Genetic Loci Contribute to the Progression of Vascular and Cardiac Hypertrophy in Salt-Sensitive Spontaneous Hypertension

Anja-Kristin Siegel, Michael Planert, Sibylle Rademacher, Ali Poyan Mehr, Peter Kossmehl, Markus Wehland, Monika Stoll, Reinhold Kreutz

Objective—The salt-sensitive Dahl rat and the spontaneously hypertensive rat develop comparable spontaneous hypertension on a low-salt diet, whereas only the salt-sensitive Dahl rat strain develops a striking increase in blood pressure and cardiovascular hypertrophy on a high-salt diet. We set out to identify quantitative trait loci (QTLs) contributing to the progression of salt-induced organ damage in hypertension by studying an F2 population derived from both strains.

Methods and Results—We determined systolic blood pressure (SBP), vascular aortic hypertrophy (AH), cardiac left ventricular (LV) hypertrophy (LVH), and LV fibrosis in 230 male F2-animals on a high-salt diet. A strong correlation between AH and LVH was found ($r=0.58$, $P<0.0001$), and genome-wide QTL mapping detected suggestive or significant QTLs in overlapping chromosomal fragments for AH and LVH on chromosomes 1, 3, and 19, respectively. A significant influence of SBP on the extent of LVH and AH was evident at all QTLs, although significant linkage to SBP (together with LVH) was only found on chromosome 9. No QTLs for LV fibrosis were detected.

Conclusions—This study demonstrates a strong correlation between AH and LVH in salt-sensitive hypertension and identifies QTLs contributing to the progression of cardiovascular hypertrophy in this condition. (Arterioscler Thromb Vasc Biol. 2003;23:1211-1217.)

Key Words: hypertension ■ hypertrophy ■ heart ■ aorta ■ genetics

A significant proportion of patients with essential hypertension demonstrate significant changes of blood pressure in response to changes in dietary salt intake and have thus been classified as salt-sensitive.1,2 The epidemiological and clinical importance of salt-sensitive hypertension is highlighted by the fact that the prevalence is high and increases with age3 and that the manifestation of target organ damage is more severe. Thus, this disease phenotype is a major contributor to overall cardiovascular risk, particularly in aging populations.4–6

Salt-sensitive hypertension plays a significant role as a factor contributing to LV hypertrophy (LVH) and LV dysfunction.7 LV fibrosis represents an additional important feature of cardiac remodeling in salt-sensitive hypertension and contributes to increased myocardial stiffness and diastolic dysfunction.8 Salt-sensitive hypertension is also associated with endothelial dysfunction and vascular hypertrophy in the aorta.9,10 Moreover, sodium-induced structural and functional changes of large conduit arteries that are independent of blood pressure and atherosclerosis may contribute, in part by increasing pulse pressure, to the increased cardiovascular mortality observed in salt-sensitive hypertension.11

It is poorly understood why subgroups of patients with essential hypertension exhibit salt sensitivity and more severe progression of hypertensive target organ damage over time. Familial aggregation3 and the higher prevalence of salt-sensitive hypertension in specific ethnic populations6,12 point to the potential importance of genetic factors. This is also supported by several genetic rat models that display salt-sensitive hypertension and related target organ damage as an inherited trait, thus representing an attractive substitute for the investigation of the polygenetic basis of the human disease.13,14 Furthermore, susceptibility loci identified in rodent models have been shown to be predictive for human genetic studies15–17 using the comparative genomic approach. Such susceptibility loci may provide target regions for subsequent studies using single nucleotide polymorphisms and linkage disequilibrium mapping in large-scale association studies in human patient populations.18–20

Here we aimed to identify quantitative trait loci (QTLs) for the progression of salt-induced vascular and cardiac organ damage. To this end we performed linkage analysis between 2 inbred genetic rat models with similar spontaneous hypertension but disparate genetic susceptibility to salt-induced
disease progression. We studied F2-progeny of a salt-resistant spontaneously hypertensive rat (SHR) strain and the contrasting salt-sensitive hypertensive Dahl rat (SS) model. Hence, both strains develop comparably hypertensive blood pressure values on a low-salt diet but show a striking difference in their susceptibility to develop salt sensitivity and cardiovascular organ damage.

Methods

Rats

The SS rats are derived from the inbred SS/Jr strain available from Harlan Sprague-Dawley (Indianapolis, Ind). SSab animals and SHRab were obtained from our colonies (established in 1997) at the Freie Universität Berlin and were maintained as reported. For the linkage analysis we generated an (SSab x SHRab) F2-cross population including 230 male animals. Experiments were performed in accordance with institutional guidelines.

Phenotyping

Blood Pressure Measurements and NaCl Loading

To unambiguously demonstrate the development of similar spontaneous hypertension in both strains under low-salt diet, we conducted blood pressure measurements by radiotelemetry. Thus, the development of spontaneous hypertension in both strains on a diet containing 0.2% NaCl by weight was studied using this method as previously reported. Transducers were implanted at 11 weeks. Blood pressures were measured in freely moving conscious rats at 14 weeks of age (n=6, respectively). In the salt-loading studies of parental and F2-animals (involving altogether more than 250 animals) it was not feasible to perform radiotelemetry measurements. Salt loading was performed in male parental animals of each strain (n=10 to 12, respectively) and in all F2 animals following a standardized protocol. In brief, at the age of 6 weeks, animals received a 4% salt by weight diet (Sniff) for 8 weeks. Systolic blood pressure (SBP) was then measured at the age of 14 weeks in awake animals by the tail-cuff method, which has been previously validated and reported. In brief, 2 training periods were performed on 2 separate days. Subsequently, the final blood pressure measurements were recorded on the 3 consecutive days. Because of 3 sets of 2 measurements at each session, the individual blood pressure phenotype was based on a quantitative phenotype representing vascular hypertrophy in the aorta, ie, aortic hypertrophy (AH), as reported.

Determination of Left Ventricular Fibrosis

For histological evaluation of LV fibrosis, a 2- to 3-mm transverse tissue slice was cut at the midpoint between the base and apex of the LV. The slices were immersed in Dubosq-Brasil solution, embedded in paraffin, and cut into 3-μm sections. Sections were subjected to H&E and Sirius-red staining as a specific dye for connective structures. Standardized staining was realized using a Robot-Stainer system (Robot-Stainer HMS 760, Microm GmbH). The amount of LV interstitial (LVIF) and LV perivascular (LVPF) fibrosis was quantified after Sirius-red staining by morphometry using a video camera combined with a separated video control system as reported.

Linkage Analysis and QTL Mapping

A complete genome screen on all 21 chromosomes except the Y-chromosome was performed as reported. The interval between the 210 polymorphic microsatellite markers was on average 10 cM. Before linkage analysis, phenotypic distribution was tested using the Kolmogorov-Smirnov test to assure normal distribution of the trait within the F2-population, as required for parametric linkage analysis. Traits failing the requirements were transformed using either a logarithmic or square root transformation and retested for normalcy.

Parametric and Nonparametric Linkage Analysis

Traits with a normal distribution were analyzed using the parametric genome scan function of the MAPMAKER/QTL computer package. The thresholds for significant and suggestive linkage were set to LOD scores of 4.3 and 2.8, respectively, as recommended. The 95% confidence interval for each QTL was determined by calculating the genetic distance based on the drop of 1.0 LOD unit from the peak. Nonparametric linkage analysis, available in MAPMAKER/ QTL (version 1.9b), was additionally applied for all traits.

Statistical Evaluation

All data are expressed as mean±SD unless stated otherwise. Genotype-phenotype correlation was performed at genetic markers closest to the QTL peak using one-way ANOVA followed by post-hoc test (Student's t test). Phenotype-phenotype correlation was performed by linear regression analysis. Except for linkage results, differences were considered significant at the level of P<0.05.

Results

Phenotypes in Parental Strains and F2 Progeny

Blood pressure measurements by radiotelemetry confirmed that the SS strain develops comparable SBP values to the SHR strain under a low-salt diet (Figure 1). In contrast, high NaCl loading leads to a striking difference in SBP and cardiovascular damage between the 2 strains (Figure 2). The overall ranges of phenotypes observed in F2-hybrids for SBP (150 to 268 mm Hg), AW (9.0 to 23.2 mg/cm), LVW (2.12 to 5.00 mg/g), LVPF (2.1% to 35.4%), and LVIF (1.1% to 18%) were in agreement with the contrasting data found between the 2 parental strains.

Linkage Analysis and QTL Mapping

Linkage analyses identified 4 chromosomes harboring QTLs for salt-sensitive SBP and cardiovascular hypertrophy phenotypes (Table, Figure 3). The only significant blood pressure QTL (LOD 4.3) was mapped to rat chromosome (RNO) RNO9, with a peak LOD score of 5.97 at D9rat10 contributing 11.5% of the genetic variance to this trait. Additional QTLs for SBP with LOD scores suggestive for linkage (LOD 2.8) were identified on RNO1 with a peak LOD score of 2.94 at D1rat9 (Figure 3) and on RNO6 with a LOD score of 2.86 at D6rat6. These 2 suggestive loci contributed an additional 12% to the genetic blood pressure variance.
When the variance of the main blood pressure QTL on RNO9 (ie, at D9Rat10) was removed from the analysis and the genome was rescanned (fixing), a fourth suggestive blood pressure QTL with 2 potential peaks (LOD 3.02 and 3.33) was identified on RNO3 (Figure 3), accounting for an additional 5% of the genetic variance.

For AW, significant linkage was found on RNO1 (LOD 5.32) and RNO3 (LOD 7.03), whereas the QTL on RNO19 was slightly below the statistical threshold for significant linkage (LOD 4.27).

Significant linkage to LVW was detected on RNO3 (LOD 7.34) and suggestive linkage on RNO1 (LOD 2.94), RNO9 (LOD 3.10), and RNO19 (LOD 3.33; Table, Figure 3). The QTLs on RNO3 and RNO9 colocaled with blood pressure QTLs, whereas RNO19 showed no linkage to SBP (P=0.11, Figure 3). No significant genetic linkage to body weight (Table) or right ventricular weight (data not shown) was observed.

Unlike the significant linkage data identified for LVW and AW, no significant QTL for either LVIF or LVPF was detected throughout the genome. Only a weak effect below the suggestive linkage threshold was found for LVIF on RNO4 (LOD 2.13). This suggests that there is no major genetic locus contributing to the variation of cardiac LV fibrosis.

Correlation Analysis and Genotype-Phenotype Analysis

We first calculated the overall contribution of SBP to the variance attributed to LVW, LVIF, LVPF, and AW in the F2-animals. SBP explained part of the phenotypic variance of LVW (28.1%) and AW (18.4%, P<0.0001). Thus, approximately 70% and 80% of the phenotypic variance of LVW and AW are explained by other factors, eg, the detected 4 QTL regions that taken together account for 40% and 30%, respectively, of the variance of each trait. LVW and AW showed a strong correlation (0.58, P<0.0001). Moreover, the increase of LVW and AW conferred by the QTLs on RNO1, 3, 9, and 19 was positively modulated, ie, enhanced, by increasing SBP at all 4 QTLs (P<0.05, respectively).

Both LVPF and LVIF showed no significant correlation with SBP (r=−0.02 and r=0.03, P>0.05, respectively); thus, overall variances of LV fibrosis were clearly independent from SBP variation. In addition, there was no significant correlation between LVW and LV fibrosis (data not shown).
Table 1. Linkage Results for SBP and Cardiovascular Hypertrophy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Locus</th>
<th>Phenotypes</th>
<th>Statistics, ANOVA, P</th>
<th>Maximal LOD Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP, mm Hg</td>
<td>D1Rat20</td>
<td>SS (185.5±16.6)</td>
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<td>198.7±25.8</td>
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<td>D3Rat47</td>
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<td>D3Rat53</td>
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<td>191.6±23.0</td>
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<td>D9Rat10</td>
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<td>191.5±22.4</td>
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<td>LW, mg/g</td>
<td>D1Rat73</td>
<td>SS (2.77±0.56)</td>
<td>(55)</td>
<td>2.59±0.29</td>
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<td></td>
<td>D3Mgh9</td>
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<td>D9Rat125</td>
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<td>AW, mg/cm</td>
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<td>12.63±1.67</td>
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<td>12.97±1.93</td>
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</table>

Discussion

To explore the genetic basis underlying the progression of salt-sensitive cardiovascular organ damage in spontaneous hypertension, we performed a genome-wide genetic analysis in the F2-progeny of SS and contrasting salt-resistant SHR rats. In a previous study we showed that the SHR strain used here is capable of maintaining SBP unchanged in response to a diet containing 4% NaCl by weight even after unilateral nephrectomy. Therefore, we decided to use the SHR strain because of its proven salt resistance of spontaneous hypertension as a contrasting strain for the SS model to study the genetics of salt-dependent disease progression in spontaneous hypertension.

At first, we identified one significant QTL linked to SBP on RNO9 and 3 chromosomal regions on RNO1, RNO3, and RNO6 demonstrating suggestive linkage. The QTLs on RNO3 and RNO9 were also linked to cardiovascular hypertrophy. The SS-allele exhibited a relatively strong effect on SBP at the significant QTL on RNO9 and accounted for a mean increase of approximately 20 mm Hg in homozygous animals compared with F2-animals homozygous for the SHR allele, which is in agreement with a previous study. In contrast to the linkage results on RNO3 and RNO9, additional QTLs were identified for cardiovascular hypertrophy on RNO1 and RNO19 that demonstrated no linkage to SBP. Consequently, these QTLs would have been missed by conventional blood pressure QTL mapping studies. Indeed, numerous blood pressure QTLs have been mapped in the last decade, but not in the corresponding regions for cardiovascular hypertrophy phenotypes on RNO1 and RNO19 identified here.

It is well established that cardiac hypertrophy results from increased hemodynamic load, but the extent of cardiac hypertrophy in hypertension exhibits significant variability and may even be absent. Both experimental and clinical studies demonstrated the impact of genetic factors contributing to variation in cardiac mass independently of blood pressure. Linkage analysis in experimental crosses between normotensive and various genetically hypertensive rat strains detected linkage to heart weight on RNO2, RNO12, RNO14, RNO17, and RNOX. No similar linkage data are available for the genetic determination of vascular hypertrophy. Recently, North et al demonstrated the influence of genetic factors on carotid artery structure and function in American Indian families, including a significant heritability of wall thickness. In addition, there is clinical and experimental evidence that pressure-independent mechanisms mediated by sodium intake directly affect both cardiac hypertrophy and compliance of large arteries. This is the first study that aimed to identify genetic factors for vascular hypertrophy in salt-sensitive spontaneous hypertension, which was stimulated by the clinical relevance of target organ damage in salt-sensitive essential hypertension in humans. The SS rat represents a valuable model organism to study the genetic basis of this disease phenotype, because it is highly susceptible to salt-induced vascular organ damage. To this end, the QTLs on RNO1 and RNO19 represent, in addition to the blood pressure–linked QTL on RNO3, the first chromosomal candidate regions linked to both vascular and cardiac hypertrophy in this condition. The corresponding candidate regions in the human genome on chromosomes 9p24 or 10q24 (corresponding to RNO1, D1rat73), 9q33-34 or 2q2 (corresponding to RNO3, D3rat53, or D3ratMgh7),
and 1p35 or 16q1 (corresponding to RNO19, D19rat18) can be identified by available comparative mapping tools (http://www.rgd.mcw.edu/) and provide the basis for future comparative genomic studies. Because of the relative imprecision of QTL mapping and the overlap or colocalization of QTLs with suggestive or significant linkage for LVW and AW on RNO1, RNO3, and RNO19, it seems possible that identical genetic factors are involved in the genetic determination of cardiovascular hypertrophy in the left ventricle and aorta. This is additionally supported by the overall strong positive correlation between LVW and AW.

Correlation analysis suggested that the overall influence of SBP accounted only for 28.1% of the variance of LVW and 18.4% of the variance of AW, whereas the identified QTLs accounted for 40% and 30% of the variance of these traits. Although no genetic linkage to SBP was identified at the cardiovascular hypertrophy QTLs on RNO1 and RNO19, additional statistical analysis indicated a positive correlation between SBP and the extent of LVW and AW at all identified QTLs. Thus, the strong correlation between AW and LVW (r=0.58) observed in our study is a consequence of both the genetic determination by common QTLs and the modulation by SBP as a crucial driving force for cardiovascular hypertrophy in the left ventricle and the aorta. The question of whether pulse pressure might have contributed some additional and SBP-independent hemodynamic impact on the extent of cardiovascular hypertrophy cannot be answered by our study, because we performed—due to the large sample size—only SBP measurements by the tail-cuff method.

Interestingly, when we investigated the modes of inheritance, it emerged that the SS-allele caused an increase of target-organ damage at the QTLs on chromosomes 1, 9, and 19, as expected from the parental phenotypes. In contrast, the SS-allele was linked to lower LVW and AW via a dominant genetic effect at the QTL on RNO3, suggesting the existence of either a protective allele in the SS or a susceptible allele in the SHR background. This finding indicates the existence of a QTL protecting against cardiovascular damage in the SS strain, the phenotypic effect of which is neutralized and can therefore only be detected by substitution of both SS-alleles by the SHR genome in recombinant F2-hybrids. Alternatively, the SHR-allele of this locus may promote salt-sensitive hypertension and target-organ damage in F2-hybrids via a recessive mode of inheritance, whereas this effect is normally
masked in SHR parental animals because of compensation by the otherwise resistant genetic background. Although the 95% confidence intervals for the LVW and AVW QTL do not overlap, it seems possible that there is one common QTL responsible for the observed effects on LVW, AVW, and SBP on RNO3. Future studies in congenic strains are needed to validate the functional relevance of the identified QTLs for AH or LVH in either the salt-resistant or salt-sensitive hypertensive background of the SHR or SS strains, respectively.44

To measure the LVW weight to body weight ratio is a well accepted method for the evaluation of LVH. Our decision to use the weight to length ratio as a parameter for aortic hypertrophy was based on previous work published by Hayakawa and Raj.10 In the latter study, it was shown that this parameter is well suited as a quantitative parameter to differentiate aortic hypertrophy between Dahl and SHR rats. The determination of wall to lumen ratios of the aortas by histological methods could have contributed additional important information to our linkage study. However, these data are not available, because histological evaluation of the aorta has not been performed. Nevertheless, the normal distribution of the aortic weight to length ratio phenotype observed in the F2-population, the significant linkage results obtained for this phenotype, and its significant correlation with LVW point overall to the validity and usefulness of this parameter in our study.

It is likely that there are differential mechanisms involved in the pathogenesis of LV fibrosis in several forms of cardiac remodeling, including pressure overload hypertrophy.27,45 In this study, we can clearly show that the quantity of LV fibrosis is not directly linked to genetic factors or blood pressure variation in the hypertensive background. Thus, secondary stimulation of either systemic or local production of vasoactive peptides such as angiotensin II or endothelin-1 or the activation of catecholamines, mineralocorticoids, and oxidative stress27,45,46 that are not primarily genetically determined seems to play a predominant role in the quantitative modulation of LV fibrosis in salt-sensitive hypertension.

In summary, our data demonstrate the importance of interactions between genetic factors and blood pressure variation for the progression of cardiovascular hypertrophy in salt-sensitive hypertension. Thus, in addition to blood pressure control, therapeutic intervention that aims to interfere with the genetic pathways of cardiovascular hypertrophy seems possible to reduce the overall higher cardiovascular risk observed in salt-sensitive hypertension. Therefore, a better understanding of the genetic mechanisms involved in the development of cardiovascular hypertrophy identified here by experimental breeding of congenic rat strains and by comparative genomic investigations in humans seems of clinical interest.

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