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Hfe acts in hepatocytes to prevent hemochromatosis

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Hereditary hemochromatosis (HH) is a common iron overload disorder that is most frequently caused by mutations in the *HFE* gene. HFE deficiency causes increased duodenal iron absorption, resulting in tissue iron overload that is a hallmark of HH. The identification of a direct interaction between HFE and the transferrin receptor 1 molecule in duodenal cells led to the hypothesis that the lack of functional HFE in the duodenum affects TfR1-mediated serosal uptake of iron and thereby causes misprogramming of the iron absorptive cells. Additional data show that HFE deficiency causes an inappropriately low expression of the hepatic iron regulatory hormone hepcidin, which in turn triggers increased duodenal iron absorption. This observation thus raised the alternate hypothesis that HFE functions in the liver, and exerts its effect secondarily on duodenal iron uptake.

To resolve this controversy, and directly investigate the role of HFE in duodenal enterocytes, *Hfe* expression was specifically ablated in mouse enterocytes using Cre/LoxP technology. Mice with an efficient, tissue-specific deletion of *Hfe* in crypt- and villi-enterocytes maintain physiological iron homeostasis with normal plasma transferrin saturation, hepatic iron levels and hepcidin mRNA expression. Furthermore, the expression of genes encoding the major intestinal iron transporters is unchanged in duodenal Hfe-deficient mice. These data demonstrate that intestinally expressed HFE is dispensable for the physiological control of systemic iron homeostasis, and exclude a role for duodenal HFE in the pathogenesis of HH.

This finding and the observation that the lack of HFE triggers inapproporiately low expression of the hepatic iron-hormone Hamp (LEAP1, Hepcidin) refocused our attention to the liver. Hamp coordinates body iron absorption and distribution by inhibiting the iron exporter ferroportin. However, it is unclear how HFE affects Hamp expression in the liver and whether HFE does so via its expression in hepatocytes, Kupffer cells and/or other cell types. To answer this question, mice lacking *Hfe* specifically in hepatocytes (Hfe^{AlbCre}) or

macrophages (*Hfe*^{LysMCre}) were generated. Southern blot analysis of genomic DNA from different tissues of *Hfe*^{AlbCre} mice shows selective recombination in the liver and highly efficient recombination in isolated hepatocytes. *Hfe*^{LysMCre} mice display *Hfe* recombination in bone-marrow derived and peritoneal macrophages. The hepatic iron content of *Hfe*^{AlbCre}(Cre+) and *Hfe*^{LysMCre}(Cre+) mice (8-10 weeks of age) was compared with Cre- littermates, mice derived from the same original line with constitutive *Hfe* deficiency (*Hfe*$^{-/-}$) and age- and sex-matched wild-type controls. As previously reported, both *Hfe*$^{-/-}$ males and females deposit significantly more hepatic iron than C57BL/6 control mice. Importantly, hepatic iron accumulation in *Hfe*^{AlbCre}(Cre+) mice of both sexes recapitulates the iron overload of *Hfe*$^{-/-}$ mice with iron predominantly accumulating in parenchymal cells of the liver. Consistent with earlier observations, *Hfe*$^{-/-}$ mice show significantly reduced splenic iron content which is likewise reflected in *Hfe*^{AlbCre}(Cre+) mice. Furthermore, the plasma iron levels and transferrin saturation are comparably elevated in *Hfe*$^{-/-}$ and *Hfe*^{AlbCre}(Cre+) mice, while *Hfe*^{LysMCre}(Cre+) mice are normal for all of the measured iron parameters. Finally hepcidin expression in *Hfe*^{AlbCre}(Cre+) but not in *Hfe*^{LysMCre}(Cre+) mice mirrors the decrease observed in *Hfe*$^{-/-}$ mice. Thus, *Hfe*-deficiency in hepatocytes (but not in macrophages) fully recapitulates the murine HH phenotype.