# YOU ARE INVITED TO ATTEND THE

# DEFENSE OF THE DOCTORAL

# DISSERTATION

**“Post-transcriptional gene regulation of Bone Morphogenetic Protein (BMP)-2 during embryogenesis and cardiovascular calcification”**

by

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**ABSTRACT**

Bone morphogenetic protein (BMP) 2 is a classical morphogen whose concentration influences myogenesis, adipogenesis, chondrogenesis, and osteogenesis. Therefore, the mechanisms that regulate BMP2 synthesis are crucial. 300 nucleotides of the Bmp2 3' untranslated region (UTR) mRNA, the "ultra-conserved sequence" (UCS), have been largely unchanged since fishes and mammals diverged. Deletion of the UCS in a reporter transgene revealed that the UCS can repress *Bmp2* in essential embryonic structures including the developing heart. Using gene targeting in mice, we generated conditional *Bmp2* alleles that enabled the deletion of the UCS. Removal of the UCS was associated with elevated *Bmp2* mRNA and BMP signaling levels, reduced fitness, and embryonic malformations.

BMP2 is increased in all forms of cardiovascular calcification. The *Bmp2* UCS represses reporter gene expression in cardiovascular cells. We determined the impact of the endogenous UCS on *Bmp2* mRNA levels, BMP signaling, and calcification in the healthy aorta and in the calcified aorta of *Klotho* mutant homozygotes with renal disease. UCS deletion induced aortic *Bmp2* mRNA and BMP signaling in control males, but not in females. UCS deletion significantly increased BMP signaling in both male and female *Klotho* homozygotes. Key differences between males and females in *Bmp2* expression and BMP signaling were discovered. Sex-specific differences in *Bmp2* gene control may partly explain the increased propensity of males to undergo cardiovascular calcification.

MicroRNAs are *trans*-regulatory factors that may repress gene expression by binding to *cis*-regulatory elements such as the 3’ UTR. Using miRNA microarrays, we profiled miRNAs in the aorta from healthy control and *Klotho* mutant mice with pathological calcification and in normal aged mice. Many miRNAs that were repressed in the calcified aorta from *Klotho* mutant mice and predicted to target *Bmp2*, were also down-regulated in human calcified cardiovascular tissues. We also have for the first time obtained aortic miRNA profiles associated with age-progression and sex. MicroRNAs that regulate the synthesis of BMP2 and down-stream proteins or factors associated with calcification are attractive biomarkers for predicting the progression of the disease and potential therapeutic targets.