Hereditary Hemochromatosis is an autosomal recessive disorder characterized by excessive intestinal iron absorption and pathological iron deposition in organs such as liver, heart and pancreas. The disease is predominantly caused by homozygosity for the p.C282Y mutation in HFE, which impairs protein association with its chaperone beta-2 microglobulin (B2M) for correct folding and traffic to the cell surface.

The role of full length HFE on iron homeostasis remains unclear. It has been postulated that it may contribute to iron metabolism regulation by acting in hepatocytes, activating hepisin synthesis and in duodenum, regulating the expression of iron metabolism related genes, thus preventing iron overload.

HFE transcripts are widely expressed and the predominant (full length) mRNA identified has +4.2 kb in length. In addition, several alternative splicing HFE transcripts have been reported but their functional significance remains elusive.1,2,3

Since we have identified a novel alternative HFE transcript due to the intron 4 inclusion4 (Fig. 1), we aimed to investigate its tissue-specific expression level, the corresponding protein structure and cellular location, as well as its putative physiological function at duodenal level.

**Results**

1. *HFE* intron 4 inclusion transcript expression in different human tissues

- RT-qPCR
  - The *HFE* intron 4 inclusion transcript has an ubiquitous expression, being its relative expression higher in duodenum and testis and lower in liver.

2. *HFE* ivs4 protein variant cellular co-localization in hepatic cells

- **Immunofluorescence (HepG2)**
  - The *HFE* full length protein co-localizes with B2M and TFR1, mostly at cell surface but not with calnexin (ER marker).
  - The *HFE* ivs4 protein does not localize with TFR1 or with calnexin.

**Conclusions**

Here we show a soluble isoform of the HFE protein (sHFE), resulting from a HFE alternative splicing transcript including the intron 4. Due to the presence of a premature termination codon within this intron, it originates a truncated protein which lacks the transmembrane and cytoplasmic domains. However, it seems to be correctly processed, since it surpasses the ER barrier and is secreted to the cell supernatant in association with B2M.

- The over expression of the sHFE in a duodenal cell line is able to modify de expression of some iron metabolism related genes. We found that it acts by repressing the expression of the duodenal cytochrome b and hephaestin, independently of the iron status, as it happens with the full-length HFE. Since those are proteins presenting well known functions in iron absorption, we can hypothesize that their decrease expression modulated by the sHFE will be translated to a reduction of dietary iron absorption in the duodenum.

- Through this study we might have unveiled the contribution of the sHFE splice variant to iron homeostasis. In fact, sHFE may be secreted by several tissues into the bloodstream and may act in the regulation of dietary iron absorption in duodenum, reducing dietary iron absorption, preventing iron overload and contributing to iron metabolism regulation.

**References**