Targeted disruption of CTCF binding in the Dahl salt-sensitive rat reveals *Renin* transcriptional dynamics

Mark Vanden Avond[1,2], Mike Grzybowski[1], Andrew S Greene[3], Mingyu Liang[4], Pengyuan Liu[4], Allen W. Cowley Jr[1] and Aron M. Geurts[1,2]

[1]Department of Physiology, [2]Cardiovascular Center, Medical College of Wisconsin, Milwaukee, WI USA; [3]The Jackson Laboratory for Mammalian Genetics, Bar Harbor, ME; [4]Department of Physiology, University of Arizona, Tucson, AZ, USA

emails: mvandenavond@mcw.edu; ageurts@mcw.edu

This work has been generously supported by NIH 5P01HL149620-03 and 2T32HL00785f2-26

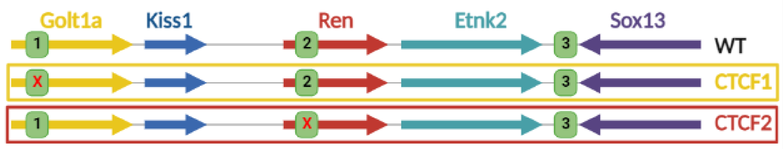
Abstract

Changes in chromatin conformation caused by common genetic variations may impact blood pressure regulation and hypertension risk. A major function of CCCTCF-binding factor (CTCF) is to bind to DNA to regulate and maintain chromatin conformation, and thereby gene expression. Cellular expression of CTCF is critical for proper *Renin* expression and the *Renin* gene is surrounded by multiple CTCF-binding motifs. In the Dahl salt-sensitive (SS) rat, CRISPR-Cas9 was utilized to create three models harboring mutations to the CTCF motif surrounding the *Renin* gene. CTCF binding was confirmed to be disrupted in all three models. The mutants and wild-type (WT) littermates were then placed on a 0.1%, 4.0% or maintained on a 0.4% NaCl diet for four days, and *Renin* mRNA and plasma renin activity (PRA) was examined. mRNA analysis showed no differences in *Renin* expression on any diet for any model compared to WT. Interestingly, two of our male mutant models failed to increase their PRA on the 0.1% NaCl diet (CTCF1=13.87±0.86; CTCF2=13.95±0.59; p<0.05 compared to WT=17.52±0.81 on 0.1% NaCl). We hypothesized that *Renin* dynamic transcriptional response to salt depletion is delayed in the mutant models compared to WT. To test this, we have utilized an ex vivo kidney slice culture approach. Kidneys were excised from SS rats fed a 4.0% NaCl diet for 1-2 days, sliced to ~1-3 mms, and cultured for 24 hrs. *Renin* expression was evaluated at 0, 1, 2, 4, 8, 12, 16, 20 and 24 hrs of incubation in basal media. *Renin* expression was elevated in SS rats at 8 hrs (3.82±0.38 compared to 0 hr = 0.93±0.32) and continued to increase until 12 hrs (7.05±1.87) and reduced at 16 hrs (1.42±0.70). CTCF1 mutant *Renin* expression was significantly reduced at 12 hrs (3.77±0.58 compared to WT=7.05±1.87), while CTCF2 mutants showed no differences in *Renin* expression compared to WT. Thus, we established an ex vivo approach for monitoring the dynamic changes in *Renin* expression by culturing kidney slices. This method could be used to evaluate other stimuli, such as therapeutics.

Introduction and Methods

Hypertension is a complex disorder that is influenced by a variety of genetic and environmental factors. GWAS have identified more than 20,000 single nucleotide polymorphisms associated with blood pressure with a majority being in non-coding, non-transcribed regions of the genome. One potential non-coding mechanism that may disrupt chromatin conformation to alter blood pressure is disrupting the ability of CTCF (CCCTC-binding factor) to bind to specific genomic sequences and organize chromatin conformation. *What is still not known is if disrupting CTCF binding is sufficient to affect complex a phenotype as blood pressure regulation.* Renin has been extensively studied in blood pressure regulation with an inverse relationship with salt intake. The Renin locus has been shown to interact with multiple CTCF sites. Three animal models were created by using CRISPRs targeting three highly conserved CTCF binding sites around the renin gene.

SNP/Mutation
↓
Altered chromatin conformation
↓
Altered (+/-) gene expression/regulation
↓
Altered (+/-) one or more intermediate physiological mechanisms
↓
Altered (+/-) blood pressure

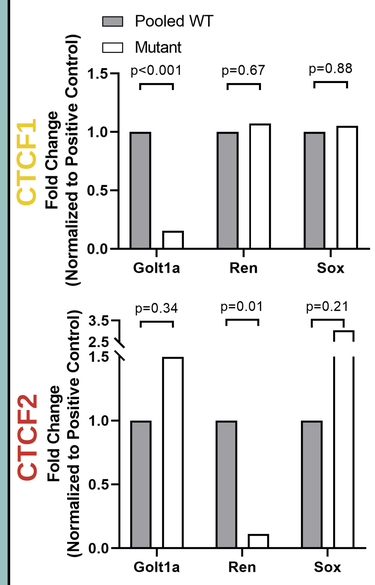


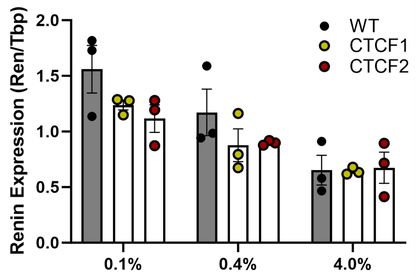
Schematic of the Renin locus of the WT Dahl Salt-Sensitive rat model and two models created using CRISPR cas9 (CTCF1 and CTCF2) with mutations in the CTCF binding sites (green boxes). The CTCF1 model has a three bp deletion in the intronic region of *Golt1a* and the CTCF2 model has an 11 bp deletion in the intronic region of *Renin*

Overall Hypothesis

Local disruption of chromatin conformation surrounding *Renin* will dysregulate *Renin* expression and alter intermediate mechanisms important for blood pressure regulation

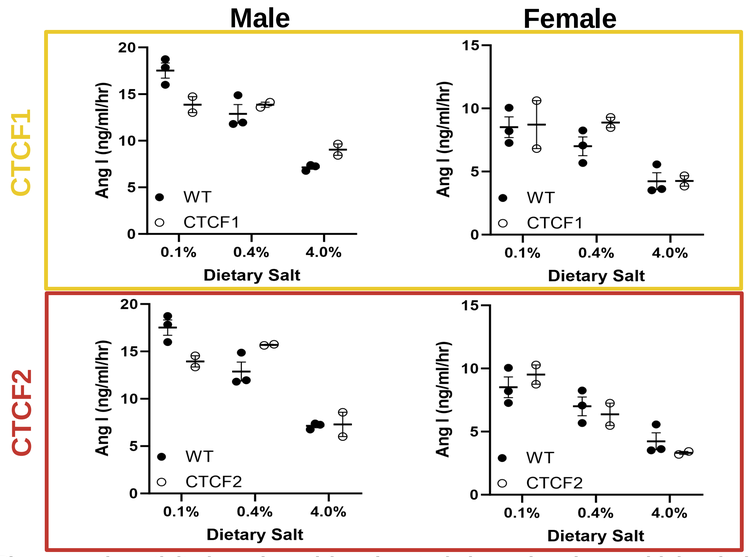
Disruption of CTCF binding at mutant loci surrounding Renin and Renin expression in the renal cortex on different salt diets





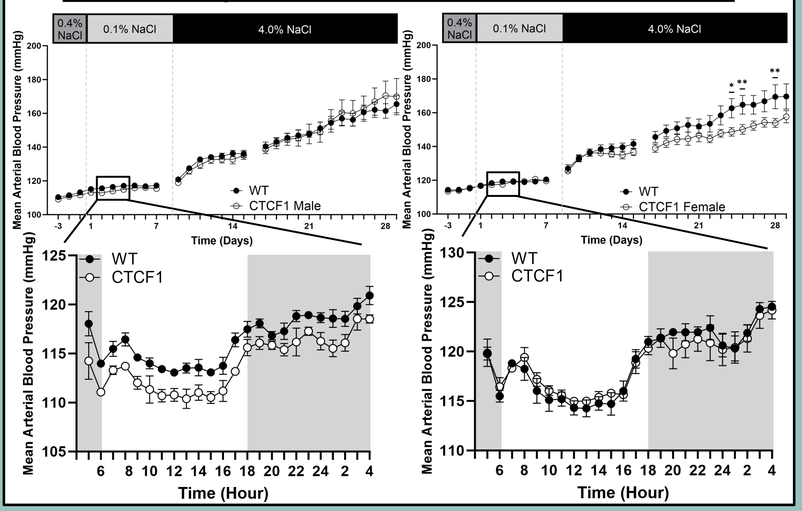
CTCF binding is decreased in mutant rats, but *Renin* expression is no different. CTCF binding was measured using a chromatin immunoprecipitation qPCR assay in liver tissue**.** CTCF binding was decreased at the intronic region of *Golt1a* in our CTCF1 model and decreased at the intronic region of *Ren* in our CTCF2 model. *Renin* expression was measured by digital PCR in the renal cortex on 0.1%, 0.4% and 4.0% NaCl. CTCF1 and CTCF2 rats showed no differences in expression compared to WT.

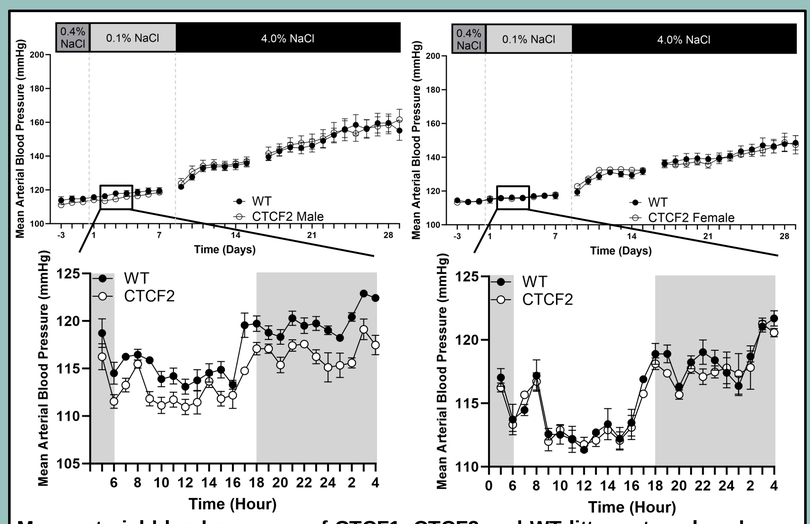
Plasma renin activity failed to reach WT levels on 0.1% NaCl in males



Plasma renin activity in male and female rats fed very low, low, or high salt diet for 4 days. CTCF1 and CTCF2 mutant males failed to reach WT levels on the very low salt diet. Male WT PRA was significantly reduced in response to an increase in dietary salt. There were no differences between female PRA compared to WT. Data is expressed as Mean (+/-) SEM, n=2-3 (2-3 rats per pool).

Diurnal blood pressure on 0.1% NaCl was reduced in males

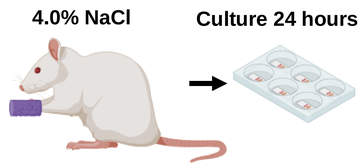




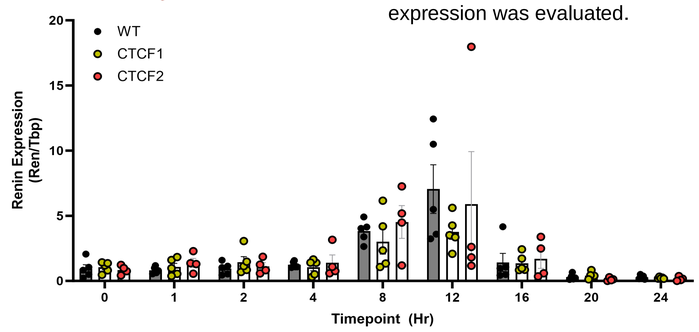
Mean arterial blood pressure of CTCF1, CTCF2 and WT littermates placed on a two-step NaCl challenge and diurnal blood pressures on 0.1% NaCl. Baseline MAP was measured on 0.4% NaCl, then switched to 0.1% for 7 days, then switched to 4.0% for 21 days. CTCF1 females showed an attenuation to salt-induced increases in BP as early as day HS day 16. Due to the differences in PRA on the 0.1% NaCl, BP was further broken down to evaluate diurnal BP. CTCF1 and CTCF2 male diurnal BP was lower than WT

Working Hypothesis

We hypothesize *Renin* transcriptional response to salt depletion is delayed in the CTCF mutant rat models.



Time course protocol used to increase *Renin* expression. SS rats were maintained on a 0.4% NaCl diet then placed on a 4.0% NaCl diet for 24 hours, then their kidneys were excised, sliced, and cultured in media for 24 hours. RNA was extracted and *Renin* expression was evaluated.



Time course of *Renin* expression in WT, CTCF1 and CTCF2 rats fed a 4.0% NaCl diet for 24 hours. WT, CTCF1 and CTCF2 rats showed increases in *Renin* expression until 12 hours of culturing in basal media. There is a trend for a decrease in *Renin* expression in the CTCF1 and CTCF2 rats at 12 hours compared to WT. *Renin* expression was reduced following 16 hours of culture. Data is expressed as Mean (+/-) SEM, n=6 pools.

Conclusions

- CRISPR modification of CTCF binding sites in the renin gene locus of SS rats disrupted CTCF binding in liver.
- Renin mRNA expression was not altered in the renal cortex as detected by RNA-seq
- CTCF1 and CTCF2 male rats failed to elevate PRA and had lower diurnal blood pressure in response to a very low salt diet.
- The induction of renin in response to lowering sodium may be delayed in CTCF mutant rats, suggesting a role for these sites in modulating transcriptional dynamics

Future Directions

- Assess changes in chromatin conformation in mutant models
- Optimize culture conditions for slices to live to 48+ hours
- Evaluate other stressors, such as Losartan's effects, on expression, PRA and MAP