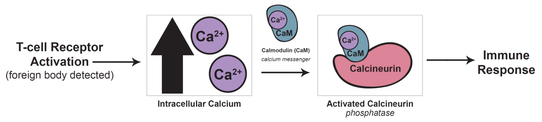
Calcineurin in the distal convoluted tubule plays a key role in tacrolimus-induced hypomagnesemia and hypercalciuria

Brittany D.K. Gratreak[1], Elizabeth A. Swanson[1], Rebecca A. Lazelle[1], Sabina K. Jelen[2], Chao-Ling Yang[1], Joost Hoenderop[2], Rene J. Bindels[2], David H. Ellison[1,3]

1 Division of Nephrology and Hypertension, Department of Medicine, Oregon Health & Science University, Portland, Oregon; **2** Radboud Institute of Molecular Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands; **3** Renal Section, Veterans Affairs, Portland Health Care System, Portland, Oregon

BACKGROUND

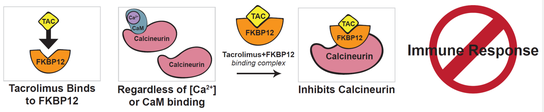
- Tacrolimus is a **calcineurin inhibitor**, a robust immunosuppressive used for solid organ transplantations to reduce graft rejection



- Unfortunately, **adverse renal side effects** increase the risk for developing metabolic diseases

MECHANISM

- Tacrolimus binds to its immunophilin **FKBP12** to inhibit calcineurin and halt the immune response



- Nijenhuis *et al*. showed that tacrolimus reduces **Mg^{2+}/Ca^{2+}** transport proteins in the kidney: *TRPV5*, *calbindin-D28K*, and *TRPM6*
- Our work sought to determine whether this is a direct result of calcineurin inhibition
- **Kidney-specific FKBP12 knockout (KS-FKBP12$^{-/-}$)** mice were utilized to study tacrolimus-induced **hypomagnesemia** and **hypercalciuria**

WHY STUDY Mg^{2+} AND Ca^{2+}?

2x INCREASED RISK FOR DIABETES MELLITUS

Hypomagnesemia is an independent predictor of **new onset-diabetes after transplant (NODAT)**

5x INCREASED RISK FOR OSTEOPOROSIS

Tacrolimus treatment is associated w/ **increased bone resorption & bone loss and hypercalciuria**

HYPOTHESIS

Renal Mg^{2+}/Ca^{2+} wasting is a direct result of calcineurin inhibition in the kidney.

- **KS-FKBP12$^{-/-}$ mice will be protected** from effects caused by tacrolimus-induced calcineurin inhibition in the kidney

METHODS

Animals Doxycycline-induced KS-FKBP12$^{-/-}$ knockout mice & age-matched/non-induced littermates as controls

Treatment Daily subcutaneous injections (3 mg/kg body weight) of tacrolimus or vehicle for 18 days

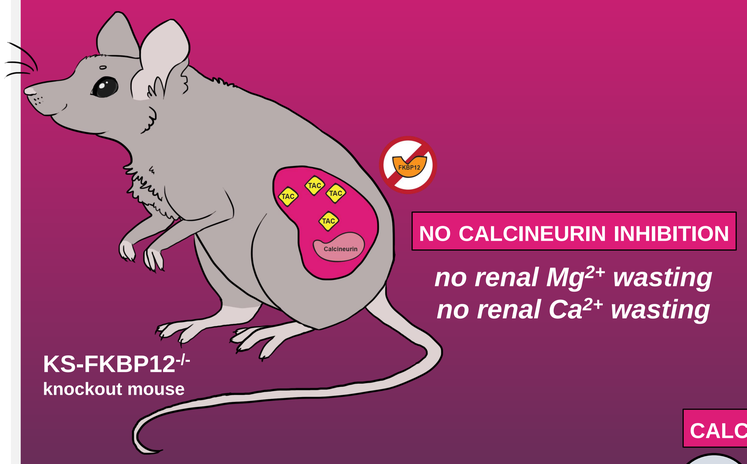
Electrolytes Whole blood and 24 hr urine analyzed via o-Cresolphthalein Complexone & Xylidyl blue assays or i-STAT

Protein Abundance Western blot analysis of snap-frozen ½ kidneys

Gene Expression mRNA transcripts measured via qPCR

OHSU

The immunosuppressive drug **tacrolimus** causes renal **calcium & magnesium wasting** by inhibiting calcineurin in the kidney.



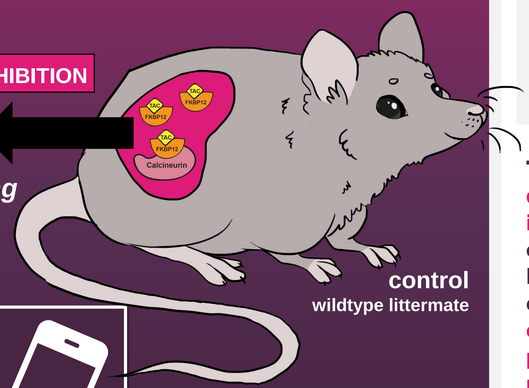
NO CALCINEURIN INHIBITION

no renal Mg^{2+} wasting
no renal Ca^{2+} wasting

CALCINEURIN INHIBITION

Mg^{2+} Ca^{2+}

renal wasting



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CORRESPONDENCE

gratreab@ohsu.edu
ellisond@ohsu.edu

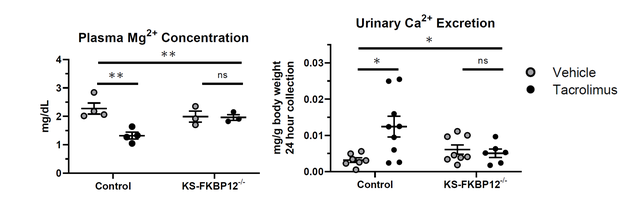

figure 1 **Deletion of renal FKBP12 *completely* prevents the development of tacrolimus-induced hypomagnesemia and hypercalciuria**

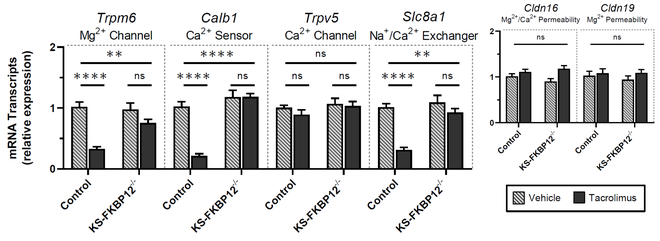

figure 2 **Calcineurin inhibition via tacrolimus alters the expression of key transport genes in the distal convoluted tubule (DCT) but not the thick ascending limb (TAL)**

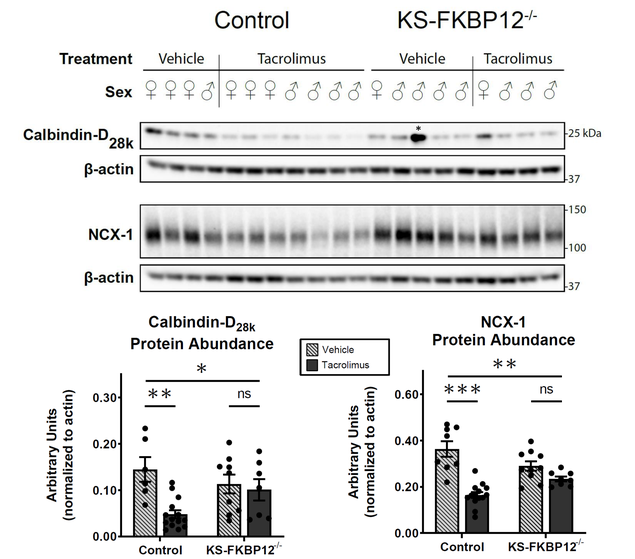

figure 3 **KS-FKBP12$^{-/-}$ mice are protected from the loss of Calbindin-D_{28k} (calcium buffer) & NCX-1 (sodium-calcium exchanger) proteins**

Thus, hypomagnesemia and hypercalciuria result directly from tacrolimus-induced calcineurin inhibition in the kidney. These results suggest that calcineurin (a phosphatase) has a direct effect on Mg^{2+} and Ca^{2+} transporters in the distal convoluted tubule. This demonstrates that calcineurin inhibition contributes to the prevalence of post-transplant diabetes and metabolic bone disease.

FUTURE DIRECTIONS

- Utilize nanotechnology for directed drug delivery ***so tacrolimus can only target T-cells***
- Develop a treatment to avoid inhibiting calcineurin in the DCT by ***delivering a calcineurin antagonist/competitive binding molecule only to the DCT*** during immunosuppressive therapy