Gene Editing Rat Resource Center
Gene Nomination Application

Round 2 Deadline: March 24, 2014 (Midnight CST time). Submit completed application to: mcwcustomrats@mcw.edu

Engineering Feasibility Score:		Comments:	Estimated Cost:
Strain Difficulty Score:			

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Date: **3/24/2014**

Name: **Dr. Investigator**

Institution: **University of XYZ**

Mailing Address: **123 Main Street**

Mailing Address: **Building 456**

City/State/Zip **Anytown, WI 12345**

Phone: **555-555-5555** Email Address: **researcher@abc.edu**

Gene Name: **Thyroid hormone receptor beta** Gene Symbol/ID (from RGD): **Thrb/3858 (as example)**

Preferred strain background: ☒ DA ☐ FHH ☐ LEW ☐ SHR ☐ SS ☐ WKY

****Note: Only inbred strains should be considered****

☒ Other (List strain and provide justification below)

DABN15 (example - consomic strain that shares the same genetic background as the DA, except chromosome 15 is replaced with the BN chromosome). We have QTL linkage data to suggest that regions on chromosome 15 are involved in renal and heart function.

Rat strain source: **DA from Harlan**

Title of Proposal: **Characterization of a Thrb knockout rat as a model for renal failure**

Hypothesis (0.5 page limit): Why is the gene important? What are the anticipated phenotypes?

"X" gene encodes a transcriptional co-activator for several nuclear receptors including the different subtypes of peroxisome proliferator-activated receptor (PPAR) (PMID: 12345 as example). Previous studies demonstrated that gene "X" exhibited ligand-dependent direct interaction with PPAR-alpha and gama (PMID: #, #). A luciferase assay showed that expression of "X" gene is required for the transcriptional activity of PPAR-alpha in mammalian cells (PMID: #). Members of PPAR regulate transcription of various genes and play central roles in the control of protective mechanisms against different tissue injuries and complex diseases (PMID: #, #). Although gene "X" has been implicated in PPAR-mediated pathway, its role in the development of disease has not been studied. By congenic mapping, our group has recently isolated a 0.7 kb region of rat DA chromosome 15 using DABN15 consomic strain that causes increased susceptibility to renal impairments and heart failure (PMID: #, #, #). Sequence analysis of this 0.7 kb region identified a deleterious amino acid substitution in gene "X" in DA rats compared to the Brown Norway (BN). qPCR analysis showed that congenic animals carrying the 0.7 kb of DA disease alleles had significant down-regulation of expression of gene "X" in the renal cortex and heart compared to control. We also found down-regulation of Ppar-alpha and differential expression for several downstream effectors of Ppar-alpha in the renal cortex of congenics and heart, suggesting that Ppar-alpha-mediated pathway may be dysregulated. We hypothesize that gene "X" is the candidate gene in the 0.7 kb congenic region, and disruption of gene "X" will cause increased susceptibility to renal and heart diseases (PMID: #). Renal impairment confers significant risk to development of cardiovascular disease (PMID: #). We expect changes in following phenotypes: cardiac hypertrophy, sensitivity of heart to ischemia, glomerulosclerosis and increased permeability of the glomerular filtration barrier.

Relevance to NHLBI (0.5 page limit): How would this model be important to the NHBLI mission?

Interestingly, the "X" gene was nominated by GWAS and replicated in African American population (PMID: #) in a large cohort of patients with kidney and heart failure. The proposed study will characterize a "X" gene knockout in a consomic DABN15 background and has high significance, as the study will (1) facilitate understanding of this compelling candidate gene in the regulation of kidney disease and heart failure, and (2) potentially identify a new player and its associated pathways that are driving renal and heart pathological phenotype.

Type of gene modification requested: *(check all that apply)*

- [x] Targeted Knockout
- [ ] Targeted Knockin
- [ ] Transgenic
- [ ] Multiplex engineering (insert lox P, large deletion)

Justification of rat: Does mouse model exist? [x] No [ ] Yes

Why should this gene be modified in rat (0.5 page limit)?

Consomic DABN15 rat shares the same genetic background with DA, except that its chromosome 15 is replaced by the BN chromosome 15. Prior linkage studies have identified multiple quantitative trait loci (QTL) for renal and heart function on rat chromosome 15 (PMID: #, #). Through congenic mapping using DABN15, our lab recently found that introgression of a 0.7 kb region of DA rat chromosome 15 onto the consomic DABN15 background significantly increased the susceptibility to renal impairments and heart failure. Based on sequence and expression studies, we identified gene "X" as the leading candidate for this region. To test if gene "X" is the causal gene, we propose to create a knockout of gene "X" using ZFNs or TALENs in the background of DABN15 consomic strain. The DA "X" allele harbors a deleterious amino acid substitution compared to BN, DABN15 animals carry the wild-type "X" allele from the BN and presents lower susceptibility to renal and heart failure susceptibility compared to the congenic strain. If gene "X" indeed contributes to heart and renal dysfunction underlying the congenic region, knockout of gene "X" in DABN15 would be expected to reverse the protective effect and recapitulate the renal impairments seen in the congenic strain. According to both PubMed and Mouse Genome Informatics, a knockout of gene "X" mouse is not available.

Rationale for strain selection (0.5 page limit):
Why was this background strain selected?

To test whether gene "X" is the causal gene underlying the congenic region, we plan to create a knockout of "X" gene in the background of DABN15 consomic strain. A consomic strain is an inbred strain with one of its chromosomes replaced by the homologous chromosome of another inbred strain. These models allow for easy screening of disease QTL and rapid generation of congenic lines. A consomic DABN15 contains chromosome 15 from BN introgressed onto the genetic background of DA. Although no phenotypic difference was observed between DABN15 and DA, we found that introgression of a1.7 Mb region of DA rat chromosome 15 onto the consomic DABN15 background significantly increased the susceptibility to renal and heart impairments. This congenic, called DABN15a, showed significantly higher level of albuminuria (72.68±9.87 vs. 34.84±5.49; $p<0.05$) and increased presence of protein cast in the renal outer medulla (13.20±1.12 vs. 7.52±1.12; $p<0.0023$) compared to DABN15 consomic. Ejection fraction after LAD occlusion was significantly impaired in congenic animals in comparison with consomic animals (26.2 +3.52 vs. 85.1+8.34). Interestingly, flow-cytometry experiments showed that DABN15a congenics had 3.2- and 2.5-fold higher number of infiltrating T cells and monocytes, respectively, in the kidney compared to DABN15. These results suggest involvement of an immune component in this congenic model, and further support gene "X" as a potential causal gene for this region. If disruption of "X" gene function indeed underlies renal impairments seen in the congenic, we expect that a knockout of gene "X" in DABN15 consomic will recapitulate the renal impairments seen in the congenic animals.

Describe the broad utility of this model to other NHLBI Investigators (0.25 page limit):

A recent human genome-wide association study identified gene "X" expression to be associated with asthma in European populations (PMID: #). Interestingly, the "X" gene was nominated by GWAS and replicated in African American population (PMID: #) in large cohort of patients with kidney and heart failure.

What phenotypes will be measured? (Do not provide detailed methods, just endpoint measurements) (0.5 page limit):

The homozygote and heterozygote knockout of gene "X" will be phenotyped for several renal and cardiovascular functional parameters and compared with the wild-type DABN15 littermates. The proposed experiments are designed to test our hypothesis that disruption of "X" gene function contributes to renal and heart impairment associated with the 1.7 Mb congenic region. Our lab has well established the protocols for the following proposed experiments, thus, we do not anticipate any problem with performing these studies.
In vivo phenotyping of renal function (PMID: #), blood pressure measurement (PMID:#), ECHO (PMID: #), kidney and heart histological examination (PMID: #), flow cytometry (PMID: #), LAD occlusion (PMID:#)

Request for MCW phenotyping: [x] No [ ] Yes

Commitment to submit data to RGD at time of publication: [x] Yes See FAQs (instructions)

Funding source(s) to support the outlined studies and rats after development:

R01 grant #, endowment fund, etc.

☐ I acknowledge that I will need to provide assurance from an authorized institutional representative that I am approved to receive genetically modified animals, including a letter of approval from my Institutional Animal Care and Use Committee (IACUC) or equivalent.

Signature Field

Date

Vet Name: **David Brown, DVM (sample)**

Vet Email: **dbrown@email.edu (sample)**

Vet Phone: **414-955-1234 (sample)**

☒ I have included a biosketch with personal statement clearly stating qualifications of applicant.