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To the editor:

Decreased hepcidin expression in murine β-thalassemia is associated with suppression of Bmp/Smad signaling

β-thalassemia is a genetic disorder of hemoglobin production characterized by ineffective erythropoiesis and anemia.[1] Iron overload, a major source of morbidity, results from inappropriately low expression of the gene encoding hepcidin (*Hamp1*).[1] Hamp1 controls plasma iron concentration by facilitating the degradation of the iron efflux protein ferroportin.[2] In healthy individuals, hepcidin istranscriptionally responsive to iron via bone morphogenetic protein (Bmp)/Smad pathway-mediated phosphorylation of Smad 1,5,8.[3] Phosphorylated Smad 1,5,8 is an essential component of the transcriptional complex that induces *Hamp1* expression in response to iron.[4] We investigated the

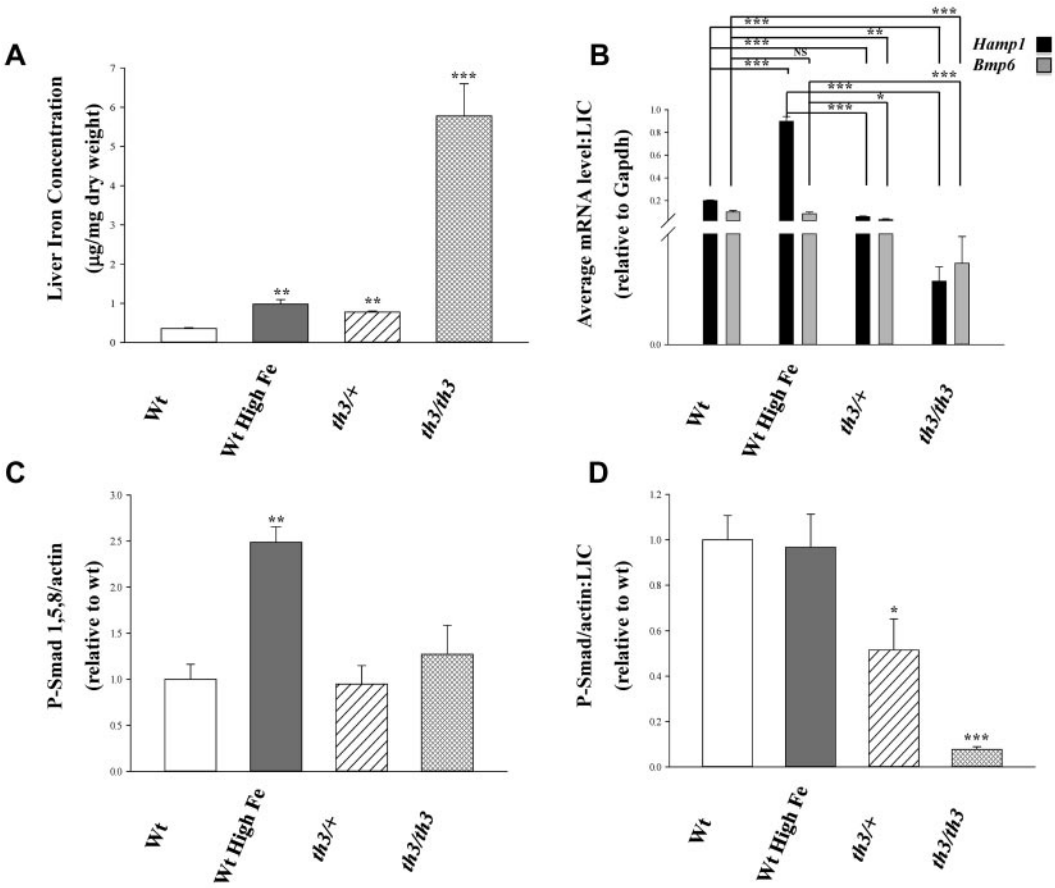

Figure 1. Bmp/Smad signaling is hyporesponsive to iron in β-thalassemic mice. (A) Liver iron concentrations (μg/mg dry weight) from wt mice fed a standard diet (200 ppm iron), wt mice fed a high iron diet (standard chow supplemented with 2.5% carbonyl iron), *th3*/+, and *th3/th3* mice were determined by atomic absorption and are expressed as mean ± SEM. (B) Average fold difference in the transcription levels of the Bmp/Smad pathway member *Bmp6* and target gene *Hamp1* were normalized to LIC. Data were normalized to mouse Gapdh and are presented as the mean ± SEM ($n \geq 3$). All averaged values are the product of duplicate determinations. (C) Chemiluminesence signals from Western blots of liver lysates reacted with antibodies to phosphorylated Smad 1,5,8 and to β-actin were quantified and data expressed as the mean ± SEM of their ratios. (D) Phosphorylated Smad 1,5,8 levels normalized to β-actin are expressed relative to LIC. Data are presented as mean ± SEM. Statistical significance was determined using the Student *t* test. $^*P \leq .05$, $^{**}P \leq .01$, $^{***}P \leq .001$. Significance with respect to wt or iron-loaded wt values are indicated by the brackets.

relationship between iron status, Bmp/Smad signaling, and *Hamp1* expression in mouse models of β-thalassemia intermedia (*th3/+*) and major (*th3/th3*).[5]

Wild-type (wt) C57BL/6 mice were placed on a high iron diet to match the elevated liver iron concentrations (LICs) observed in *th3/+* mice (Figure 1A). The severity of iron overload in *th3/th3* was even higher, by 5-fold, relative to iron-loaded wt mice (Figure 1A). Dietary iron loading of wt mice resulted in the expected increase in *Hamp1* and *Bmp6* expression (measured by quantitative RT-PCR). However, despite the similarly elevated LICs in *th3/+* mice, neither *Bmp6* nor *Hamp1* expression was significantly and proportionally increased. Moreover, the highly iron loaded *th3/th3* mice demonstrated a decrease in expression of both *Bmp6* and *Hamp1*. Thus, relative to hepatic iron load, expression of these genes is significantly decreased in both β-thalassemia models (Figure 1B). Furthermore, hepatic phosphorylated Smad 1,5,8 protein concentration is significantly increased only in liver samples from wt mice fed a high-iron diet relative to controls (Figure 1C). When normalized to LIC, our data reveal significantly decreased phosphorylated Smad 1,5,8 in *th3/+* and *th3/th3* samples compared with wt and wt fed a high-iron diet (Figure 1D). These results present strong evidence of a blunted relationship between LIC and *Bmp6* mRNA expression in β-thalassemic mice.

No prior studies have examined *Bmp6* expression or Smad signaling in models of β-thalassemia. Suppressed *Hamp1* transcription relative to the degree of iron overload in β-thalassemic mice has been postulated to occur as a consequence of expanded or ineffective erythropoiesis and effects of certain erythroid factors (eg, GDF15 or TWSG1) on hepatocellular hepcidin expression.[6-8] Our results suggest that the effects of ineffective erythropoiesis on *Hamp1* expression include a suppression of *Bmp6* mRNA expression relative to LIC, with consequent decreased Smad signaling. In contrast, studies in mice examining the acute effects of erythropoietin administration on *Bmp6* and *Hamp1* demonstrated decreased *Hamp1* expression but no effect on *Bmp6* expression.[9] This observation supports the proposal that different erythroid signals regulate hepcidin in normal and ineffective erythropoiesis.[8] Whether or not as a consequence of an erythroid factor, our findings demonstrate that in β-thalassemic mice the normal relationship between iron status and liver *Bmp6* mRNA expression is blunted. This dysregulation results in low *Hamp1* levels relative to iron stores and excess iron absorption relative to tissue demands. Continued exploration of the mechanisms underlying blunted Bmp/Smad signaling in β-thalassemia will enhance understanding of the iron overload that is central to the pathophysiology of this disease.

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