Salt Susceptibility Maps to Chromosomes 1 and 17 With Sex Specificity in the Sabra Rat Model of Hypertension

Chana Yagil, Marina Sapojnikov, Reinhold Kreutz, Gurion Katni, Klaus Lindpaintner, Detlev Ganten, Yoram Yagil

Abstract—Random genome screening was initiated in the Sabra rat model of hypertension in search of genes that account for salt sensitivity or salt resistance in terms of the development of hypertension. Female salt-sensitive Sabra hypertension-prone (SBH/y) rats were crossed with male salt-resistant Sabra hypertension-resistant (SBN/y) rats, resulting in an F2 cohort consisting of 100 males and 132 females. Systolic blood pressure (BP) was measured in rats at 6 weeks of age under basal conditions and after 4 weeks of salt loading. Genotypes for 24 polymorphic microsatellite markers localized to chromosome 1 and for 8 markers localized to chromosome 17 were determined in F2 and cosegregation with BP was evaluated by ANOVA and multipoint linkage analysis. Basal BP did not cosegregate with any locus on chromosomes 1 or 17. In contrast, BP after salt loading showed significant cosegregation with three QTLs, two on chromosome 1 and one on chromosome 17, designated SS1a, SS1b, and SS17, respectively; the maximal logarithm of the odds (LOD) scores were 4.71, 4.91, and 3.43, respectively. Further analysis revealed sexual dimorphism. In male F2, BP response to salt loading cosegregated with one QTL (LOD score 4.52) and a second QTL (LOD score 2.98), both on chromosome 1 and coinciding with SS1a and SS1b, respectively. In female rats, BP response cosegregated with one QTL on chromosome 1 (LOD score 3.08) coinciding with SS1b, and with a second QTL on chromosome 17 (LOD score 3.66) coinciding with SS17. In males, the additive effects of the two QTLs on chromosome 1 accounted for most of the BP variance to salt loading, whereas in females the additive effects of the QTLs on chromosomes 1 and 17 accounted for over two thirds of the variance. These results identify three putative gene loci on chromosomes 1 and 17 that contribute importantly to salt sensitivity and/or resistance and uncover sex specificity in the role that salt susceptibility genes fulfill in the development of hypertension. (Hypertension. 1998;31[part 1]:119-124.)

Key Words: salt susceptibility ■ rats ■ sex ■ genome screen ■ microsatellites ■ genes ■ quantitative trait loci ■ linkage analysis

Among the environmental factors that contribute to the variance in BP in the general population and to the development of hypertension is the composition of dietary intake in general and the amount of salt intake in particular. The BP response to dietary salt intake varies widely, and at the two ends individuals are categorized either as salt sensitive or salt resistant. Experimental and clinical data suggest that salt sensitivity and salt resistance represent inherited phenotypic traits that are determined by “salt susceptibility genes,” that is, genes which, depending on the molecular variant present, modify the effect of the environmental exposure to dietary salt. The limited ability to study the relation between salt and hypertension in humans and the observation that salt sensitivity and resistance are genetically transmitted led to the development of experimental models of salt susceptibility that have served as useful investigational tools. Currently, the two major genetic models that are available for studies of salt sensitivity and salt resistance are inbred rat strains derived from the original Dahl and Sabra colonies. Earlier, primarily comparative studies in these models of salt susceptibility attempting to identify the genetic basis of salt susceptibility have more recently been supplemented by programmed breeding experiments and cosegregation studies, using updated molecular genetic tools. Such experiments have been carried out so far primarily in the Dahl-derived rats: Crosses between the Dahl rat and other strains have revealed BP-relevant QTLs on chromosomes 1, 2, 3, 5, 7, 9, 10, 13, and 17. Which of these QTLs relates to salt susceptibility per se, however, remains unclear because the Dahl-derived rats, in addition to expressing salt sensitivity and resistance, develop spontaneous hypertension as a function of age, even in the absence of dietary salt exposure. It is thus difficult to differentiate from these crosses between QTLs that encode genes for salt susceptibility from QTLs that encode genes for spontaneous hyper-
tension. These and other considerations have led us to initiate investigation of the genetic basis of salt susceptibility in the other major model of salt-related hypertension, the Sabra rats, which have the distinct advantage of developing hypertension only after salt loading without ever developing spontaneous hypertension. The fact that in the absence of salt loading BP remains unchanged throughout the lifetime of both SBH/y and SBN/y rats, the highly predictable BP response to salt loading of each strain, and the availability of a large number of polymorphic markers among the salt-sensitive SBH/y and salt-resistant SBN/y strains enabled and favored the use of these specific strains for efficient and informative cosegregation studies of salt susceptibility.

In the current study, we randomly screened the genome of the Sabra rat in search of genes that cosegregate with salt sensitivity or salt resistance in terms of the development of hypertension. We present our findings with respect to chromosomes 1 and 17, in which significant cosegregation was found with the blood pressure response to salt.

Methods

Animals
SBH/y and SBN/y rats from the colony inbred at the Barzilai Medical Center (Ashkelon, Israel) were used for the studies detailed below. Breeding of animals for genetic analyses was initiated by crossing seven female SBH/y rats with two male SBN/y rats. F1 progeny were crossed to produce F2 rats. The F2 population was weaned at 1 month of age.

Animals were housed in the Center’s animal facility in strict compliance with institutional regulations and with the guidelines set forth by the American Physiological Society. Climate-controlled conditions were maintained and temperature was set at 22°C to 24°C. Regular 12-hour diurnal cycles were kept with the use of an automated light switching device. Unless stated otherwise, tap water and standard rat chow containing 0.65% NaCl (Kosfof) were provided ad libitum.

Phenotyping
When the rats were 6 weeks old, basal BP (on standard chow and before salt loading) was measured. Animals were then salt loaded by subcutaneous implantation in the back of the neck of a 25 mg desoxycorticosterone acetate pellet (Innovative Research) and providing 1% NaCl as drinking water (hereforth salt loading) and standard rodent chow ad libitum. After 4 weeks of salt loading, BP was measured for the second time.

BP (systolic) was measured at ambient temperature (27°C to 28°C) in awake animals by the tail-cuff method with the use of an IITC photoelectric oscillatory detection device (IITC Life Science), as previously described. At least three replicate BP measurements were obtained on 3 consecutive days (ie, at least 9 measurements over 3 days) at both baseline and after salt loading. The average of all measurements at each experimental time-point was taken as representative of systolic BP.

DNA Extraction
Genomic DNA was prepared from tail clipping of each animal by salt precipitation, followed by phenol-chloroform cleaning. Purity and quantity of extracted DNA were assessed spectrophotometrically (GeneQuant II, Pharmacia Biotech).

Microsatellite Markers
Microsatellite markers for chromosomes 1 and 17 were obtained from Research Genetics. Of the 40 rat chromosome 1 markers tested, 20 produced polymorphic bands when amplified from SBH/y and SBN/y rats. Four mouse chromosome 7 markers that map to rat chromosome 1 by homology were also informative for our strains. Thus a total of 24 microsatellite markers were available for mapping on chromosome 1 of the F2 cross. Of the 15 rat chromosome 17 markers tested, 8 were polymorphic for our strains and thus available for mapping of chromosome 17.

Genotyping
Genotyping was carried out by polymerase chain reaction (PCR) amplification, as previously described. In brief, genomic DNA (50 ng) was amplified by PCR in a final reaction volume of 10 μL containing 100 nmol/L of each primer, 200 nmol/L dNTP, 1.5 mmol/L MgCl2, and 0.05 U of Taq DNA polymerase (Promega). The forward primer was labeled with 32P-ATP (Dupont, NEN) using T4 polynucleotide kinase (Promega). The PCR reactions were processed on an MJ Research PTC 100 thermal cycler, using the following protocol: Initial denaturation at 92°C for 3 minutes, followed by 34 cycles of denaturation at 92°C for 15 seconds, annealing for 1 minute at a temperature between 50°C and 55°C (depending on primer characteristics), extension at 72°C, followed by a final extension step at 72°C for 7 minutes. To each reaction tube, 10 μL of denaturing loading buffer was added, followed by heating at 94°C for 5 minutes and snap-cooling on ice. The product of each reaction (3 μL) was loaded onto a 7% polyacrylamide gel containing 37.5% formamide, 8 mol/L urea, 90 mmol/L Tris-borat, and 2 mol/L EDTA. Gels were run with the use of a Base Ace apparatus (Stratagene) at 60 W (Feathervolt 3000, Stratagene) for 4 hours and exposed to Kodak XAR-5 film for autoradiography.

Linkage and Statistical Analysis
Basal BP is consistently 12 mm Hg higher in SBH/y than in SBN/y, and the BP response to salt loading differs between SBH/y and SBN/y by 50 mm Hg. The data were therefore analyzed to determine if (a) basal BP and (b) BP after salt loading in the F2 progeny cosegregated with the chromosome 1 and 17 markers tested. Analysis was for the entire F2 cohort for effects of genotype and sex by two-way ANOVA (Complete Statistical Software, StatSoft) and after stratification by sex by one-way test. Summary data are represented as mean±SD. Multipoint linkage analyses were carried out with the MAPMAKER/EXP 3.0 and MAPMAKER/QTY 1.1 programs obtained from Dr Eric Lander (Whitehead Institute, Cambridge, Mass), using the free default model for calculations.

Results
The F2 cohort resulting from the cross between SBH/y and SBN/y rats consisted of 232 animals (132 female and 100 male rats). Blood pressure data are provided separately for values obtained at baseline and after salt loading, for the overall F2 cohort, and for females and males separately. Linkage maps for the chromosome 1 and 17 data for the entire F2 cohort are shown in Fig 1 and Fig 3, respectively. LOD tracings for chromosomes 1 and 17 for the entire cohort and for males and females in separate are shown in Fig 2 and Fig 4, respectively. BP values by genotype at microsatellite markers demarcating the respective QTLs are shown in the Table. Genotype distributions at all molecular markers tested were in general in
the expected proportions of 1:1:2 for the two homozygous variants and the heterozygous state, respectively.

**Chromosome 1**

**Basal BP**

Analysis of basal BP by genotype for the entire F2 cohort at each of the tested chromosome 1 marker loci revealed no cosegregation with any of the markers tested, neither by ANOVA nor by analysis with MAPMAKER/QTL (data not shown).

**BP Response to Salt Loading**

Analysis by ANOVA revealed on chromosome 1 significant cosegregation of 12 microsatellite markers (demarcated by D1Mgh2 and D1Mgh9) with BP after salt loading. Two peaks were detected, one at CYPE and the other at D1Mit12 (Fig 1). Multipoint linkage analysis of the entire F2 cohort using the MAPMAKER/QTL programs detected two distinct QTLs (Figs 1 and 2). One QTL is demarcated by D1Mit8 and D1Mit11, it has a peak LOD score of 4.71 between D1Mit1 and D1Mgh5, and spans over 64.9 cm (max LOD score ± 1). This QTL, henceforth referred to as SS1a, accounts for 12.6% of the explained genetic variance and 43% of the difference in the BP response to salt loading between SBH/y and SBN/y.

The second QTL is demarcated by D1Mgh7 and D1Mgh8, it has a peak LOD score of 4.91 between D1Mit2 and D1Mit12, and spans over 17.2 cm. Henceforth referred to as SS1b, this QTL accounts for 10.4% of the explained genetic variance and 35% of the difference in the BP response to salt loading between SBH/y and SBN/y.

Analysis of the BP data after stratification by sex revealed unexpected sexual dimorphism. In the male F2 progeny, ANOVA showed significant cosegregation with the BP response to salt loading of a region demarcated by the microsatellite markers D1Mit1 and D1Mit12. Two peaks were detected, one between D1Mit1 and D1Mgh7 and the other between D1Mit2 and D1Mit12. Multipoint linkage analysis confirmed these results and detected one QTL demarcated by D1Mgh2 and D1Mit11 and a second QTL demarcated by D1Mit11 and D1Mit4 (Fig 2). The first QTL has a peak LOD score of 4.52 in the vicinity of D1Mit1, spans over 43.1 cm, and accounts for 23.9% of the explained variance and for 59% of the difference in the BP response to salt loading between SBH/y and SBN/y. This QTL is at the same chromosomal location as QTL-SS1a and its peak LOD score (Fig 2) accounts in full for the LOD score found in the overall cohort analysis. The second QTL has a peak LOD score of 2.98 (borderline in terms of statistical significance), is demarcated by D1Mgh7 and D1Mit2, spans over 18 cm, and accounts for 44% of the BP response to salt loading. This second QTL is at the same chromosomal location as QTL-SS1b but does not fully account for the LOD score calculated in the overall cohort.

**Figure 1.** Linkage map for rat chromosome 1 based on data from 232 rats (100 male and 132 female, overall effect) and generated by the MAPMAKER/QTL programs.19,20 The BP effect was calculated as the difference in BP between the homozygous for the H (salt-sensitive hypertensive) allele and those homozygous for the N (salt-resistant normotensive) allele. Statistical analysis was by two-way ANOVA accounting for genotype (or zygosity) and sex. Arrows indicate maximum LOD score; QTL vertical bar indicates ±1 LOD for linkage to BP.

**Figure 2.** Tracing of LOD score along chromosome 1 for BP after salt loading as generated by MAPMAKER/QTL, based on analysis of the entire F2 cohort (top), male progeny (lower left) and female progeny (lower right). Dashed horizontal lines represent LOD = 3, the level above which linkage is taken as statistically significant. Dotted curved lines in the lower panels represent the entire cohort analysis on which the LOD scores by sex are superimposed as continuous curved lines.
analysis (Fig 2). In the female F2 cohort, ANOVA revealed significant cosegregation with BP of a region demarcated by the microsatellite markers D1Mit2 and D1Mit12 (Figs 1 and 2). Multipoint linkage analysis detected one QTL demarcated by D7Mit87 and D1Mit4 (Figs 1 and 2) with a peak LOD score of 3.08 between D1Mit2 and D1Mgh8, a span of about 18 cM (max LOD score 6.1), and accounting for 12.7% of the explained genetic variance and for 30% of the difference in the BP response to salt loading between SBH/y and SBN/y. This QTL in the female is at the same chromosomal location as SS1b but does not fully account for nor explain the LOD score found in the analysis of the overall cohort (Fig 2).

Chromosome 17

**Basal BP**

Analysis of basal BP by genotype for the entire F2 cohort at each of the tested chromosome 17 marker loci revealed no cosegregation of any of the markers tested, neither by ANOVA nor by analysis with MAPMAKER/QTL (data not shown).

**BP Response to Salt Loading**

Analysis by ANOVA revealed significant cosegregation of four microsatellite markers (demarcated by D17Mgh4 and D17Mit4) with BP after salt loading, with one peak at D17Mit3 (Fig 3). Multipoint linkage analysis of the entire F2 cohort using the MAPMAKER/QTL programs detected one QTL (Fig 4) with a peak LOD score of 3.66 between D17Mgh4 and D17Mit3, spanning over 16.3 cM and accounting for 12.6% of the explained genetic variance and for 38% of the difference in the BP response to salt loading between SBH/y and SBN/y.

After stratification by sex, analysis of the male F2 progeny data by ANOVA revealed no significant cosegregation of any region on chromosome 17 with the BP response to salt loading. In contrast in the female F2 cohort, analysis of the data by ANOVA revealed significant cosegregation of a region demarcated by the markers D17Mit2 and D17Mit4 with BP. Multipoint linkage analysis detected a second QTL (Fig 4) demarcated by the markers D17Mgh4 and D17Mit4, with a peak LOD score of 3.66 between D17Mgh5 and D17Mit3, spanning over 16.3 cM and accounting for 12.6% of the explained genetic variance and for 38% of the difference in the BP response to salt loading between SBH/y and SBN/y.

**Figure 3.** Linkage map for rat chromosome 17 based on data from 232 rats (100 male and 132 female, overall effect). Data are presented as in Fig 1.

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<th>Systolic BP by Genotype in the F2 Cohort for Microsatellite Markers Demarcating the QTLs on Chromosomes 1 and 17</th>
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Data are represented as mean±SD; between-group analysis was by ANOVA; n designates number of animals with given genotype; HH stands for homozygosity for the H (salt-sensitive hypertensive) allele, NN for homozygosity for the N (salt-resistant normotensive), and HN for heterozygosity.
Salt sensitivity and salt resistance are likely to be determined by “susceptibility genes” that contribute significantly to the development of hypertension and cardiovascular disease. The search for the salt susceptibility genes has made, until very recently, almost exclusive use of the Dahl rats as the representative experimental animal model of salt-sensitive hypertension. Even though the Dahl rats have generally been regarded as a classic model of salt-sensitive hypertension and have been studied extensively as such, their usefulness in the study of salt susceptibility is limited by the fact that these animals also develop spontaneous hypertension over time. Thus any QTL found in the Dahl rats may be related to spontaneous hypertension, to salt sensitivity, or to both, and it may not possible in that model to differentiate between the three possibilities. The other major experimental model of salt susceptibility, the SBH/y and SBN/y rats, in which the genetic basis of hypertension has not been studied until now, differs from the Dahl rats in that these animals have been selectively inbred to maintain normotension at baseline and to express specifically salt sensitivity or salt resistance, according to the strain. Consequently, any QTL that is found to cosegregate in the Sabra rat with the development of hypertension after salt loading is more likely to be related directly to salt susceptibility than in similar cosegregation studies in the Dahl rat or in any other experimental model of salt susceptibility currently available.

Our present data were collected as part of a total genome-wide screen with 210 microsatellite markers distributed over the 20 rat autosomes and the X chromosome, and covering 96.8% of the rat genome, that is, the percentage of the genome that fell within 20 cM of the markers tested based on Jacob’s linkage map of the laboratory rat. The genome screen was conducted in an F2 cohort bred from a cross between the Sabra salt-sensitive SBH/y and salt-resistant SBN/y rats and identified two loci on chromosome 1 and one locus on chromosome 17 as carrying genes that contribute to the genetic variance of the BP response to dietary salt intake. On chromosome 1, two QTL (SS1a and SS1b) were identified that are about 20 cM apart, that do not overlap, and that most likely represent two distinct gene loci. An additional QTL (SS17) was identified on chromosome 17 that is likely to represent a third gene. Thus salt susceptibility in the Sabra rats appears to map to three distinct gene loci on chromosomes 1 and 17. Comparison of these findings with respect to salt susceptibility–related gene loci in other crosses, such as those involving the Dahl model, reveals intriguing parallels. Two separate QTLs were detected on rat chromosome 1 by24, and one QTL on chromosome 17 by25 among male F2-hybrids derived from a cross of the Dahl SS with the Lewis rats (LEW/NCrLBR). The reported localization of the QTLs on chromosome 1 at cytochrome P450 2B10,22 and 4A10,23 loci correspond well with the placement estimates of SS1a and SS1b, respectively, from our present study. A number of additional studies in a variety of other crosses have demonstrated linkage of chromosome 1 loci in the approximate location of SS1b with blood pressure phenotype, primarily after excess dietary sodium loading.24,25 The QTL detected in the cross of the Dahl SS with the Lewis rats on chromosome 17 also corresponds well with the placement of SS17 from our study. While the phenotype difference among the progenitor strains of these crosses was much less specific for dependence on dietary salt excess than among the SBH/y and SBN/y, these results may be seen as a confirmation of our data and as indicating the presence of several genes that appears to fulfill a ubiquitous role in salt susceptibility.

Analysis of the data after stratification for sex reveals sexual dimorphism in the genetic basis of salt susceptibility, a novel finding. In the male rats, two culprit QTLs were localized to chromosome 1, coinciding with SS1a and SS1b. In the female rats, one QTL was detected on chromosome 1 coinciding with SS1b, and a second QTL on chromosome 17. These findings suggest that the QTL designated SS1a is sex specific for males, that the QTL designated SS17 is sex specific for females, whereas the QTL designated SS1b is not sex specific. They further suggest that in male Sabra rats, the BP response to salt loading can be entirely accounted for by the two QTLs detected on chromosome 1, if the effect of the two genes encoded within these QTLs is additive. In the female Sabra rats, chromosomes 1 and 17 contribute together to nearly 70% of the BP response to salt loading. Thus while the combined effects of SS1a and SS1b account for a large portion of the overall phenotype variance in male rats, it would appear that other, yet unidentified genetic loci must contribute additionally to the genetic variance in females, indicating the need to pursue the search for additional gene loci at other chromosomal locations.

The findings of a sexual specificity with respect to the effects of QTLs SS1a and SS17 raises interesting questions as to the level of effect modification and imply that different sets of autosomal genes may contribute to salt susceptibility and hypertension among both sexes. Sex specificity of QTLs in other strains of genetically hypertensive rats has been reported in the past.26 While epistatic and ecogenetic interactions of a gene with the Y chromosome may come into play,27 hormonal interaction at the transcriptional as well as posttranslational levels represent an alternative possibility that needs to be
Salt Susceptibility Maps to Chromosomes 1 and 17

The large number of potential candidate genes already identified in the areas swept by the placement confidence intervals for SS1a, SS1b, and SS17, which include among others the genes encoding the α-3 subunit of Na-K-ATPase28 and cystatin D,29 virtually precludes further evaluation of the present results with respect to gene identification, until higher resolution linkage information, forthcoming from congenic substrains currently under construction, is obtained. The congenic strains will also help clarify the significance of the identified QTLs and to determine if they encode the putative genes for salt sensitivity or for resistance in terms of the development of hypertension.

In summary, we have detected in a cross between SBH/y and SBN/y rats two QTL for salt susceptibility on chromosome 1 and one QTL on chromosome 17. One QTL is specific for males only, one is specific for females only, and a third involves both males and females. Even though it may be reasonable to interpret our findings in a more general sense, it is important to note that the linkages detected apply only to the specific strains and experimental conditions studied. Thus extrapolations to other models, and certainly to human traits, should be made with great caution. Nonetheless, these data suggest that different susceptibility genes for hypertension might come into play between the sexes. Our study demonstrates more directly than previous ones, based on the availability of a very selectively salt-sensitive model of hypertension, that the three gene loci on chromosomes 1 and 17 contribute importantly to the development of hypertension in response to dietary salt exposure. Further exploration of the Sabra model of salt susceptibility, the eventual identification of causative genes, and the decoding of the mechanism of action may ultimately provide clinically relevant tools for novel diagnostic and therapeutic approaches that will benefit an epidemiologically important group of patients with hypertension.

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