Iron deficiency drives an autosomal dominant hypophosphatemic rickets (ADHR) phenotype in fibroblast growth factor-23 (Fgf23) knock-in mice


Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN 46202; Laboratory of Endocrinology and Metabolism, West China Hospital, Sichuan University, Chengdu, Sichuan, China; Department of Foods and Nutrition, Purdue University, West Lafayette, IN 47907; and Departments of *Anatomy and Cell Biology, *Pathology and Laboratory Medicine, *Pediatrics, and *Medicine, Indiana University School of Medicine, Indianapolis, IN 46202

AUTHOR SUMMARY

The study of disorders caused by single gene defects that alter how phosphate is processed by the body can provide critical insights into normal phosphate metabolism. Autosomal dominant hypophosphatemic rickets (ADHR), a condition that can result in bone fracture, is characterized by low levels of phosphate and vitamin D in the blood serum (1). ADHR is caused by missense mutations (2) in the gene encoding fibroblast growth factor-23 (FGF23), a hormone produced by the bones that helps maintain normal phosphate levels (i.e., phosphate homeostasis) by causing the kidney to decrease reabsorption of phosphate so that less phosphate enters the bloodstream. The missense mutations that cause ADHR effectively replace arginine (R) residues at positions 176 or 179 with glutamine (Q) or tryptophan (W) residues. The mutated region of the protein, known as the RXXR (176/179/Q/W) subtilisin-like proprotein convertase (SPC) site, normally helps stabilize the intact protein (2–4). ADHR is unique among the disorders involving FGF23, because individuals with this disease carry gain-of-function FGF23 mutations that can manifest as disease states ranging from mild or no symptoms to late-onset of hypophosphatemic bone disease. Delayed onset often occurs in physiological states associated with iron deficiency, including puberty and pregnancy.

Our goal was to test iron deficiency as the cause of late-onset ADHR and to determine the interaction of genes, proteins, and molecules that contribute to this delayed disease phenotype. Here, we demonstrate that iron deficiency substantially increases Fgf23 mRNA levels in the bone of both wild-type mice (controls) and mice genetically altered to carry an Fgf23 mutation that causes ADHR in humans (these mice have been designated “ADHR R176Q-Fgf23 knock-in” mice). We showed that increased expression of Fgf23 mRNA likely occurs through the activation of key pathways involved in sensing iron. Importantly, wild-type mice proteolytically cleaved excess Fgf23 protein into smaller pieces to maintain normal phosphate metabolism. In contrast, the proteolyis in R176Q-Fgf23 knock-in mice was compromised, resulting in elevated levels of intact serum Fgf23 and hypophosphatemic bone disease, consistent with the late-onset ADHR phenotype. Thus, our work supports the concept that iron deficiency controls Fgf23 and phosphate metabolism and that ADHR is caused by gene–environment interactions.

The ADHR spectrum contains two subgroups of affected individuals: One subgroup consists of patients who present during childhood with phosphate wasting, rickets, and lower limb deformity; the second subgroup consists of those who are unaffected as children but present clinically later, during adolescence or adulthood (1). To test the role of iron deficiency in the regulation of Fgf23 and ADHR onset in vivo (i.e., in the organism itself), wild-type and R176Q-Fgf23 knock-in (ADHR) mice were placed on normal (control) or low-iron diets for 8–12 wk. The low-iron diet decreased serum iron levels only in the ADHR mice receiving the low-iron diet but not in the other groups. Further, alkaline phosphatase (a marker for bone formation) was only increased in both wild-type and ADHR mice and caused similar degrees of iron-deficiency anemia in both groups compared with controls. Interestingly, the low-iron diet resulted in significant hypophosphatemia and bone osteomalacia (improper mineralization) only in the ADHR mice receiving the low-iron diet but not in the other groups. Further, alkaline phosphatase (a marker for bone formation) was only increased in both wild-type and ADHR mice and caused similar degrees of iron-deficiency anemia in both groups compared with controls. Interestingly, the low-iron diet resulted in significant hypophosphatemia and bone osteomalacia (improper mineralization) only in the ADHR mice receiving the low-iron diet but not in the other groups.
hypophosphatemic bone disease) was elevated only in the ADHR mice on the low-iron diet, and no changes in serum calcium or parathyroid hormone, which promotes increases in serum calcium, were observed. These skeletal and biochemical phenotypes parallel those of patients with late-onset ADHR.

To test the mechanisms for late-onset ADHR, serum Fgf23 was measured in the treated mice by using serum assays that measured either intact, bioactive Fgf23 or intact and C-terminal Fgf23 protein. Of significance, both the wild-type and ADHR mice receiving the low-iron diet had markedly elevated C-terminal Fgf23; however, the wild-type mice on the low-iron diet displayed normal levels of intact Fgf23, indicating that these animals were capable of regulating phosphate homeostasis through active proteolysis of the Fgf23 protein. In contrast, the ADHR mice on the low-iron diet had elevated intact and C-terminal Fgf23, demonstrating that intact Fgf23 cleavage was impaired by the Fgf23 ADHR R176Q mutation, resulting in hypophosphatemic bone disease. To test the potential sources for the elevated serum Fgf23, we next examined Fgf23 expression in the bones of the mice receiving the two different diets. Wild-type and ADHR mice on the low-iron diet showed marked increases in Fgf23 mRNA and protein levels at 8 and 12 wk compared with the respective control mice (summarized in Fig. P1).

To isolate the responses of bone cells to iron deficiency, we treated a cell line derived from bone tissue (the UMR-106 osteoblastic cell line) with the therapeutic iron chelator deferoxamine (DFO) to reduce total iron content. DFO treatment resulted in a significant increase in Fgf23 mRNA and elevated intracellular signaling; both these effects were blocked by inhibitors. Furthermore, the hypoxia-induced factor (HIF) transcription factor HIF1α, known to respond to reductions in cellular iron, was stabilized with DFO and with a specific HIF activator, l-mimosine (l-MIM). In parallel, l-MIM treatment also significantly stimulated Fgf23 mRNA expression, implicating an increase in Fgf23 mRNA transcription by HIF1α as the molecular basis for elevated Fgf23 during iron deficiency (Fig. P1).

This work supported the following concepts: (i) iron deficiency can induce ADHR biochemical and skeletal phenotypes in a model animal in vivo; (ii) the 176RXXR179 SPC site is critical for the normal control of circulating Fgf23 at a secondary regulatory step; and (iii) Fgf23 production is downstream of (i.e., responds to or is controlled by) transcriptional pathways associated with iron-deficiency sensing. Our findings show that ADHR, unlike other diseases with elevated FGF23, is a syndrome that can be caused by gene–environment interactions, whereby the combined presence of an ADHR mutation and altered iron status are capable of producing the disease phenotype.

Since the identification of the FGF23 ADHR mutations, reconciling our current knowledge of phosphate handling with the development of ADHR in patients has been difficult. Under normal circumstances, the expression of FGF23 is reduced strongly by hypophosphatemia; therefore, as serum phosphate levels decrease, serum FGF23 concentrations should normalize through transcriptional repression of the FGF23 gene. The fact that ADHR patients cannot suppress FGF23 suggests that a stimulus outside the traditional calcium–phosphate endocrine axis, namely, iron deficiency, can stimulate Fgf23 production strongly despite frank hypophosphatemia, as we observed in the ADHR mice. These findings could lead to novel diagnostic monitoring and therapeutic approaches for ADHR and for common disorders of impaired phosphate metabolism, such as chronic kidney disease-mineral bone disorder.