Consomic strategies to localize genomic regions related to vascular reactivity in the Dahl salt-sensitive rat

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Consomic strategies to localize genomic regions related to vascular reactivity in the Dahl salt-sensitive rat. Mol Gen Genomics 26: 218–225, 2006. First published June 13, 2006; doi:10.1152/physiolgenomics.00004.2006.—Chromosomal substitution strains afford the opportunity to discover regions of the rat genome that contain genes related to cardiovascular traits with the long-range goal of linking these genes to physiological function. PhysGen (Programs for Genomic Applications) created a consomic panel of rats derived from the introgression of a single chromosome (≥95% of the BN chromosome, one at a time) of the Brown Norway (BN/NHsd-Mcwi) rat onto the homogeneous genetic background of the Dahl salt-sensitive rat (SS/JrHsdMcwi). For 3 wk before the experiment, the rats were maintained on a low-salt diet (0.4% NaCl). The dose response of aortic rings from each strain of rat to phenylephrine, acetylcholine, sodium nitroprusside, and three different levels of tissue bath hypoxia (10, 5, and 0% O2) was measured and compared with the parental SS rat. To maximize the possibility that differences among the strains would become apparent, each strain of rat including the parental SS and BN was also studied after being maintained on a high-salt diet (4.0% NaCl) for 3 wk. If the response of the aortic ring from a consomic strain to these vasoactive substances was different from that of the SS parental strain, it was concluded that the introgressed chromosome contained a gene or genes that contributed to that difference. Because the BN chromosome is removed from its native background and the SS rat loses a native chromosome, it is necessary to consider a contribution of changes in gene-to-gene interaction.

aortic ring; high-salt diet; chromosomal substitution

Numerous studies have attempted to elucidate the relative contributions of genetic background and environment on the development of hypertension and its associated phenotypes. However, there are many obstacles to segregating the influences of these factors, especially in complex polygenic diseases (e.g., hypertension) in human populations. This is because multiple genes, genetic heterogeneity, moderate gene penetrance, and environmental interactions each play a role in the differential expression of phenotypes. A widely used approach to addressing these issues is the use of inbred rat strains, since the rat is a well-recognized model for studying physiological traits. This is due, in part, to a high degree of conserved gene order between rat and human. In addition, disease genes identified in the rat can be predicted to be in regions of synteny (homologous chromosomes) with humans and mice (6). Furthermore, inbred rat strains minimize the effects of genetic heterogeneity in determining physiological phenotypes.

The goal of PhysGen, the National Heart, Lung, and Blood Institute-funded Program for Genomic Applications (PGA) study at the Medical College of Wisconsin (MCW), is to link genes to physiological function to identify chromosomes that contain a gene or genes that code for proteins that are involved in the regulation of cardiovascular phenomena. The ultimate goal of this strategy is to gain insight into the identity of genes and proteins that may be implicated in the development and maintenance of hypertension and its related phenotypic traits.

The parental strains of rats used in this study, Dahl salt-sensitive (SS/JrHsdMcwi) and Brown Norway (BN/NHsd-Mcwi) rats, express very different cardiovascular phenotypes. The SS rat develops a low-renin form of hypertension when fed a high-salt diet and is also insulin resistant and hyperlipidemic (7, 23, 30, 35, 40). In contrast, the BN rat is normal for these phenotypes. PhysGen has developed a panel of chromosomal substitution strains (consomic panel) of these two parental strains in which single chromosomes from the BN rat (≥95% of the BN chromosome) are introgressed onto the homogeneous genetic background of the SS rat, one chromosome at a time (6). These consomic rats are then identified as SS-NBN with “N” identifying the BN chromosome that was introgressed (http://pga.mcw.edu/). In a panel of consomic rats, it is possible to assess the contribution of genes on each chromosome by phenotyping the consomic strain for the traits of interest, using the advantage of the uniform genetic background. Because each strain is genetically equivalent to the SS parental strain except for the introgressed chromosome, the consomic rat is an ideal control animal for the parental rat (in this case the SSMcwi). A few of the rat strains studied in the present investigation were congenic strains in which a portion of the BN chromosome was introgressed into the SS genetic background. The nomenclature for these rats identifies the segment of the BN chromosome that was introgressed.

A common denominator in hypertension (human or experimental) is an increase in vascular resistance that maintains the elevation in mean arterial pressure in the face of a cardiac output that is within normal limits (5). An enhanced constriction to stimuli such as angiotensin II, norepinephrine, endothelin, and increased tissue oxygen delivery (3, 13, 15, 24, 27, 34) and an impaired relaxation of blood vessels to vasodilator stimuli such as acetylcholine (ACh) and hypoxia (1, 16, 17, 29, 31–33) have been observed in microvessels, resistance arteries, and conduit vessels of genetic and experimentally induced hypertension in animals. Studies of isolated middle cerebral arteries (MCAs) and skeletal muscle resistance arteries of the SS rat (10, 18) and studies of the in situ microcirculation of the SS rat (1–2, 9, 34) demonstrate that these vessels have an...
vascular reactivity of consomic strains of Dahl SS rat

impaired relaxation to hypoxia, ACh, and sodium nitroprusside (SNP) and an enhanced vasoconstriction in response to elevated oxygen levels in the superfusion solution. These changes in vascular reactivity of course could either contribute to or result from the high blood pressure. In addition, the factors that influence these changes in vascular tone and the mechanisms of the changes of vascular reactivity induced by a high-salt diet are not fully known.

The vascular protocol of PhysGen was designed to characterize the responses of aortic rings from a complete panel of consomic rats and their parental strains (SS/JrHsdMcwi and BN/NHsdMcwi) to phenylephrine (PE), ACh, SNP, and reduced Po2. High-throughput studies of these aortic rings using established and widely employed experimental protocols for force measurements in isolated aortic segments (14, 19, 21, 25, 26, 36, 39, 42) provide a potent tool that could permit the localization of the genes that are important in the regulation of vascular reactivity. In addition, since each strain of rat was on a low-salt (0.4% NaCl) and a high-salt diet (4.0% NaCl) we could study the contribution of chromosomes to salt-induced changes in vascular reactivity. Since January 2001, we have studied 19 different strains of male rats from a consomic panel created from a SS/JrHsdMcwi and BN/NHsdMcwi cross.

This work was a beginning effort to identify the chromosomal location of genes that might play a role in the vascular response to vasoconstrictors and vasodilators. In this study, aortic rings from each group of parental and consomic rats were studied after the rats were maintained for 3 wk on a low-salt diet (unstressed condition) or a high-salt diet (stressed condition). The high-salt diet was intended to increase the likelihood that differences among strains would become apparent and unmask linkages between genes and function. In addition, it has been demonstrated that a high-salt diet per se has major effects on reactivity of arterioles, resistance vessels, and aortas of rats, e.g., an increased constriction to elevated O2, a decreased dilation to ACh and hypoxia, and a decreased production of nitric oxide (17, 28, 37, 41, 43).

METHODS

Animals. All rats (n = 304 for low salt and n = 303 for high salt) were produced and housed at the MCW Animal Resource Transgenic Barrier Facility. Rats were cared for according to established guidelines for the care and use of laboratory animals (National Institutes of Health, 1996). The rats were part of a consomic rat panel, in which each full-length chromosome (95% of the chromosome) from the inbred BN rat was substituted one at a time into the homogeneous background of the inbred SS rat. In this case, BN and SS rats were mated to create a heterozygotic F1 generation. F1 progeny were backcrossed to the SS parental rat to obtain the SS genetic background. Offspring from this mating that were determined by genotyping to be heterozygotic for the target chromosome (i.e., the chromosome to be introgressed into parental genomic background) were backcrossed with the SS parental strain for four to eight generations using marker-assisted selection to keep the target chromosome heterozygous. Brother-sister intercrosses of these rats yielded rats that were homozygous at the target chromosome (BN/BN) and homozygotic for SS at all other chromosomes (6). (Note: For a complete genotype of each consomic and congenic rat we direct the reader to http://pga.mcw.edu/.)

Rats used in the present PhysGen study were housed in an American Association for Accreditation of Laboratory Animal Care-accredited animal care facility, and all procedures received prior Institutional Animal Care and Use Committee approval. All rats were maintained from birth on 0.4% NaCl Teklad chow (3075S; Madison, WI). Three weeks before the study, at age 7 wk, one group of consomic rats (10 male rats of each consomic strain and 2 male parental SS rats for sentinels) were placed on a 4.0% NaCl diet (Teklad, TD01454). Another group of consomic rats and SS sentinels of the same strain and number were maintained on a low-salt Teklad diet for 3 wk before the study.

Aortic rings from two male SS rats were studied simultaneously with the aortic rings of each strain of 10 male rats. These sentinel rats allowed us to determine the internal consistency of the experimental protocol from strain to strain and week to week. Phenotypes were measured in four groups of the SS parental rat in four time periods over the year to account for any seasonal variations. The variations did not exceed normal variation.

Experimental protocol. Equipment used in these studies included a 16-tissue bath system with reservoirs and circulators (Radtomi Glass Technology, Monrovia, CA), Digi-Med tissue force analyzers (Micro Med DMSI-210; Louisville, KY), and Grass FT-03 force transducers. Gas tanks for delivery of 95%, 10%, 5%, and 0% oxygen mixtures (with 5% CO2-balance N2) were obtained from Praxair (Burlington, WI).

The 10-wk-old rats were anesthetized with an injection of pentobarbital sodium (60 mg/kg ip) to produce deep anesthesia. The chest of the rat was opened, and a 3- to 5-cm length of aorta was removed and placed in a labeled petri dish containing room temperature physiological salt solution (PSS, in mM: 119 NaCl, 4.7 KCl, 1.17 MgSO4·7H2O, 1.6 CaCl2·2H2O, 1.18 NaH2PO4, 24 NaHCO3, 0.03 EDTA, 5.5 glucose, and 5.0 HEPES). The remaining blood was washed from the tissue by gently moving it back and forth in the PSS. The ends of the aorta were pined to the Sylgard resin in a petri dish. Under a dissecting microscope, fat and connective tissue were removed and the ends of the vessel were cut away with fine #5 Dumont forceps and small Vannas scissors. The aorta was then divided into 3-mm-wide rings. Triangular wire holders were inserted through the lumen of the vessel and connected to the force transducer and tissue holder rod in the vessel bath. Two aortic rings were mounted in fresh PSS for each rat. One ring was mounted in the eight-bath setup for the contraction studies and another was mounted in the eight-bath setup for the relaxation studies. The remaining vessel rings were used as spares.

Preload and equilibration. The tension on the rings was adjusted to 1.5-g passive force using the tension adjustment dial for each transducer and allowed to equilibrate for 30 min in the bath with a 21% O2-5% CO2-balance N2 gas mixture. The rings were washed with fresh PSS every 10 min. Passive force was readjusted to 1.5 g as needed during this period. When rings were stable at 1.5 g of passive force, the baseline reading on the tissue force analyzers was set at 0 g.

Preconditioning of aortic rings. PE at a final concentration of 10−5 M was added to the bath to contract the ring, and force was allowed to stabilize for 5 min. Then ACh at a final concentration of 10−5 M was added to the precontracted rings to test for endothelial integrity (5 min). If a ring failed to contract in response to PE or to relax in response to ACh, it was replaced with one of the spare rings. After the initial test for vessel viability and endothelial integrity, the ring was washed three times with PSS, allowed to equilibrate, and then re-washed with fresh PSS at 10-min intervals until the measured active force stabilized at 0 g. The maximum contraction achievable by the ring was then determined by filling the bath with 80 mM K+ and adding PE at a final concentration of 10−5 M.

Maximal contractile force generated in response to the combination of 80 mM K+ and 10−5 M PE was normalized to the wet weight of the aortic ring (determined at the end of the experiment). After determining the maximum contraction of the aortic rings, we allowed the vessels to stabilize and washed them with PSS every 10 min until the measured active force returned to 0 g.
**Contraction protocol.** Figure 1A shows a typical concentration-response curve for PE. The contraction protocol was performed on a single aortic ring from each rat. Ten male rats from each strain were studied within a 1-wk period (maximum of eight rings per day). Cumulative concentration-response curves to PE were created by increasing the PE concentration in the tissue bath by successive addition of appropriate dilutions of stock solutions to achieve final bath concentrations of 1 nM to 300 µM PE. The rings were then washed with PSS, allowed to equilibrate, and rewashed with fresh PSS at 5- to 10-min intervals until active force returned to a stable value of 0 g.

**ACh and SNP protocol.** The relaxation protocol was performed on a single aortic ring from each rat. Ten male rats from each strain were studied within 1 wk (maximum of eight rings per day). With few exceptions, rings studied in the relaxation protocols were from the same animals studied for the contraction experiments.

Figure 1B shows a typical concentration-response curve generated for ACh. Cumulative concentration-response curves to ACh were created by increasing the ACh concentration in the tissue bath by successive addition of appropriate dilutions of stock solutions to achieve final bath concentrations of 1 nM to 30 µM ACh.

Figure 1C shows a typical dose response curve generated for SNP. Cumulative concentration-response curves to SNP were created by increasing the SNP concentration in the tissue bath by successive addition of appropriate dilutions of stock solutions to achieve final bath concentrations of 0.1 nM to 3 µM SNP. Aortic responses to SNP and hypoxia (see below) were not studied in several of the consomic strains because those two parts of the protocol were eliminated in 2002–2003 to expedite the movement of the large groups of rats demanded by the high-throughput design of the PGA.

**Hypoxic relaxation protocol.** The rings were precontracted with PE at a final concentration of 0.1 µM and allowed to stabilize at a maximum response (~10 min). Then the gas equilibration mixture in the tissue bath was changed from 21% O2-5% CO2-balance N2 to one containing 95% O2-5% CO2. After 10 min at 95% O2-5% CO2, the oxygen concentration in the bath was reduced in a step-wise fashion at 20-min intervals to mixtures containing 10% O2, 5% O2, and 0% O2, with 5% CO2 and the balance N2. To verify that the ring was viable at the end of the hypoxic relaxation protocol, the oxygen concentration in the bath was returned to 95% O2-5% CO2 for 20 min. If the aortic ring did not contract and develop a force approximately equal to the initial force development either in response to PE or 95% O2, the force values obtained during exposure to hypoxia were not included in the data summary.

**Data and statistical analyses.** Analog-to-digital conversions of force waveforms were accomplished with a Digi-Med System Integrator model 210. The converted data were automatically transferred from the system integrator into a spread sheet and analyzed with GraphPad Prism software. All data are summarized as means ± SE. Differences between means were assessed by a conventional ANOVA or, if Levene’s test showed that the groups had unequal variances, by a nonparametric ANOVA. This was followed by Dunnett’s test to compare all consomic strains to the SS parental strain. P < 0.05 was considered to be statistically significant. Comparisons were between an individual strain and the SS parental strain on the same diet or between the same strain on a different diet. All the raw data for the consomic and congenic strains used in each protocol (vascular, renal, cardiac, respiratory, and biochemistry) of the PGA are available, along with statistical analyses, on the PhysGen website (http://pga.mcw.edu).

**RESULTS**

[Note: The SS-(D8rat163-D8rat81)BN discussed in the following is a congenic not a consomic strain. The segment of chromosome 8 that carries the BN extends from D8rat163-D8rat81[SS.BN-(D8rat163-D8rat81)]. This spans from 31,517,289 bp to 124,946,614 bp. The total length of chromosome 8 is 129,061,546 bp. We have defined a consomic as having substituted at least 95% of the chromosome; therefore, if we have missed >6.5 Mb of chromosome 8 (the first ~30 Mb in this case), we must define it as a congenic.]
Of the rats on a high-salt diet, the aortas of the SS-18BN, SS-XBN, SS-7BN, SS-(D8rat163-D8rat81)BN, SS-13BN, SS-15BN, SS-11BN, SS-10BN, SS-16BN, and SS-8BN were significantly more sensitive to PE than those of the SS, whereas the aortas of the BN and SS-17BN were significantly less sensitive to PE than those of the SS. Three weeks of a high-salt diet did not affect the sensitivity of the aortic rings to PE of the SS but significantly decreased the PE sensitivity in aortic rings of the SS-16BN. In contrast, aortic sensitivity to PE was increased by high-salt diet in the SS-18BN, SS-XBN, SS-(D8rat163-D8rat81)BN, SS-13BN, SS-15BN, SS-11BN, SS-10BN, SS-16BN, and the SS-19BN.

Comparison of the maximum force generated by the aortic rings is summarized in Figure 3. Aortic rings of the BN on a low-salt diet generated significantly more force per wet weight of aorta than the SS, whereas aortic rings of the SS-2BN, SS-11BN, SS-18BN, SS-3BN, SS-17BN, SS-15BN, SS-12BN, SS-19BN, and SS-5BN generated significantly less force. Of the rats on a high-salt diet, aortic rings of the BN and SS-XBN generated significantly more force per wet weight of aorta than the SS, whereas aortic rings of the SS-11BN, SS-5BN, SS-13BN, SS-3BN, SS-14BN, SS-4BN, SS-15BN, SS-12BN, SS-17BN, and SS-19BN generated significantly less force than those of the SS.

A high-salt diet reduced the maximum force obtained in the aortic rings of the BN and SS-17BN, SS-10BN, SS-4BN, SS-6BN, and SS-13BN, whereas the maximum force was significantly increased in the SS-XBN strain on a high-salt diet.

Sensitivity to ACh and SNP. Figure 4 compares the sensitivity of the aortic rings to ACh in the various strains. Aortic rings of the SS-16BN, SS-YBN rats on low-salt diet were more sensitive to ACh than the SS, whereas the SS-20BN, SS-9BN, SS-13BN, and strain were less sensitive to ACh than those of SS rats on low-salt diet. Of the rats on high-salt diet, aortic rings of the SS-(D8rat163-D8rat81)BN and SS-2BN were significantly more sensitive to ACh than those of the SS, whereas aortics of the SS-XBN, SS-13BN, SS-15BN, SS-10BN, SS-18BN, SS-4BN, and SS-20BN were less sensitive to ACh than those of the SS. Sensitivity increased in the aortic rings of the SS-8BN, SS-YBN and SS-5BN when the rats were fed a high-salt diet, while sensitivity to ACh decreased in the SS-YBN and SS-16BN.

The sensitivity of the aortic rings to SNP is summarized in Fig. 5. Aortic rings of the BN on low-salt diet were significantly more sensitive to SNP than those of the SS on low-salt diet. Aortic rings of the SS-16BN on low-salt diet were significantly less sensitive to SNP than those of the SS parental strain. Aortic rings of the SS-(D8rat163-D8rat81)BN and the SS-18BN rats on high-salt diet had an increased sensitivity to SNP compared with SS rats on high-salt diet. Aortic rings of the SS-(D8rat163-D8rat81)BN and the SS-18BN rats on high-salt diet were significantly less sensitive to SNP than those of the SS. A high-salt diet enhanced the sensitivity of the SS-16BN and SS-YBN strain to ACh, while it attenuated the reactivity in the SS-(D8rat163-D8rat81)BN strain.

Sensitivity to reduced PO2. Figure 6, A–C, summarizes the responses of the aortic rings to reduced PO2. For 10% O2 (Fig. 6A), the aortic rings of the SS-(D8rat163-D8rat81)BN, SS-9BN, and the BN on low-salt diet relaxed significantly more than those of the SS. Of the rats on high-salt diet, the aortic rings of SS-(D8rat163-D8rat81)BN and SS-9BN, and SS-2BN that had been on a high-salt diet for 3 wk were significantly less sensitive to hypoxia compared with their counterparts maintained on a low-salt diet. The aortic ring of the SS rat was more sensitive to 10% O2.
Hypertension is a complex cardiovascular disease, and it is a daunting task to determine the degree to which each of a multitude of interrelated and environmentally influenced molecular and biochemical pathways contributes to the pathologic rise in blood pressure. The overall purpose of the PhysGen studies, using chromosomal substitution techniques in the rat, is to expand our knowledge base related to the interrelationships among genes, environmental factors, and the regulatory systems that control blood pressure.

The specific purpose of the vascular protocol, described herein, was to uncover broad genomic regions that contain genes that may be involved in the regulation of vascular tone and the changes in vascular reactivity that occur in experimental models of hypertension when maintained on a high-salt diet. The summarized data presented here are derived from a first-pass high-throughput screening of several consomic strains of male rats and their parental strains. As such, these studies should provide an important first step in linking phenotypic data to genomic data.

Overall, the results of this study can be interpreted in two ways. The first is as an evaluation of the relative contributions of the BN chromosomes to the control of the responses of aortic smooth muscle to selected vasodilators and vasoconstrictors in the rat, independent of the BN genomic background. The second is as an evaluation of the effect of the removal of a single parental SS chromosome on vascular reactivity. However, caution must be exercised in the interpretation and analysis of these data. Because the BN chromosome is introgressed onto a different chromosomal background and the normal SS chromosomal background loses a native chromosome, either the gain of a new gene or the loss of native gene-to-gene interactions could contribute to the differences between the consomic and the parental strains. Nonetheless, the SS-NBN consomic strains provide a powerful control animal compared with the parental SS, since the only difference between the SS parental strain and the consomic strain is the introgressed chromosome. The SS-13BN has only a 1.95% allelic variation from the parental SS/JrHsdMcwi strain (6) yet exhibits striking differences in several physiological phenotypes. For example, the substitution of BN/Mcwi chromosome 13 on the isogenic background of the inbred SS/JrHsdMcwi rat attenuates the rise of arterial pressure and associated microalbuminuria observed with a high-salt diet in the parental SS/JrHsdMcwi (8) and restores the dilation of MCAs in re-
response to several vasodilator stimuli that fail to cause vascular relaxation in SS rats on a low-salt diet (10).

The amount of data gathered in these experiments can be overwhelming and has the potential to be bewildering. However, if the data are analyzed in discrete amounts, they can be used effectively to design macro- and microvascular experiments that help elucidate the changes in vascular reactivity that often accompany salt-sensitive hypertension and high-salt diets alone. Drenjancevic-Peric and coworkers (9–12) have conducted several studies of the microcirculation and resistance arteries of the SS parental, the SS-13BN, and the SS-16BN strains and report substantial differences in the reactivity of skeletal muscle arterioles and cerebral resistance arteries between the consomic strains and the SS parental rat. For example, in situ arterioles of the cremaster muscle of the SS rat on a 4.0% NaCl diet for 4 wk were significantly less sensitive to ACh than those of the SS rat on a 0.4% NaCl diet and those of the SS-13BN on the high-salt diet. The high-salt diet did not affect the response of cremasteric arterioles to ACh in the SS-13BN (9). The MCA of the SS rat on a low-salt diet did not relax to either ACh or 0% oxygen in the bath, in contrast to the MCA of the SS-13BN. However, the MCAs from the SS-13BN fed a high-salt diet for 3 days lost the ability to relax to ACh and hypoxia (10). It is also interesting to note that introgression of the BN chromosome 16 onto the SS background did not restore the relaxation to hypoxia or ACh in the corresponding consomic rats, indicating that some gene or genes particular to chromosome 13 are involved in the differences in the vascular response to ACh and hypoxia between the SS and the consomic SS-13BN (10). Previous studies have demonstrated that the SS rat has an impaired ability to regulate the renin-angiotensin system (RAS) (8, 20). Chromosome 13 from the BN rat contains a normally functioning renin gene (8), and the authors of the previously cited studies (9–12) indicated that the results of their experiments suggest that a properly functioning RAS is necessary to maintain normal vascular relaxation mechanisms.

These studies offer important insights into the cellular mechanisms related to vasoreactivity that may be altered in salt-sensitive hypertension. They also demonstrate the value of the consomic model in identifying genomic regions that may contain a gene or genes that code for proteins that regulate vascular responses.

In this regard, it is of interest to note that the responses to ACh in the aortas of the SS and SS-13BN do not parallel the responses of the resistance arterioles of these strains. The aortic ring of the SS rat on a low-salt diet was more sensitive to ACh than that of the SS-13BN on a low-salt or a high-salt diet (Fig. 4), and the maximum relaxation to ACh was greater in the aortic ring of the SS on a low-salt diet than that of the SS-13BN (data not shown). A high-salt diet did not affect the aortic response to ACh in either strain. The sensitivity of the BN aortic ring to ACh was not different from the SS rat when maintained on a low-salt diet, and aortas of BN rats were less sensitive to ACh than the SS when maintained on a high-salt diet.
diet. MCAs of the SS-16BN rats constricted to ACh (10), but aortic rings of the SS-16BN relaxed to ACh and were significantly more sensitive to ACh than those of the SS parental strain. These regional differences in vascular responses to ACh could reflect the fact that although ACh-induced dilation is endothelium dependent and generally mediated by the release of nitric oxide, other mechanisms have been suggested to contribute to ACh-induced relaxation, including arachidonic acid metabolites and endothelium-derived relaxing or hyperpolarizing factor (4). In addition, the regulation of endothelial and smooth muscle responses could vary very well be different from vascular bed to vascular bed (22, 38).

The results of the present study are a first step in the localization of genes that may be involved in the response of vascular smooth muscle to vasoconstrictors and vasodilators. Substantial work remains before the relationship between functional pathways and specific genes are established. Nonetheless, the present studies are an important first attempt to link genes with known physiological functions, in this case, the complex pathways that regulate vascular tone and vascular sensitivity to vasoconstrictor and vasodilator stimuli. The chromosomal substitution technique has provided some broad genomic regions that are likely to contain genes that are involved in those pathways.

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GRANTS

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