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ABSTRACT

Hypertension, diabetes and obesity, along with genetic predisposition, contribute to the growing number of chronic kidney disease patients. Our novel congenic model [S.SHR(11)] was developed through genetic modification of the Dahl salt-sensitive (S) rat, a model of hypertension related renal disease. The S.SHR(11) strain exhibits accelerated kidney injury compared to the already highly susceptible S rat. On either a low or high-salt diet, the S.SHR(11) model predominately exhibited more tubulointerstitial fibrosis compared to the S rat (17.1 1.29% vs. 12.9 1.22%). Increased α -SMA and macrophage infiltration was also observed. The S and S.SHR(11) had similar blood pressure (week 12), despite an early reduction in renal function in the S.SHR(11); however at an advanced age the S.SHR(11) demonstrated significantly higher blood pressure than the S (215 6.6 mm Hg vs. 183 5.9, respectively). This suggests that increased kidney injury is driving the development of hypertension later in life. Since these two animal models are identical with exception of chromosome 11, the causative genetic variants contributing to decreased renal function must reside within this region. The Dahl S and SHR genomes have been sequenced; this data provides a catalog of all the genetic variants between the two models. The 95% confidence interval of the genomic locus contains 28 non-synonymous SNP, with 15 of these SNP occurring within only three genes: *Retnlg*, *Trat1* and *Myh15*. Two of these genes, *Retnlg* and *Trat1*, are known to play a role in immune response leading to our hypothesis that genetic variants in these genes alter protein function and lead to an increased immune response. Bone marrow transplant studies have been initiated to test our hypothesis and preliminary data shows that S rats who receive S.SHR(11) bone marrow have kidney function measurements similar to the S.SHR(11). The sequencing information has also led to the development of nine new, more refined congenic strains. Through functional analysis of these new congenic animals, identification of the causative genetic variations will be expedited. In summary, we are employing a model of accelerated kidney disease to identify genes or genetic variants responsible for reduced kidney function and hypertension.

OBJECTIVE

To characterize the development of kidney injury in the S.SHR(11) animal model and identify the causative genetic variants using a sequence-driven approach. Specifically, whether two immune related genes that exhibit a large number of genetic variants within the transferred genomic segment play a role in the increased renal injury observed in the model.

INTRODUCTION

- An earlier genetic analysis involving the Dahl salt-sensitive rat (S) and spontaneous hypertensive rat (SHR) identified a region on rat chromosome 11 responsible for increased kidney injury and decreased kidney function.
- The genomic region on chromosome 11 from the S rat (protective) was replaced with a genomic segment from the SHR (susceptibility) generating our new model (Fig. 1). This S.SHR(11) model shows increased kidney injury (glomerulosclerosis and interstitial fibrosis) and reduced kidney function compared to the Dahl S.
- Work is now being completed to isolate and identify the gene or genetic variants causative of the decreased renal function seen in our S.SHR(11) congenic strain.

Graphical Representation of Genetic Model Being Investigated

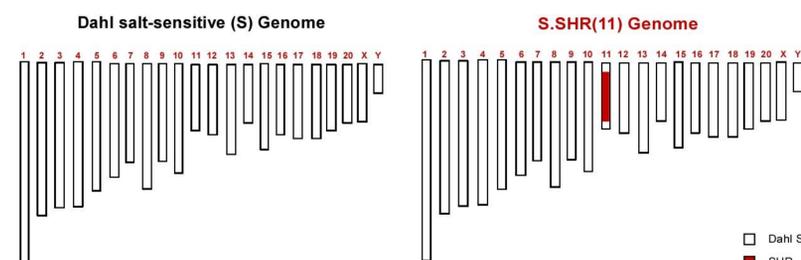


Figure 1: The S.SHR(11) has the Dahl S genetic background with a genomic region transferred from the SHR.

BACKGROUND

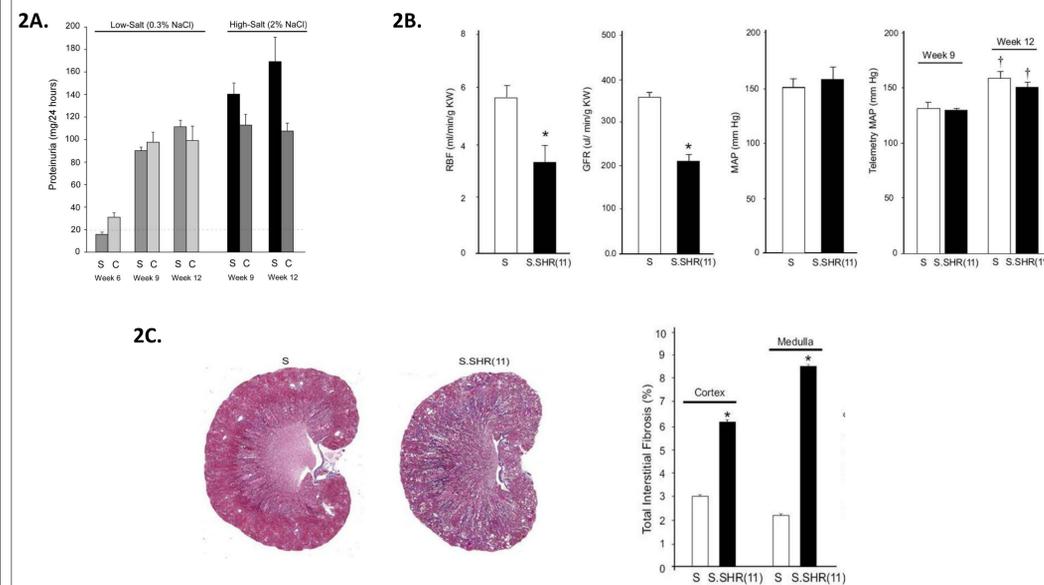


Figure 2A: Proteinuria comparison between the Dahl S and the S.SHR(11) congenic strain. As early as week 6, the congenic model showed an increase in proteinuria that plateaued around week 9 (either low and high salt diet). The S rat, however, demonstrated a gradual and consistent increase in proteinuria on both the low and high salt diets. The decline in proteinuria seen in the S.SHR(11) rat can be attributed to the decline in GFR (Fig. 2B). **Figure 2B:** MAP and renal hemodynamics between the S and S.SHR(11) animal models. These measurements show that while the S.SHR(11) rat exhibits significantly less renal function than the Dahl S rat, the decline in kidney function is independent of blood pressure as the two strains do not show significantly different MAP. **Figure 2C:** Masson's trichrome whole kidney histology from the S and S.SHR(11) models at an advanced age (week 35). The S.SHR(11) model shows substantial interstitial fibrosis among other pathology in comparison to the Dahl S rat (Regner, Harmon et al., *Physiol. Genomics*, 2012).

SEQUENCING DATA

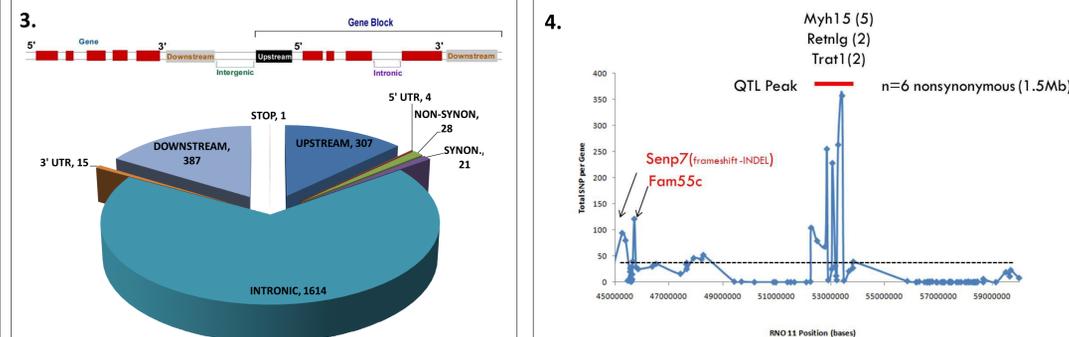


Figure 3. Sequence analysis within chromosome 11 renal function locus. The entire genomes of both S and SHR have been sequenced. The 95% confidence interval for the renal function locus spans 15Mb and contains 108 genes. SNP variation was analyzed within the 15Mb regions. The vast majority (93%) of the SNP were either in the intergenic or intronic regions. 58 genes contain SNP in at least one of these regions. 12 genes contain non-synonymous SNP, but 3 of these genes contain more than half of the total 28 non-synonymous SNP identified.

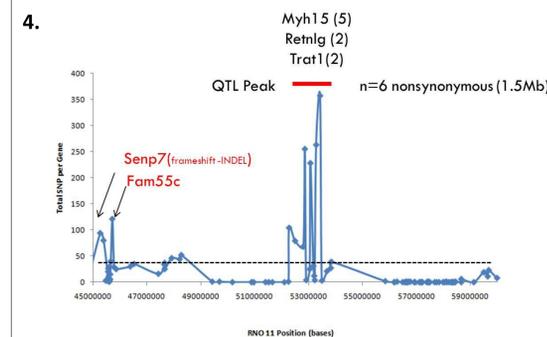


Figure 4: Graphical representation of the total number of SNP per gene. Within the 95% CI of chromosome 11, 28 non-synonymous SNP were identified. Fifteen of these 28 SNP were observed within three genes, *Retnlg*, *Trat1* and *Myh15*, and six of these non-synonymous SNP are located within just 1.5 Mb of one another. The location of the majority of the non-synonymous SNP corresponds perfectly with the known QTL peak of the renal function locus, further leading us to believe that the causative gene is located within this small Mb region.

PRELIMINARY WORK

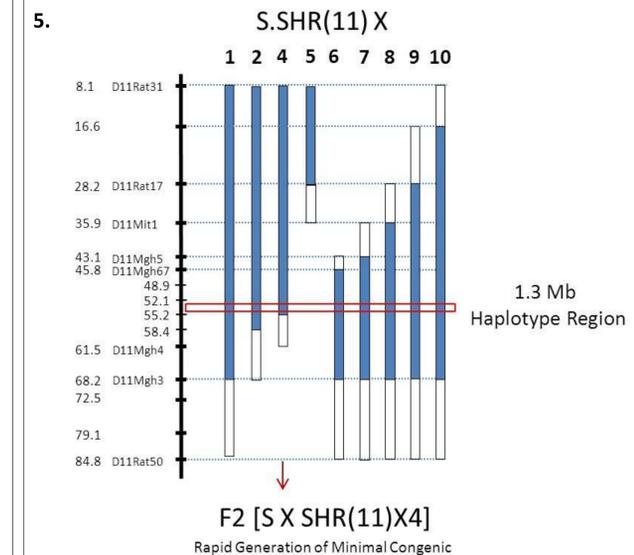


Figure 5: New congenic substrains created for the purpose of testing candidate genes. Nine new substrains with refined SHR genomic regions have been developed and will soon be phenotyped in order to refine the genomic interval. The regions in which the immune candidate genes reside will be tested first because they contain the 1.3 Mb haplotype region. The S.SHR(11)protX4 strain will also be back crossed to the Dahl S rat in order to narrow the SHR genomic region to contain only the 1.3 Mb haplotype region (*Retnlg*, *Trat1* and *Myh15* genes).

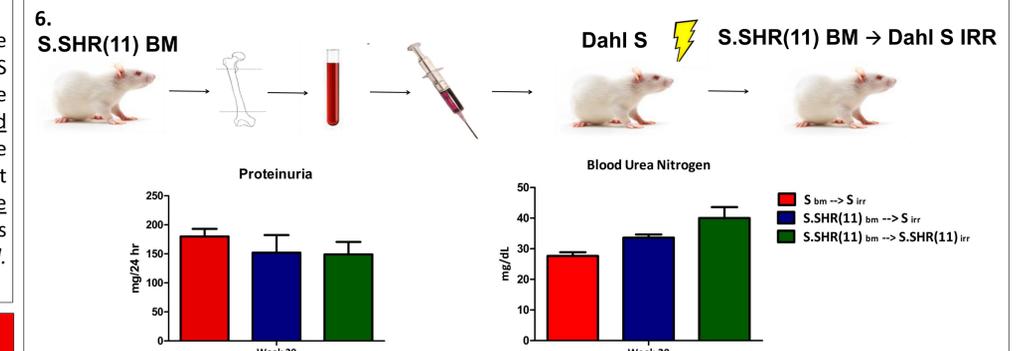


Figure 6: Bone marrow transplant procedure and preliminary data. Since *Retnlg* and *Trat1* are both immune related genes, we conducted bone marrow transplants on three groups of animals to test the impact of these two candidate genes on proteinuria, BUN and creatinine clearance. While not conclusive, these preliminary data show that the experimental group of animals [S.SHR(11) bone marrow into irradiated Dahl S rats] have renal measurements similar to that of the S.SHR(11) model which suggest that one, or both, of these candidate genes is contributing to the decline in kidney function seen in our congenic strain. Further studies with a larger group of animal is underway to provide for definite conclusion.

CONCLUSION

- Complete genomic sequencing of S and SHR have identified a catalogue of genetic difference within the chromosome 11 renal function locus. Future work will involve testing the functional significance of these variants.
- The candidate genes *Retnlg* and *Trat1* are being evaluated as causative to reduced renal function exhibited by S.SHR(11) congenic strain using bone marrow transplant studies.
- The sequencing data has helped select congenic strains and target regions for specific congenic strain development. These animals will be phenotyped for renal function in order to better refine, localize and identify causative gene variants.

ACKNOWLEDGEMENTS

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