

# Allison Howard

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Major: Biochemistry  
University: University of Missouri-Columbia  
Faculty Mentor: Dr. Charlotte Phillips  
Mentor Department: Biochemistry and Child Health  
Funded by: Life Sciences Undergraduate Research Opportunity Program

## Investigation of type I collagen deposition in the glomeruli of COL1A2 deficient mice

Allison Howard, Amanda Brodeur, David Wirth, Brent Pheiffer and Charlotte Phillips

Type I collagen is the most abundant structural protein in the body, playing a major role in the strength and integrity of connective tissues. Alterations in the synthesis and structure of type I collagen result in a number of connective tissue disorders, such as osteogenesis imperfecta and Ehlers-Danlos syndrome. Type I collagen is normally a heterotrimeric type I collagen molecule composed of three pro  $\beta 1(I)$  collagen chains and one pro  $\beta 2(I)$  collagen chain. The COL1A2 deficient mouse produces only homotrimeric molecules, composed of three pro  $\beta 1(I)$  collagen chains resulting from a functional null mutation in the COL1A2 gene. Recently our lab discovered a novel type I collagen glomerulopathy in the COL1A2 mouse. The novel glomerulopathy demonstrated increased collagen deposition in the renal glomerular mesangium. The collagen accumulation is similar to what is observed in the secondary progression of renal damage, caused by a variety of kidney disorders. This project entails the measurement of COL1A2 and COL1A1 mRNA levels discerning whether increased collagen deposition in the glomeruli mesangium in COL1A2 deficient mice is related to increased type I collagen mRNA expression (pretranslational mechanism). These studies required harvesting kidneys from the COL1A2 deficient, heterozygous, and wildtype mice at one week, two weeks, and one month of age. Total RNA was isolated from harvested kidneys using the Qiagen RNAeasy RNA isolation kit, post mortar and pestle homogenization. The isolated total RNA was then analyzed for amount and quality via spectrophotometer at 260nm and 280nm, and 80ng of total RNA was used for cDNA generation via reverse transcriptase polymerase chain reaction using Promega ImpromII reagents. PCR products were generated for mouse COL1A2 transcripts using primers optimized for Real Time PCR analysis (RT-PCR). Quantitation of the COL1A1 and COL1A2 transcripts was obtained on the Roche LightCycler, with SYBR green monitoring after each amplification cycle. Previous in situ hybridization data suggests that there may not be an increase in mRNA. The study will attempt to gain a greater understanding of the molecular mechanism leading to type I collagen accumulation in the renal mesangium, with future application to understanding the role of extracellular matrix deposition in renal pathology and disease.