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Proof-of-concept of gene therapy for X-linked hypophosphatemia

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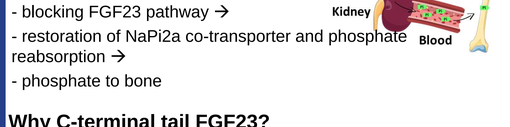
INTRODUCTION

- X-linked hypophosphatemia (XLH) is a rare skeletal disorder due to mutation in *PHEX* gene leading to increased levels of fibroblast growth factor 23 (FGF23) (1,2)



- Hypophosphatemia and low levels of active vitamin D due to high FGF23 result in skeletal and osteo-articular abnormalities (1,2)
- The conventional substitutive treatment (phosphate+active vitamin D analog) is associated with severe long-term side effects (1,2)
- Monoclonal antibody against FGF23 has been approved for XLH but still remaining a high-cost lifelong therapy (3-7)

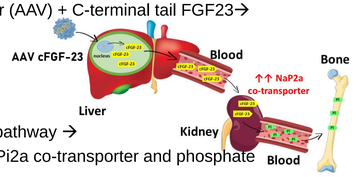
AIM

Proof-of-concept of gene therapy:
one injection to rescue the bone phenotype in a murine model of XLH

METHOD

Gene therapy approach for XLH

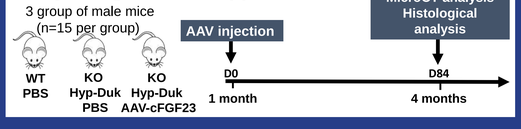
- Adenovirus vector (AAV) + C-terminal tail FGF23→
- liver-targeting→
- blood→
- kidney→
- binding FGFR1→
- blocking FGF23 pathway →
- restoration of NaPi2a co-transporter and phosphate reabsorption →
- phosphate to bone



Why C-terminal tail FGF23?

it is biologically inactive but it is capable to bind to FGFR1 (the main receptor of FGF23), thus blocking the action of native FGF23

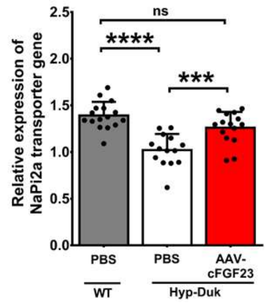
Study design: *in-vivo* approach



RESULTS

1/ Restoration of NaPi2a co-transporter in the kidney

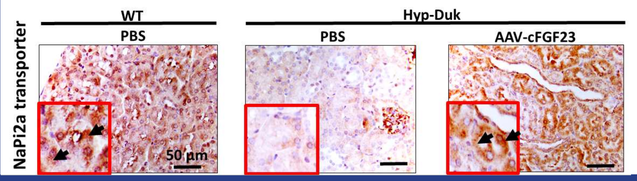
Figure 1: Expression of the NaPi2a transporter mRNA in kidney



- ✓ One-month-old Hyp-Duk mice were injected with AAV8 expressing sp7-cFGF23co-clFIX-Alb (AAV-cFGF23) and sacrificed three months after injection
- ✓ PBS-injected WT and Hyp-Duk mice served as controls
- ✓ AAV-cFGF23 treatment led to the restoration of expression of the NaPi2a transporter mRNA **(Fig.1)** as well as of the NaPi2a transporter protein in kidney obtained with immunohistochemistry **(Fig.2, arrows)**

Statistical analyses were performed by ANOVA (*** p < 0.001; **** p < 0.0001; ns: not significant). All data are shown as mean ± SD (n=14-16 mice per group)

Figure 2: Expression of the NaPi2a transporter in the kidney, obtained with immunohistochemistry



2/ Correction of bone abnomalities

Figure 3: Trabecular and cortical bone microstructure (femur)

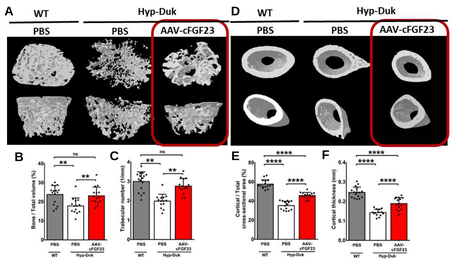
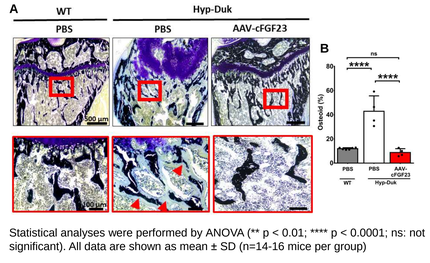
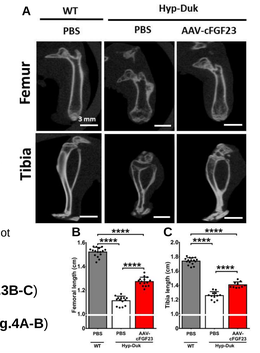


Figure 4: Von Kossa staining (proximal tibia)



Statistical analyses were performed by ANOVA (** p < 0.01; **** p < 0.0001; ns: not significant). All data are shown as mean ± SD (n=14-16 mice per group)

Figure 5: Femur and tibia length

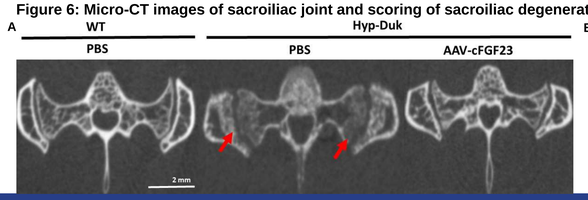


Three months after AAV-cFGF23 treatment, micro-computed tomography (micro-CT) analysis and histological analysis revealed:

- ✓ Complete restoration of the trabecular bone (**Fig.3A**), in particular, the bone volume–to–total volume ratio and the number of trabeculae (**Fig.3B-C**)
- ✓ Improvement of the cortical bone (**Fig.3D**), in particular, the ratio cortical to total cross-sectional area and the cortical thickness (**Fig.3E-F**)
- ✓ Increased bone mineralization and decreased amount of osteoid (non-mineralized collagenous matrix (arrows), a feature of osteomalacia) (**Fig.4A-B**)
- ✓ Elongation of femur and tibia together with a general amelioration of the distorted epiphysis and diaphysis (**Fig.5A-C**)

3/ Correction of osteo-articular abnomalities

Figure 6: Micro-CT images of sacroiliac joint and scoring of sacroiliac degeneration



- ✓ The treatment rescued signs of sacroiliac arthritis both in terms of morphology (**Fig.6A**) and sacroiliac score (**Fig.6B**): multiple erosions and irregular cortical board are present in untreated Hyp-Duk mice and indicated by red arrows on **Fig.6A**, but completely disappear after AAV–cFGF23 treatment, **Fig.6A-B**)

Statistical analyses were performed by ANOVA (** p < 0.01; **** p < 0.0001; ns: not significant). All data are shown as mean ± SD (n=14-16 mice per group)

CONCLUSIONS

1. Through the restoration of NaPi co-transporter in kidney, the AAV-cFGF23 gene therapy corrects the abnormal skeletal phenotype in Hyp-Duk mice:
   - ✓ bone mineralization and microstructure
   - ✓ bone elongation and growth
   - ✓ osteo-articular manifestations
2. Our project opens new perspectives on the treatment of skeletal diseases by gene therapy targeting the liver

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