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

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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 regulate and maintain chromatin conformation, and thereby gene expression. Cellular expression of CTCF is critical for *Renin* expression with multiple CTCF-binding motifs. In the Dahl salt-sensitive (SS) rat, CRISPR-Cas9 was utilized to create three models harboring mutations to 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 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 male mutant models failed to increase PRA on 0.1% NaCl diet (CTCF1=13.87±0.86; CTCF2=13.95±0.59; $p<0.05$ compared to WT=17.52±0.81). We hypothesized that *Renin* transcriptional response to salt depletion is delayed in mutant models. To test this, we 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), 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.