Susceptibility to Hypertensive Renal Disease in the Spontaneously Hypertensive Rat Is Influenced by 2 Loci Affecting Blood Pressure and Immunoglobulin Repertoire

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Abstract—High blood pressure exerts its deleterious effects on health largely through acceleration of end-organ diseases. Among these, progressive loss of renal function is particularly important, not only for the direct consequences of kidney damage but also because loss of renal function is associated with amplification of other adverse cardiovascular outcomes. Genetic susceptibility to hypertension and associated end-organ disease is non-Mendelian in both humans and in a rodent model, the spontaneously hypertensive rat (SHR). Here, we report that hypertensive end-organ disease in the inbred SHR-A3 line is attributable to genetic variation in the immunoglobulin heavy chain on chromosome 6. This variation coexists with variation in a 10 Mb block on chromosome 17 that contains genetic variation in 2 genes involved in immunoglobulin Fc receptor signaling. Substitution of these genomic regions into the SHR-A3 genome from the closely related, but injury-resistant, SHR-B2 line normalizes both biomarker and histological measures of renal injury. Our findings indicate that genetic variation leads to a contribution by immune mechanisms hypertensive end-organ injury and that, in this rat model, disease is influenced by differences in germ line antibody repertoire. (***Hypertension*. 2018;71:700-708. DOI: 10.1161/HYPERTENSIONAHA.117.10593.)** • **Online Data Supplement**

Key Words: biomarkers ■ genetics ■ hypertension ■ immunoglobulin ■ proteinuria

Hypertension is associated with progressive renal disease, and the most effective predictor of renal disease is the presence of a close relative that has required renal dialysis.[1,2] This genetic underpinning has prompted exhaustive studies in humans[3–8]; however, explanation of genetic risk remains limited. The spontaneously hypertensive rat also shows strong genetic influences on renal injury susceptibility.[9,10] The SHR-A3 line experiences progressive renal disease and has much shortened life span, whereas other spontaneously hypertensive rat (SHR) lines with overlapping genetic susceptibility to hypertension, such as SHR-B2, resist renal injury and experience normal life spans.[11] The emergent pattern of injury is focal regions of combined glomerular and tubulointerstitial damage along with albuminuria. Much normal tissue architecture remains between focal regions of injury, and disease progression includes both increasing severity of injury in injury foci and increased numbers of injury foci.[11] The close genetic similarity between these lines (87% identical by descent) provides an opportunity to discover and prove the genetic basis of this susceptibility.[12]

In previous studies, we have addressed whether the injury-prone SHR-A3 line shares the same genetic architecture controlling hypertension as the SHR-B2 line.[13] These lines are descended from the same founder pair.[14,15] Selective breeding fixed hypertension before the lines were separated. However, SHR-A3 is generally recognized to have higher systolic blood pressure (SBP) than injury-resistant lines.[16,17] We showed that these 2 lines differ in SBP and mapped a single haplotype block encompassing 10 Mb on chromosome 17 that accounts for the SBP difference between these 2 lines.[13] In the present study, we have created a congenic line in which this chr17 block has been transferred from SHR-B2 into the SHR-A3 genetic background. We have used this to prove the effect of this segment on SBP and to investigate whether reduction of SBP to a level not different from the injury-resistant line influences the emergence of renal damage.

SHR-A3 and SHR-B2 also have extreme genetic divergence in the immunoglobulin heavy chain (IgH) locus, and the haplotype block containing IgH is also associated with renal injury.[18,19] We have further tested whether this genetic

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difference contributes to the emergence of renal injury by creating a congenic line in which the IgH locus has been transferred from SHR-B2 into SHR-A3. We have used this congenic line to confirm that renal injury is determined, in part, by genetic variation in the IgH locus.

Finally, by crossing these 2 single congenic lines to create a double congenic line, we have examined concurrent effects of these 2 loci on renal injury.

Methods

Transparency

The data that support the findings of this study are available from the corresponding author on reasonable request.

Animals

Studies were performed on male rats of the injury-prone spontaneously hypertensive-A3 (SHR-A3, SHRSP/Bbb) and the injury-resistant SHR-B2 inbred lines and progeny derived from crosses between these lines. The lines are maintained as closed colonies in our facility, and the genetic integrity of the lines is verified using high-throughput genotyping of genome-wide single-nucleotide polymorphisms (SNP). The lines and their origins before transfer to our laboratory have been recorded at the Rat Genome Database (rgd.mcw.edu), which has applied the following identifiers: SHR-A3–RGD ID=8142383; symbol=SHRSP/BbbUtx; SHR-B2–RGD ID=8142385, symbol=SHR/Utx. Animals used in these studies were housed in an AAALAC-approved animal facility with sentinel monitoring to confirm the absence of transmissible pathogens in the colony. They were provided a standard rodent chow diet and drinking water ad libitum.

Genome Sequencing

We have previously described the assembly and analysis of Illumina short-read genome sequences from SHR-A3 and SHR-B2.[12] Variant call files are available on request to the corresponding author.

Congenic Line Construction

SHR-A3 (males) and SHR-B2 (females) parental lines were crossed to generate the F1 progeny. This progeny was backcrossed into SHR-A3 animals. Backcrossed animals were genotyped at each generation using a previously described panel of ≈200 SNP markers to allow speed congenic selection of optimal animals (highest loss of SHR-B2 background alleles while retaining the introgressed chromosome 6 or 17 SHR-B2 haplotype blocks).[11] Each congenic line was then created by mating the best 2 male and female animals from the previous backcross and selecting their progeny that were homozygous for SHR-B2 alleles at the target loci and for SHR-A3 alleles at any locus at which heterozygosity remained in the previous backcross. Transfer of the entire chromosome 17 introgressed block was verified by genotyping the 3 SNP panel markers in the block as well as 3 additional SNP markers located at the center and extreme ends of this block. Transfer of the entire chr6 IgH haplotype block from SHR-B2 into the SHR-A3 genetic background was also verified by SNP markers located at the extreme ends of this block as well as an additional marker in the center of the block.

Serum Immunoglobulin γ Subclass Measurement

We have previously reported the ELISA systems we used to measure serum immunoglobulin γ subclass levels.[18]

Blood Pressure

Blood pressure was measured by radiotelemetry devices (Data Sciences, St. Paul, MN) in adult animals implanted at 16 to 17 weeks of age. Catheters (Model PA-C40) were implanted under isoflurane anesthesia into the abdominal aorta above the bifurcation and below the renal arteries. Postoperative analgesic treatment with buprenorphine was provided for 2 days. Animals were allowed to recover from implantation for at least 7 days before blood pressure measurement began. Implants were calibrated under pressure (120, 160, and 200 mm Hg) at 37°C before implantation and again after removal, and observed blood pressures were adjusted, if needed, to compensate for calibration drift. Blood pressure was measured for 24 hours once per week. During each 24-hour recording period, pressures were sampled for 30 seconds every 30 minutes.

Histological Assessment of Renal Injury and ELISA Determination of Albuminuria

The divergence of histological measures of renal injury across time in SHR-A3 and SHR-B2, supported by representative photomicrographs, has been previously published.[11] Quantitative histological measures of renal injury were assessed following methods we have previously described that use extensive random sampling to assess injury in both affected and unaffected renal tissue.[11] Spot urines were collected because of divergent behavioral adaptation to metabolism cages observed between the 2 SHR lines. Urinary albumin excretion was measured by ELISA as previously described and normalized for urine creatinine levels determined by high-performance liquid chromatography.[11]

Renal Biomarker Studies

We investigated urine levels in spot urine samples of KIM-1 (kidney injury molecule 1, Havcr1 [hepatitis A virus cell receptor 1]), Lcn2 (lipocalin 2, also known as NGAL [neutrophil gelatinase-associated lipocalin]), and OPN (osteopontin) using the Kidney Injury Panel 1 (rat) Assay Kit manufactured by Meso Scale Diagnostics (Gaithersburg, MD). The multiplex assay plate was read on a Meso Scale Diagnostics SECTOR Imager 2400 electrochemiluminescence plate reader. Biomarkers were determined in 8 animals per group and normalized to urine creatinine levels measured by high-performance liquid chromatography.[11]

Statistical Analysis

Study groups comprised 6 to 26 animals; please refer to figure and table legends for more information on group sizes. Group means±SEM are shown. Multiple group comparisons were performed by ANOVA followed by Scheffé test. Histological scores are arbitrary nonparametric data and were tested to verify normality using Kolmogorov–Smirnov Test. Kruskal–Wallis test was used to identify differences in histological scores across groups. Statistical significance of nonparametric data was estimated by Scheffé test for the normally distributed multiple group comparison samples.

Results

Confirmation of the Physical Extent of the Mapped Chromosome 17 Blood Pressure Block by Genome Sequencing

The genomes of SHR-A3 and SHR-B2 are descended from the same outbred founder pair and shared ancestors of both lines were inbred for 8 generations before the 2 lines were isolated. As a result, SHR-A3 and SHR-B2 are 87% identical by descent. The 13% of the SHR-A3 and SHR-B2 genome that is descended from different ancestors has a haplotype block structure.[13] A single haplotype block located from chr17:7.2 Mb to chr17:15.8 Mb (Rn5 assembly) was mapped and shown to explain the difference in SBP observed between these 2 SHR lines at 18 weeks of age. This age precedes the onset of progressive renal injury, and, therefore, this difference in blood pressure does not result from renal injury.[13] Using next-generation whole-genome sequence assemblies obtained for these 2 SHR lines, we are able to define this block with high resolution

(chr17:6,765,076–16,742,956, Rn5 assembly), examine the genes contained within it, and tabulate the genetic variation associated with those genes (Table S1 in the online-only Data Supplement). We have previously reported renal gene expression differences between SHR-A3 and SHR-B2 associated with genes in the chr17 block.[13]

Creation and Confirmation of Congenic State of SHR-A3(chr17-SHR-B2) Rat Line

A congenic line was created to test the hypothesis that the chr17 block influences blood pressure in the SHR-A3 genetic background.[20,21] We used a panel of ≈200 dimorphic SNP markers to determine genotypes at each generation of backcrossing during creation of the SHR-A3(chr17 SHR-B2) congenic line. Congenic animals were homozygous for SHR-B2 alleles at 4 SNP markers spanning the introgressed block (marker positions chr17:7,151,820, chr17:11,561,842, chr17:13,705,857, and chr17:15,630,391, Rn5 assembly) and were further verified with PCR interrogating the extreme ends of the block (SNPs located at chr17:6,815,854 and chr17:16,288,148; Figure S1). SNP genotyping across the blocks of nonidentity by descent allows us to conclude that, outside the 10 Mb introgressed chromosome 17 segment, this congenic line is >99.7% genetically identical with the SHR-A3 parental line. A single region of introgressed SHR-B2 ancestry was detected outside of the chr17 target block lying between chr11:15,415,747 and 22,927,323 (Rn5).

Effect of Congenic Introgression of the Mapped Chromosome 17 Block on Blood Pressure

The Table indicates that there is a significant difference (+16.1 mm Hg) in SBP between the SHR-A3 parental line and the SHR-A3(chr17 SHR-B2) congenic line but no difference between the congenic line and SHR-B2. The Table also indicates levels of mean and diastolic blood pressure and heart rate. In our earlier SHR-A3×SHR-B2 F2 intercross mapping study, this locus was detected as a highly significant SBP locus and was not a significant diastolic blood pressure locus. This is confirmed in the congenic. We estimated that homozygosity in F2 animals for the SHR-A3 chr17 haplotype resulted in SBP 13.6 mm Hg greater than homozygosity for SHR-B2 alleles.[13] Difference in SBP between the parental SHR-A3 and SHR-B2 lines is 19 mm Hg (Table). Thus, the chr17 block seems to account for the SBP difference between these 2 SHR lines, and the congenic line proves the role of allelic variation at the chr17 locus uncovered by mapping.

Except for the chr17 locus, no BP loci segregate between SHR-A3 and SHR-B2.[13] This indicates that the remaining polygenic basis for hypertension is shared by SHR-A3 and SHR-B2, a finding consistent with the known genealogy and observations of hypertensive trait fixation in SHR.[15] We infer that the congenic SHR-A3(chr17 SHR-B2) and SHR-B2 share an identical genetic mechanism of hypertension so that traits in which they diverge arise from the action of other genetic variation.

Effect of Congenic Introgression in Chromosome 17 on Renal Injury

SHR-B2 strongly resists hypertensive renal injury.[11] We proposed the null hypothesis that, if renal injury is driven solely by SBP differences between SHR-A3 and SHR-B2, no injury difference would be present in SHR-A3(chr17 SHR-B2) and SHR-B2. Figure 1 indicates 3 measures of renal injury assessed at 40 weeks of age in SHR-A3(chr17 SHR-B2) and its progenitor lines SHR-A3 and SHR-B2. Glomerular injury in the congenic line is reduced compared with SHR-A3 and is not significantly different from SHR-B2. Thus, concerning glomerular injury, our null hypothesis is supported. In contrast, tubulointerstitial injury in the congenic line is greater than that in SHR-B2 but significantly less than that in SHR-A3. Finally, proteinuria as reflected in urinary albumin/creatinine ratio is not different between SHR-A3 and the congenic line. This supports the conclusion that SBP has an important role in determining glomerular and tubulointerstitial injury in SHR-A3. However,

Table. Blood Pressures in SHR Lines at 17 to 18 wk of Age

SHR Line	n	SBP	SEM	*P* Value	MBP	SEM	*P* Value	DBP	SEM	*P* Value	HR	SEM	*P* Value	ANOVA Comparison
SHR-A3	26	205.7	3.9	*	177.4	3.4	*	149.0	3.1	†	330.1	2.9	NS	SHR-A3 vs SHR-B2
SHR-A3(chr17 SHR-B2)	25	190.0	2.6	†	173.8	2.5	NS	156.0	2.9	NS	321.0	3.4	NS	SHR-A3 vs SHRA3(chr17 SHR-B2)
SHR-B2	20	186.7	2.5	NS	159.1	2.6	†	135.8	2.1	*	324.9	2.8	NS	SHR-B2 vs SHRA3(chr17 SHR-B2)
LOD score in BP mapping F2 A3×B2	…	5.23			3.57			2.97			…	…	…	…
LOD score *P* value	…	*			‡			NS			…	…	…	…

n=number of animals per group. BP and HR multiple group comparisons by ANOVA followed by Scheffé test when ANOVA *f* statistic was significant (insignificant *f* statistic only for HR). LOD scores and *P* values obtained in F2 intercross mapping of SBP, MBP, and DBP was previously reported.[13] BP indicates blood pressure; DBP, diastolic arterial blood pressure; HR, heart rate; MBP, mean arterial blood pressure; NS, not significant; and SBP, systolic arterial blood pressure.

*$P<0.001$.

†$P<0.01$.

‡$P<0.05$.

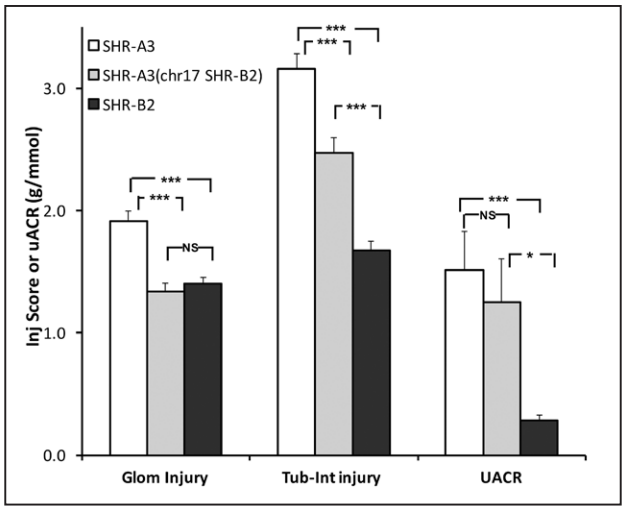

Figure 1. Histologically assessed glomerular and tubulointerstitial injury scores measured in Periodic acid–Schiff–stained kidney sections and urinary albumin-creatinine ratios (UACR) and from 40-wk-old SHR-A3, SHR-B2, and congenic SHR-A3(chr17 SHR-B2) animals (SHR-A3, SHR-A3(chr17 SHR-B2), SHR-B2, n=8, 12, and 21, respectively). *P<0.05 and ***P<0.001 (ANOVA, Scheffé test). NS indicates no significant difference.

the effect is only partial regarding tubulointerstitial injury, and no effect to reduce proteinuria was observed. This may indicate other genetic influences on proteinuria arising outside of this locus.

Confirmation of the Physical Extent of the Chromosome 6 Haplotype Block Containing the IgH Locus by Whole-Genome Sequencing and Haplotype Differences in Coding Sequences

A haplotype block is located at the distal end of chromosome 6 at which genomic sequences of SHR-A3 and SHR-B2 are highly divergent. We defined the extent of this block from chr6:146,030,387 to 154,214,590 (Rn5 assembly of the rat genome). This block contains all components (VDJ genes, Fc isotype genes) of IgH.[19] We also confirmed that the remainder of chr6 is identical by descent across the 2 SHR lines with the exception of a small block from chr6:2,028,567 to 4,036,845. A summary of non-IgH gene coding variation in the distal chr6 locus is provided in Table S2. Excluding IgH genes, only 18 other genes are present in this block, and only 3 of these are affected by amino acid substitution. In contrast, of 230 IgH amino acid coding genes in the block, 112 IgH genes contain nonsynonymous variation. There is also extensive structural variation (gene duplication and deletion) differentially affecting IgH genes in SHR-A3 compared with SHR-B2.[19]

Creation and Confirmation of Congenic State of SHR-A3(IgH SHR-B2) Rat Line

A congenic line was created by backcrossing as above, targeting the IgH haplotype block. We genotyped animals from the resulting SHR-A3(IgH SHR-B2) congenic line to verify preservation of the entirety of the introgressed haplotype block. Our genome-wide SNP panel indicated that, outside the ≈8 Mb introgressed chr6 segment, the congenic line is genetically identical with the SHR-A3 parental line.

Effect of Congenic Introgression of the IgH Locus on Serum IgG Levels

The rat IgG isotype exists as 4 subclasses. Serum levels of 3 of these subclasses (IgG1, IgG2b, and IgG2c) persistently differ between SHR-A3 and SHR-B2. Our genetic mapping studies indicated a strong influence of the IgH block on serum IgG subclass levels, indicating that both coding sequence variation and regulation of immunoglobulin serum levels were determined by variation the IgH locus between SHR-A3 and SHR-B2.[18] Congenic substitution of IgH from SHR-B2 into SHR-A3 may confer not only the coding sequences of SHR-B2 immunoglobulins on the congenic line but also shift the serum levels of IgG subclasses away from those observed in SHR-A3 and toward those in SHR-B2. Measurement of serum IgG subclasses confirms the effect of the IgH locus on serum IgG subclass levels (Figure 2).

Effect of Congenic Introgression of the IgH Locus on Blood Pressure

Recent evidence suggests the involvement of B lymphocytes in hypertension.[22] Antigen-directed immunoglobulin affinity maturation and secretion of immunoglobulin are unique functional specializations of the B-cell lineage. B-lymphocyte effects on blood pressure may be affected by IgH genetic variation acting through either serum immunoglobulin levels or functional effects of IgH coding sequence differences, or both. Therefore, we used the IgH congenic line to test for an effect of the chromosome 6 IgH block on blood pressure. We compared blood pressure measured by telemetry in SHR-A3 and the congenic line starting before the age when renal injury is present (17–18 weeks) until injury becomes established at 25 to 26 weeks (Figure S2). No effect on blood pressure was observed to result from substitution of the IgH locus in SHR-A3.

Effect of Congenic Introgression of the IgH Locus on Renal Injury

Because administration of an immunosuppressive drug (mycophenolate mofetil) that impedes proliferation and maturation of T and B lymphocytes also reduces renal injury in SHR-A3,[12] we considered it possible that IgH variation might provide a genetic influence on renal injury in SHR-A3. Figure 3 shows that glomerular injury is reduced at 40 weeks of age in the SHR-A3(IgH SHR-B2) line and approaches levels observed in SHR-B2 (SHR-A3=1.91±0.08, SHR-A3(IgH SHR-B2)=1.36±0.08 and SHR-B2 1.20±0.07). Tubulointerstitial injury score is also significantly reduced in the congenic line (SHR-A3=3.16±0.12, SHR-A3(IgH SHR-B2)=2.35±0.09 and SHR-B2 1.32±0.12). However, no significant difference between uACR in SHR-A3 and SHR-A3(IgH SHR-B2) was observed.

Creation and Confirmation of Double Congenic State of SHR-A3(chr17, IgH SHR-B2) Rat Line

The capacity of the IgH and chr17 loci to influence renal injury independently leads us to investigate the effect of the combined actions of these 2 loci. We mated animals from the 2 single congenic lines and bred to homozygosity animals that contained the SHR-A3 genetic background

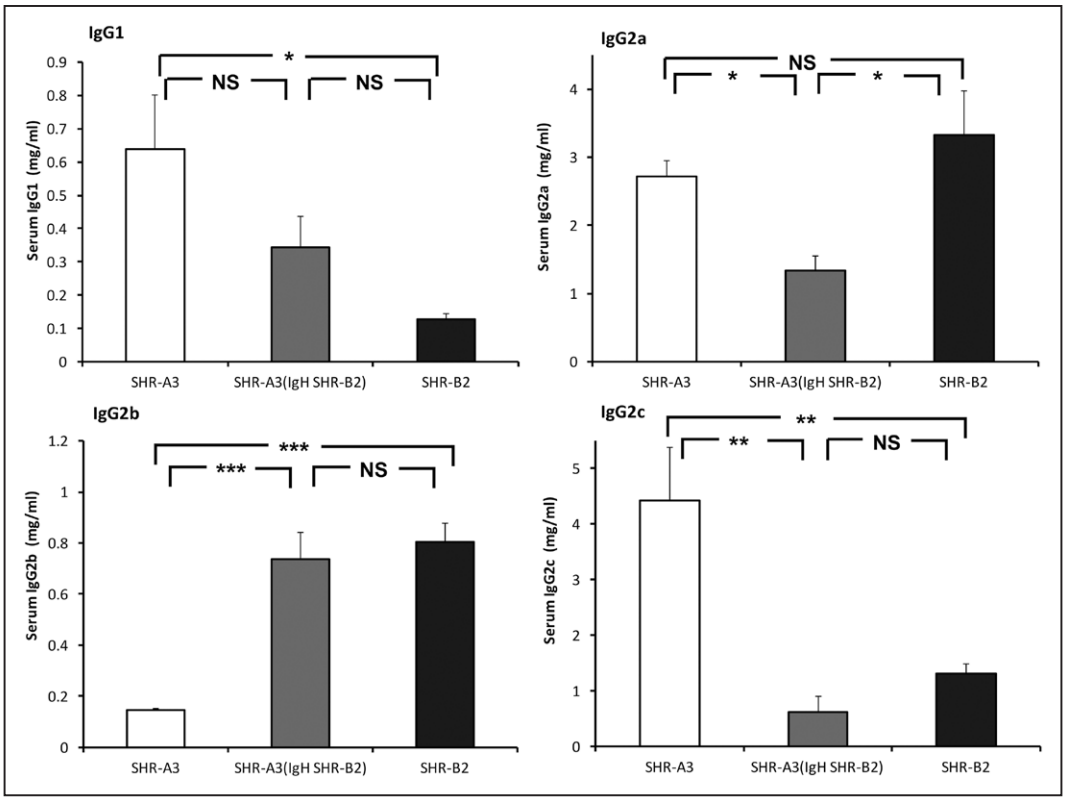

Figure 2. Serum IgG subclass levels were assessed by ELISA in 30-wk-old SHR-A3, SHR-B2, and SHR-A3(IgH SHR-B2), n=6 per group. Significant differences in IgG1, IgG2b, and IgG2c in SHR-A3 versus SHR-B2 were observed. In the congenic line, transfer of the SHR-B2 IgH segment into SHR-A3 resulted in serum IgG subclass levels that closely resembled the donor (SHR-B2) levels for IgG2b and IgG2c. For IgG2a, no effect of congenic transfer was predicted based on prior mapping, though a reduction was observed in the congenic line. For IgG1, the congenic levels were intermediate between the donor and recipient levels and not significantly different from either (*P<0.05, **P<0.01, ***P<0.001). NS indicates no significant difference.

but were homozygous at both the IgH and chr17 loci for SHR-B2 alleles. We examined the development of renal injury in these double congenic animals at 40 weeks of age. Figure 4 shows that histologically assessed glomerular and tubulointerstitial injury in the double congenic are both reduced to levels not different from SHR-B2. However, proteinuria was not reduced in the double congenic and seems to be controlled by genetic variation outside these 2 loci.

Urinary Biomarkers of Renal Injury

We assessed urinary levels of 3 established biomarkers of acute renal injury (OPN, NGAL, and KIM-1) in SHR-A3, SHR-B2, the SHR-A3(IgH SHR-B2) congenic line, the SHR-A3(chr17 SHR-B2) congenic line, and the double congenic line at 30 weeks of age. There was extreme divergence in each of the 3 biomarker levels between the parental lines, SHR-A3 and SHR-B2 (Figure 5). The 2 single congenic lines had intermediate levels of each of the biomarkers, whereas the double congenic line biomarker levels were all close to those observed in SHR-B2. Although the precise biological significance of these markers is not fully clear, they seem to reflect predominantly injury to the proximal renal tubules and thus extend our measurement of urinary albumin excretion from what is predominantly a filtration and barrier function to a reflection of renal epithelial cellular damage.[23,24] Our observations suggest their relevance to progressive renal injury in the SHR-A3 model and support and extend the conclusion from our histological studies that the BP effect of the chr17 locus and effects arising from germ line variation in IgH are key elements of the emergence of progressive renal disease in this model of hypertension.

Discussion

SHR-A3 and SHR-B2 are descended from a single founder pair of Wistar rats whose progeny were selectively bred to fix the trait of hypertension. Separation of distinct SHR-A and SHR-B lineages occurred after fixation of hypertension so it can be expected that alleles causing hypertension in SHR are shared across SHR lines.[14,15,25,26] We have shown by high-density single-nucleotide polymorphism analysis that the genome-wide extent of shared ancestry across these 2 lines is ≈87% and that it comprised blocks of shared ancestry interspersed with blocks of ancestry arising from genetically divergent progenitors.[13] This structure facilitates mapping of traits at which the 2 lines diverge because of genetic variation. We performed mapping studies to determine whether the higher blood pressure levels in SHR-A3 than other SHR lines might be attributed to an additional genetic effect that is absent in SHR-B2. This work indicated that SHR-A3 alleles in the chr17 block segregated with blood pressure in an SHR-A3 and SHR-B2 F2 intercross when blood pressure was measured before the emergence of renal injury. This indicates that hypertension alleles are

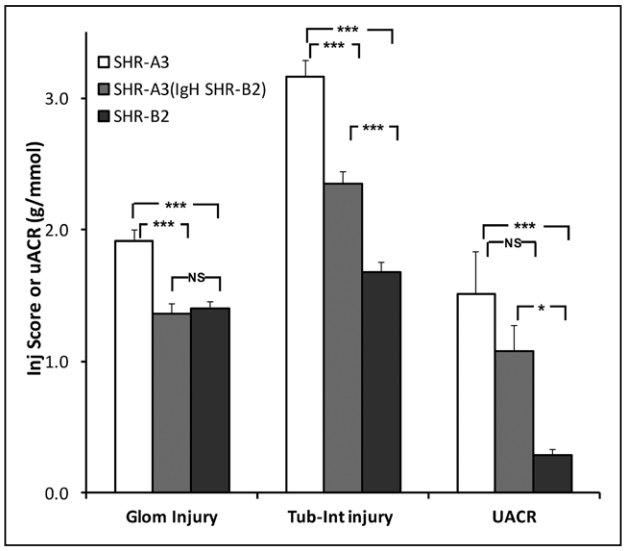

Figure 3. Histologically assessed glomerular and tubulointerstitial injury scores and urinary albumin-creatinine ratios (UACR) and from 40-wk-old SHR-A3, SHR-B2, and the congenic SHR-A3(IgH SHR-B2) lines, n=8, 19, and 21, respectively. Glomerular and tubulointerstitial injury were both significantly reduced in the congenic line, compared with SHR-A3; UACR was not different. *P<0.05 and ***P<0.001 (ANOVA, Scheffé test). NS indicates no significant difference.

shared by both lines and that an additional BP effect exists only in SHR-A3, which is confined to this ≈10 Mb block that constitutes 0.36% of the rat genome.[13] The chr17 congenic line confirms the role of this short segment of chr17 in the higher BP levels seen in SHR-A3. The present study clearly indicates that the chr17 block produces a combined effect on both blood pressure and renal injury. The injury effect may arise secondarily to the further elevation of blood

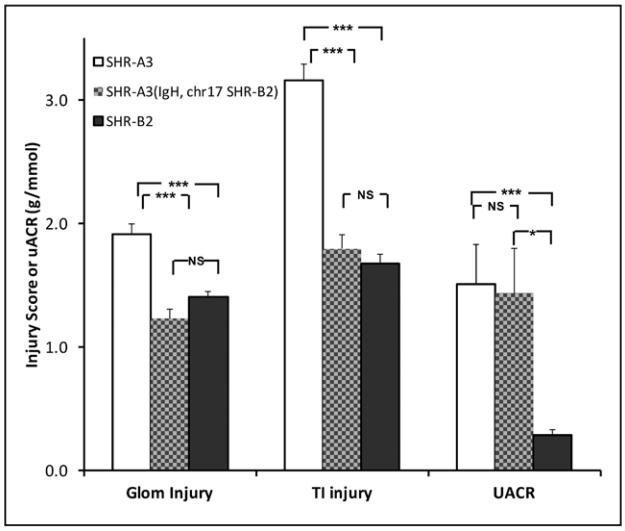

Figure 4. Histologically assessed glomerular and tubulointerstitial injury and urinary albumin-creatinine ratios (UACR) and from 40-wk-old SHR-A3, SHR-B2, and the congenic SHR-A3(IgH, chr17 SHR-B2) double congenic line, n=8, 20, and 21, respectively. Glomerular and tubulointerstitial injury scores were not significantly different in the congenic line compared with SHR-B2; however, UACR was different from SHR-B2 but not from SHR-A3. *P<0.05 and ***P<0.001 (ANOVA, Scheffé test). NS indicates no significant difference.

pressure, and it is notable that levels of blood pressure in SHR-A3 exceed the autoregulatory range of renal blood flow and may contribute to injury initiation by disrupting renovascular function as has been suggested for other rat renal injury models.[27–30]

Our previous work indicates that difference in injury susceptibility seems to result, at least in part, from genetic variation in SHR-A3 that influences renal inflammation.[12] This raises the question of whether substitution of the chr17 block affecting SBP and renal injury alters an immune component of renal injury in SHR-A3. Although the chr17 block is relatively rich in genes, 2 immune-signaling genes (*Syk* and *Dok3*) lie in this block and exist as dimorphic alleles across SHR-A3 and SHR-B2. Furthermore, these genes both participate in inflammatory signaling networks arising from Fc receptors for which immunoglobulins are the activating ligand.[31–33] Further refinement of this locus by subcongenic line creation may clarify the possible involvement of one or both of these genes in contributing to blood pressure elevation and renal injury.

There is extensive genetic variation between the germ line IgH sequences across SHR-B2 and SHR-A3.[19] Immunosuppression reduces hypertensive renal injury in SHR-A3 line,[12] and this suggested the possible involvement of B-cell–mediated immunity in hypertensive renal injury. The SHR-A3(IgH SHR-B2) congenic line provides clear evidence that germ line genetic variation in IgH influences hypertensive renal injury and that such influence does not arise from a resulting difference in SBP between SHR-A3 and SHR-B2.

Congenic substitution approaches are subject to confounding effects arising from undetected gene variants transferred during backcrossing from outside the targeted congenic segment. Such a possibility seems to be small in this case because of the high levels of genetic identity between the SHR-A3 and SHR-B2 lines and because we have extensively genotyped across the haplotype blocks from which the SHR-A3 and SHR-B2 lines arise from divergent ancestors. IgH sequence variation seems likely to be the cause of trait variation in the comparisons made, because IgH comprises essentially all of the protein-coding variation in the transferred block. Analysis of whole-genome sequences for SHR-A3 and SHR-B2 has allowed us to determine the extent of nonsynonymous protein-coding variation between these 2 closely related rat lines. Across the entire genome, excluding the IgH locus, we have identified 652 genes containing nonsynonymous sequence variation comparing these lines with each other (≈3% of all genes). In contrast, there are 112 immunoglobulin gene segments with nonsynonymous variation in the IgH locus (49% of IgH genes) and potentially even more variation arising from the frequent structural genetic variation and gene duplication known to occur in the IgH locus and that is poorly resolved by next-generation, short-read sequencing. Thus, at the whole-genome level, at least 17% of nonsynonymous genetic variation between SHR-A3 and SHR-B2 occurs in the 0.3% of the genome that constitutes the IgH locus. An assembled, structurally complete genome sequence of the highly variable IgH locus in humans has only recently

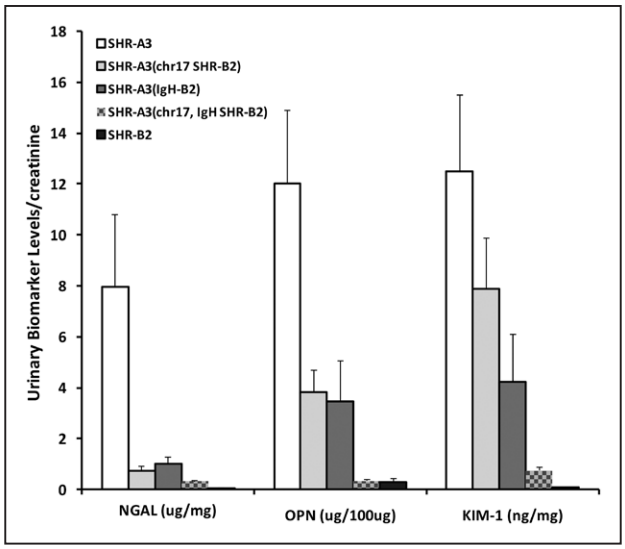

Figure 5. Urinary biomarker levels normalized to urine creatinine in 30-wk-old SHR-A3, SHR-B2, the SHR-A3(chr17 SHR-B2) congenic line, the SHR-A3(IgH SHR-B2) congenic line, and the double congenic SHR-A3(IgH, chr17 SHR-B2) line, n=8 per group. Although presented in a single figure for clarity, the hypotheses tested by comparing each of the congenic lines with the 2 parental strains are independent, and statistical significance testing (ANOVA, Scheffé test) reflects this. Comparing SHR-A3(IgH SHR-B2) to its parental strains: NGAL; SHR-A3 vs SHR-B2 P=0.03, SHR-A3 vs SHR-A3(IgH SHR-B2) P=NS, SHR-A3(IgH SHR-B2) vs SHR-B2 P=NS: OPN; SHR-A3 vs SHR-B2 P=0.001, SHR-A3 vs SHR-A3(IgH SHR-B2) P=0.02, SHR-A3(IgH SHR-B2) vs SHR-B2 P=NS: KIM-1; SHR-A3 vs SHR-B2 P=0.001, SHR-A3 vs SHR-A3(IgH SHR-B2) P=0.03, SHR-A3(IgH SHR-B2) vs SHR-B2 P=NS. Comparing SHR-A3(chr17 SHR-B2) to its parental strains: NGAL; SHR-A3 vs SHR-B2 P=0.01, SHR-A3 vs SHR-A3(chr17 SHR-B2) P=0.03, SHR-A3(chr17 SHR-B2) vs SHR-B2 P=NS: OPN; SHR-A3 vs SHR-B2 P=0.002, SHR-A3 vs SHR-A3(chr17 SHR-B2) P=0.04, SHR-A3(chr17 SHR-B2) vs SHR-B2 P=NS: KIM-1; SHR-A3 vs SHR-B2 P=0.002, SHR-A3 vs SHR-A3(chr17 SHR-B2) P=NS, SHR-A3(chr17 SHR-B2) vs SHR-B2 P=0.05. Comparing SHR-A3(IgH, chr17 SHR-B2) to its parental strains: NGAL; SHR-A3 vs SHR-B2 P=0.01, SHR-A3 vs SHR-A3(IgH, chr17 SHR-B2) P=0.01, SHR-A3(IgH, chr17 SHR-B2) vs SHR-B2 P=NS: OPN; SHR-A3 vs SHR-B2 P=0.0003, SHR-A3 vs SHR-A3(IgH, chr17 SHR-B2) P=0.0003, SHR-A3(IgH, chr17 SHR-B2) vs SHR-B2 P=NS: KIM-1; SHR-A3 vs SHR-B2 P=0.0003, SHR-A3 vs SHR-A3(IgH, chr17 SHR-B2) P=0.0005, SHR-A3(IgH, chr17 SHR-B2) vs SHR-B2 P=NS. NS indicates no significant difference.

been achieved, and none is available for the rat.[34] Germ line immunoglobulin sequence diversity provides the starting point for antigen recognition.[35] Germ line variation in IgH in SHR-A3 may favor the development of antibodies to antigens exposed in the hypertensive kidney.

The double congenic line we have created indicates that renal injury in SHR-A3 can be reduced to the same low levels observed in SHR-B2 by substitution of just 2 genomic loci comprising 18 Mb of the 2870 Mb rat genome. SBP levels in SHR-A3 that are nearly 20 mm Hg greater than SHR-B2 may play an important role in the initiation of injury. This additional degree of SBP elevation may overwhelm renal blood flow autoregulatory processes, which seem to retain function until ≈180 mm Hg.[36] The resulting damage might initiate injury in both glomerular and tubulointerstitial compartments that is amplified when the germ line SHR-A3 IgH sequences are present compared with those in SHR-B2. The persistence of high levels of proteinuria in the double congenic is unexpected and indicates a potential genetic influence from the SHR-A3 genome outside of the loci we have isolated, although other explanations may be possible.

In conclusion, these studies have identified two loci that determine susceptibility to renal injury in this rat model of hypertension. The pathway seems to involve both increased injury because of genetic variation driving higher blood pressure and to the involvement of genetic variation in immunoglobulin biology arising from within the IgH locus. There is possible interaction between gene variation affecting renal injury because two diallelic immunoglobulin signaling genes are present in the BP locus. These studies pose questions on the potential interaction between immunoglobulin genetic variation and hypertensive renal injury in humans. The difficulty of addressing this possibility is highlighted by the fact that the extreme genetic diversity of the immunoglobulin loci reduces the capacity of contemporary genome-wide association or genome-sequencing studies to detect this involvement.[37,38] This may contribute to the missing heritability observed in genome-wide association studies of renal function.[39] It is well recognized that there is common genetic variation in humans that influences the likelihood of formation of self-reactive antibodies.[40–44] Such variation might interact with germ-line immunoglobulin variation to contribute to risk of disease in hypertensive patients. This rat model proposes novel, discrete disease targets and mechanisms for investigation in humans.

Perspectives

SHR lines exist that differ in susceptibility to hypertensive end-organ injury and provide an opportunity to advance understanding of genetic mechanisms that create injury susceptibility. We have identified 2 loci that control the emergence of renal injury in the SHR strain. One is associated with a greater rise in blood pressure (chr17:7.2–15.8 Mb). The other (chr6:146.0–154.2 Mb) includes all immunoglobulin VDJ and constant genes of the IgH locus, which are highly divergent between SHR lines. The presence of genetic variation in the chr17 block that affects genes involved in B-cell immune signaling also suggest a role for immunoglobulin structural and functional divergence in the mechanism of disease. These observations tie the suspected role of immune-mediated damage in end-organ disease to specific aspects of immune function. Interestingly, the structural complexity of the immunoglobulin locus has prevented it from being investigated in human population genetic studies of end-organ disease, and it may be an important source of unexplained heritability of disease susceptibility.

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Disclosures

None.

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Novelty and Significance

What Is New?

- Hypertensive kidney injury has a strong heritable basis that remains largely undefined.
- In a hypertensive rat model, we show that injury in the susceptible SHR-A3 line is strongly attenuated by replacement of 2 loci representing <0.7% of the genome from the SHR-B2 injury-resistant line.
- One of these loci (chr17) contributes to additional elevation of blood pressure.
- The other locus contains genes encoding the immunoglobulin heavy chain (chr6 and IgH).

What Is Relevant?

- These studies indicate that previous observations implicating immunologic processes in hypertensive renal disease can arise genetically.
- The IgH has a complex structure that cannot be systematically investigated in human genome-wide association studies.

Summary

These studies show interaction between increased blood pressure and renal injury and indicate that genetic variation in the antibody repertoire can contribute to hypertensive renal disease.

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Table S1.

Structure of the chromosome 17 haplotype block in WKY, SHR-A3 and SHR-B2. showing distribution of ancestral alleles, gene positions, and non-synonymous coding sequence variation. Alleles present in SHR-A3 are shown at the left side of the panel in red, those present in SHR-B2 are shown in green. Alleles shared by SHR-A3, SHR-B2 and the normotensive WKY line are shown in green, those shared by SHR-A3 and WKY are shown in red. A small block including the genes Sfxn1 and Drd1a appears to comprise a single shared ancestral haplotype common to all three lines. Across this 10Mb Chr17 block the normotensive inbred WKY rat strain appears to have fixed the same alleles as SHR-B2 except for a small block commencing near Chr17:13.3Mb and extending to Chr17:15.5Mb where SHR-A3 and WKY share the same single nucleotide haplotype structure. Genes containing non-synonymous coding sequence variation comparing each of these lines to the reference are indicated in the right column. When SHR-A3 alleles differ from the rat reference the gene is identified by pink color, where SHR-B2 genes contain different coding sequences from the rat reference these are indicated in pale green. Gene positions are with reference to the Rn5 genome assembly.

WKY	SHR-A3	SHR-B2	Gene name	Chr	Start	End	Non-synonymous
			Isca1	17	7,507,069	7,519,772	N
			Naa35	17	7,636,171	7,688,566	N
			Agtpbp1	17	7,735,244	7,837,762	N
			Ntrk2	17	8,156,432	8,464,507	Y
			Slc28a3	17	8,665,223	8,715,359	N
			Hnrnpk	17	8,871,937	8,881,027	N
			Mir7a1	17	8,879,389	8,879,485	N
			Kif27	17	8,905,688	8,975,053	N
			Gkap1	17	8,989,135	9,028,910	N
			Ubqln1	17	9,044,249	9,080,136	N
			Mir874	17	9,222,188	9,222,263	N
			Trpc7	17	10,316,807	10,456,371	N
			Smad5	17	10,489,082	10,499,276	N
			Tgfbi	17	10,576,347	10,605,606	N
			Lect2	17	10,665,529	10,671,747	N
			Il9	17	10,733,181	10,736,304	N
			Pitx1	17	11,043,913	11,050,071	N
			Catsper3	17	11,065,031	11,090,130	N
			Txndc15	17	11,147,137	11,548,898	N
			Cxcl14	17	11,271,210	11,279,231	N
			Neurog1	17	11,315,715	11,317,234	N
			Tifab	17	11,395,058	11,398,075	N
			H2afy	17	11,440,883	11,502,758	N
			Mir3542	17	15,527,603	11,527,719	N
			Ddx46	17	11,582,992	11,626,966	N
			Camlg	17	11,631,232	11,642,058	N
			B4galt7	17	11,657,450	11,666,049	N
			Tmed9	17	11,668,161	11,672,674	N
			Fam193B	17	11,705,724	11,737,150	N
			Ddx41	17	11,740,661	11,746,053	N
			Dok3	17	11,748,066	11,752,826	Y
			Pdlim7	17	11,762,297	11,777,443	N
			Dbn1	17	11,788,597	11,802,569	Y
			Grk6	17	11,814,901	11,830,526	N
			Fl2	17	11,845,561	11,853,600	N
			Slc34a1	17	11,856,946	11,872,100	Y
			Rgs14	17	11,887,270	11,901,352	N
			Lman2	17	11,907,481	11,925,373	N
			Mxd3	17	11,940,006	11,943,732	N
			Prelid1	17	11,941,925	11,948,965	N
			Rab24	17	11,944,939	11,951,132	N
			Nsd1	17	11,953,813	12,062,583	N
			Fgfr4	17	12,099,383	12,113,632	Y
			Zfp346	17	12,130,923	12,168,871	N
			Uimc1	17	12,173,068	12,239,750	N
			Hk3	17	12,246,816	12,261,635	Y
			Unc5a	17	12,262,647	12,317,433	N
			Fbxo23	17	12,482,940	12,490,522	N
			Sncb	17	12,510,521	12,518,728	N
			Rnf44	17	12,589,090	12,596,031	N
			Faf2	17	12,613,753	12,653,474	N
			Cltb	17	12,665,188	12,682,747	N
			Higd29	17	12,684,712	12,685,636	N
			Nop16	17	12,683,809	12,692,726	N
			Arl10	17	12,694,957	12,701,201	N
			Thoc3	17	12,803,773	12,812,964	N
			Cplx2	17	12,883,653	12,892,106	N
			Hrh2	17	13,041,793	13,042,869	N
			Sfxn1	17	13,157,818	13,193,253	N
			Drd1a	17	13,212,535	13,214,770	N
			Msx2	17	13,785,024	13,790,688	N
			Sptlc1	17	13,756,140	13,995,224	N
			Ror2	17	14,050,995	14,228,982	Y
			Nfil3	17	14,359,252	14,374,102	N
			Auh	17	14,413,757	14,501,296	N
			Syk	17	14,703,903	14,759,160	N
			Diras2	17	14,854,091	14,855,291	N
			Gadd45g	17	15,469,297	15,471,042	N
			Sema4d	17	15,590,977	15,613,214	Y
			Secisbp2	17	15,630,050	15,661,255	N
			Cks2	17	15,662,365	15,667,466	N
			Shc3	17	15,743,125	15,862,911	N
			Nxnl2	17	16,120,216	16,124,963	Y

Table S2

Non-synonymous gene variation in the IgH locus among non-immunoglobulin genes

In addition to the IgH genes present in the congenic segment there are 18 potentially protein coding non-immunoglobulin genes. Many of these genes are potentially deleted by immunoglobulin class switch recombination in B cells. Only three of these genes have non-synonymous variation between SHR-A3 and SHR-B2. Details are provided in the table below. Wild type refers to the allelic state of the rat genome reference sequence.

Gene	SNP position	Amino acid position	Wild type	SHR-A3	SHR-B2
Gpr132	146,650,044	189	Asp	Asp	Lys
Gpr132	146,650,532	26	Thr	Ile	Thr
Gpr132	146,654,278	5	Pro	Ser	Pro
Adam6	147,428,273	531	Val	Val	Met
Wdr60	153,055,240	44	Thr	Ala	Thr

Figure S1

Chromosome 17 has 3 haplotype blocks that are non-identical by descent and are indicated here. The shaded block is the bloc transferred into the SHR-A3(chr17 SHR-B2) congenic line. The positions of markers, genotyped to ensure the integrity of the transferred block, are indicated.

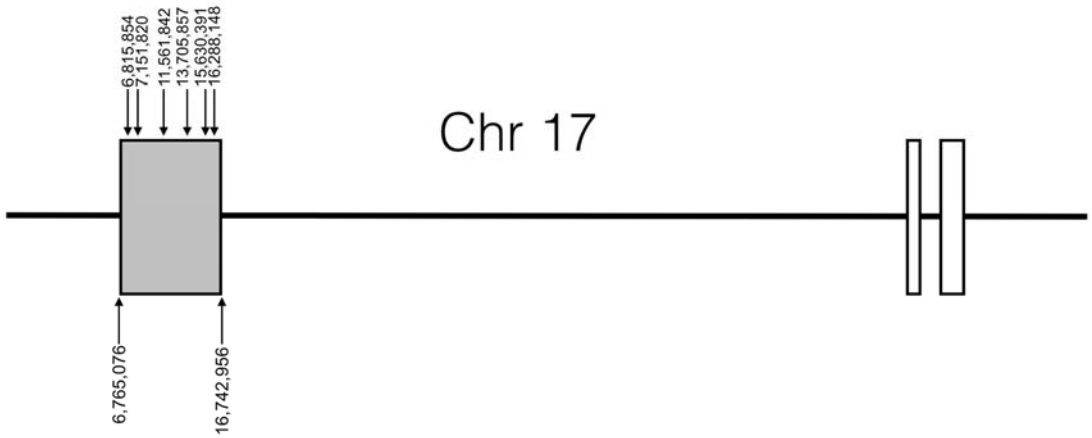

Figure S2

Blood pressure was measured by telemetry in SHR-A3 (n = 10) and SHR-A3(IgH SHR-B2) (n = 6) congenic rats. No significant difference was observed in systolic blood pressure between the two lines.