



The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

112. THALASSEMIA AND GLOBIN GENE REGULATION

Bone Marrow Sinusoidal Endothelial Cells Are a Site of *Fgf23* Upregulation in Murine β -ThalassemiaJackie Fretz, PhD¹, Eileen Chua², Xiuqi Li, PhD², Karin E Finberg, MDPHD²¹ Department of Orthopaedics & Rehabilitation, Yale School of Medicine, New Haven, CT² Department of Pathology, Yale School of Medicine, New Haven, CT

Fibroblast growth factor-23 (FGF23) is a bone-derived hormonal regulator of mineral metabolism with emerging functions in the bone marrow. FGF23 has been reported to negatively regulate erythropoiesis [PMID:24509850] and promote hematopoietic progenitor mobilization from the bone marrow [PMID:33512467]. Elevated FGF23 levels in the circulation have been described in iron-deficient subjects and also in subjects with the iron-loading anemia β -thalassemia. Recently, we reported bone marrow sinusoidal endothelial cells (BM-SEC) as a novel site of *Fgf23* upregulation in mice with chronic iron-deficiency anemia and acute, phlebotomy-induced anemia [PMID:37417950].

We and others have detected *Fgf23* mRNA elevation in total bone marrow of *Hbb*^{th3/+} mice, a model of non-transfusion dependent β -thalassemia that exhibits systemic iron loading. In the present study, we sought to clarify sites of *Fgf23* promoter activity in *Hbb*^{th3/+} bone marrow using a reporter allele in which the *enhanced green fluorescent protein* (eGFP) coding sequence is knocked into the endogenous *Fgf23* locus (*Fgf23*^{eGFP}). We generated *Hbb*^{+/+} and *Hbb*^{th3/+} female littermates that carried either one (*Fgf23*^{+/eGFP}) or zero (*Fgf23*^{+/+}) copies of the *Fgf23* reporter allele, which were raised on a standard rodent diet (200 mg/kg iron, 1.0% calcium, 0.7% phosphorus). *Fgf23*^{eGFP/eGFP} homozygotes were not generated due to known early growth retardation and mortality in *Fgf23* null mice. After blood and tissue collection at 8 weeks of age, phenotypes of the 4 *Hbb*-*Fgf23* genotype combinations were compared by two-way ANOVA.

Compared to mice with intact *Hbb* and *Fgf23* alleles (*Hbb*^{+/+} *Fgf23*^{+/+}), *Hbb*^{th3/+} *Fgf23*^{+/+} mice showed markedly elevated plasma levels of "total" FGF23 (sum of intact, active hormone and C-terminal cleaved fragments). Plasma total FGF23 levels in *Hbb*^{th3/+} *Fgf23*^{+/+} and *Hbb*^{th3/+} *Fgf23*^{+/eGFP} mice were similar, indicating utility of the latter genotype for interrogating sites of increased *Fgf23* expression in murine β -thalassemia. We therefore used anti-GFP immunohistochemistry in formalin-fixed bone marrow sections to assess *Fgf23*^{eGFP} reporter allele expression in the context of tissue architecture. *Hbb*^{th3/+} *Fgf23*^{+/eGFP} mice showed GFP expression in BM-SEC throughout the bone marrow, which was more intense than in non-anemic *Hbb*^{+/+} *Fgf23*^{+/eGFP} mice; controls lacking the *Fgf23*^{eGFP} allele confirmed anti-GFP antibody specificity. *Hbb*^{th3/+} mice also showed *Fgf23*^{eGFP} reporter expression in the thymic vasculature but not vasculature of the liver, spleen, or kidney.

In contrast to their marked elevation in plasma total FGF23, *Hbb*^{th3/+} *Fgf23*^{+/+} mice showed plasma levels of the intact, biologically-active FGF23 hormone that overlapped with levels in *Hbb*^{+/+} *Fgf23*^{+/+} controls. Interestingly, compared with *Hbb*^{th3/+} *Fgf23*^{+/+} mice, *Hbb*^{th3/+} *Fgf23*^{+/eGFP} mice showed 45% lower plasma intact FGF23, suggesting an effect of *Fgf23* gene dosage. Given prior reported effects of FGF23 on hematopoiesis, we assessed if *Fgf23* gene dosage modulated the severity of ineffective erythropoiesis in *Hbb*^{th3/+} mice. Compared to *Hbb*^{+/+} *Fgf23*^{+/+} controls, *Hbb*^{th3/+} *Fgf23*^{+/+} mice showed reductions in RBC, hemoglobin, and mean corpuscular hemoglobin, as well as increases in RDW and in spleen weight (reflecting extramedullary hematopoiesis). However, the severity of these parameters was not modified by heterozygous *Fgf23* disruption (*Hbb*^{th3/+} *Fgf23*^{+/eGFP}). While FGF23 has been reported to induce left ventricular hypertrophy [PMID:21985788], *Hbb*^{th3/+} *Fgf23*^{+/+} and *Hbb*^{th3/+} *Fgf23*^{+/eGFP} mice showed similarly increased heart-to-body weight ratios compared to *Hbb*^{+/+} *Fgf23*^{+/+} controls. In non-thalassemic mice, heterozygous *Fgf23* disruption (*Hbb*^{+/+} *Fgf23*^{+/eGFP}) caused a trend ($P=0.10$) towards lower plasma intact FGF23 while not impacting hematological parameters or spleen weight.

In summary, by showing that *Fgf23* gene dosage modulates plasma intact FGF23 levels in *Hbb*^{th3/+} mice without altering severity of anemia or splenomegaly, our data suggest that moderate reductions in circulating levels of the intact FGF23 hormone do not alter the severity of ineffective erythropoiesis in *Hbb*^{th3/+} mice at 8 weeks of age. Our finding that β -thalassemic mice exhibit *Fgf23* upregulation in BM-SEC lays the groundwork for future investigation of the functional effects of locally-produced FGF23 in the thalassemic hematopoietic stem cell niche.

Disclosures No relevant conflicts of interest to declare.

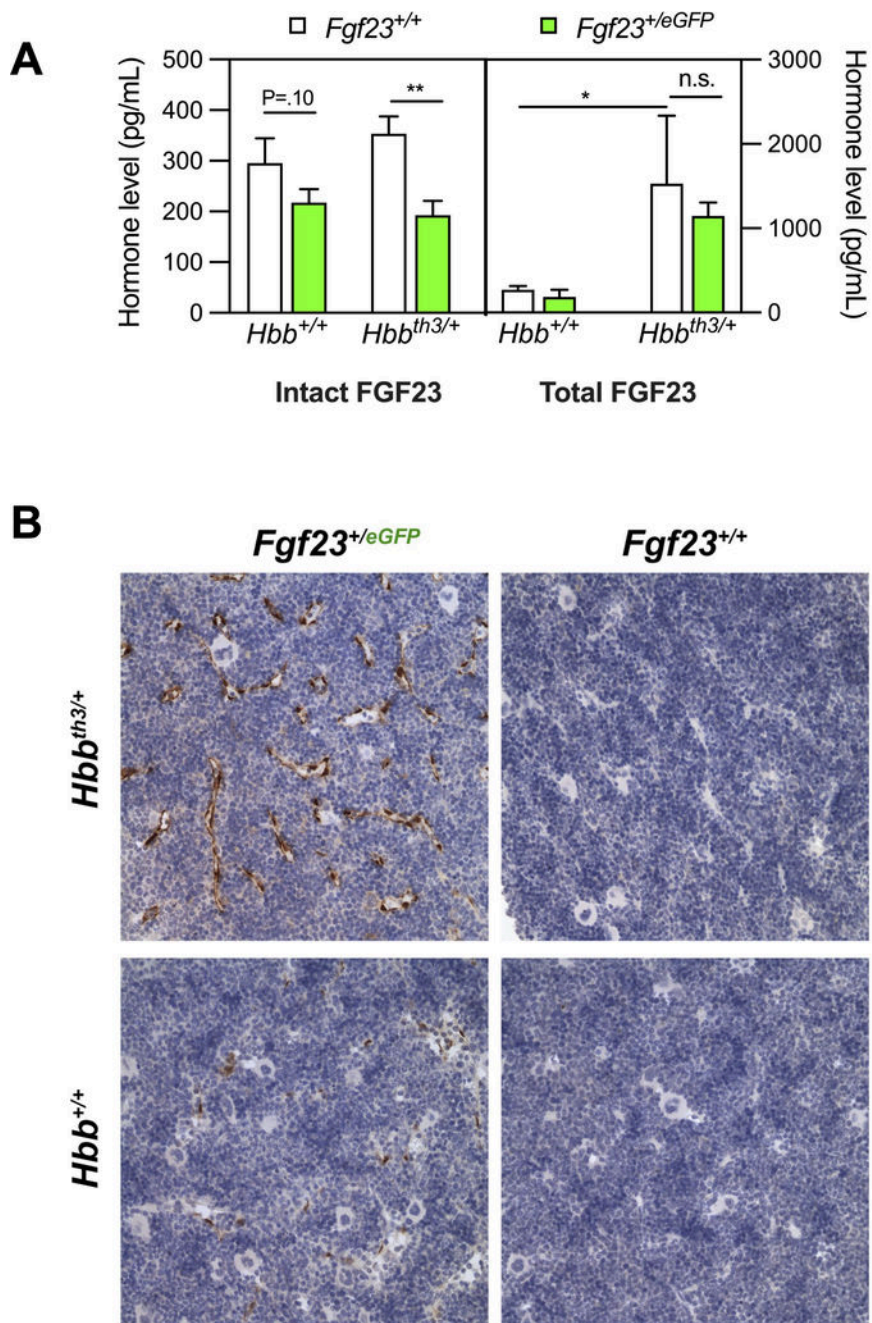


Figure. Phenotypic characterization of 8-week-old female mice of different *Hbb-Fgf23* genotype combinations. (A) Intact and total FGF23 levels in plasma. **P* < .05, ***P* < 0.01 by 2-way ANOVA with Tukey's post-hoc test. (B) Localization of *Fgf23*-GFP reporter expression in fixed bone marrow sections by anti-GFP immunohistochemistry (original magnification 40X).

Figure 1

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