Sex Differences in the Kidney Circadian Transcriptome

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Background: Circadian rhythms are endogenous 24-hour oscillations regulated by a molecular clock present in nearly every tissue of the body, orchestrating physiological and metabolic processes. Premenopausal women are relatively protected from kidney disease, hypertension, and cardiovascular disorders compared to men. Our prior work showed male mice lacking the circadian protein PER1 develop salt-sensitive, non-dipping hypertension, while females do not. This led us to investigate sex-specific circadian mechanisms in the kidney. We aimed to characterize the male and female kidney circadian transcriptome and found lipid metabolism prominently involved in female circadian genes, prompting analysis of SREBP1.
Goal: We aimed to perform a novel comparison between male and female circadian kidney transcriptomes, using the number of rhythmically expressed genes (REGs).
Methods: Seventy-two 6 month old wild type C57BL/6 mice (36 female, 36 male) were acclimated for four weeks in a normal 12:12 light:dark cycle. The mice were subjected to total darkness for 18 hours and maintained in that condition for the duration of the study. Starting at Circadian Time (CT) 18 (Midnight), kidneys were collected and snap frozen from three females and three males every four hours for 48 hours. Whole kidneys were processed for total RNA. N=3 mice per sex and per time point were pooled and processed for bulk RNAseq (UF ICBR and Novagene). Data were analyzed to identify REGs. Pathway analysis was then performed.
Results: Our data reveal that females exhibit 3,303 rhythmic genes ($p < 0.05$), whereas males display 2,201 rhythmic genes, with only 624 genes overlapping between sexes. From this dataset, the candidate gene *Srebf1* emerged as a particularly notable female-specific REG. Srebf1 encodes SREBP1, a key transcription factor that regulates lipid metabolism. SREBP1 mRNA was expressed at a significantly higher level in females than males, regardless of time of day (1.5 fold, $p=0.01$), and its expression was significantly rhythmic in females ($p=0.001$) but not males ($p=0.276$). Preliminary Western blot data indicate that renal SREBP1 protein expression is higher in female vs male.
Conclusion: We found that kidneys from female mice have a higher number of genes that are rhythmic compared to male mice, suggesting that females have a stronger clock mechanism in the kidney.. Furthermore, lipid metabolism synthesis pathway was represented more in female circadian genes than male. These results highlight a potential mechanistic link between circadian regulation and sex differences in renal physiology, providing a foundation for future studies exploring how circadian-driven lipid metabolism influences cardiovascular and renal health.