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics.* 121:185–199.

38. Jacob, H. J., K. Lindpaintner, S. E. Lincoln, K. Kusumi, R. K. Bunker, Y. Mao, D. Ganten, V. Dzau, and E. S. Lander. 1991. Genetic mapping of a gene causing hypertension in the stroke-prone spontaneously hypertensive rat. *Cell.* 67:213–224.

39. Levan, G., J. Szpirer, C. Szpirer, K. Kinga, C. Hanson, and M. Q. Islam. 1991. The gene map of the Norway rat (*Rattus norvegicus*) and comparative mapping with mouse and man. *Genomics.* 10:699–718.

40. Yamada, J., T. Kuramoto, and T. Serikawa. 1994. A rat genetic linkage map and comparative maps for mouse or human homologous rat genes. *Mamm. Genome.* 5:63–83.

41. Chen, P. Y., and P. W. Sanders. 1991. L-Arginine abrogates salt sensitive hypertension in Dahl/Rapp rats. *J. Clin. Invest.* 88:1559–1567.

42. Sawada, Y., T. Sakamaki, T. Nakamura, K. Sato, Z. Ono, and K. Murata. 1994. Release of nitric oxide in response to acetylcholine is unaltered in spontaneously hypertensive rats. *J. Hypertens.* 12:745–750.

43. Chen, P. Y., and P. W. Sanders. 1993. Role of nitric oxide synthesis in salt-sensitive hypertension in the Dahl/Rapp rats. *Hypertension (Dallas).* 22:812–818.

44. Radomski, M. W., R. M. J. Palmer, and S. Moncada. 1990. Glucocorticoids inhibit the expression of an inducible, but not the constitutive, nitric oxide synthase in vascular endothelial cells. *Proc. Natl. Acad. Sci. USA.* 87:10043–10047.

45. Daghigh, F., J. M. Fukoto, and D. E. Ash. 1994. Inhibition of rat liver arginase by an intermediate in NO biosynthesis N^{-G}-hydroxy-L-arginine: implications for the regulation of nitric oxide biosynthesis by arginase. *Biochem. Biophys. Res. Commun.* 202:174–180.

46. Tonolo, G., R. Frasker, J. M. C. Connell, and C. J. Kenyon. 1988. Chronic low-dose infusions of dexamethonasone in rats: effects on blood pressure, body weight and plasma atrial natriuretic peptide. *J. Hypertens.* 6:25–31.

47. Hattori, Y., E. B. Campbell, and S. S. Gross. 1994. Argininosuccinate synthetase mRNA and activity are induced by immunostimulants in vascular smooth muscle. *J. Biol. Chem.* 269:9405–9408.

48. Gu, L., H. Dene, A. Y. Deng, and J. P. Rapp. 1994. Mapping of two blood pressure quantitative trait loci on rat chromosome 1. *Hypertension (Dallas).* 24:373. (Abstr.)

49. Hilbert, P., K. Lindpaintner, J. S. Beckmann, T. Serikawa, F. Soubrier, C. Dubay, P. Cartwright, B. De Gouyon, D. Julier, S. Takahasi, et al. 1991. Chromosomal mapping of two genetic loci associated with blood-pressure regulation in hereditary hypertensive rats. *Nature (Lond.).* 353:521–529.

50. Jeunemaitre, X., R. P. Lifton, S. C. Hunt, R. R. Williams, and J. M. Lalouel. 1992. Absence of linkage between the angiotensin converting enzyme locus and human essential hypertension. *Nature Genet.* 1:72–75.

51. Schmidt, S., I. M. S. van Hooft, D. E. Grobbee, D. Ganten, and E. Ritz. 1993. Polymorphism of the angiotensin I converting enzyme gene is apparently not related to high blood pressure: Dutch Hypertension and Offspring Study. *J. Hypertens.* 11:345–348.

52. Harrap, S. B., H. R. Davidson, J. M. Connor, F. Soubrier, P. Corvol, R. Fraser, C. Foy, and C. M. Watt. 1993. The angiotensin I-converting enzyme gene and predisposition to high blood pressure. *Hypertension (Dallas).* 21:455–460.

53. Zee, R. Y. L., Y-K. Lou, L. R. Griffiths, and B. J. Morris. 1992. Association of a polymorphism of the angiotensin I-converting enzyme gene with essential hypertension. *Biochem. Biophys. Res. Commun.* 184:9–15.

54. Morris, B. J., R. Y. L. Zee, L.-H. Ying, and L. R. Griffiths. 1993. Independent marked associations of alleles of the insulin receptor and dipeptidyl carboxypeptidase-1 genes with essential hypertension. *Clin. Sci. (Lond.).* 85:189–195.

55. Morris, B. J., and R. Y. L. Zee. 1994. Dipeptidyl carboxypeptidase-1 gene polymorphism paradox in hypertension explained by "deletion depletion." *J. Hypertens.* 12:1199–1201.

56. Kuramoto, T., T. Maihara, M. Masu, S. Nakanishi, and T. Serikawa. 1994. Gene mapping of NMDA receptors and metabotropic glutamate receptors in the rat (*rattus norvegicus*). *Genomics.* 19:358–361.

57. Kunieda, T., E. Kobayashi, M. Tachibana, H. Ikadai, and T. Imamichi. 1992. Localization of the interleukin-3 gene to rat chromosome 10 by linkage analyses with a variable number of tandem repeats (VNTR). *Mamm. Genome.* 3:464–466.