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Chromosome substitution modulates resistance to ischemia reperfusion injury in Brown Norway rats

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Brown Norway rats (BN, BN/NHsdMcwi) are profoundly resistant to developing acute kidney injury (AKI) following ischemia reperfusion. To help define the genetic basis for this resistance, we used consomic rats, in which individual chromosomes from BN rats were placed into the genetic background of Dahl SS rats (SS, SS/JrHsdMcwi) to determine which chromosomes contain alleles contributing to protection from AKI. The parental strains had dramatically different sensitivity to ischemia reperfusion with plasma creatinine levels following 45 min of ischemia and 24 h reperfusion of 4.1 and 1.3 mg/dl in SS and BN, respectively. No consomic strain showed protection similar to the parental BN strain. Nine consomic strains (SS-7^{BN}, SS-X^{BN}, SS-8^{BN}, SS-4^{BN}, SS-15^{BN}, SS-3^{BN}, SS-10^{BN}, SS-6^{BN}, and SS-5^{BN}) showed partial protection (plasma creatinine about 2.5–3.0 mg/dl), suggesting that multiple alleles contribute to the severity of AKI. *In silico* analysis was performed using disease ontology database terms and renal function quantitative trait loci from the Rat Genome Database on the BN chromosomes giving partial protection from AKI. This tactic identified at least 36 candidate genes, with several previously linked to the pathophysiology of AKI. Thus, natural variants of these alleles or yet-to-be identified alleles on these chromosomes provide protection against AKI. These alleles may be potential modulators of AKI in susceptible patient populations.

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Physicians who care for acutely ill people have long observed that individual patients, with otherwise similar comorbidities, have differential degrees of susceptibility to developing acute renal failure or acute kidney injury (AKI).[1] This well-recognized phenomenon raises the question of whether an underlying genetic background may significantly affect an individual's susceptibility or resistance to developing AKI brought on by a specific insult.

Ischemia reperfusion (I/R) injury is a frequent cause of clinical AKI and therefore has been commonly used in rats and mice to model the pathophysiology of AKI. A complex interaction of pathophysiological pathways influences the severity of I/R-induced AKI, such as pathways that affect cellular oxidant stress, metabolism, inflammation, immune cell activation, cell death, and vascular tone.[2–6] In addition, the activation of cell stress response factors (e.g., Hmox1, Hif1a, and heat-shock proteins (HSPs)) may protect against ischemic injury and are thought to mediate, in part, ischemic preconditioning.[7–16] Clearly, diverse biochemical and physiological pathways integrate to generate the final profile of renal injury in response to I/R.

Differences in the sensitivity to injury among rodent strains provide the potential for a powerful experimental system to study allelic contributions to kidney injury. Previously, our group identified the Brown Norway rat (BN/NHsdMcwi) as being strongly resistant to renal damage induced by I/R.[17] Independently, another group has shown that BN rats have reduced inflammatory responses and accelerated healing following I/R relative to Sprague–Dawley rats.[18] In our initial study, BN rat kidneys had higher basal levels of inducible HSPs compared with the unprotected strain.[17] However, it is unlikely that the modestly increased HSP expression is the sole contributing factor that conveys protection in the BN kidney. Indeed, Nilikantan *et al.*[19] demonstrated a favorable profile of antioxidant proteins in BN rat kidneys, and Saenz-Morales *et al.*[18] demonstrated attenuated proinflammatory gene expression in the kidney and in circulating cells following I/R in BN versus SD rats. Taken together, multiple pathways affected by the unique

genetic background of the BN likely work in concert to afford the profound resistance to injury observed in this strain.

One way to determine the genetic contribution to disease severity has been to make use of a chromosomal substitution paradigm in which one chromosome from an inbred disease-resistant strain is introgressed into the background of an alternate inbred, disease-prone strain.[20] The feasibility of this approach was verified when a panel of consomic rats was developed by introgressing individual chromosomes from the normotensive BN/Mcwi rat strain into the genetic background of the Dahl SS strain (SS/JrHsdMcwi), which exhibits salt-sensitive hypertension. These consomic strains were initially developed to validate linkage studies identifying quantitative trait loci (QTLs) in a chromosomal region. This strategy provides a strain to test the functional role of two sets of alleles within a narrowed down genomic region, an entire chromosome in this case. Although the use of consomics fails to validate all QTLs, the ability to run groups of genetically identical animals may be advantageous when measuring traits with some physiological variability.

Although it is unlikely that a complex trait is attributable to a single allelic difference, differences in complex traits can be observed by substitution of single chromosomes using this strategy.[20] For example, substitution of individual chromosomes of the Dahl SS rat can attenuate the severity of hypertension and proteinuria.[21] Once so defined, informative consomic strains can be used to generate congenic strains to narrow down the region of interest on a chromosome and facilitate positional cloning of candidate genes. Recently, this strategy was used to define regions on chromosome 13 that can attenuate the development of hypertension in Dahl S rats.[22]

Our hypothesis, then, was that the marked resistance to ischemic renal injury observed in BN rats is the result of contributions from multiple BN alleles present on separate chromosomes. The goal of this study was to identify protective BN alleles using the two-allele system available through the SSxBN consomic panel and the model of ischemia-induced renal injury. We found differential susceptibility to I/R injury in several distinct consomic SS-BN lines, suggesting that multiple alleles on separate chromosomes influence the susceptibility to injury in the SS rat and the full profound resistance to AKI in the BN rat.

RESULTS

Dahl SS/Mcwi salt-sensitive rats (SS) were used as the background strain for the generation of a panel of consomic rats by introgressing individual chromosomes from the AKI-resistant BN/Mcwi (BN).[20] We studied this consomic panel to define which specific BN chromosomes carried genes that afford protection against renal I/R injury. When subjected to 45 min of renal ischemia and then allowed to recover for 24 h, SS rats developed significant AKI, as illustrated by the marked increase in the level of serum creatinine (Cr) to $\sim 4.1 \pm 0.2$ mg/dl. This level of serum Cr is similar to that which we previously reported using this model of I/R injury

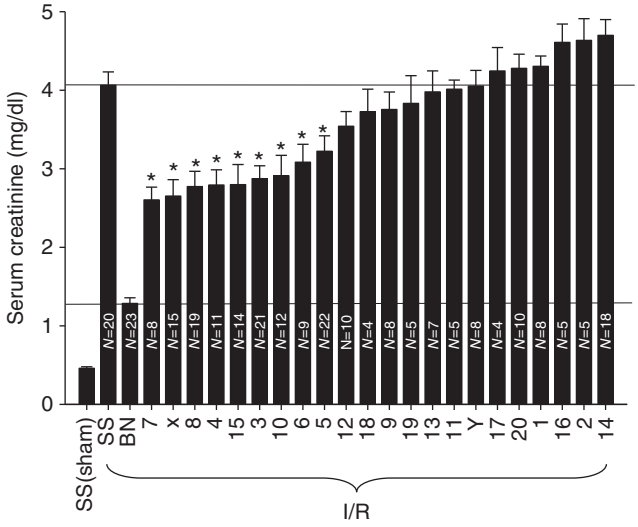

Figure 1 | Resistance to ischemia reperfusion (I/R)–induced acute kidney injury (AKI) in Brown Norway (BN) and SS-BN consomic rats. Except for SS sham, rats were subjected to 45 min of bilateral renal ischemia with 24 h reflow. Data are presented as mean ± 1 s.d. of the serum creatinine values at 24 h. The *N* for each group is indicated in individual bars; *indicates $P < 0.001$ versus SS rats post I/R. Numbers on the x axis are the BN chromosome introgressed into the SS background (e.g., 7 is SS-7^{BN}).

for the commonly studied, outbred Sprague–Dawley rat.[17] It is important to highlight that these results have been derived from multiple cohorts spread out over a 5-year period as consomic strains became available. The individual mean of each surgical cohort of the parental SS strain was always between 3.5 and 4.5 mg/dl and remained consistent over the study period. In comparison, and as we previously reported, the increase in serum Cr in the BN rat subjected to the same ischemic insult was significantly less profound at 24 h of reperfusion (1.3 ± 0.1 mg/dl, Figure 1). This resistance to injury in BN rats also remained stable across all surgical cohorts over the 5-year study period, with no cohort manifesting a mean 24 h Cr level > 1.6 mg/dl.

All consomic strains showed sensitivity to I/R-induced injury that was significantly greater than that of the parental BN strain ($P < 0.05$, by analysis of variance). Most of the consomic strains had equivalent or worse AKI, as measured by serum Cr, compared with the parental SS strain. However, several consomic strains manifested renal insufficiency following I/R that was intermediate between the ischemia-susceptible SS and the resistant BN parental strains. Consomic animals from nine different BN chromosome substitutions showed a statistically significant reduction in serum Cr level at 24-h reperfusion relative to the parental SS strain subjected to the same ischemic insult. Nine consomic strains (SS-7^{BN}, SS-X^{BN}, SS-8^{BN}, SS-4^{BN}, SS-15^{BN}, SS-3^{BN}, SS-10^{BN}, SS-6^{BN}, and SS-5^{BN}) had mean serum Cr values < 3.0 mg/dl at 24 h recovery, but no strain had serum Cr values less than 2.5 mg/dl. Therefore, all of the protected consomic strains showed only partial protection against ischemia-induced AKI when compared with the marked