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# Erythroferron as a Regulator of Erythropeisis and its Role in Anemia of Chronic Kidney Disease Children Patients

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**Abstract**

Erythroferrone (ERFE) is a recently identified erythroid hormone that bridges erythropoiesis and iron metabolism by regulating hepcidin, the master iron-regulatory peptide. Since its discovery, ERFE has drawn attention for its potentiality as a biomarker of erythropoietic activity, particularly in conditions such as chronic kidney disease (CKD) and β-thalassemia.

ERFE, encoded by the FAM132B gene on chromosome (2q37.3) is a glycoprotein member of the C1q/TNF-related protein family. It comprises an N-terminal signal peptide, a collagen-like linker, and a C-terminal globular domain, with high structural conservation across species. ERFE is secreted by erythroblasts in response to erythropoietin stimulation, especially during anemia or hypoxia. Functionally, it suppresses hepatic hepcidin expression mainly through inhibition of the bone morphogenetic protein (BMP)–Smad signaling pathway, possibly by acting as a "BMP trap" that sequesters BMP2, BMP6, or their heterodimers.

Under physiological conditions, ERFE ensures iron mobilization to sustain erythropoiesis. In β-thalassemia and other states of ineffective erythropoiesis, excessive ERFE leads to chronic hepcidin suppression and systemic iron overload. In CKD, elevated ERFE reflects increased erythropoietic drive, yet its effect is counteracted by inflammation-induced hepcidin elevation, resulting in functional iron deficiency.

Serum ERFE levels are primarily measured using enzyme-linked immunosorbent assays (ELISA), while newer chemiluminescent and multiplex immunoassays enhance sensitivity and enable integration with other biomarkers. However, standardization of measurement protocols and establishment of reference ranges remain essential for clinical application.

**Keywords:** Erythroferrone, Hepcidin, Iron metabolism, Erythropoietin, Chronic kidney disease, Anemia, Biomarker

## Introduction

Chronic kidney disease (CKD) is defined as persistent structural or functional impairment of the kidneys lasting for at least three months. This impairment may present as a sustained reduction in the estimated glomerular filtration rate (GFR), persistent proteinuria, or both **(Vaidya & Aeddula, 2023)**.

In the pediatric population, congenital anomalies of the kidney and urinary tract (CAKUT) represent the predominant cause of CKD, accounting for nearly half of all cases. Other major etiologies include hereditary renal disorders and glomerulonephritis **(Chevalier, 2023)**.

The distribution of CKD causes varies across age groups and ethnic backgrounds; CAKUT is more frequent in children below 12 years, whereas focal segmental glomerulosclerosis is commonly observed among

black adolescents. Additionally, infants born with low birth weight or those who are small for gestational age carry an increased risk of progressing to end-stage renal disease (ESRD) later in life **(Yu et al., 2021)**.

Anemia is one of the most frequent complication of CKD, particularly during advanced stages of the disease. Its pathogenesis is multifactorial but primarily involves reduced erythropoietin synthesis and disturbances in iron metabolism due to chronic inflammation **(Shaikh, Hashmi, & Aeddula, 2024)**.

Adequate iron stores are vital for optimizing the response to erythropoiesis-stimulating agents (ESAs). Iron deficiency is a key contributor to ESA hyporesponsiveness. Recent research has emphasized the role of growth/differentiation factor 15 (GDF-15) in the regulation of anemia, particularly in kidney transplant recipients, through modulation of hepcidin expression **(Farag, Mousa, Elsayed, & Ismeil, 2023)**.

Several investigations have examined the association between ESA therapy and biomarkers of iron metabolism, particularly hepcidin, in patients undergoing hemodialysis. ESA administration has been shown to reduce circulating levels of hepcidin and ferritin, promoting more efficient erythropoiesis. Prolonged use of ESAs enhances red blood cell production, facilitates iron mobilization, and improves anemia management, ultimately contributing to a better quality of life in patients with CKD-associated anemia **(Gutiérrez et al., 2022)**.

Erythroferrone (ERFE), encoded by the FAM132B gene, plays a pivotal role in maintaining systemic iron homeostasis. It is secreted by erythroblasts in response to both endogenous and exogenous erythropoietin (EPO) stimulation. By suppressing hepcidin synthesis, ERFE promotes intestinal iron absorption and mobilization from stores, particularly under conditions of increased erythropoietic demand such as anemia or stress erythropoiesis **(Srole & Ganz, 2021)**.

This study aims to evaluate the alterations in serum ERFE levels among pediatric patients with CKD in relation to the presence, degree, and severity of anemia, as well as their iron profile.

**Erythroferrone Structure and Properties**

ERFE is a protein synthesized by erythroblasts in the bone marrow and encoded by the ERFE gene located on chromosome 2q37.3. It belongs to the C1q/tumor necrosis factor-related protein (CTRP) family and exhibits a characteristic four-domain architecture, consisting of a distinct N-terminal region and a conserved C-terminal domain. The mature human ERFE protein comprises 354 amino acids, with an estimated molecular weight ranging from 35 to 40 kDa. This molecular weight variation is mainly attributed to glycosylation of a specific asparagine residue and the cleavage of signal peptides during protein translation **(Srole, Jung, Waring, Nemeth, & Ganz, 2023)**.

The globular C-terminal region of ERFE is predicted to form a well-defined TNF/C1q-like head domain, whereas the N-terminal segment is thought to adopt an extended and flexible secondary structure. These two major domains are connected by a short, proline-rich collagen-like linker that likely contributes to the protein's ability to multimerize. Additionally, ERFE may occur in several cleaved isoforms, as it contains two potential recognition sites for the proprotein convertase PCSK3 enzyme **(Srole & Ganz, 2021)**.

ERFE is encoded in the genomes of all vertebrate species, underscoring its evolutionary importance in iron regulation. Comparative analyses of amino acid sequences reveal strong conservation of ERFE across species, extending even to Xenopus tropicalis. Each ERFE ortholog possesses an N-terminal signal peptide responsible for protein secretion, followed by a highly conserved segment of approximately 100 amino acids exhibiting similar charge and polarity characteristics. This conserved region contains hydrophobic motifs, positively charged residues, and a glycine/proline-rich collagen-like linker that likely contributes to the protein's structural stability and functional activity. Although the C-terminal tumor necrosis factor (TNF)-like domain represents the most conserved region of the molecule, it appears not to be the main determinant of ERFE's inhibitory action on hepcidin expression **(Melchert et al., 2020)**.

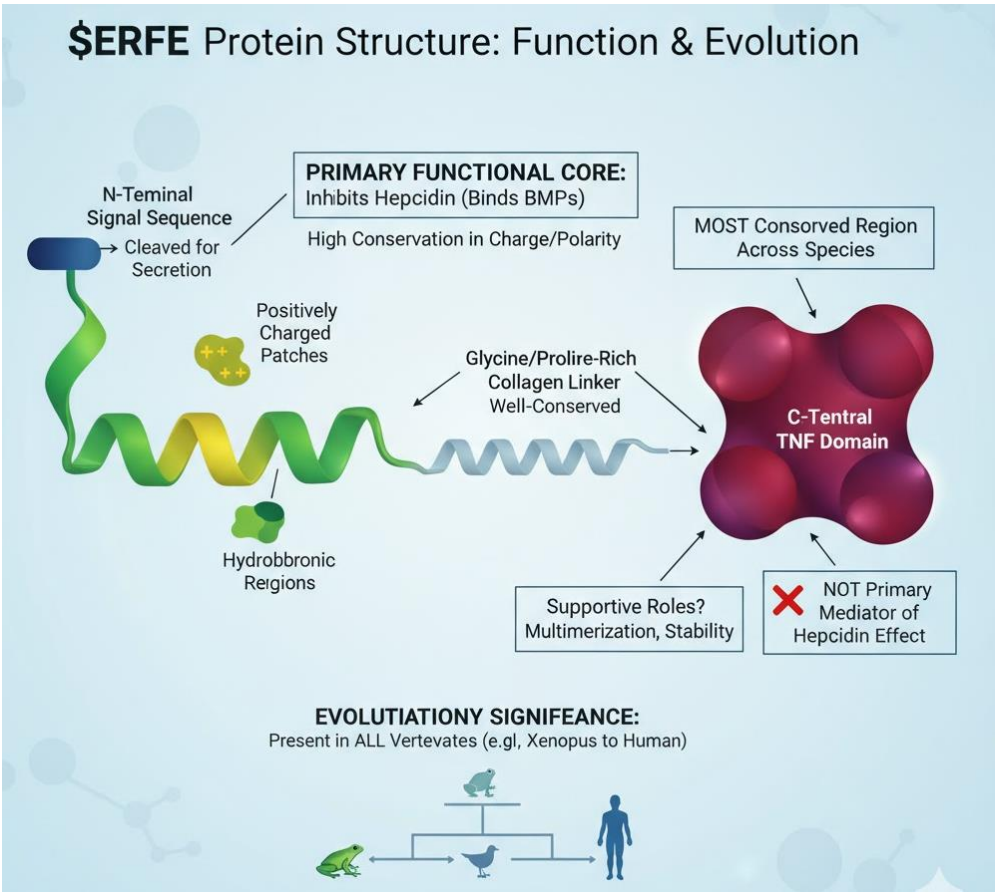


Figure (1): Structure of ERFE (**Srole et al., 2023)** .

**Mechanism of Action of ERFE**

ERFE plays a central role in maintaining systemic iron balance by downregulating hepcidin expression through several interconnected mechanisms. Under conditions of elevated plasma and hepatic iron levels, hepcidin synthesis is stimulated via activation of the Smad1/5/8 signaling cascade, triggered by bone morphogenetic protein (BMP) signaling—primarily through the interaction of BMP2/6 heterodimers with their receptors. ERFE antagonizes this pathway by effectively suppressing BMP–Smad signaling, a phenomenon confirmed in both in vivo experimental models and in vitro studies using Hep3B hepatocyte cells **(Xiao, Alfaro-Magallanes, & Babitt, 2020)** .

Several mechanisms have been proposed to explain ERFE's inhibitory effect on hepcidin synthesis. One possibility is that ERFE exerts its action through specific receptor-mediated signaling and interacts with matriptase-2 (TMPRSS6), a serine protease known to suppress hepcidin transcription by cleaving membrane-bound hemojuvelin, a key co-receptor that amplifies BMP signaling. In addition, ERFE functions as a "BMP trap," binding and sequestering bone morphogenetic protein (BMP) ligands—particularly BMP2, BMP6, and the BMP2/6 heterodimer—thereby preventing their association with BMP receptors such as ALK3 and attenuating hepcidin expression. Similar BMP-binding properties have also been observed among other members of the C1q/TNF-related protein (CTRP) family, suggesting that this mechanism may represent a conserved physiological feature within the group **(Ganz et al., 2023)**.

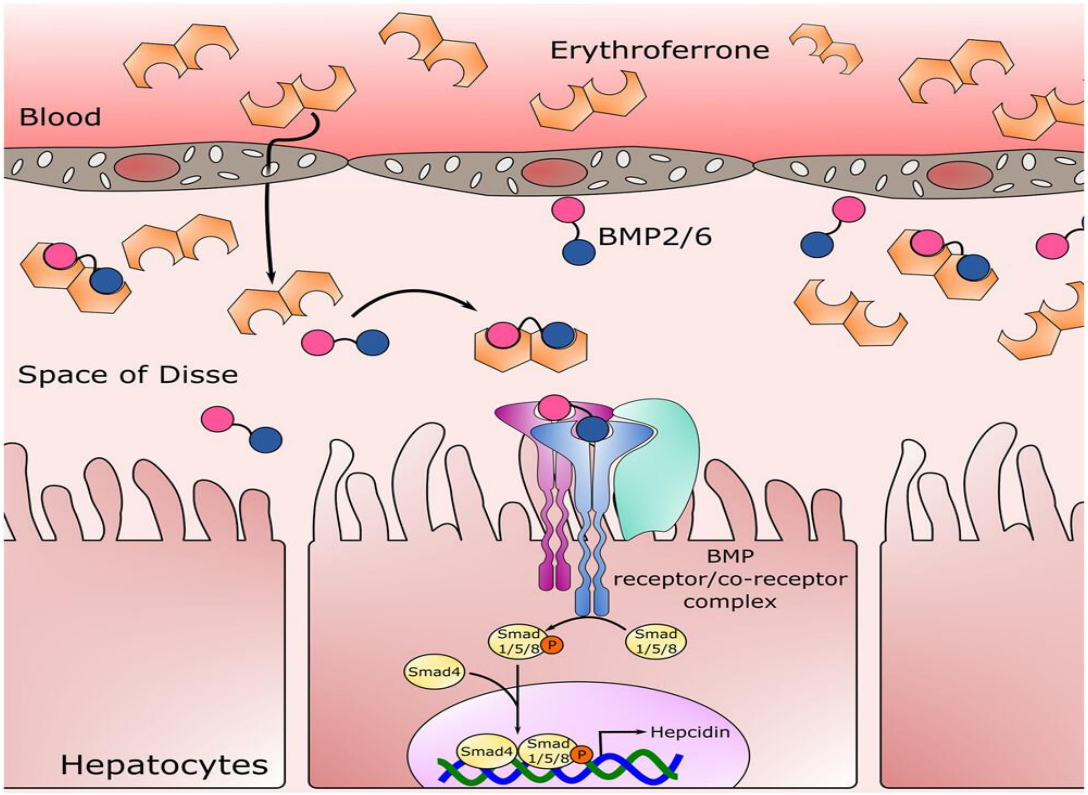


Figure (2): Mechanism of action of ERFE **(Coffey & Ganz, 2018)**.

**ERFE Pathophysiology**

**ERFE in Regular and Stress Erythropoiesis**

Under normal physiological conditions, the bone marrow continuously generates new red blood cells to replace senescent or damaged ones. Most of the iron required for this process is recycled by macrophages in the spleen and liver. When anemia or hypoxia occurs, tissue oxygen delivery decreases, prompting the kidneys to increase erythropoietin (EPO) production. Elevated plasma EPO levels promote the survival and proliferation of erythroid precursors and stimulate erythroblasts to secrete ERFE. ERFE, in turn, suppresses hepatic hepcidin synthesis, thereby enhancing iron availability. Reduced hepcidin levels facilitate iron export from storage sites, increase dietary iron absorption, and promote mobilization of iron from macrophages and hepatocytes to support heme and hemoglobin synthesis in newly formed erythrocytes **(Vogt et al., 2021)**.

In conditions of cellular hypoxia, interstitial fibroblasts in the kidney and hepatocytes respond by upregulating erythropoietin (EPO) gene expression through activation of hypoxia-inducible factor 2 (HIF-2). The increased EPO production stimulates proliferation and differentiation of erythroblasts in the bone marrow, leading to enhanced synthesis of ERFE. ERFE then acts to suppress hepatic hepcidin transcription, thereby facilitating iron mobilization and maintaining adequate iron availability for erythropoiesis **(Watts et al., 2020)**.

During anemia, ERFE production rises through two primary mechanisms: expansion of the erythroid precursor population and increased ERFE synthesis by individual cells. In conditions characterized by ineffective erythropoiesis, the erythroid compartment is markedly enlarged and continuously stimulated by erythropoietin; however, most precursor cells fail to mature into functional red blood cells. These immature erythroid cells secrete excessive amounts of ERFE, persistently suppressing hepcidin expression and leading to systemic iron overload. Elevated levels of non–transferrin-bound iron promote the generation of reactive oxygen species, resulting in oxidative tissue injury. Iron toxicity primarily affects the liver, myocardium, and endocrine organs and heightens susceptibility to infections. To mitigate these complications, iron chelation therapy is employed to remove excess iron via urinary and fecal excretion **(Olivera, Zhang, Nemeth, & Ganz, 2023)**.

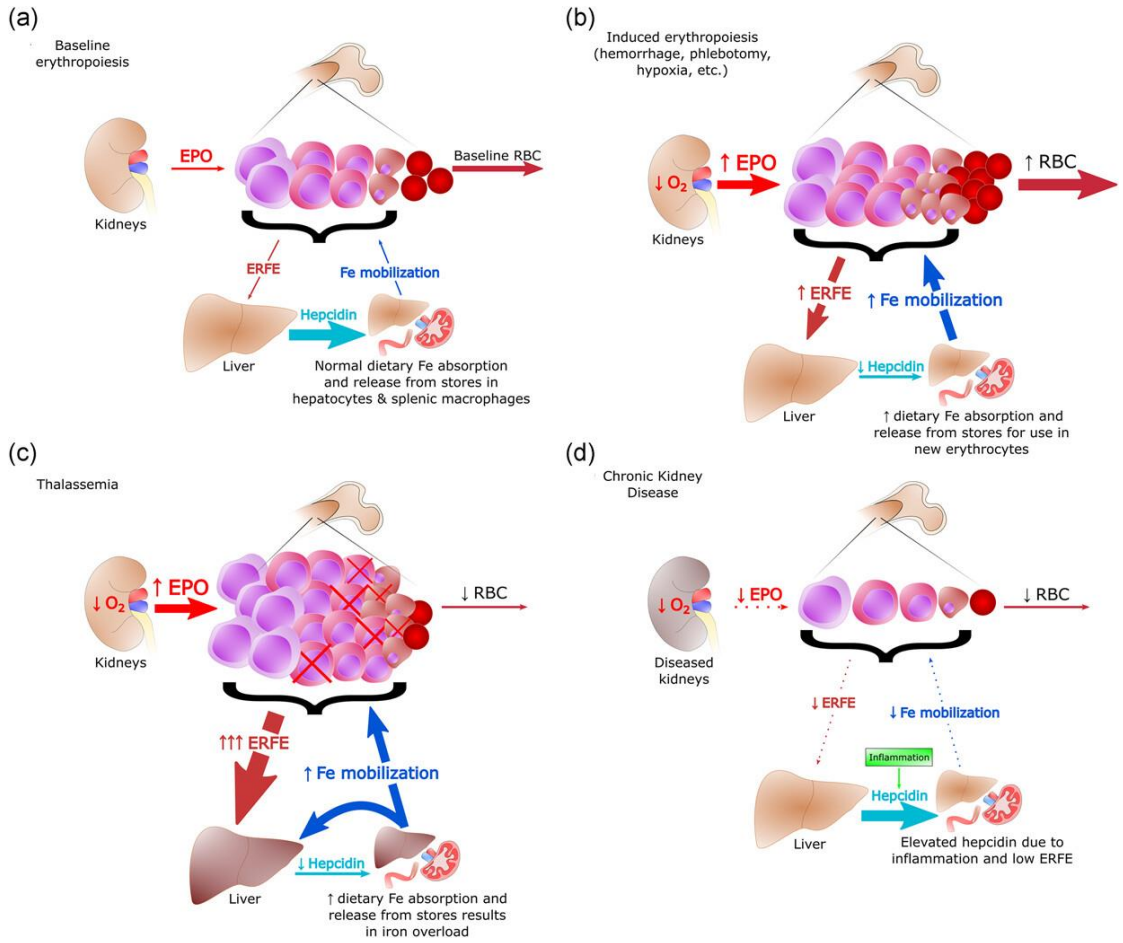


Figure (3): Effective and Ineffective Red Blood Cell Production **(Lanser, Fuchs, Kurz, & Weiss, 2021)**.

**Serum levels of ERFE**

ERFE concentrations differ markedly between healthy and diseased children, reflecting its key role in the regulation of iron metabolism and erythropoiesis. In healthy individuals, circulating ERFE levels are typically low—ranging from approximately 0.5 to 2 ng/mL—consistent with balanced erythropoietic activity and stable iron homeostasis. In contrast, conditions that stimulate erythropoiesis, such as anemia or acute blood loss, are associated with elevated ERFE levels, often reaching 10–20 ng/mL, which act to suppress hepcidin and increase iron availability for hemoglobin synthesis. Markedly higher ERFE concentrations, frequently exceeding 50 ng/mL, are seen in disorders characterized by ineffective erythropoiesis, such as β-thalassemia. In these cases, sustained ERFE overproduction leads to persistent hepcidin suppression and systemic iron overload. Therefore, assessing ERFE levels in both physiological and pathological states provides valuable information about iron regulation and the erythropoietic response to various hematologic conditions **(Dulkadir, Turna Saltoğlu, & Güneş, 2024)**.

**ERFE in children with anemia of CKD**

In pediatric patients with CKD–related anemia, circulating ERFE levels are generally elevated compared with those of healthy children, reflecting an increased erythropoietic drive in response to inadequate erythropoietin production and chronic inflammation. Reported ERFE concentrations in this group typically range from 5 to 15 ng/mL, higher than the 0.5–2 ng/mL observed in healthy counterparts. However, despite this compensatory rise, hepcidin levels often remain elevated in CKD due to the persistent inflammatory environment, which diminishes ERFE's inhibitory effect on hepcidin synthesis. This imbalance contributes to functional iron deficiency and ongoing anemia. A clearer understanding of ERFE regulation in CKD-associated anemia may help elucidate the intricate relationship between inflammation, iron homeostasis, and

erythropoiesis, thereby supporting the development of targeted therapeutic strategies to optimize iron utilization and improve anemia management **(Shaikh et al., 2024)**.

**Methods of measurement of ERFE**

Serum ERFE concentrations are typically assessed using highly sensitive and specific immunoassay techniques, with enzyme-linked immunosorbent assay (ELISA) being the most widely applied method. ELISA employs antibodies that selectively bind to ERFE, allowing quantitative detection through colorimetric or fluorescence signals directly proportional to protein concentration in serum samples. Recent technological advancements have introduced chemiluminescent immunoassays, which provide superior sensitivity and an expanded dynamic range, making them particularly useful for detecting low basal ERFE levels in healthy individuals and monitoring elevated concentrations in pathological states. Although less commonly utilized, Western blot analysis serves as a qualitative approach to verify ERFE expression and to differentiate among its isoforms. Emerging multiplex assay platforms are being developed to simultaneously measure ERFE alongside other iron-regulatory biomarkers, aiming to enhance diagnostic accuracy in conditions such as anemia associated with chronic kidney disease and β-thalassemia. However, standardization of assay methodologies and establishment of reference ranges are essential to ensure reproducibility and comparability across clinical and research settings **(Arezes et al., 2020)**.

**Conclusion**

ERFE is a pivotal regulator linking erythropoietic demand and systemic iron availability. A deeper understanding of its structure, signaling mechanisms, and pathological variations may enhance diagnostic accuracy and guide personalized management of anemia in CKD and related disorders

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