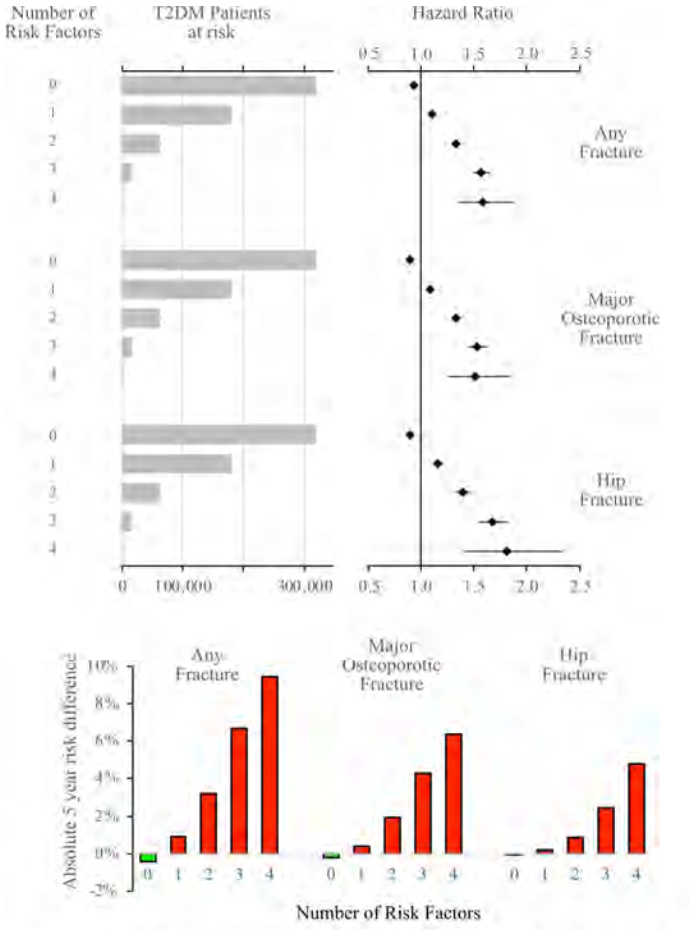

Relative and absolute risk difference in T2DM patients per number of risk factors compared to matched controls using a multivariable adjusted Cox regression model. The four diabetes related risk factors were: (i) BMI<25, (ii) Time since diabetes diagnosis > 15 years, (iii) Insulin treatment, (iv) Absence of physical activity

***Disclosures: Kristian Axelsson**, None*

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Long-Acting PTH/PTHrP Hybrid Analogue Rectifies Hypocalcemia in a Mouse Model for Autosomal Dominant Hypocalcemia Type-1 (ADH1)
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Autosomal dominant hypocalcemia type-1 (ADH1), caused by gain-of-function calcium-sensing receptor (CaSR) mutations, leads to lifelong hypocalcemia that is symptomatic in ~50% of patients, reduced PTH secretion, and normal or increased urine calcium excretion. Current therapies with oral calcium and vitamin D analogues are unsatisfactory and predispose patients to hypercalciuric renal disease. Recombinant PTH injections are effective for symptomatic ADH1, although their ability to maintain long-term steady state increases in serum calcium is unknown. However, a long-acting PTH/PTHrP hybrid analogue, termed AZP-3601, is reported to induce sustained increases in serum calcium, and we therefore evaluated its effectiveness in treating ADH1 in a mouse model with a heterozygous gain-of-function CaSR mutation, Leu723Gln (L723Q). All studies were conducted in age-matched adult ADH1 mice+/Q723 and in accordance with institutional welfare guidelines. Male and female ADH1 mice were treated with once-daily s.c. injections of 0, 1, 2, or 4 nM/kg AZP-3601 for 14 days (n=6-7 mice per dose). Plasma ionised calcium (iCa) was measured at 6hr post-dose on day 7 and 14. Plasma and 24hr urine was obtained on day 14 for analysis of minerals, renal function, 1,25-dihydroxyvitamin D, and bone turnover. Renal gene expression was assessed by quantitative reverse transcriptase (qRT)-PCR. AZP-3601 treatment for 7 days caused dose-dependent increases in plasma iCa. Thus, control (untreated) ADH1 mice were hypocalcemic (iCa=0.8+/-0.1 mmol/L), whilst ADH1 mice treated with 1 or 2 nM/kg AZP-3601 became normocalcemic (iCa of 1.2+/-0.1 or 1.3+/-0.1 mmol/L, respectively, p<0.01 versus controls), and ADH1 mice treated with 4 nM/kg AZP-3601 became hypercalcemic (iCa=1.6+/-0.2 mmol/L, p<0.001 versus controls). These increases in iCa were maintained on day 14. AZP-3601 did not alter plasma phosphate, magnesium, creatinine, 1,25-dihydroxyvitamin D, or 24hr urine calcium, but AZP-3601 at 2 and 4 nM/kg doses increased CTX-1 and P1NP bone turnover markers in ADH1 mice. AZP-3601 had no effect on the expression of 8 genes (CASR, PTHR1, CYP27B1, CYP24A1, NKCC2, TRPV5, CLDN16, and CLDN19) which mediate renal calcium metabolism. In summary, AZP-3601 causes stable and dose-dependent elevations in plasma calcium in ADH1 mice, without adverse effects on urine calcium excretion or renal calcitropic gene expression. These findings support the potential utility of AZP-3601 for the long-term management of ADH1.

***Disclosures: Fadil Hannan**, None*

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Genome-wide CRISPR Screens Reveal Regulators of Osteogenic Phosphate Sensing
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Organismal homeostasis of inorganic phosphate (Pi) is tightly controlled to maintain a consistent serum Pi level, disruption of which can have major consequences for the body. We currently lack a mechanistic understanding of the molecular pathways in osteogenic cells that sense altered serum Pi and transmit this information to induce release of FGF23. In this work, we utilized cell-based genome-wide approaches to identify factors in bone cells that function in this regulation.To do so, we initially utilized several genome-wide approaches. We profiled the temporal transcriptional responses of an osteoblast (MC3T3-E1) and an osteocyte (OCY454) cell line to increases in extracellular Pi with RNA-seq and detect clear transcriptional signatures, including an initial induction of immediate early response genes within 30 min, and a late phase response at 24-48 hr with induction of EGF and MAPK signaling factors, as well as osteogenic genes.Based on these findings, we developed two flow cytometry-based readouts of the response to increased extracellular Pi; a fluorescent reporter MC3T3-E1 line with Dmp1-2A-mCherry and a cell surface antigen stained with an antibody. To identify novel factors involved in Pi sensing, we utilized these reporters to conduct unbiased, genome-wide CRISPR-interference screens. After sorting into percentiles (bottom, mid, and top 15%) of reporter response, we sequenced the sgRNAs in each population and compared abundancies. In addition to identifying genes previously implicated in Pi sensing, including Casr, Gnaq, and Fgf23, our screens revealed a large set of novel potential Pi regulators. We compared hits with genes associated with serum Pi levels by genome-wide association studies (GWAS), and identified a set of Pi GWAS hits enriched in our screen, including calcium and PTH signaling factors, suggesting these cell-based screens can identify regulators of organismal Pi. Using gene knockout and inhibitor studies in cells, we have investigated several of these potential regulators, and among others, we find that TGFB2 may be involved in Pi sensing and regulation. Not only are TGF beta targets upregulated by high Pi, TGF beta pathway members were identified as hits, and addition of TGFB2 stimulates the Pi response in osteogenic cells.To conclude, this work has revealed novel phosphate sensing factors identified in osteogenic cells, and we hope understanding these pathways could reveal novel treatments for misregulated Pi and FGF23.

***Disclosures: Dylan Kuennen**, None*

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Intestinal Epithelial Cell-Deletion of Cyp24a1 Reduces Renal Cyp27b1 mRNA and Enhances Trpv6 mRNA Induction by Low Dietary Calcium
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Cyp24a1 is an enzyme that initiates the catabolism of 1,25 Hydroxyvitamin D3 (1,25(OH)2D), the active hormonal form of Vitamin D that regulates bone and calcium homeostasis. In the intestine, Cyp24a1 may regulate local 1,25(OH)2D action and also contribute to whole-body vitamin D metabolism. To evaluate the differential role of Cyp24a1 in local versus whole-body vitamin D metabolism, we generated mice with intestinal epithelial cell (IEC)-specific deletion of Cyp24a1 (villin-Cre x Cyp24a1flox/flox, IEC KO). Control (Cyp24a1flox/flox) and IEC KO mice were fed a standard Chow diet from weaning until 11 weeks of age, at which time they were randomized to AIN93G diets (0.4% P, 200 IU/kg Vitamin D3) with either adequate (0.5%) or deficient (0.2%) calcium (Ca) levels (n=5/sex/group). One week later, all mice were euthanized, and tissues were collected and stored for RNA isolation. Duodenal (Dd) and renal (Kd) mRNA levels were quantified using qPCR. As expected, the low Ca diet increased Dd Trpv6 as well as Kd Cyp27b1 and Cyp24a1 mRNA levels 2-fold in control mice. In IEC KO mice, Dd Trpv6 mRNA was not altered in the 0.5% Ca diet group, but induction by the 0.2% Ca diet was 45% higher than control mice fed the low Ca diet. This shows that the reduction in local 1,25(OH)2D degradation increases 1,25(OH)2D-mediated gene expression in mice under dietary calcium stress. IEC-deletion also affected whole-body vitamin D metabolism; Kd Cyp27b1 mRNA was reduced by 50% in the IEC KO regardless of the diet fed. However, diet-mediated induction of Kd Cyp24a1 mRNA was not altered in IEC KO mice. This suggests the need for circulating 1,25(OH)2D is reduced when Cyp24a1 is deleted from the intestine. We conclude that Cyp24a1 in IEC mediates both local 1,25(OH)2D degradation and is involved in an endocrine signaling path-