Candidate genes for hypertension: Insights from the Dahl S rat

Nathan P. Rudemiller¹ and David L. Mattson¹

Department of Physiology¹

Medical College of Wisconsin¹

Running title: Investigating candidate genes for hypertension

Correspondence to:

Nathan P. Rudemiller
Department of Physiology
Medical College of Wisconsin
8701 Watertown Plank Road
Milwaukee, WI  53226
Phone: 414-955-4304
FAX: 414-955-6546
E-mail: nrudemiller@mcw.edu

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ABSTRACT

Human genetic linkage and association studies have nominated many genes as possible contributors to disease. Mutating or deleting these genes in a relevant disease model can validate their association with disease and potentially uncover novel mechanisms of pathogenesis. Targeted genetic mutagenesis has only recently been developed in the rat, and this technique has been applied in the Dahl salt-sensitive (S) rat to investigate human candidate genes associated with hypertension. This mini-review communicates the findings of these studies and displays how targeted genetic mutagenesis may contribute to the discovery of novel therapies for patients.

IDENTIFYING GENETIC CONTRIBUTORS TO DISEASE

Despite a variety of available medications, hypertension remains uncontrolled in many patients (19), eliciting considerable interest in defining its genetic basis. Polygenic diseases such as hypertension are postulated to arise from epistatic interactions of many common single nucleotide polymorphisms (SNPs) (17). Genetic linkage and association studies identify SNPs, or loci, that significantly segregate with disease in human populations. This unbiased approach in nominating loci (28) has led to many obscure associations – no intuitive link exists between the gene(s) within the loci and the associated disease. Many loci have been linked to hypertension (13, 16, 20), and experimental validation of these associations may uncover novel pathogenic pathways and highlight therapeutic targets to reduce blood pressure.

THE DAHL SALT-SENSITIVE RAT – A RELEVANT MODEL FOR TESTING GENETIC CONTRIBUTION TO HYPERTENSION
The Dahl salt-sensitive (S) rat is well known for its rapid elevation of blood pressure and extensive renal injury when placed on a high salt diet (4-8% NaCl)(3, 18). This genetic model of human hypertension also displays vascular dysfunction (5, 29). Extrapolation of experiments performed in the Dahl S rat is not limited to salt-sensitive hypertension, since renal and vascular dysfunction are postulated to contribute to much of the hypertension in humans (12, 21, 22). The mechanisms that cause renal and vascular dysfunction remain unclear, though many hypotheses implicate genetic factors that exacerbate tissue damage and/or alter cellular pathways that affect tissue function (27). Since the Dahl S is genetically homogeneous, this strain serves as a powerful model to uncover genetic determinants of cardiovascular and renal pathology that ultimately augment blood pressure.

The ability to create targeted mutations in the rat genome was long unavailable. This changed in 2009 when zinc finger nucleases (ZFNs) were used to create the first knockout (KO) rat (9). ZFNs are a class of engineered DNA-binding proteins that cause a double-stranded break at a user specified location in the genome. The intrinsic DNA-repair mechanism of non-homologous end joining often results in the addition or deletion of base pairs. Shifts in the reading frame alter the functionality and/or expression of the gene’s protein product. Injecting ZFNs into a fertilized embryo results in pups harboring total body mutation at the desired genetic location (10). This technique has been implemented in the Dahl S rat to validate candidate genes of hypertension and to explore the mechanisms by which they modulate pathogenesis.

VALIDATING CANDIDATE GENES OF HYPERTENSION
Many candidate genes for hypertension have no intuitive link to blood pressure regulation (1, 13, 16). ZFN mutation of a handful of these candidate genes in the Dahl S rat has illuminated novel pathways potentially involved in cardiovascular and renal pathology (Table 1).

ADAMTS16 is a secreted metalloproteinase of previously unknown function, which has recently been shown to play an important role in early kidney development (14). Human linkage analysis, followed by positional cloning in the rat, nominated \textit{Adamts16} as a candidate gene for hypertension (15, 24). ZFN mutation of \textit{Adamts16} in the Dahl S significantly attenuated hypertension (11). Furthermore the mutant rats had reduced pulse wave velocity, a surrogate for arterial stiffness in humans, and showed decreased vessel media thickness compared to Dahl S controls. Due to the altered vascular structure in the mutant rat – increased endothelial cilia length and splitting of glomerular capillaries – the authors suggest that ADAMTS16 may alter blood pressure through its contribution to the structural integrity of vasculature. This model may reveal structural biomarkers for susceptibility to future CVD.

\textit{Plekha7}, a GWAS candidate gene for hypertension, encodes an adherens junction protein with little known function (23). Mutation of \textit{Plekha7} in the Dahl S blunted hypertension and renal disease, which may be attributed to improvements in vascular function (7). In vitro assays revealed enhanced endothelium-dependent and flow-mediated dilation in the mutant vessels compared to wild-type (WT) controls. The authors showed that mutation of \textit{Plekha7} augmented intracellular calcium release in endothelial cells, leading to increased bioavailability of nitric oxide (NO) – a potent vasodilator. Further investigation into the pathways that link PLEKHA7, calcium release,
and NO production may uncover therapeutic options to reduce vascular resistance and lower blood pressure.

Recent work in our lab has focused on inflammatory genes nominated by human linkage and association studies. *Cd247*, a blood pressure candidate gene (6), encodes the zeta chain of the T cell receptor. ZFN-mediated deletion of CD247 resulted in no cellular expression of the T cell receptor complex proteins; therefore, *Cd247* KO rats have no functional T cells. Deletion of CD247 significantly attenuates Dahl S hypertension and renal disease and reduces the infiltration of T cells into the kidneys in response to high salt diet (25). This model further validates a role for T cells in the pathogenesis of hypertension and may serve as an ideal model for adoptive transfer studies to elucidate mechanisms by which T cells mediate hypertensive pathology.

*Sh2b3*, a GWAS candidate for hypertension and renal disease (1, 16), encodes SH2B adaptor protein 3, which functions in many cellular processes, including inflammatory signaling (4). ZFN mutation of *Sh2b3* significantly attenuated Dahl S hypertension and renal disease (26). Subsequent bone marrow transplant experiments showed that reconstituting Dahl S rats with *Sh2b3* mutant bone marrow blunted Dahl S pathology and vice versa, confirming that the mutation was eliciting a protective effect via alterations in immune cell function. Furthermore *Sh2b3* mutant rats have increased proportions of T regulatory cells among all T cells in the circulation and spleen – a phenomenon that may be alleviating the deleterious inflammation associated with the development of hypertension (2). Better understanding of the pathways involved in this process may lead to insights on shifting the immune system to a more anti-inflammatory state – a possible therapy for human disease.
As mentioned above, genetic association studies link SNPs to a disease. These SNPs signify genetic loci, which often contain multiple genes in linkage disequilibrium. An example of this is the Agtrap-Plod1 locus, which contains six genes (Agtrap, Mthfr, Clcn6, Nppa, Nppb, and Plod1). SNPs within this locus have been linked to blood pressure via GWAS (16, 20); however, the close proximity of the genes makes it difficult to ascertain which SNP is affecting gene function to modulate disease. Utilizing ZFNs, Flister et al. individually mutated all six genes within the Agtrap-Pod1 locus on the Dahl S background to determine their relative contributions to the development of hypertension and renal disease (8). The results showed that mutation of 5 of the 6 genes have direct effects on blood pressure and/or proteinuria, indicating that multiple genes at a single candidate locus can modulate disease development independently. These data reaffirm the importance of experimental validation of candidate genes in linkage disequilibrium.

CONCLUSION

The newfound ability to collect and interpret vast amounts of human genetic data has nominated many loci as possible genetic determinants of disease severity. Because of its genetic homozygosity and its robust hypertensive phenotype, the Dahl S rat serves as a powerful tool in determining the genetic contribution to this pathology. Utilizing targeted genetic mutation, the studies reviewed herein have experimentally validated candidate genes for hypertension and have begun to explore their mechanistic roles in pathogenesis. Further experimentation on these and additional mutant models (see http://rgd.mcw.edu/wg/physgenknockouts) may uncover targetable pathways for the treatment of human disease.
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245 protein with a tissue distribution and subcellular localization distinct from ZO-1 and E-


Table 1. Published mutations of candidate genes for hypertension in the Dahl S rat

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Dahl S mutant strain</th>
<th>DNA deletion</th>
<th>Effect on hypertension</th>
<th>Effect on kidney injury</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adams16</td>
<td>Adams16mutant</td>
<td>17-bp frameshift deletion in exon 1</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Plekha7</td>
<td>SS-Plekha7em4Mcwi</td>
<td>19-bp frameshift deletion in exon 6</td>
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<td>↓</td>
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<tr>
<td>Cd247</td>
<td>SS-Cd247em1mcwi</td>
<td>11-bp frameshift deletion in exon 1</td>
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<td>↓</td>
</tr>
<tr>
<td>Sh2b3</td>
<td>SS-Sh2b3em1mcwi</td>
<td>6-bp in-frame deletion in exon 2</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Agtrap</td>
<td>SS-Agtrapem1mcwi</td>
<td>100-bp deletion of part of exon 3 and the splice acceptor</td>
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<tr>
<td>Mthfr</td>
<td>SS-Mthfrem1mcwi</td>
<td>28-bp frameshift deletion in exon 2</td>
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<tr>
<td>Clcn6</td>
<td>SS-Clcn6em2mcwi</td>
<td>15-bp frameshift deletion in exon 13</td>
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<td>↓</td>
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<td>Nppa</td>
<td>SS-Nppaem4mcwi</td>
<td>22-bp frameshift deletion in exon 2</td>
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<td>–</td>
</tr>
<tr>
<td>Nppb</td>
<td>SS-Nppbem4mcwi</td>
<td>138-bp deletion of part of intron 1 and exon 2</td>
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<td>–</td>
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<tr>
<td>Plod1</td>
<td>SS-Plod1em1mcwi</td>
<td>10-bp frameshift deletion in exon 4</td>
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<td>↑</td>
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