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Loss-of-function ferroportin disease: novel mechanistic insights and unanswered questions

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Ferroportin, a 571 amino acid cation transporter encoded by the SLC40A1 gene, is the only known human cellular iron exporter and primarily expressed in the basolateral membrane of duodenal enterocytes, macrophages, and hepatocytes.¹² By regulating ferroportin-mediated iron export in these cells, the hepatocyte-derived peptide hormone hepcidin plays a central role in intestinal iron resorption, macrophage iron recycling and hepatic iron storage (Figure 1A).¹ Functional studies reveal the internalization of ferroportin upon hepcidin binding with subsequent ubiquitination of the protein resulting in the diminution of cellular iron export. The mechanism of the inhibition of cellular iron efflux is not fully elucidated.¹⁴ Genetic ferroportin variants result in autosomal dominant inherited hereditary hemochromatosis (HH) type 4 or ferroportin disease which is traditionally divided into two entities primarily based on the pattern of cellular iron distribution. Classical ferroportin disease (type 4A) is characterized by macrophage iron retention and decreased circulating iron availability for erythropoiesis, and is clinically recognized by the presence of elevated serum ferritin concentrations with low to normal transferrin saturation (TSAT) and poor tolerance to phlebotomy. Non-classical (atypical, type 4B or ferroportin-associated HH) ferroportin disease is characterized by parenchymal (hepatocellular) iron overload with both elevated ferritinemia, a normal TSAT and the heterozygous presence of iron homeostasis modulating co-morbiti- ties such as alcohol consumption and liver steatosis, but also by the limited applicability of in silico prediction models in the absence of a fully elucidated three-dimensional (3D) structure of ferroportin. In the most widely accepted secondary structure, ferroportin comprises 12 helices located in 12 transmembrane (TM) domains bound via six extracellular (ES) and five intracellular (IS) segments with a large intracellular loop between the 6th and 7th transmembrane helix and an intracellularly located N- and C-terminus.¹⁵ The available 3D models are based on a comparison with membrane transport proteins from a wide range of other species with only a 10 - 24% sequence homology and a maximal 40% similarity. Two studies, using E. coli glycerol-3-phosphate transporter GlpT and lactose permease LacY or the Bdellovibrio bacteriovorus Bd2019 iron transporter as template, respectively, reveal an open inward and an open outward structure with an intra- and extracellular gate between the 6th and 7th transmembrane domain.⁶ Site mutagenetic and conformational studies revealed the residues, located at IS1, IS2 and IS5, that are important in intracellular gate interaction and the residues, located at TM1, TM12 and ES1 and ES4, that are involved in extracellular gate interaction. These studies also identified ferroportin residues that are essential for binding and ubiquitination of hepcidin and may be involved in iron binding and egress. The unequivocally hepcidin-resistant gain-of-function ferroportin variants Cys526Ser, Tyr501Cys, Asp504Asn and Tyr644Asn and His507Arg are reported to have impaired hepcidin binding and hepcidin-dependent ubiquitination, respectively. In the open outward structure, variants causing impaired hepcidin binding were found to be localized within the extracellular gate, while variants causing impaired ubiquitination were found in the peripheral loop of the molecule, suggesting that these latter variants interfere with appropriate folding after hepcidin binding.⁶ These findings provide a molecular basis for the observed cellular distribution pattern of the type 4B iron overload in patients and the behavior in functional tests performed for these hepcidin-resistant gain-of-function variants (Figure 1B).

The study by Ka et al., published in this issue, provides interesting insights into the pathophysiologic mechanisms involved in ferroportin disease caused by loss-of-function variants. They describe 22 patients from six independent families with hyperferritinemia, a normal TSAT and the heterozygous presence of the Arg178Gln variant. The serum hepcidin levels determined in two patients were above the reference range. The hepatic iron content, estimated by MRI methodology in three of the patients, was mildly elevated and a liver biopsy, performed in one patient, revealed predominant iron deposition in Kupffer cells. Although Arg178Gln displayed a reduced export of ⁵⁷Fe out of transfected HEK293T cells, the variant was properly localized on the cellular membrane with disappearance after...
hepcidin exposure. Based on both the clinical phenotype and functional tests the variant was assigned as a hepcidin sensitive loss-of-function variant. In the open outward conformation of the 3D structure, using *Bdellovibrio bacteriovorus* Bd2019 as the template, the demonstrated non-covalent interaction between Arg178 and Asp473 (located on the N- and C-lobe, respectively) was assumed to be involved in the stabilization of the open outward conformation needed to preserve iron egress. Indeed, the Asp473Ala ferroportin variant was also properly expressed on the membrane with a nearly total loss of iron export capacity. In most loss-of-function variants the abolished iron transport is attributed to defective expression of ferroportin on the membrane. Ka et al., however, provide evidence for interference in the stabilization of the conformation state, i.e., the open outward state, in the Arg178Gln variant as an alternative mechanism for diminished iron transport, despite the proper membrane expression of this variant. Further exploration of the amino-

![Figure 1. Model displaying cellular iron flows in patients with loss-of-function ferroportin variants as compared to physiological conditions and patients with gain-of-function ferroportin variants and IRIDA.](image-url)