Hypertensive Renal Injury Is Associated With Gene Variation Affecting Immune Signaling

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Background—The spontaneously hypertensive rat (SHR) strain exists in lines that contrast strongly in susceptibility to renal injury in hypertension. These inbred lines share common ancestry, and only 13% of their genomes arise from different ancestors.

Methods and Results—We used next gen sequencing to detect natural allelic variation in 5 genes of the immunoreceptor signaling pathway (IgH, Dok3, Src, Syk, and JunD) that arise from different ancestors in the injury-prone SHR-A3 and the resistant SHR-B2 lines. We created an intercross between these lines, and in the F2 progeny, we observed that the inheritance of haplotype blocks containing the SHR-A3 alleles of these 5 genes correlated with increased albuminuria and histological measures of renal injury. To test whether accumulated genetic variation in this pathway may create a therapeutic target in hypertensive renal injury, rats of both lines were treated with the immunosuppressant mycophenolate mofetil (MMF). MMF reduced proteinuria (albumin to creatinine ratio) from 6.6 to 1.2 mg/mg (P<0.001) in SHR-A3. Glomerular injury scores were reduced in MMF-treated SHR-A3 from 1.6 to 1.4 (P<0.002). Tubulo-interstitial injury was reduced in MMF-treated SHR-A3 from 2.62 to 2.0 (P=0.001). MMF treatment also reduced renal fibrosis in SHR-A3 (3.9 versus 2.0; P<0.001).

Conclusions—Polygenic susceptibility to renal injury in hypertension arises in association with genetic variation in genes that participate in immune responses and is dramatically improved by reduction of immune system activity.


Key Words: genome ■ hypertension ■ kidney ■ renal disease

Chronic progressive kidney disease (CKD) risk is strongly predicted by CKD occurrence in family members. Among incident dialysis patients, 23% have a relative with end stage renal disease.1 Essential hypertension is a major correlate of CKD, but does not predict renal disease risk, as well as the occurrence of an affected relative. Rather hypertension seems to play a permissive role on which other factors determine disease risk. The heritable risk of CKD is well replicated in a result of Fc receptor binding. Additional genetic variation in heavy chain locus in this rat model of hypertensive renal disease.6,7 In SHR lines differing in CKD risk, injury susceptibility may arise within the genes participating in immune responses triggered by disturbed vascular autoregulation. We have recently reported extensive functional natural allelic variation affecting the immunoglobulin heavy chain locus in this rat model of hypertensive renal disease.8,9 This variation results in functional alteration to immunoglobulin G (IgG) sequence that is associated with severity of albuminuria. In addition to antigen recognition, IgG is a signaling ligand that drives downstream immune responses as a result of Fc receptor binding. Additional genetic variation in African Americans, suggesting an important role of genetic evolution of the host immune response to pathogens in the origin of susceptibility to CKD in hypertension.8

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When systemic blood pressure is elevated, autoregulation of renal capillary perfusion can break down.3 This may disturb the relationship between renal perfusion and local metabolic demand and initiate danger signals that in turn are activators of inflammation and immunity.6,7 In SHR lines differing in CKD risk, injury susceptibility may arise within the genes participating in immune responses triggered by disturbed vascular autoregulation. We have recently reported extensive functional natural allelic variation affecting the immunoglobulin heavy chain locus in this rat model of hypertensive renal disease.8,9 This variation results in functional alteration to immunoglobulin G (IgG) sequence that is associated with severity of albuminuria. In addition to antigen recognition, IgG is a signaling ligand that drives downstream immune responses as a result of Fc receptor binding. Additional genetic variation in
the Fc receptor signaling pathway may add to injury effects initiated by immunoglobulin.

In the present article, we identify additional genes in the immunoglobulin-signaling pathway that arise from different ancestors across injury-prone and resistant lines and show that they contain functional regulatory and structural genetic variation across the SHR lines. By examining the inheritance in SHR-A3 × SHR-B2 intercross F2 progeny of the haplotype blocks containing these immunoreceptor signaling alleles, substantial differences in renal injury susceptibility can be explained, supporting the conclusion that additive immune system genetic variation can be a key determinant of the renal injury response to hypertension. The hypothesis that heritable differences in immune signaling influence hypertensive renal injury in this genetic model was tested by comparing the response of renal injury–resistant and susceptible SHR lines to treatment with the immunosuppressant, mycophenolate mofetil (MMF).

Methods

Animals and Treatments

Studies were performed on male rats of the stroke-prone spontaneously hypertensive-A3 (SHR-A3, SHRSP/Bbb) and the injury-resistant SHR-B2 line. The origin of these inbred lines has been previously reported. Animals were housed in an American Association for the Advancement of Laboratory Animal Care–approved animal facility and provided a standard rodent chow diet and drinking water ad libitum. No stroke-enhancing dietary manipulations were used. The 2 lines are derived from a single pair of founders common to both lines. The number of the genome-shared identical by descent across these lines is partitioned into discrete haplotype blocks. SHR-A3 (males) and SHR-B2 (females) were crossed to generate an F1 progeny. The F1 progeny was further crossed to generate a freely segregating F2 progeny (n=210), as previously reported. All animal use was prospectively reviewed and approved by the University’s Animal Welfare Committee.

Genome Sequencing and Bioinformatic Analysis

Genomic DNA was extracted from samples of liver tissue obtained from male representatives of SHR-A3 and SHR-B2 (n=1 per SHR line) and quantified by UV spectroscopy. DNA samples were submitted to a commercial genome sequencing company (Axeq Technologies, Rockville, MD) for sequencing. Sequencing was performed using the Illumina HiSeq 2500 platform. An average of 1.37 billion reads of 100 bp was obtained from each sample, providing an average genome coverage of ≈45×. We mapped the paired-end reads to the rat assembly (RGSC3.4, Ensembl release 69) using Novoalign software (Novocraft.com, version V2.08.01) using default settings. After generating binary sequence alignment/map files, the mapped reads were sorted by coordinate location using SamTools and Picard tools. Finally, we used the Genome Analysis Tool Kit (GATK version v2.3-9-g5ebf34) to perform local realignment, recalibration, and variant calling. We removed false-positive calls by using a postcalling filter that enforces that each variant has a mapping quality >30, a base quality >20, and a coverage ≥10, with ≥3:7 ratio of variant to reference and the presence of the variant in reads from both orientations. The resulting variant call format files (vcf version 4.1) were annotated using Annovar software together with the rat genome annotations (RGSC3.4.69). Finally, to identify location of haplotype blocks, we visually inspected the paired-end read data in the integrative genomics viewer.

Blood Pressure Measurement

Blood pressure was measured by implanted telemetry (Data Sciences, St. Paul, MN) as previously reported. Animals were implanted at 16 weeks of age and allowed to recover for 1 week before blood pressure recordings began. Blood pressure was measured by continuous sampling for 30 seconds every 30 minutes for 24 hours. Blood pressure recording began 3 to 4 days after the initiation of drug/vehicle treatment and was repeated 1 day per week throughout the study.

Mycophenolate Mofetil Study

After implantation of blood pressure telemetry devices and a 1 week recovery period, singly housed male SHR-A3 and SHR-B2 were divided into 2 groups per line (n=8–10 per group): one group received 25 mg/kg/day MMF (CellCept, Genetech) as a single dose by gavage, whereas the other received a similar volume of water by gavage. Treatment commenced at 17 weeks of age, before emergence of histological renal injury.9 was administered 6 days per week and continued for 8 weeks. Animals were weighed once per week to allow adjustment of dosage as animals grew.

Histological Assessment of Renal Injury

At the conclusion of the drug/vehicle treatment period, kidneys were harvested from isoflurane-anesthetized rats by ventral laparotomy. Kidneys were cut into radial segments by hand, fixed in 4% buffered formalin, and embedded in paraffin using standard techniques. Five micron serial sections were stained with Periodic-Acid-Schiff’s stain for assessment of renal injury and Picro-sirus red to evaluate the extent of collagen accumulation as previously described.

Lymphocyte Infiltration

We assessed kidney lymphocyte infiltration using immunohistochemistry of paraffin-embedded kidney blocks from control- and MMF-treated animals of both rat lines. An antibody against the lymphocyte cell surface protein sialophorin (anti-CD43, Clone: W3/13; ThermoFisher Scientific, Waltham, MA) was used to detect lymphocytes; this antibody detects T lymphocytes, granulocytes, monocytes, and some B lymphocytes. Lymphocyte infiltration was assessed by counting CD43 positive cells present in 15 high power (×400) cortical fields per sample.

Albuminuria

We assessed albuminuria as urinary albumin:creatinine ratio. Urinary creatinine concentrations were determined by high-performance liquid chromatography in urine samples collected by direct puncture of the urinary bladder. Urine was collected during implantation of the telemetry device and again at the conclusion of the study. Urine albumin was measured by an ELISA specific for rat serum albumin (Bethyl Labs, Montgomery, TX).

Statistical Analysis

Normality testing of parametric data was performed using the Kolmogorov–Smirnov test as implemented in the package StatPlus (AnalystSoft, Inc., Vancouver, BC, Canada.) No data transformation was used. Comparison of data obtained from treated and untreated SHR lines was analyzed by analysis of variance in the same software. Non-parametric data were analyzed by Kruskal–Wallis test after which significance testing of multiple groups was performed by Mann–Whitney U test with P values corrected for multiple testing using the Bonferroni correction. The effect of allelic variation in multiple immune signaling genes on renal injury was examined in F2 progeny. The F2 progeny was further crossed to generate a freely segregating F2 progeny (n=210), as previously reported. All animal use was prospectively reviewed and approved by the University’s Animal Welfare Committee.

Results

We have reported extensive functional sequence variation in the Fc region of the immunoglobulin gamma heavy chains of SHR-A3 and its association with renal injury. Our analysis of the whole genome sequences for the 2 SHR lines reveals
that the IgH locus contains extensive variation between the 2 SHR lines; for SHR-B2, this is the most divergent region of the entire genome. Variation at the IgH locus results in highly divergent serum levels of 3 of the 4 rat IgG subclasses and total serum IgG levels in SHR-A3. IgG can drive inflammation by binding to and activating Fc receptors. Others have reported that genetic variation in the locus containing the immunoglobulin receptor gene, FCGR3, strongly influences susceptibility to renal immune disease. These findings suggest that the Ig-Fc receptor signaling pathway may be relevant to hypertensive renal disease in the SHR model in which extensive renal infiltration with immune cells occurs.

We sought to identify which genes in the Ig-Fc immunoreceptor signaling pathway might contain functional variation between SHR-A3 and SHR-B2. The FCGR3 gene lies in the 87% of the genome shared identical between SHR-A3 and SHR-B2. Targeted re-sequencing to detect copy number variation led us to conclude that this locus does not differ across our SHR lines (data not shown). Pro-inflammatory Fc immunoreceptors are coupled to the protein tyrosine kinase, Syk. Coupling between Fc receptors and Syk occurs after phosphorylation of the immunoreceptor tyrosine activation motif domains of the Fc receptor by Src kinases. A scaffold protein, Dok3, is involved in spatial coordination of Src signaling. Important outputs of this signaling pathway include the proliferation of immune cells and increased reactive radical production. Both of these responses involve the transcription factor JunD. Each of these immunoreceptor signaling genes (Src kinase, Dok3, Syk, and JunD) lies in the small part of the genome that has arisen from different ancestors in SHR-A3 and SHR-B2. Our analysis of the genome sequences of the 2 SHR lines in these regions is presented in Table I in the Data Supplement. We defined the haplotype blocks containing each of these genes and used analytical methods to determine which genes in these blocks contain amino acid variation across the 2 SHR lines. In aggregate, these 4 blocks contain the 5 immunoreceptor signaling genes that comprise 28 Mb of the entire genome. Variation in introns and no nonsynonymous variation occurs in introns and no nonsynonymous variation was detected. However, sequence variation was detected in the proximal promoter of the gene that may influence transcriptional control of expression.

Verification of Allelic Variation in Other SHR Lines
The selective breeding of distinct SHR lines has led to several injury-resistant SHR lines that are not genetically identical. We examined genomic sequence variation (Rat Genome Database) from 2 additional injury-resistant SHR lines and 1 additional SHR-A3 line to determine whether sequence variation identified in the immunoreceptor signaling pathway was consistently observed in other lines that share the same end organ injury phenotype (see Data Supplement). We found that injury-resistant SHR lines share the same haplotype IgG, Dok3, Syk, and JunD, whereas the 2 injury-prone SHR-A3 lines share the contrasting haplotype of these genes. For Src, the majority of variants observed also fell into dichotomous haplotypes distinguishing injury-prone from injury-resistant SHR lines (see Data Supplement).

Association of Immunoreceptor Gene Variation With Renal Injury Traits in the F2 Progeny of an SHR-A3 x SHR-B2 Intercross
We hypothesized that the accumulation of allelic variation in the immunoreceptor signaling pathway may cause differences in hypertensive renal injury observed in SHR-A3 compared with SHR-B2. To test this hypothesis, we examined the

JunD (chr 16)
We resequenced the JunD promoter and its single coding exon in SHR-A3 and SHR-B2 and identified extensive variation in the promoter. Altered JunD expression has been previously reported in rats in association with a promoter single nucleotide polymorphism. SHR-B2 was found to share this promoter variant, whereas SHR-A3 possessed the genotype associated with reduced expression. We examined our previously reported Affymetrix gene expression array data from the kidneys of SHR-A3 and SHR-B2 and observed abundant expression of JunD in kidney RNA from SHR-B2. In contrast, JunD expression in SHR-A3 was not detected. In F2 animals in which free segregation of these 2 JunD alleles can occur, the level of JunD gene expression assessed by Affymetrix array correlated strongly with the inheritance of 2, 1, or 0 SHR-A3 JunD alleles (see Figure I in the Data Supplement).

Dok3 (chr 17)
Two nonsynonymous amino acid substitutions were detected in SHR-A3 (Ile71Val and Ala83Val). These substitutions are located in the pleckstrin homology domain of Dok3. Dok3 is an adapter molecule that recruits molecules to the immunoreceptor signaling complex. The Ile71Val substitution replaces an isoleucine that occurs uncommonly at this position in mammalian Dok3 with a valine that is the most common mammalian residue at this location. The alanine/valine substitution at position 83 replaces a highly conserved residue among higher vertebrates, suggesting potential deleterious consequences of substitution in SHR-A3.

Syk (chr 17)
Syk is biallelic across SHR-A3 and SHR-B2. Most genetic variation occurs in introns and nonsynonymous variation was detected. However, sequence variation was detected in the proximal promoter of the gene that may influence transcriptional control of expression.

 Src Kinase (chr 3)
This gene is biallelic across the 2 SHR lines and contains 12 single nucleotide polymorphism variations. The SHR-A3 has a substitution in the conserved-3 position of the Kozak sequence. The substitution has been assessed in vitro and found to alter protein translation efficiency.
relationship in the F2 progeny of an SHR-A3 × SHR-B2 intercross, between renal injury and inheritance of the 4 haplotype blocks containing SHR-A3 alleles of IgH (chr6), Src (chr3), Dok3 and Syk (chr17), and JunD (chr16). We used single nucleotide polymorphism genotypes at the following positions to represent inheritance of these blocks: chr3:146,943,779; chr6:143,652,058; chr16:18,825,153; and chr17: 14,963,716 (Rn4). Table 1 contrasts the phenotypic values observed for distinct renal injury phenotypes in SHR-A3 and SHR-B2 animals at 25 weeks of age. The table also shows what portion of the parental line trait difference was explained by accumulation of SHR-A3 alleles at these 4 blocks in the F2 progeny. For fibrosis and albuminuria, two thirds of the trait difference could be attributed to SHR-A3 alleles at these 4 loci. About half of the parental line trait difference for glomerular and tubulo-interstitial injury was explained. These findings suggest that accumulation of genetic variation in loci encoding genes of the immunoglobulin signaling pathway can influence susceptibility to hypertensive renal disease in this rat model.

**Effect of Immunosuppression on Renal Injury and Lymphocyte Infiltration in SHR-A3 and SHR-B2**

**Albuminuria**

Treatment for 8 weeks with MMF of SHR-A3 animals resulted in strong suppression of albuminuria compared with untreated SHR-A3 controls (Figure 1). In untreated SHR-A3, albuminuria had become severe at 25 weeks of age. However, in SHR-A3 treated with MMF, albuminuria was markedly reduced (Figure 1). In injury-resistant SHR-B2, the age-related increase in albuminuria was much less and was unaffected by MMF treatment (gray lines). Group sizes: SHR-A3 Control 16 and 25 weeks old, n=10 and 8, respectively; SHR-B2 Control 16 and 25 weeks old, n=8; SHR-A3 MMF treated 16 and 25 weeks old, n=9; and SHR-B2 MMF treated 16 and 25 weeks old, n=9 and 8, respectively. CTRL indicates control; uACR, urinary albumin to creatinine ratio.

**Figure 1.** Urine samples were collected during implantation of blood pressure telemetry devices at 16 weeks of age and at the conclusion of the study. In untreated spontaneously hypertensive rat (SHR)-A3, albuminuria had become severe at 25 weeks of age. However, in SHR-A3, mycophenolate mofetil (MMF) treatment dramatically reduced albuminuria (broken black line; $P<0.001$). In injury-resistant SHR-B2, the age-related increase in albuminuria is much less and is unaffected by MMF treatment (gray lines). Group sizes: SHR-A3 Control 16 and 25 weeks old, n=10 and 8, respectively; SHR-B2 Control 16 and 25 weeks old, n=8; SHR-A3 MMF treated 16 and 25 weeks old, n=9; and SHR-B2 MMF treated 16 and 25 weeks old, n=9 and 8, respectively. CTRL indicates control; uACR, urinary albumin to creatinine ratio.

**Figure 2.** Tubulo-interstitial injury was assessed on a 1 to 5 scale as described in periodic acid–Schiff’s stained sections of kidney at the conclusion of the treatment period. Injury was more severe in untreated spontaneously hypertensive rat (SHR)-A3 than in SHR-B2. Treatment with mycophenolate mofetil (MMF) was associated with a marked reduction in tubulo-interstitial injury in SHR-A3 ($P<0.001$), but had no effect in SHR-B2. Group sizes: SHR-A3 control, n=10; SHR-A3 MMF treated, n=9; SHR-B2 control, n=9; and SHR-B2 MMF treated, n=8. Ctrl indicates control.
SHR-A3 animals (Figure 1). In contrast, MMF had no effect on urine albumin-creatinine ratios in injury-resistant SHR-B2 animals, which increased only slightly during the treatment interval in both the MMF-treated and control animals.

**Histologically Assessed Injury**

Similar effects of MMF to reduce renal injury in SHR-A3 were observed when kidney tissue harvested at the completion of the study was examined histologically. Tubulo-interstitial renal injury is prominent in the control SHR-A3 animals at 25 weeks of age. However, injury scores in MMF-treated SHR-A3 animals were not different than in SHR-B2 animals compared with tubulo-interstitial injury, glomerular damage is less extensive in SHR-A3. MMF treatment was associated with significant reductions in glomerular injury in SHR-A3 (P<0.002), but had no effect in SHR-B2. Group sizes as given in Figure 2. Ctrl indicates control.

**Glomerular Injury**

Glomerular injury was assessed on a 1 to 5 scale as described in periodic acid-Schiff's stained sections of kidney at the conclusion of the mycophenolate mofetil (MMF) treatment period. Injury was more severe in untreated spontaneously hypertensive rat (SHR)-A3 than in SHR-B2. Treatment with MMF reduced glomerular injury in SHR-A3 (P<0.002), but had no effect in SHR-B2. Group sizes as given in Figure 2. Ctrl indicates control.

**Renal Fibrosis**

Renal fibrosis was assessed on a 1 to 5 scale as described in Picro-sirius red-stained sections of kidney at the conclusion of the treatment period. Fibrosis was more severe in untreated spontaneously hypertensive rat (SHR)-A3 than in SHR-B2. Treatment with mycophenolate mofetil (MMF) was associated with a marked reduction in tubulo-interstitial injury in SHR-A3 (P<0.001), but had no effect in SHR-B2. Group sizes: SHR-A3 control, n=10; SHR-A3 MMF treated, n=10; SHR-B2 control, n=8; and SHR-B2 MMF treated, n=11. Ctrl indicates control.

**Renal Lymphocyte Infiltration**

Renal lymphocyte infiltration was assessed by immunohistochemistry using a rat anti-CD43 antibody. Lymphocyte infiltration was extensive in untreated 25-week-old spontaneously hypertensive rat (SHR)-A3 and was minimal in untreated SHR-B2. Treatment of SHR-A3 rats with mycophenolate mofetil (MMF) was associated with significant reductions in lymphocyte infiltration to levels indistinguishable from that in untreated SHR-B2 rats (P<0.001). Group sizes: SHR-A3 control, n=10; SHR-A3 MMF treated, n=6; SHR-B2 control, n=8; SHR-B2 MMF treated, n=8. Ctrl indicates control; and HPF, high power field.

**Blood Pressure (BP)**

Blood pressure (BP) was recorded throughout the treatment period, starting 2 to 4 days after gavage with mycophenolate mofetil (MMF) or water. BP increased progressively in untreated spontaneously hypertensive rat (SHR)-A3, whereas MMF-treated SHR-B2 animals, only small changes in BP were noted that were significant only at 4 weeks of treatment (*P<0.05, **P<0.01, ***P<0.001). Group sizes: SHR-A3 control, n=10; SHR-A3 MMF treated, n=10; SHR-B2 control, n=8; SHR-B2 MMF treated, n=9. Polynomial regression (degree 2) analysis of the BP trends over time was performed using R. Coefficient values and their significance are included as supplemental data. NS indicates not significant; and SHR, spontaneously hypertensive rat.
a significant reduction in glomerular damage in the SHR-A3 line (Figure 3). No effect on glomerular injury scores was observed as a result of MMF treatment in the SHR-B2 line. Examination of renal fibrosis using Picro-sirius red staining indicated that MMF treatment significantly reduced fibrosis in SHR-A3, but was without effect on the already low levels of fibrosis observed in SHR-B2 (Figure 4). To demonstrate that MMF altered immune cell infiltration into the kidney, we quantitated CD43-immunoreactive cells in paraffin-embedded sections of renal cortex. MMF treatment was associated with strongly reduced lymphocyte infiltration in SHR-A3. Lymphocyte infiltration in SHR-B2 was sparse and was not affected by MMF (Figure 5; Figure II in the Data Supplement).

**Blood Pressure Effects**

We began our treatment with MMF at a time when renal injury is beginning to emerge in SHR-A3.2 Progression of renal injury during the following weeks may result in additional increase in blood pressure as renal inflammation increases and function declines. We hypothesized that, if immune system genetic variation contributes to increasing blood pressure during the emergence of renal injury, then treatment with MMF would reduce the increase in blood pressure during the period when renal injury progresses in SHR-A3. We observed that in vehicle-treated SHR-A3 animals, blood pressure continued to rise over the 8 week study interval (Figure 6). Treatment of SHR-A3 with MMF prevented elevation of blood pressure in SHR-A3 during the period from 18- to 25 weeks when renal injury develops in untreated animals.2 Blood pressure in 17- to 18-week-old SHR-B2 animals is lower than that in SHR-A3.9 In SHR-B2, treatment with MMF produced no consistent significant effect on blood pressure in SHR-B2. Thus, immunosuppression in SHR-A3, during the period when renal injury is developing, was associated with both reduced injury and a suppression of the progressive elevation in blood pressure seen in untreated SHR-A3 animals during this period.

**Discussion**

Susceptibility to hypertensive renal disease in both human populations and animal models of disease indicates the role of risk factors that are separate from those producing hypertension.2,33,34 The heritability of risk implies that studies to identify genetic variation associated with susceptibility may shed light on disease mechanism. However, with the exception of a locus that has major effects on disease risk in African Americans,35,36 little progress has been made in defining the elements of genetic risk. This presumably reflects a complex genetic pathogenesis, including a polygenic inheritance, so that studies of even large human populations have yielded little substantial insight. The missing heritability in genome-wide association studies of human populations may also reflect uneven sampling of genetic variation. This is a substantial problem at the highly divergent immunoglobulin heavy chain locus.27

Hypertensive renal injury susceptibility in SHR-A3 is also a polygenic trait, and the power of quantitative trait mapping to define loci involved is constrained by a genetic architecture that may involve multiple variant genes and functional interactions among these genes.2 Our hypothesis that hypertensive renal injury may reflect genetic variation in immune mechanisms arises from our prior work showing that injury-prone SHR-A3 have extensive immune cell infiltration in the kidney and foam cells in the glomeruli.2 It is further substantiated by the presence of extensive functional genetic variation, compared with SHR-B2, in the immunoglobulin heavy chain.2 This variation in the IgH locus alters the preimmune immunoglobulin repertoire (the germ-line encoded combinations of VDJ gene segments that provide initial interaction with antigen in the B cell lineage).20 IgH locus variation in SHR-A3 compared with SHR-B2 also determines large differences in serum immunoglobulin levels (and therefore Fc receptor ligand concentrations) and disrupts some IgG–Fc receptor interactions.4 The immunoreceptor signaling pathway is enriched with genetic variation that is much greater than expected by chance alone. Overall, only ≈1 in 8 genes arise from different ancestors across the 2 SHR lines we studied. The finding that 5 of the genes in this pathway arise from different ancestors is unexpected and may reflect the effect of trait selection during the fixation of hypertensive end-organ injury susceptibility in SHR-A3.31

In the present study, we have proposed that genetic variation in additional genes of the immunoglobulin signaling pathway links the injury initiated by hypertension to severity of renal damage. Fc receptor variation in other rat strains involving the presence or absence of genomic sequence encoding a decoy Fc gamma receptor illustrates how genetic variation can modulate immunoreceptor signaling to affect renal disease risk.38 These observations prompted our investigation to uncover further genetic variation in this pathway as a mechanism influencing renal injury in hypertension. Sequence analysis of these genes has uncovered functional genetic variation in them that may contribute to differences in renal disease susceptibility in SHR lines. We have used both targeted re-sequencing of genes in the immunoglobulin signaling pathway as well as whole genome sequencing of both SHR lines. The former was performed in multiple animals of each SHR line, whereas the latter used DNA from 1 representative animal of each line. Such whole genome sequencing is not error free, but we have been able to confirm variation in SHR sequenced by other laboratories. The accumulation of functional genetic variation in the pathway suggested to us that immune signaling may be a specific therapeutic target by which hypertensive renal disease could be modulated. The capacity of MMF to markedly suppress distinct elements of renal disease in the injury-prone SHR line supports the involvement of natural allelic variants involved in immune signaling on loss of renal function and progression of tissue damage in hypertension.

MMF prevented renal injury–associated elevation of blood pressure in injury-prone SHR-A3, but was without consistent effects on blood pressure in injury-resistant SHR-B2. MMF is not a blood pressure lowering drug: it does not lower blood pressure in hypertensive SHR-B2 animals, and clinical reports linking this drug to lower blood pressure occur only when immune-mediated renal disease coexists. The capacity of MMF to prevent the progressive elevation of blood pressure as renal injury emerges in SHR-A3 can be reasonably attributed to its immunosuppressive effect and the reduction in progression of renal injury that occurs when MMF is administered.
Early evidence of the role of immunity in hypertensive kidney injury has been extended by the introduction of knockout models, modern immunosuppressant drugs, and growing evidence of interactions between angiotensin II, hypertension, and immune function. The observations we report in the present study focus attention specifically on the role of B cell-mediated immunity. However, MMF-mediated immunosuppression may also suppress immune mechanisms involving T cells, macrophages, and possibly other cellular components of the immune system. Several of the genes we have investigated are known to also function in other components of the cellular immune system, and genetic variation has been associated with altered immune cell function. Finally, our F2 intercross studies suggest that a large fraction, but not all, of the heritable risk of renal injury is attributable to loci containing the genes we have identified, but disease risk unaccounted in our studies may lie outside these genomic regions.

In conclusion, we report here that functional genetic variation is enriched in the immunoreceptor signaling pathway of an injury-prone SHR line beyond that expected by chance. Examination of the inheritance of this variation, by tracking the transmission of the loci containing the variation in an F2 intercross, provides support that this variation is associated with susceptibility to renal injury. Finally, the potential causative role of these genes is supported by the effect of immunosuppression with MMF to reduce renal injury.

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Disclosures

None.

References


**CLINICAL PERSPECTIVE**

Hypertension is a major correlate of progressive renal disease. However, the presence of end-stage renal disease in a relative is the strongest predictor of risk of disease, indicating heritable risk of renal disease. Genetic studies indicate that susceptibility to renal disease arises independently from susceptibility to hypertension. Genetic models of hypertensive renal disease may shed light on the mechanisms of renal damage because they allow deeper phenotypic characterization of disease and its progression. Here we report studies from 2 lines of spontaneously hypertensive rats that differ in their susceptibility to hypertensive renal injury, but share 87% of their genomes identical by descent. Our studies reveal an important role for genetic variation in pathways involved in immune signaling in the genesis of renal disease. This pathway presents a druggable target, and pharmacological suppression of immune function using mycophenolate mofetil markedly reduces albuminuria and histological measures of renal damage in the injury-prone rat line.
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Supplemental Table 1. Location and extent of blocks of non-IBD containing genes of the immunoreceptor pathway and effects of ancestral variation on protein coding genes in block. The table indicates only genes with non-synonymous variation from the Brown-Norway (BN) reference genome in each block and omits the immunoglobulin heavy chain (chr 6) because of the extreme variation observed in this gene. The table also indicates confirmation of alleles in whole genome sequence from another SHR-A3 colony (Glasgow) and whether the variant allele is present in either of 2 injury-resistant (SHRSR) lines for which whole genome sequence data is available.

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**IgG Fc variants (positions from rn5):**

1. **Location:** chr6:147108547 intron
   SHR-A3 TGGAGCTTGGTCCACACCTGTAGACAAAT
   SHR-B2 TGGAGCTTGGTCCACACCTGTAGACAAAT
   SHR-B2 different from BN reference

2. **Location:** chr6:147107456 exon
   SHR-A3 ACCTTCAGATGCAAGGTCCACCAGTCAGCTTTCCATCC
   SHR-B2 ACCTTCAGATGCAAGGTCCAACAGTCAGCTTTCCATCC
   SHR-B2 different from BN reference, synonymous

3. **Location:** chr6:147141845 intron
   SHR-A3 AGACAAAATGATCACGCATACTTATTCTTGTAGCTGAAACAAC
   SHR-B2 AGACAAAATGATCACGCATACTTTTTCTTGTAGCTGAAACAAC
   SHR-A3 different from BN reference

4. **Location:** chr6:14714178 intron
   SHR-A3 TAAGGCTATGACGTACACAGACACGTTGCGGAACAG
   SHR-B2 TAAGGCTATGACGTACACAGACACGTTGCGGAACAG
   SHR-B2 different from BN reference

5. **Location:** chr6:147141081 intron
   SHR-A3 ACAGCTTATGACGTATGCAAGACCTATTCATCTGTC
   SHR-B2 ACAGCTTATGACGTATGCAAGACCTATTCATCTGTC
   SHR-B2 different from BN reference, non-synonymous, Gly to Val

6. **Location:** chr6:147141059 exon
   SHR-A3 TTTCCTTTGCATCTAAGCTAGAGTATCATCTGTC
   SHR-B2 TTTCCTTTGCATCTAAGCTAGAGTATCATCTGTC
   SHR-A3 different from BN reference, non-synonymous, Ser to Pro

7. **Location:** chr6:147141057 exon
   SHR-A3 TCTTCTTCACTAAACAGGCTAGCTGCTGCTCCTATCTC
   SHR-B2 TCTTCTTCACTAAACAGGCTAGCTGCTGCTCCTATCTC
   SHR-A3 different from BN reference, non-synonymous, Ser to Pro

8. **Location:** chr6:147141051-2 exon
   SHR-A3 TCTACCTACGGCTACTGTCTATGTCTGTCCTCATCTCTG
   SHR-B2 TCTACCTACGGCTACTGTCTATGTCTGTCCTCATCTCTG
   SHR-A3 different from BN reference, non-synonymous, Glu to Asp, Val to Ile

9. **Location:** chr6:147140508-9 exon
   SHR-A3 TCTATCCACACACACACACACACATCTGCTGCTGCTG
   SHR-B2 TCTATCCACACACACACACACACATCTGCTGCTGCTG
   SHR-A3 different from BN reference, non-synonymous, Tyr to Val

10. **Location:** chr6:147086792 intron
    SHR-A3 GACAGAAGGTGCACTACAGAAAGGGGTAGGTAGGAACAG
    SHR-B2 GACAGAAGGTGCACTACAGAAAGGGGTAGGTAGGAACAG
    SHR-A3 different from BN reference

11. **Location:** chr6:147086612 intron
SHR-A3 CACTGAAGATCAAGGCTCCTGGGCTGGAGAGTTGGCTC
SHR-B2 CACTGAAGATCAAGGCTCCTGGGCTGGAGAGTTGGCTC

SHR-A3 different from BN reference

12. Location: chr6: 147086388 intron
SHR-A3 AAAAGTCAAGAGGTCTCAAGGCTCCTGGGACCTGCTGACACCTTA
SHR-B2 AAAAGTCAAGAGGTCTCAAGGCTCCTGGGACCTGCTGACACCTTA

SHR-A3 different from BN reference

13. Location: chr6: 147086179 exon
SHR-A3 GATACACCCAGCTCCACCCGGTGAGGTAGGGTCTGGGATGCCTGGTCAAGGG
SHR-B2 GATACACCCAGCTCCACCCGGTGAGGTAGGGTCTGGGATGCCTGGTCAAGGG

SHR-A3 different from BN reference

14. Location: chr6: 147086108 exon
SHR-A3 GATACAACCAGCTCCACCCGGTGAGGTAGGGTCTGGGATGCCTGGTCAAGGG
SHR-B2 GATACAACCAGCTCCACCCGGTGAGGTAGGGTCTGGGATGCCTGGTCAAGGG

SHR-A3 different from BN reference, synonymous

15. Location: chr6: 147086092 exon
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SHR-B2 GATACAACCAGCTCCACCCGGTGAGGTAGGGTCTGGGATGCCTGGTCAAGGG

SHR-A3 different from BN reference, non-synonymous, Ala to Pro

16. Location: chr6: 147086065 exon
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SHR-A3 different from BN reference, synonymous

17. Location: chr6: 147085968 exon
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SHR-B2 GATACAACCAGCTCCACCCGGTGAGGTAGGGTCTGGGATGCCTGGTCAAGGG

SHR-A3 different from BN reference, synonymous

18. Location: chr6: 147085957 intron
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SHR-B2 GATACAACCAGCTCCACCCGGTGAGGTAGGGTCTGGGATGCCTGGTCAAGGG

SHR-A3 different from BN reference

19. Location: chr6: 147085842 intron
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SHR-B2 GATACAACCAGCTCCACCCGGTGAGGTAGGGTCTGGGATGCCTGGTCAAGGG

SHR-A3 different from BN reference

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SHR-B2 GATACAACCAGCTCCACCCGGTGAGGTAGGGTCTGGGATGCCTGGTCAAGGG

SHR-A3 different from BN reference

21. Location: chr6: 147085482 intron
SHR-A3 GATACAACCAGCTCCACCCGGTGAGGTAGGGTCTGGGATGCCTGGTCAAGGG
SHR-B2 GATACAACCAGCTCCACCCGGTGAGGTAGGGTCTGGGATGCCTGGTCAAGGG

SHR-A3 different from BN reference
22. Location: chr6: 147085758 intron
   SHR-A3 AGACATGATCCCTCTGGATGATAGTCTGTCATGTATAGGATCATAC
   SHR-B2 AGACATGATCCCTCTGGATGATAGTCTGTCATGTATAGGATCATAC
   SHR-A3 different from BN reference

23. Location: chr6: 147085750 intron
   SHR-A3 TGGTATAGTGTCTGTGTCATGCATAGGATCATACCAGGGACAAA
   SHR-B2 TGGTATAGTGTCTGTGTCATGCATAGGATCATACCAGGGACAAA
   SHR-A3 different from BN reference

24. Location: chr6: 147085643 exon
   SHR-A3 CTCTACAGACGCCGAAATGGGGGCACTGGACACAAATG
   SHR-B2 CTCTACAGGGGCAAAATGGGGGCACTGGACACAAATG
   SHR-B2 different from BN reference, non-synonymous, Ile to Asn

25. Location: chr6: 147085580 intron
   SHR-A3 GCCCAGTAAATCTGACTGACCTGGGCCCTCGACTCGAGACG
   SHR-B2 GCCCAGTAAATCTGACTGACCTGGGCCCTCGACTCGAGACG
   SHR-A3 different from BN reference

26. Location: chr6: 147085489 exon
   SHR-A3 CATTTCTATCTCTCCTCATGCTCGGAGAGGAGGAGCCAGACGTCCAG
   SHR-B2 CATTTCTATCTCTCCTCATGCTCGGAGAGGAGGAGCCAGACGTCCAG
   SHR-B2 different from BN reference

27. Location: chr6: 147085464 exon
   SHR-A3 CCATCTGGACATCTTCCCCAAAGCCCAAGGACATCTCCGTTTGA
   SHR-B2 CCATCTGGACATCTTCCCCAAAGCCCAAGGACATCTCCGTTTGA
   SHR-A3 different from BN reference, synonymous

28. Location: chr6: 147085449 exon
   SHR-A3 CCATCTGGACATCTTCCCCAAAGCCCAAGGACATCTCCGTTTGA
   SHR-B2 CCATCTGGACATCTTCCCCAAAGCCCAAGGACATCTCCGTTTGA
   SHR-A3 different from BN reference, synonymous

29. Location: chr6: 147085379 exon
   SHR-A3 ACCTGTGGTGGATGGATGGATGGAGAGCCAGACGTCCAG
   SHR-B2 ACCTGTGGTGGATGGATGGATGGAGAGCCAGACGTCCAG
   SHR-A3 different from BN reference, synonymous

30. Location: chr6: 147085368 exon
   SHR-A3 CTGATGAGCAGGAGCGAGGCAGGCTACGTTTCGAGCCTTGGTTT
   SHR-B2 CTGATGAGCAGGAGCGAGGCAGGCTACGTTTCGAGCCTTGGTTT
   SHR-A3 different from BN reference, synonymous

31. Location: chr6: 147085102 intron
   SHR-A3 CTCTGAGATGCTTATGAGATGTGCTCTGTCATGTATAGGATCATAC
   SHR-B2 CTCTGAGATGCTTATGAGATGTGCTCTGTCATGTATAGGATCATAC
   SHR-A3 different from BN reference, synonymous

31. Location: chr6: 1470850352 exon
   SHR-A3 GTCAGAAACACAGCAGGAGCTGCTGCTCCAGGACAGACGAG
   SHR-B2 GTCAGAAACACAGCAGGAGCTGCTGCTCCAGGACAGACGAG
   SHR-A3 different from BN reference, synonymous
32. Location: chr6: 147084991-5001 exon
SHR-A3 GACAGAGCAGTTGACTGAGAAAGCGGTCATTTTGACCTGCTTGA
SHR-B2 GACAGAGCAGTTGACTGAGAAACGGGTCATTTTGACCTGCTTGA
SHR-A3 different from BN reference, non-synonymous Gln to Lys

33. Location: chr6: 147084818 exon
SHR-A3 GGAGCAGGTGGGATAGCAGAGCGTCCTTCGTCTGCTCCGTGGTCCACGA
SHR-B2 GGAGCAGGTGGGATAGCAGAGCGCCCTTCGTCTGCTCCGTGGTCCACGA
SHR-A3 different from BN reference, non-synonymous, Pro to Ser

34. Location: chr6: 147084778 exon
SHR-A3 GTCCACGAGGGTCTGCACAATCGCCACGTGGAGAAGAGCATCTC
SHR-B2 GTCCACGAGGGTCTGCACAATCGCCACGTGGAGAAGAGCATCTC
SHR-A3 different from BN reference, non-synonymous, His to Arg

35. Location: chr6: 147189280 exon
SHR-A3 AGCCGTAACCGTAAAATGGAACTCTGGAGCCCTGTCCAGCGGTGTG
SHR-B2 AGCCGTAACCGTAAAATGGAACTATGGAGCCCTGTCCAGCGGTGTG
SHR-B2 different from BN reference, non-synonymous Ser to Tyr

36. Location: chr6: 147189135 exon
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SHR-B2 AGCCCACCCAGCCACCAAAAGCAACTTGATCAAGAGAATTGGTA
SHR-B2 different from BN reference, non-synonymous Asp to Asn

37. Location: chr6: 147189069 intron
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SHR-B2 AATATTTAATCAGGAGGTCAGGCTGGGGTCAACCCCTTGTATAGAACA
SHR-B2 different from BN reference

38. Location: chr6: 147188923 intron
SHR-A3 CTCTTCCCCAGGCCCCATTAATGACCAGTCTTTTCTCTGC
SHR-B2 CTCTTCCCCAGGCCCCATTAATGACCAGTCTTTTCTCTGC
SHR-B2 different from BN reference

39. Location: chr6: 147188913 intron
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SHR-B2 CTCCTGGAATGCTCCTGGGAATGCTCCTACATCAGGGAG
SHR-B2 different from BN reference

40. Location: chr6: 147188913 intron
SHR-A3 CTCTTCCCCAGGCCCCATTAATGACCAGTCTTTTCTCTGC
SHR-B2 CTCTTCCCCAGGCCCCATTAATGACCAGTCTTTTCTCTGC
SHR-B2 different from BN reference

41. Location: chr6: 147188770 intron
SHR-A3 GGACGAGGATCCCTAGAGAGCTACATCTGACACACAG
SHR-B2 GGACGAGGATCCCTAGAGAGCTACATCTGACACACAG
SHR-B2 different from BN reference

42. Location: chr6: 147188379 exon
SHR-A3 GTGAGTACCGTCCCATATCCAGCAACGGACTGGA
SHR-B2 GTGAGTACCGTCCCATATCCAGCAACGGACTGGA
43. Location: chr6: 147188230 intron
SHR-A3 CTGGGATGGGCGTAAGAATTAATGCTGCGTGGACG
SHR-B2 CTGGGATGGGCGTAAGAATGAATGCTGCGTGGACG
SHR-B2 different from BN reference, synonymous

44. Location: chr6: 147188078 exon
SHR-A3 ACCAGCTTCTACCCCGATCCATCAGTGGAGT
SHR-B2 ACCAGCTTCTACCCCGATCCATCAGTGGAGT
SHR-A3 different from BN reference (Ala to Pro)

45. Location: chr6: 147187959 exon
SHR-A3 ACAGCAAGCTCAGTGTGGACACGGACAGTTGGATGCGAG
SHR-B2 ACAGCAAGCTCAGTGTGGACACAGACAGTTGGATGCGAG
SHR-B2 different from BN reference, synonymous
Haplotype structure in SHR strains across this region. SHR-A3 and SHRSP/Gla are injury-prone strains. SHR-B2, SHR and SHR/NHsd are injury resistant strains. SHR lines with the same genotype as SHR-A3 at each variant site are colored pink at that site. SHR lines with the same genotype as SHR-B2 are shaded green when this genotype differs from the injury-prone lines. Genome sequence variation for SHRSP/Gla, SHR, SHR/NHsd was provided by the Rat Genome Database.

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**Dok3 variants (positions from rn5):**

1. Location: chr17:11747889 proximal promoter
   SHR-A3 AAGCTCCCATAGGCTAACGTGGTTCTTGGAAACACAGCAAGG
   SHR-B2 AAGCTCCCATAGGCTAACGTGGTTCTTGGAAACACAGCAAGG
   SHR-B2 differs from BN reference seq

2. Location: chr17:11748271 intron
   SHR-A3 GCTGAAGTGAAAGCTGGAGACGGGAGGTTCTTGGCCCAAGAA
   SHR-B2 GCTGAAGTGAAAGCTGGAGACGGGAGGTTCTTGGCCCAAGAA
   SHR-A3 differs from BN reference seq

3. Location: chr17:11748474 proximal promoter
   SHR-A3 CAGGCTGGTTCTCCTGGAGAAGAGATGGGCTGGGAATCTAT
   SHR-B2 CAGGCTGGTTCTCCTGGAGAAGAGATGGGCTGGGAATCTAT
   SHR-A3 differs from BN reference seq

4. Location: chr17:11749072 exon 2
   SHR-A3 GCCGTAGAGGGAAAGGCCTCATACGCCTGGCTGACTGT
   SHR-B2 GCCGTAGAGGGAAAGGCCTCATACGCCTGGCTGACTGT
   SHR-A3 differs from BN reference seq, non-synon Ile to Val (I71V)

5. Location: chr17:11749109 exon 2
   SHR-A3 CTGTGTATCTCTTCTGCCGGTGGATGGTGAGAGTTGCCCCA
   SHR-B2 CTGTGTATCTCTTCTGCCGGTGGATGGTGAGAGTTGCCCCA
   SHR-A3 differs from BN reference seq, non-synon Ala to Val (A83V)

6. Location: chr17:11749790 intron
   SHR-A3 TCTTCTCAGCACTGCTCATAGGCTGGAGGATGGTGAGTGCTGC
   SHR-B2 TCTTCTCAGCACTGCTCATAGGCTGGAGGATGGTGAGTGCTGC
   SHR-B2 differs from BN reference seq

7. Location: chr17:11750490 intron
   SHR-A3 CTAATACCTGAACTGGGACTCTTTAAAATAGTAATGTCC
   SHR-B2 CTAATACCTGAACTGGGACTCTTTAAAATAGTAATGTCC
   SHR-B2 differs from BN reference seq

8. Location: chr17:11750514 intron
   SHR-A3 TTAAAATAGTAATGTCCAGGGTGAGAAATGACTCCGTAG
   SHR-B2 TTAAAATAGTAATGTCCAGGGTGAGAAATGACTCCGTAG
   SHR-B2 differs from BN reference seq

9. Location: chr17:11750578 intron
   SHR-A3 AGATGATAGTTCAATTCCAGCAACACCAGATGGCAGCTC
   SHR-B2 AGATGATAGTTCAATTCCAGCAACACCAGATGGCAGCTC
   SHR-B2 differs from BN reference seq

10. Location: chr17:11750642 intron
    SHR-A3 AGAGGACTGACACCCTCAACAATCCAATCAACACACAAAA
    SHR-B2 AGAGGACTGACACCCTCAACAATCCAATCAACACACAAAA
    SHR-B2 differs from BN reference seq

11. Location: chr17:11750740 intron
SHR-A3 TGTAGACAAGGTTTCTCCATATAGCCCTGGCTGTTCTAGAA
SHR-B2 TGTAGACAAGGTTTCTCCATGTAGCCCTGGCTGTTCTAGAA
SHR-B2 differs from BN reference seq

12. Location: chr17:11751049 intron
SHR-A3 CTGTCTTGAAAAACAAAAAGGAAAAAGTCAGGTAG
SHR-B2 CTGTCTTGAAAAACAAAAAGGAAAAAGTCAGGTAG
SHR-B2 differs from BN reference seq

13. Location: chr17:11751393 intron
SHR-A3 CCTGGGAGTGGTGGTCAGAGTGAGCTGATGGCGGAGAGTG
SHR-B2 CCTGGGAGTGGTGGTCAGAGCGAGCTGATGGCGGAGAGTG
SHR-B2 differs from BN reference seq

14. Location: chr17:11752923 3'-UTR
SHR-A3 TGCTCAGTGTAGCAAGCCTGGCTGGACCTGAG
SHR-B2 TGCTCAGTGTAGCAAGCCTGGCTGGACCTGAG
SHR-A3 differs from BN reference seq

Haplotype structure in SHR strains across this region. SHR-A3 and SHRSP/Gla are injury-prone strains. SHR-B2, SHR and SHR/NHsd are injury resistant strains. SHR lines with the same genotype as SHR-A3 at each variant site are colored pink at that site. SHR lines with the same genotype as SHR-B2 are shaded green when this genotype differs from the injury-prone lines. Genome sequence variation for SHRSP/Gla, SHR, SHR/NHsd was provided by the Rat Genome Database.

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Src variants (positions are rn5):

1. Location: chr3:158138524 Promoter
   SHR-A3  CAGGACCTTATCTGCTCCTATCTGTTAGTGGGTAGGGTGTCTTGTCTT
   SHR-B2  CAGGACCTTATCTGCTCCTATCTGTTAGTGGGTAGGGTGTCTTGTCTT
   SHR-A3 differs from BN reference sequence

2. Location: chr3:158138450 Promoter Kozak consensus sequence
   SHR-A3  GGGATTTCTGTCGCCCCCAGCCAGGGCCATGGGCAGCAACAAGAGCAAGC
   SHR-B2  GGGATTTCTGTCGCCCCCAGCCAGGGCCATGGGCAGCAACAAGAGCAAGC
   SHR-A3 differs from BN reference sequence

3. Location: chr3:158136014 Intron
   SHR-A3  TATTCACCCCTCAAGATCCAGGGTGGGGCTGCCCTCCCTGCAAGCAGGT
   SHR-B2  TATTCACCCCTCAAGATCCAGGGTGGGGCTGCCCTCCCTGCAAGCAGGT
   SHR-A3 differs from BN reference sequence

4. Location: chr3:158134950 Intron
   SHR-A3  ACAGTGTGTGTCTCAGAGAACTACTGAGCTCTGGGGGGCCGGGGCAGTACAG
   SHR-B2  ACAGTGTGTGTCTCAGAGAACTACTGAGCTCTGGGGGGCCGGGGCAGTACAG
   SHR-A3 differs from BN reference sequence

5. Location: chr3:158131765 Intron
   SHR-A3  ATGGCAACCAAGCCAGGCTGCTTCCTGTGGGCCTGGGGCTCAGCAGGAGA
   SHR-B2  ATGGCAACCAAGCCAGGCTGCTTCCTGTGGGCCTGGGGCTCAGCAGGAGA
   SHR-A3 differs from BN reference sequence

6. Location: chr3:158129031 Intron
   SHR-A3  TGTGGCCAGGTCTCACACCAAGCCTCAGCCTCCTGATGTGAACAC
   SHR-B2  TGTGGCCAGGTCTCACACCAAGCCTCAGCCTCCTGATGTGAACAC
   SHR-A3 differs from BN reference sequence

7. Location: chr3:158128929 Intron
   SHR-A3  AGTGCTGTGGCGAGGGAGTAGTGGGAAGTAGGTGTCCGGTCTCCCG
   SHR-B2  AGTGCTGTGGCGAGGGAGTAATGGGAAGTAGGTGTCCGGTCTCCCG
   SHR-A3 differs from BN reference sequence

8. Location: chr3:158127846 Intron
   SHR-A3  CAGGCTGACCTCTGTCAGATGCCGCACACGGGAGACTGAGGGTAAC
   SHR-B2  CAGGCTGACCTCTGTCAGATGCCGCACACGGGAGACTGAGGGTAAC
   SHR-A3 differs from BN reference sequence

9. Location: chr3:158127694 Intron
   SHR-A3  GCCTCGGGTGTGACCGTGGTGTGTGTTGGTGTGTGTTGGT
   SHR-B2  GCCTCGGGTGTGACCGTGGTGTGTGTTGGTGTGTGTTGGT
   SHR-A3 differs from BN reference sequence

10. Location: chr3:158127366 Intron
    SHR-A3  TACACAGCTCGGGAACAGGGGTATCTGTACGGTGTATCGG
    SHR-B2  TACACAGCTCGGGAACAGGGGTATCTGTACGGTGTATCGG
    SHR-A3 differs from BN reference sequence

11. Location: chr3:158126261 Intron
    SHR-A3  TACACAGCTCGGGAACAGGGGTATCTGTACGGTGTATCGG
    SHR-B2  TACACAGCTCGGGAACAGGGGTATCTGTACGGTGTATCGG
    SHR-A3 differs from BN reference sequence
SHR-B2  TACACAGCTCGCAAGGGTGGAGTGGCGGTTATCTGATGGTATCGG
SHR-A3 differs from BN reference sequence
12. Location: chr3:158125852 Intron
SHR-A3 TGCCTCTGGTGAGCTAGCTTCAAGGCTGACG
SHR-B2 TGCCTCTGGTGAGCTAGCTTCAAGGCTGACG
SHR-A3 differs from BN reference sequence

Haplotype structure in SHR strains across this region. SHR-A3 and SHRSP/Gla are injury-prone strains. SHR-B2, SHR and SHR/NHsd are injury resistant strains. SHR lines with the same genotype as SHR-A3 at each variant site are colored pink at that site. SHR lines with the same genotype as SHR-B2 are shaded green when this genotype differs from the injury-prone lines. Genome sequence variation for SHRSP/Gla, SHR, SHR/NHsd was provided by the Rat Genome Database.

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Syk variants (positions from rn5):

1. Location: chr17:14759592 proximal promoter
   SHR-A3 GTTTCCTATTTTTTAGCCTCAGAGTTTTTTTTTCATGTTACT
   SHR-B2 GTTTCCTATTTTTTAGCCTCAGAGTTTTTTTTTCATGTTACT
   SHR-A3 differs from BN reference seq

2. Location: chr17:14730906 Intron
   SHR-A3 GTGTCTACATCCCCAGGCTAGATCCAGATGTTAC
   SHR-B2 GTGTCTACATCCCCAGGCTAGATCCAGATGTTAC
   SHR-B2 differs from BN reference seq

3. Location: chr17:14728977 Intron
   SHR-A3 CTGAGCCTCGCTGCCCCTGCACAAGATCGACTCGCTG
   SHR-B2 CTGAGCCTCGCTGCCCCTGCACAAGATCGACTCGCTG
   SHR-B2 differs from BN reference seq

4. Location: chr17:14721420 Exon
   SHR-A3 GCCCTGCCCATGGACACCGAGGTATATGAGAGTCCTTACGCTG
   SHR-B2 GCCCTGCCCATGGACACCGAGGTATATGAGAGTCCTTACGCTG
   Synonymous, codon is Thr, third base of codon affected
   SHR-A3 differs from BN reference seq

5. Location: chr17:14706170 3’UTR
   SHR-A3 GGTCACGTGGTATCCACACAGAGGAAGCGAAGAATGAACAGGAA
   SHR-B2 GGTCACGTGGTATCCACACTGAGGAAGCGAAGAATGAACAGGAA
   SHR-A3 differs from BN reference seq

6. Location: chr17:14704598 3’UTR
   SHR-A3 GGTTTGAGACTGACCTCTCTGCTATCCAGATGCTGACACGAGG
   SHR-B2 GGTTTGAGACTGACCTCTCTGCTATCCAGATGCTGACACGAGG
   SHR-B2 differs from BN reference seq

7. Location: chr17:14704464 3’UTR
   SHR-A3 TCTGTGTGAAATTCGTGTCGGATAGGTTAACATCTCTCCTCCTG
   SHR-B2 TCTGTGTGAAATTCGTGTCGGATAGGTTAACATCTCTCCTCCTG
   SHR-B2 differs from BN reference seq

8. Location: chr17:14704198 3’UTR
   SHR-A3 CCCTGATCTCGGCTGCACCACATCCACTGACCTCACCACACACATACC
   SHR-B2 CCCTGATCTCGGCTGCACCACATCCACTGACCTCACCACACACATACC
   SHR-B2 differs from BN reference seq

9. Location: chr17:14704196 3’UTR
   SHR-A3 CCCTAATCTCGCCCTGCCACACATCCACTGACCTCACCACACACATACC
   SHR-B2 CCCTAATCTCGCCCTGCCACACATCCACTGACCTCACCACACACATACC
   SHR-A3 differs from BN reference seq
Haplotype structure in SHR strains across this region. SHR-A3 and SHRSP/Gla are injury-prone strains. SHR-B2, SHR and SHR/NHsd are injury resistant strains. SHR lines with the same genotype as SHR-A3 at each variant site are colored pink at that site. SHR lines with the same genotype as SHR-B2 are shaded green when this genotype differs from the injury-prone lines. Uncolored boxes indicate no genomic sequence variation data for SHRSP/Gla, SHR or SHR/NHsd. Genome sequence variation for SHRSP/Gla, SHR, SHR/NHsd was provided by the Rat Genome Database.

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JunD variants (positions from rn5):

1. Location: chr16:20344584 proximal promoter
   SHR-A3: TGAGATCTTGTCTTTAAGCCCAAAAACACAGATTAAA
   SHR-B2: TGAGATCTTGTCTTTAAGCCCAAAAACACAGATTAAA
   SHR-B2 differs from BN reference seq

2. Location: chr16:20344303 proximal promoter
   SHR-A3: ACCCTGGTTGCGCCCGAGGGGCGGCGGCTGCTAT
   SHR-B2: ACCCTGGTTGCGCCCGAGGGGCGGCGGCTGCTAT
   SHR-A3 differs from BN reference seq

3. Location: chr16:20344265 proximal promoter
   SHR-A3: ACCCTGGTTGCGCCCGAGGGGCGGCGGCTGCTAT
   SHR-B2: ACCCTGGTTGCGCCCGAGGGGCGGCGGCTGCTAT
   SHR-A3 differs from BN reference seq

4. Location: chr16:20344104-5 proximal promoter
   SHR-A3: GAGAGAGAGAGAGAGAGAGACAAGAAACAGCAGGGGA
   SHR-B2: GAGAGAGAGAGAGAGAGAGACAAGAAACAGCAGGGGA
   SHR-A3 differs from BN reference seq

5. Location: chr16:20344084-5 proximal promoter
   SHR-A3: GGGAGGAAGAGAGAGGG--GAGAGAGAGAGAGAGAGA
   SHR-B2: GGGAGGAAGAGAGAGGGTAGAGAGAGAGAGAGAGAGA
   SHR-B2 differs from BN reference seq

6. Location: chr16:20343878 proximal promoter
   SHR-A3: CCCGCCCCTTTATGCAAATCCACGACGTCACCTCGAGCC
   SHR-B2: CCCGCCCCTTTATGCAAATCCACGACGTCACCTCGAGCC
   SHR-B2 differs from BN reference seq

7. Location: chr16:20342252-4 3’-UTR
   SHR-A3: TTTTTAAGAAACCGGGGAAG---AAAAAAAAATCCTCCC
   SHR-B2: TTTTTAAGAAACCGGGGAAG---AAAAAAAAATCCTCCC
   SHR-A3 differs from BN reference seq

Haplotype structure in SHR strains across this region. SHR-A3 and SHRSP/Gla are injury-prone strains. SHR-B2, SHR and SHR/NHsd are injury resistant strains. SHR lines with the same genotype as SHR-A3 at each variant site are colored pink at that site. SHR lines with the same genotype as SHR-B2 are shaded green when this genotype differs from the injury-prone lines. Uncolored boxes indicate no genomic sequence variation data for SHRSP/Gla, SHR or SHR/NHsd. Genome sequence variation for SHRSP/Gla, SHR, SHR/NHsd was provided by the Rat Genome Database.

<table>
<thead>
<tr>
<th>Variant #</th>
<th>SHR-A3</th>
<th>SHRSP/Gla</th>
<th>SHR-B2</th>
<th>SHR</th>
<th>SHR/NHsd</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T</td>
<td>T</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>2</td>
<td>T</td>
<td>T</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>3</td>
<td>G</td>
<td></td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>GA</td>
<td></td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>--</td>
<td></td>
<td>TA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>C</td>
<td>T</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>7</td>
<td>---</td>
<td>AAG</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Supplemental statistical analysis for Figure 6.

**SHR-A3 ctrl BP vs time**

Call:
`lm(formula = sample1$BP ~ poly(sample1$Week, 2, raw = TRUE))`

Residuals:
<table>
<thead>
<tr>
<th></th>
<th>Min</th>
<th>1Q</th>
<th>Median</th>
<th>3Q</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-24.5763</td>
<td>-7.7596</td>
<td>-0.4215</td>
<td>7.9176</td>
<td>24.1168</td>
</tr>
</tbody>
</table>

Coefficients:

|                | Estimate | Std. Error | t value | Pr(>|t|) |
|----------------|----------|------------|---------|----------|
| (Intercept)    | 198.1606 | 5.0170     | 39.498  | < 2e-16 *** |
| poly(sample1$Week, 2, raw = TRUE)1 | 8.6372 | 2.5579 | 3.377 | 0.00115 ** |
| poly(sample1$Week, 2, raw = TRUE)2 | -0.6258 | 0.2774 | -2.256 | 0.02693 * |

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 11.37 on 77 degrees of freedom
Multiple R-squared: 0.3089, Adjusted R-squared: 0.2909
F-statistic: 17.21 on 2 and 77 DF, p-value: 6.644e-07

**SHR-A3 +MMF BP vs time**

Call:
`lm(formula = sample2$BP ~ poly(sample2$Week, 2, raw = TRUE))`

Residuals:
<table>
<thead>
<tr>
<th></th>
<th>Min</th>
<th>1Q</th>
<th>Median</th>
<th>3Q</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-30.8104</td>
<td>-3.2991</td>
<td>0.8181</td>
<td>6.3479</td>
<td>28.5903</td>
</tr>
</tbody>
</table>

Coefficients:

|                | Estimate | Std. Error | t value | Pr(>|t|) |
|----------------|----------|------------|---------|----------|
| (Intercept)    | 190.2577 | 4.9032     | 38.803  | <2e-16 *** |
| poly(sample2$Week, 2, raw = TRUE)1 | 3.0319 | 2.4999 | 1.213 | 0.229 |
| poly(sample2$Week, 2, raw = TRUE)2 | -0.2889 | 0.2711 | -1.065 | 0.290 |

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 11.11 on 77 degrees of freedom
Multiple R-squared: 0.02247, Adjusted R-squared: -0.002918
F-statistic: 0.8851 on 2 and 77 DF, p-value: 0.4168

**SHR-B2 ctrl BP vs time**

Call:
`lm(formula = sample3$BP ~ poly(sample3$Week, 2, raw = TRUE))`

Residuals:
<table>
<thead>
<tr>
<th></th>
<th>Min</th>
<th>1Q</th>
<th>Median</th>
<th>3Q</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-24.0196</td>
<td>-5.0269</td>
<td>-0.6765</td>
<td>5.2464</td>
<td>13.5330</td>
</tr>
</tbody>
</table>

Coefficients:

|                | Estimate | Std. Error | t value | Pr(>|t|) |
|----------------|----------|------------|---------|----------|
| (Intercept)    | 164.9447 | 3.4117     | 48.346  | <2e-16 *** |
| poly(sample3$Week, 2, raw = TRUE)1 | 4.1114 | 1.7395 | 2.364 | 0.0213 * |
| poly(sample3$Week, 2, raw = TRUE)2 | -0.3412 | 0.1887 | -1.809 | 0.0754 . |

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 6.917 on 61 degrees of freedom
Multiple R-squared: 0.1513, Adjusted R-squared: 0.1235
F-statistic: 5.437 on 2 and 61 DF, p-value: 0.006719

**SHR-B2 +MMF BP vs time**
Call:
`lm(formula = sample4$BP ~ poly(sample4$Week, 2, raw = TRUE))`

Residuals:
```
  Min  1Q Median  3Q Max
```

Coefficients:
```
                      Estimate Std. Error   t value  Pr(>|t|)
(Intercept)                     164.9861      4.5248    36.462   <2e-16 ***
poly(sample4$Week, 2, raw = TRUE)1     2.7700       2.3527     1.177     0.243
poly(sample4$Week, 2, raw = TRUE)2   -0.3237       0.2573    -1.258     0.213
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Residual standard error: 9.597 on 63 degrees of freedom
Multiple R-squared:  0.02533, Adjusted R-squared:  -0.005608
F-statistic: 0.8188 on 2 and 63 DF,  p-value: 0.4456
Renal JunD expression determined by Affymetrix array in F2 rats inheriting contrasting JunD alleles, is determined in cis by the JunD allele inherited.

$R^2 = 0.67$, $p=0.004$
Supplemental Figure 2.

Representative photomicrographs of CD43 immunohistochemistry in SHR-A3 and SHR-B2 animals at 25 wks of age with (+ MMF) and without (CTRL) 8 weeks of MMF treatment
Supplemental Figure 3.

Divergence at the nucleotide level between the rat reference genome sequence derived from the Brown Norway strain and SHR-A3 and SHR-B2 in the haplotype block on chromosome 3 that contains Src (chr3:148,157,256-148,170,524). The vertical axis indicates the number of bases that differ in each rat strain compared to the reference. This value was calculated by dividing the region into bins of 100,000 bases and summing the number of variants for each bin across the entire region. In regions of identity by descent SHR-A3 and SHR-B2 are descended form a common ancestor and share the same divergence from the rat reference genome. In this block of non-identity by descent SHR-A3 is more divergent from the rat reference strain than SHR-B2 and differences in divergence vary across this region.
Supplemental Figure 4.

Divergence at the nucleotide level between the rat reference genome sequence derived from the Brown Norway strain and SHR-A3 and SHR-B2 in the haplotype block on chromosome 16 that contains JunD (chr16:19,239,694-19,241,529). In this block of non-identity by descent the degree of divergence is less between SHR-A3 and SHR-B2, possibly indicating that this region of the chromosome is derived from a more recent common ancestor that is shared by the two lines than is suggested by Supplemental Figure 3 where the nucleotide divergence is greater.
Supplemental Figure 5.

Divergence at the nucleotide level between the rat reference genome sequence derived from the Brown Norway strain and SHR-A3 and SHR-B2 in the haplotype block on chromosome 17 that contains Dok3 (chr17: 15,154,414-15,159,609) and Syk (chr17: 18,440,672-18,498,001).