Rare autosomal dominant hereditary hemochromatosis associated with *SLC40A1* gene: ferroportin disease or type 4 hereditary hemochromatosis?

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Introduction

Ferroportin (**FPN1**) is the only **iron exporter** so far identified in mammals, encoded by the *SLC40A1* gene (2q32.2). The main function of FPN1 is to transfer dietary iron from the enterocytes of the duodenum and from cells of iron storage, known as macrophages, into the bloodstream. FPN1 function is **negatively** regulated by the hormone **hepcidin**. Hepcidin binds to FPN1 and triggers its internalization and degradation, limiting iron export (Fig. 1).

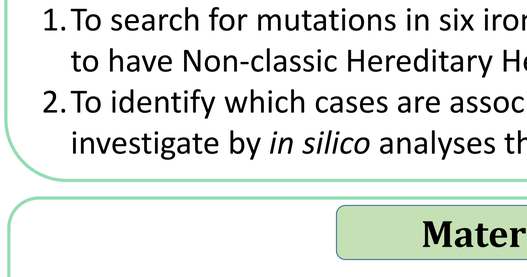

Fig. 1: Effect of hepcidin on iron transport in the duodenum and in macrophages
A) After dietary iron is taken up by duodenal enterocytes, **ferroportin** mediates iron export across the basolateral membrane into the plasma.
B) After red blood cells are phagocytosed by macrophages, hemoglobin-derived iron is exported by ferroportin into the plasma.
In both of these cell types, hepcidin limits iron export by triggering the internalization and degradation of ferroportin in lysosomes. Adapted from [1].

Mutations in *SLC40A1* gene may affect differently the FPN1 structure and function, originating **two phenotypically distinct rare hereditary autosomal dominant diseases of iron overload** [1,2].

Gain-of-function of FPN1	Loss-of-function of FPN1
Hyperferritinemia	
High serum iron and **transferrin saturation**	**Normal/low serum iron** and **transferrin saturation**
FNP1 has full iron export capacity and is insensitive to hepcidin down-regulation – high cellular iron export into the plasma	**FNP1 is unable to export iron from cells** leading to cellular (especially macrophage from spleen) iron accumulation
Iron accumulation preferentially in hepatocytes (liver iron overload)	Iron accumulation preferentially in splenic and hepatic macrophages (spleen and liver iron overload)
Hereditary Hemochromatosis type IV	**Ferroportin Disease**

Aims

1. To search for mutations in six iron metabolism related genes in patients suspected to have Non-classic Hereditary Hemochromatosis.
2. To identify which cases are associated with *SLC40A1* pathogenic mutations and to investigate by *in silico* analyses the underlying pathophysiologic mechanisms.

Material and Methods

Subjects analyzed: 335 individuals suspected of having **Non-Classical Hereditary Hemochromatosis** (HH).

Six genes related with iron metabolism (*HFE, HAMP, HJV, TFR2, SLC40A1* and *FTL*) were analysed by SSCP, dHPLC or NGS.

NGS used *TruSeq* or *Nextera XT* libraries and a *MiSeq* platform (*Illumina*)

Validation of genetic variants found was performed by Sanger sequencing.

Predictive consequences of variants at protein level were evaluated using *PolyPhen-2* and *SIFT softwares.*

Results and Discussion

Three *SLC40A1* rare pathogenic variants were detected in three different patients, presenting two distinct phenotypes:

1. Ferroportin Disease (FD)

One missense mutation, c.238G>A, located in exon 3 and **one deletion c.485_487delTTG in exon 5**, were found in **heterozygosity** in two women presenting with hyperferritinemia and **low** transferrin saturation.

p.Gly80Ser

- *PolyPhen-2* software predicts p.Gly80Ser is probably a damaging mutation (*score* = 1.000; Fig. 2);

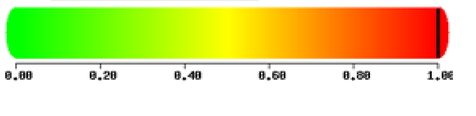

Fig. 2: Prediction of the pathogenic effect of p.Gly80Ser alteration on FPN1 protein by *PolyPhen-2* software.

- It is located at the cytosolic bottom of the second intermembrane segment of FPN1 [3];
- Reported *in vitro* studies have shown a reduced iron export capacity and less presence at cell surface [3,4,5].

p.Val162del

- *SIFT Indel* software predicts p.Val162del is damaging for the structure and function of FPN1 (*score* = 0.858);
- It is located in a cytosolic loop of FNP1 between transmembrane domains 4 and 5 [3];
- Reported *in vitro* studies have shown the deletion impairs the correct folding of the protein and reduces its iron export function [3,4,5].
- p.Val162del has been described in several families of different ethnic origin suggesting it is the most common mutation of FNP1 [6,7].

FPN1 Loss-of-function → Ferroportin disease

2. Hereditary Hemochromatosis type IV

The **other missense mutation, c.610G>A**, was also found in **heterozygosity** in a woman presenting hyperferritinemia **high** transferrin saturation and liver iron overload.

p.Gly204Ser

- Affects an extracellular segment between transmembrane domains 5 and 6 [3];
- *PolyPhen-2* classifies this mutation as probably damaging to protein structure and function (*score* = 1.000; Fig. 3);

Fig. 3: Prediction of the pathogenic effect of p.Gly204Ser alteration on FPN1 protein by *PolyPhen-2* software.

- p.Gly204Ser does not appear to cause FD. Reported functional studies indicate that this mutation does not affect the presence of the protein on the cell surface but causes hepcidin resistance [7].

FPN1 Gain-of-function → Hereditary Hemochromatosis type IV

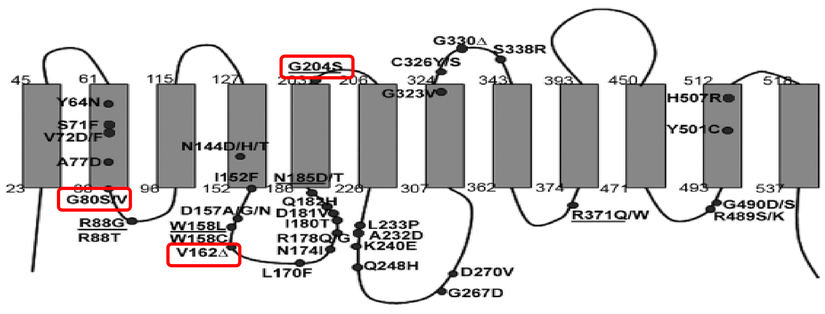

Fig. 4: Schematic representattion of mutations on FPN protein (model of Liu *et al*, 2005 [8]). The three mutations reported in this study are underlined.

Conclusions

- ✓ Although these three *SLC40A1* genetic variants are already reported in public databases (*Ensembl*, *ClinVar*), they were not known to be present in the Portuguese population.
- ✓ Detailed clinical evaluation of patients in addition to *in silico* analyses and *in vitro* functional studies are useful to reveal the pathogenic effect of mutations in FPN1 function, expression and mechanism of regulation by hepcidin.
- ✓ As these diseases have an autosomal-dominant inheritance with incomplete penetrance, family screening is mandatory since siblings and offspring have a 50% chance of carrying the pathogenic mutation.

References

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