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VPS33B IS REQUIRED IN THE *DROSOPHILA* TUBULE AND HINDGUT FOR NORMAL EXCRETION

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INTRODUCTION:
Arthrogryposis-renal dysfunction-cholestasis (ARC) syndrome is a rare, autosomal recessive disease in which most affected individuals do not live past infancy. This condition is accompanied by several severe symptoms including renal tubular dysfunction, which our lab is specifically interested in. All known ARC patients possess mutations in the genes *VPS16B* or *VPS33B*, which code for Vacuolar Protein Sorting (VPS) proteins 16B and 33B respectively. However, aside from their involvement in membrane trafficking, not much is known about these proteins. Since *VPS16B* and *VPS33B* are conserved in *Drosophila melanogaster*, our lab can use the fly as a model organism to study these proteins and their function in the renal epithelium. We hypothesized that *Vps16B* and *Vps33B* are important to renal function in the fly.

METHODS:
In order to test this hypothesis, we established the "poop-drop assay" which allows us to monitor excretion of the flies. Doing so offers us insight into how our proteins of interest are impacting secretion and reabsorption in the fly renal system. Decreased secretion will result in less excretion while decreased reabsorption will result in more excretion. We began the assay by using complete genomic knockout mutant flies along with genomic rescue flies for each of our proteins of interest. After these flies are collected and sorted by gender, we starve them in humidity vials for 7 hours before transferring them into vials with a normal diet or a diet supplemented with high salt (0.3M NaCl). After an incubation period of 18 hours at 25°C, we mouth-pipette flies in groups of five into empty vials for two hours. Next, we clear the vials and count the excretion drops. We repeated this process with knockdown flies. We knocked down *Drosophila Vps16B* and *Vps33B* in different parts of the fly excretory system. Using the GAL4/UAS-RNAi system, we knocked down *Drosophila Vps16B* and *Vps33B* in either the Malpighian tubules or the hindgut, the two parts of the fly excretory system, and measured excretory ("poop") drops per fly. This process allows us to target specific tissues within the renal epithelium to see where the proteins function.

RESULTS:
Here we report that there is no significant excretion phenotype in *Vps16B* knockout mutants or when *Vps16B* is knocked down in the hindgut or in the tubules. However, *Vps33B* genomic knockout females express an increased excretion phenotype when exposed to high salt. In order to verify that this is caused by *Vps33B* and not some underlying factor, we perform genomic rescue by reinserting our gene of interest back into the genome on a different chromosome. Performing the poop drop assay on our genomic rescue flies resulted in a partial rescue, indicating that *Vps33B* was responsible, in part, for the increased excretion. A similar, but not

statistically significant, trend is observed in males on high salt. We next observe that genomic knockdown males express a reduced excretion phenotype with *uro-GAL4*, which is localized in principal cell of the tubule, when fed high salt diet. Furthermore, we observe a significant increase in excretion in *Vps33B/c601* females exposed to a normal diet. The *c601-GAL4* is expressed in the hindgut.

CONCLUSION:
Collectively, our data suggest that the VPS33B protein affects excretion and functions in both the tubule and hindgut, while VPS16B has not been proven to play a significant role in either tissue.