This article can be cited before page numbers have been issued, to do this please use: L. Jin, D. M. Frazer, Y. Lu, S. J. Wilkins, S. Ayton, A. I. Bush and G. Anderson, Metallomics, 2019, DOI: 10.1039/C8MT00370J.

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Significance to metallomics statement

Manganese is an essential metal, but how its trafficking is regulated within the body remains incompletely understood. Ferroportin is best known as an iron export protein, although it can also transport a number of other divalent metal ions, including potentially manganese. However, the literature is divided in this area, and in particular, there are few data on how ferroportin might contribute to manganese homeostasis under physiological conditions. By taking advantage of two knockout mouse models with altered ferroportin levels, we have been able to provide new insights into the contribution this transporter makes to manganese distribution in vivo.
Mice overexpressing hepcidin suggest ferroportin does not play a major role in Mn homeostasis

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Manganese is an essential metal that is required for a wide range of biological functions. Ferroportin (FPN), the only known cellular exporter of iron, has also been proposed to play a role in manganese export, but this relationship is incompletely understood. To investigate this in more detail in vivo, we examined the relative distributions of manganese and iron in Tmprss6 deficient mice, which are characterized by constitutively high expression of the iron regulatory hormone hepcidin and, consequently, very low FPN levels in their tissues. Tmprss6\textsuperscript{−/−} mice showed frank iron deficiency and reduced iron levels in most tissues, consistent with FPN playing an important role in the distribution of this metal, but manganese levels were largely unaffected. Associated studies using intestine-specific FPN knockout mice showed that loss of FPN significantly reduced the dietary absorption of iron, but had no effect on manganese intake. Taken together, our data suggest that FPN does not play a major role in Mn transport in vivo. They do not exclude a minor role for FPN in manganese homeostasis, nor the possibility that the transporter may be relevant at high Mn levels, but at physiological levels of this metal, other transport proteins appear to be more important.

Introduction

Metals such as iron (Fe) and manganese (Mn) are essential for a wide range of biological functions. Iron, for example, is required for oxygen delivery, energy production and DNA synthesis,\textsuperscript{1} and manganese is required for the activity of many enzymes, including those involved in glucose and lipid metabolism and antioxidant defence.\textsuperscript{2} Both Fe and Mn levels in the body must be strictly controlled as a deficiency or excess of either metal can be harmful. Iron deficiency can lead to anemia and its associated pathological consequences, including compromised neurological development when present in infancy.\textsuperscript{3, 4} In contrast, since Fe can catalyze the formation of toxic oxygen radicals, excess iron can damage vital organs including the liver and heart. Mn is also toxic when present in excess, and its neurotoxicity is particularly notable.\textsuperscript{2} Significantly, high Mn levels have been associated with neurological disorders such as hepatic encephalopathy and parkinsonism.\textsuperscript{5, 6} Because of this dual nature, being essential, yet being toxic in excess, sophisticated mechanisms have developed to maintain the cellular and body levels of Fe and Mn within the optimum physiological range. This is largely achieved by regulating the cellular intake and efflux of the metals. For both Fe and Mn, the liver plays a central regulatory role.\textsuperscript{8, 9}

In broad terms, the regulation of Fe traffic in the body is better studied than that of Mn. Dietary Fe is taken up by intestinal enterocytes, then released into the circulation across the enterocyte basolateral membrane through ferroportin (FPN; SLC40A1), the only known mammalian iron export protein.\textsuperscript{10} In the circulation, iron binds to transferrin and is then distributed to cells throughout the body. In addition to being important for intestinal Fe absorption, FPN is required for Fe export from most body cells. This iron export process is regulated by the hepatic hormone hepcidin, which binds to FPN and leads to its internalization and degradation.\textsuperscript{11} When body iron levels rise, hepcidin production is increased and FPN levels decline.\textsuperscript{12} This in turn reduces iron absorption and iron accumulates in intestinal enterocytes. Iron also accumulates in other cells in the body. Conversely, when body iron levels are depleted, hepcidin expression is downregulated to allow for increased dietary Fe absorption and mobilization of body Fe stores, both of which require active FPN on the cell surface.\textsuperscript{13, 14} Pathological disturbances leading to either increased or decreased hepcidin expression lead to anemia and hemochromatosis respectively.\textsuperscript{15-17}

FPN has been shown to transport several divalent metal ions other than Fe\textsuperscript{2+}, including Cu\textsuperscript{2+} and Zn\textsuperscript{2+}, but not others such as Co\textsuperscript{2+} or Cd\textsuperscript{2+}.\textsuperscript{18} The literature on whether FPN is able to transport Mn is confusing and apparently contradictory. Some \textit{in vitro} studies have suggested that FPN transports minimal amounts of Mn,\textsuperscript{18, 19} but others have suggested otherwise.\textsuperscript{20, 21} \textit{In vivo} studies have generally suggested that FPN is able to...
transport Mn, but its influence is usually modest. For example, it has been reported that the flatiron mouse (ffe/+), a model of genetic FPN deficiency, albeit with an unusual underlying mechanism, has a reduced capacity to absorb orally administered Mn and has reduced Mn levels in the blood, liver and bile.\textsuperscript{22} In addition, there is evidence that intestinal absorption of Mn is increased in Hfe knockout mice, a mouse model of HFE-related hemochromatosis\textsuperscript{23} where FPN expression is increased secondary to diminished hepcidin expression.\textsuperscript{24, 25} While these studies provide some evidence to implicate FPN in Mn homeostasis, its relative contribution at physiological levels of Mn remains unclear.

FPN is only one of more than ten transmembrane metal transport proteins that have been implicated in Mn homeostasis.\textsuperscript{26} These include proteins involved in Mn import, such as DMT1 (NRAMP2),\textsuperscript{27, 28} SLC39A8 (ZIP8)\textsuperscript{29} and SLC39A14 (ZIP14).\textsuperscript{30-33} and those involved in Mn export, such as SLC30A10 and SLC39A14 play particularly important roles in coordinately regulating body Mn levels. Patients with loss of function mutations in SLC30A10 and SLC39A14,\textsuperscript{37-40} or mice lacking this protein,\textsuperscript{43, 44} show accumulation of Mn in the blood, liver and brain, and associated parkinsonian-like symptoms. The localization of SLC30A10 to the canalicular membrane of HepG2 cells\textsuperscript{34} is consistent with it being involved in Mn excretion into the bile. Mice with a liver-specific knockout of Slc30a10 show minimal alterations in body Mn,\textsuperscript{45} implying that other Mn export pathways exist. The gut is a strong candidate for an alternative excretion route as SLC30A10 is also expressed on the apical membrane of mature enterocytes and appears to be responsible for effluxing Mn into the intestinal lumen.\textsuperscript{46} SLC30A10 does not appear to be involved in regulating brain Mn levels under basal conditions, but it can protect against neurotoxicity when levels of the metal are increased.\textsuperscript{45} Disruption of the metal importer SLC39A14 in both humans and mice has also been associated with body Mn accumulation in multiple organs, including the brain, with associated adverse neurological consequences.\textsuperscript{30-33} However, in this case, Mn levels in the liver are low. Radioactive Mn\textsuperscript{44} distribution studies comparing Slc39a14 null and wild-type mice indicate that this protein is important for hepatic and pancreatic Mn uptake, and for its gastrointestinal excretion.\textsuperscript{31} Taken together, these data suggest that SLC39A14 and SLC30A10 work together in hepatocytes to facilitate Mn excretion into the bile, and studies with Slc39a14/Slc30a10 double knockout mice are consistent with this.\textsuperscript{44} The loss of this major excretion pathway leads to the accumulation of Mn in the body and its associated adverse consequences. Interestingly, loss of SLC39A14 specifically in the liver in mice does not lead to substantive extrahepatic Mn loading,\textsuperscript{33, 44} implying that Mn can be excreted through other routes, most likely via the pancreas and intestinal epithelium.

In this study, we have taken advantage of transmembrane serine protease 6 (TMPRSS6) knockout mice to further explore the links between FPN and Mn metabolism and to compare this to the homeostasis of iron. TMPRSS6 is a protease which acts upstream of hepcidin to reduce its expression.\textsuperscript{46} Tmprss6\textsuperscript{–/–} mice lack the protease and consequently hepcidin is expressed at a constitutively high level.\textsuperscript{47} This in turn reduces FPN expression and leads to iron deficiency in the mice. These animals represent an ideal model in which to study the effects of increased hepcidin (and hence reduced FPN) on Mn metabolism. Using both these mice and mice carrying an intestine-specific deletion of FPN, we showed that FPN likely plays a minimal role in body Mn homeostasis. Metal transporters other than FPN appear to be more important in regulating Mn flux in the body under normal physiological conditions.

**Experimental**

**Animal care and procedures**

All experimental procedures were approved by the QIMR Berghofer Medical Research Institute Animal Ethics Committee. Homozygous Tmprss6 knockout mice (Tmprss6\textsuperscript{–/–})\textsuperscript{47} on a C57BL/6 background were used as a model of constitutively high hepcidin production. This strain has been validated previously in our hands to show that Hamp1 mRNA levels and plasma hepcidin levels are constitutively high and that FPN protein is reduced in the proximal small intestine.\textsuperscript{48} Heterozygous littermates (Tmprss6\textsuperscript{+/–}) were used as controls as we observed no phenotypic differences between wild-type mice and heterozygotes.\textsuperscript{48} In some studies, we also used mice lacking FPN specifically in the enterocytes which were created and characterized as previously described.\textsuperscript{48} Briefly, they were produced by crossing FPN floxed mice with vil-Cre-ERT2 mice (both on a C57BL/6 background), which express tamoxifen-inducible Cre recombinase under the control of the villin promoter. Cre recombinase expression was induced in these mice by injecting tamoxifen (75 μg/g body weight) subcutaneously at 8-10 days of age. Ferrophin floxed littermates lacking Cre recombinase also were injected with tamoxifen as the control group. Our previous work has shown that these animals have a 90% reduction in intestinal iron absorption and that this is associated with loss of FPN protein in the small intestine.\textsuperscript{46} For consistency in our experiments, only male mice were used. Some sex differences in Fe and Mn homeostasis have been observed in mice,\textsuperscript{31, 43, 49} but, where present, these are almost always quantitative in nature and do not represent fundamental differences between the responses of males and females. Thus, we anticipate that the findings will be equally applicable to females. The animals were weaned 3 weeks after birth, then provided with unlimited access to a standard pellet diet (Norco Stockfeed, Lismore, NSW, Australia; Metal ion content: Fe 120mg/kg; Mn 80mg/kg; Zn 200mg/kg; Cu 16mg/kg) and tap water.

In initial studies, Tmprss6\textsuperscript{–/–} mice and Tmprss6\textsuperscript{+/–} controls were examined at 2, 4, 8 and 12 weeks of age. Animals were anesthetized (200mg/kg ketamine and 10mg/kg xylazine) before euthanasia. Blood was taken by cardiac puncture and mice were perfused with trace-metal grade saline (Sigma). Organs, including the liver, brain, heart, kidney and spleen...
were collected, snap frozen in liquid nitrogen and stored at -80°C for subsequent investigation. Intestine-specific Fpn knockout mice were processed similarly at the age of 8-10 weeks. Perfusion with trace-metal grade saline was performed. In this case the liver was the only tissue collected.

In some experiments Mn was administered by oral gavage (100mg Mn/kg body weight) for 5 consecutive days. This represents approximately 5 times the daily intake of Mn each mouse would receive from a standard pellet diet. Both Tmprss6+/− and Tmprss6−/− mice were studied (n=6). Five days after the final gavage, mice were euthanised and blood and other tissues collected as described above.

Real time quantitative PCR
Trizol reagent (Thermo Fisher Scientific, Scoresby, Victoria, Australia) was used to extract total RNA according to the manufacturer’s instructions. Complementary DNA was synthesized using M-MLV Reverse Transcriptase (Thermo Fisher Scientific) and an oligo (dt) primer according the manufacturer’s instructions. Real-time quantitative PCR was performed on a CFX384 Touch Real-Time PCR Detection System (Bio-Rad, Gladesville, NSW, Australia). Each sample was analyzed in triplicate, and gene expression was calculated from the Ct value using the standard curve method. Gene expression levels were expressed relative to the housekeeping gene, hypoxanthine guanine phosphoribosyl transferase (Hprt). Validation of primers and analyses were consistent with the MIQE guidelines.50 The primer pairs used were: Hamp1 forward: AGAGCTGCAAGCCTTGCCAC; Hamp1 reverse: ACACGGGAAAGTTGTAAGCATTT; Hprt forward: GGACTGATTATGGACAGGA; Hprt reverse: GAGGGGCAATATGTGATG.

ICP-MS
Tissue for ICP-MS was dried at 110°C, weighed, and wet-digested42 prior to metal analysis. Briefly, 50 µL of HNO3 (14.4M, 65% Suprapur, Merck) was added to the dried sample and digestion was allowed to proceed overnight at room temperature. The temperature was then raised to 90°C using a heating block and incubation continued 20 mins. After the addition of an equivalent volume of hydrogen peroxide (9.9 M, 30% Aristar, BDH), the samples were incubated for 30 mins at room temperature, followed by a further 15 mins at 70°C. The volume of each sample was then determined and 1% HNO3 (0.22M) added to a total volume of 500 µL. Trace element levels were measured using an Agilent 7700 series ICP-MS instrument under routine multi-element operating conditions using a Helium Reaction Gas Cell. Metal concentrations were expressed as µg/g dry tissue for tissue samples or µM for serum samples.

Statistical analysis
Each experimental group contained 5-10 animals, and data are presented as the mean ± SEM. Statistical differences between treatment groups were determined using t tests when comparing two groups or one-way analysis of variance (ANOVA) followed by Sidak’s multiple comparisons test when three or more groups were involved. Differences were considered significant when p < 0.05. All analyses were carried out with GraphPad Prism 7 software (GraphPad Software, San Diego, CA, USA).

Results and discussion
Tmprss6 deficiency affects iron and manganese metabolism differently
In this study, we systemically compared iron and manganese homeostasis in Tmprss6 deficient mice. We first confirmed that these animals had constitutively high hepcidin expression by examining Hamp1 mRNA levels in mice aged 2, 4, 8 and 12 weeks (Fig. 1) and we have previously shown that Hamp1 mRNA expression correlates with plasma hepcidin protein levels and intestinal FPN protein in these mice8. Hepatic Hamp1 expression increased in both Tmprss6−/− and littermate controls as the mice aged, but at all ages hepcidin expression was significantly elevated in Tmprss6 knockout mice compared with their heterozygous littermates. These results are consistent with published data on hepcidin expression in this mouse strain.47

Hamp1 mRNA levels were assessed in the liver of Tmprss6 knockout mice (KO)(open squares) and heterozygous littermates (Het)(solid circles) at 2, 4, 8 and 12 weeks of age. Gene expression was analysed using qPCR and the data were normalized to the Hamp1 mRNA level of 8 week-old heterozygous mice. Data are shown as means ± SEM, n=6 and differences between Tmprss6 knockout and heterozygous mice were determined by t test separately at each age. (**p < 0.01; ***p < 0.001; ****p < 0.0001)

Tmprss6 knockout mice also showed obvious anemia as indicated by reduced hemoglobin concentration, hematocrit and mean corpuscular volume (MCV) at all ages studied (Fig. 2A-C). Red blood cell (RBC) number was not influenced by the loss of Tmprss6 (Fig. 2D). This anemia is consistent with
elevated hepcidin expression and with previous descriptions of Tmprss6(-/-) mice.47,48

![Graphs of hematological and iron status](image)

Fig. 2 Hematology and iron status of Tmprss6(-/-) mice (open squares) and heterozygous littermate controls (solid circles) at various postnatal ages. (A) Hgb: hemoglobin concentration; (B) Hct: hematocrit; (C) MCV: mean corpuscular volume; (D) RBC: red blood cell count; (E) serum iron; and (F) hepatic iron. Data are shown as means ± SEM, n=6 and differences between Tmprss6 knockout and heterozygous mice were determined by t test separately at each age. (**p < 0.01; ****p < 0.0001).

Tmprss6(-/-) mice also had lower serum and hepatic iron levels at all ages (Fig. 2E,F) which is to be expected in the presence of elevated hepcidin as the peptide binds to FPN on the surface of target cells, thereby reducing the donation of iron to the plasma. This might be expected to lead to iron accumulation within the tissues, but since intestinal enterocytes are a major site of hepcidin action, dietary iron absorption is reduced when hepcidin levels are high, and hence the iron content of most tissues is reduced (Figs 2F and 3A-C). An exception to this pattern is seen in 2 week-old mice hence the iron content of most tissues is reduced (Figs 2F and 3A-C).

We also examined the effects of increased hepcidin expression on Fe levels in several tissues other than the liver. A marked decline in Fe levels was observed in the brain and heart of Tmprss6(-/-) mice (Fig. 3A,B), although the decrease was not as striking as it was in the liver (Fig. 3B). In the kidney, a significant drop in Fe level was only observed in 12 week-old Tmprss6 knockouts (Fig. 3C), and no significant differences in splenic iron were observed between the two genotypes at any of the ages studied (Fig. 3D). The latter results may reflect slower iron turnover in the kidney and spleen.

Some studies have suggested that FPN contributes to Mn homeostasis and may be involved in cellular Mn export, but others have suggested otherwise. Since Tmprss6(-/-) mice have greatly reduced tissue FPN levels as a result of hepcidin overexpression, we were able to use them as an in vivo model to investigate whether altered FPN expression affected Mn distribution. We thus measured Mn levels in various tissues of Tmprss6(-/-) mice. In most cases, no significant differences were observed in Mn concentrations between Tmprss6(-/-) mice and controls in the serum, brain, heart, kidney, spleen and liver (Fig. 4A-F). Small, but significant, differences were observed in the heart of 8 week old mice (Fig. 4C), the spleen of 12 week old mice (Fig. 4E) and the liver of 8 week old mice (Fig. 4F), but in each case Mn levels were increased. (In the same samples, Fe levels were decreased.) Whether these changes are sufficiently large to be physiologically relevant is difficult to determine.

Interestingly, Mn levels in the livers of 2 week old Tmprss6(-/-) mice were significantly decreased (Fig. 4F). Mn concentrations have previously been reported to be similar between fetal and suckling mice, suggesting that the amount of Mn in the suckling pup depends largely on how much of the metal was transferred across the placenta before birth. Placental transport of Mn to the fetus could be impaired by a reduction in FPN caused by high fetal hepcidin in knockout fetuses, but this seems unlikely since Fe levels are far more sensitive to hepcidin action and they were not reduced. Further studies are required to examine the mechanisms operating in suckling mice. Since no decreases in Mn levels were seen in older mice, our data suggest that intestinal Mn absorption is not impaired in response to increased hepcidin and argue against a strong role for FPN in Mn transport in the gut. These data highlight a significant difference between Mn and Fe.
Other metals such as copper (Fig. 5) and zinc (Fig. 6) showed similar behaviour to Mn, with no or minimal differences between Tmprss6−/− and wild-type mice. A previous in vitro study⁶ showed that FPN was unable to transport significant amounts of Cu²⁺, but it was able to transport Zn²⁺, albeit far less efficiently than iron. These findings are largely in line with our data.

Taken together, our findings with Tmprss6−/− mice suggest that FPN levels are not a major contributor to Mn distribution, as they are for Fe. These data do not exclude some role for FPN in the cellular export of Mn, but suggest that it is likely to be a minor role and that other (and potentially Mn-specific) Mn transport pathways are involved. Further investigations into the contributions of known or suspected Mn transporters are required to clarify the mechanisms underlying the physiological distribution of this metal.
knockout and heterozygous mice were determined by t test separately at each age. (**p < 0.01)

To provide some support for the data obtained with mice overexpressing hepcidin, we examined Fe and Mn levels in mice with specific deletion of FPN in the intestine.\(^{32}\) In intestinal Fpn\(^{-/-}\) mice, Fe levels in the liver were strongly decreased relative to those of control mice, whereas the hepatic Mn concentration was unchanged (Fig. 7). These findings are consistent with our observations in Tmprss6 deficient animals.

As loss of Tmprss6 did not affect Mn distribution under normal physiological conditions where body Mn levels are relatively low, we investigated whether we could see any effects when wild-type and Tmprss6\(^{-/-}\) mice were challenged with a large dose of Mn. When Mn was administered by oral gavage at a dose of 100 mg/kg, both Tmprss6\(^{-/-}\) and heterozygous control mice accumulated more Mn in their liver, kidney and brain than saline treated mice (Fig. 8A-C). However, only small increases were observed in the serum, spleen and heart, and these were not statistically significant (Fig. 8D-F). Previous studies have shown that in Mn overload situations, the metal preferentially accumulates in the liver and brain, consistent with our findings.\(^ {52}\) With one exception (see below) tissue Mn concentrations did not differ between Tmprss6\(^{-/-}\) and heterozygous control mice, consistent with our earlier observations that FPN does not appear to play a significant role in Mn distribution in vivo. The exception was the brain, where Mn levels in Tmprss6 deficient mice were significantly lower than those of heterozygous control animals, but only under Mn overload conditions (Fig. 8B). The reason for this surprising finding (which is not consistent with the effects of elevated hepcidin expression on FPN) is not immediately obvious. However, Mn transport in the brain is complicated, with a range of transporters likely to be involved and the potential for crosstalk between these transporters.\(^ {26, 53, 54}\)
This, combined with high local concentrations of Mn in the incubation solutions, likely does not accurately reflect in vivo conditions. However, one cellular study where endogenous FPN was knocked down suggested that this led to a reduction in Mn-associated toxicity, but neither Mn levels nor transport per se were investigated\textsuperscript{20}. Our work with the Tmprss6\textsuperscript{-/-} and Fpn knockout mice is one of the very small number of studies to address this issue in vivo. Experiments with the flatiron mouse, which has dominant negative mutation in FPN that leads to altered body iron distribution,\textsuperscript{58} provided some evidence that FPN could contribute to Mn export, although the differences observed were smaller than those generally found in in vitro studies.\textsuperscript{22} In a mouse model of HFE-related hemochromatosis, there were either no or only very small effects on Mn.\textsuperscript{23} In this model, levels on Mn are reduced, but some of the transporter is likely to remain, so the weakness of the effect may not be considered surprising. In our own work, we found no clear evidence that overexpression of hepcidin, the normal physiological repressor of FPN, had a significant influence on Mn distribution, nor that loss of FPN itself in the gut. Taken together, these data suggest that if FPN does contribute to Mn transport in the body, it only does so in a very limited way.

If FPN does not play a major role as a Mn export protein, at least at physiological concentrations of the metal, then other transporters must be involved in Mn traffic around the body. As noted above, a range of other transporters have either been demonstrated to export Mn from cells/organelles or are likely to perform this function. Current attention is particularly focused on the coordinated roles of SLC30A10 and SLC39A14 in maintaining body Mn homeostasis, with SLC39A14 acting to transport Mn into hepatic, intestinal and pancreatic cells from which it is excreted via SLC30A10.\textsuperscript{44} The roles of other Mn transporters, such as the lysosome protein ATP13A2 and the Golgi protein ATP2C1 in Mn efflux have yet to be fully explored.

Conclusions

In this study we have demonstrated that Mn and Fe behave quite differently in the presence of constitutively high hepcidin expression (and hence low tissue FPN levels). Fe export into the body from the intestinal epithelium is significantly reduced when hepcidin is high, and hence Fe levels in internal tissues are low. In contrast, Tmprss6 deletion had little effect on Mn levels in any of the tissues studied, consistent with the conclusion that FPN does not make a major contribution to Mn distribution under these conditions. The use of intestine-specific FPN knockout mice confirmed that FPN had little effect on dietary Mn absorption. Several other Mn transport proteins have been identified and these are likely to be more important than FPN in the homeostasis of this metal, but further investigations are required to determine the relative contributions of these transporters and whether they have tissue-specific effects.

Conflicts of interest
There are no conflicts to declare.

Acknowledgements

This work was supported by Project Grant APP1051882 from the National Health and Medical Research Council of Australia (NHMRC). LJ was supported by a University of Queensland International Scholarship and GJA was supported by a Senior Research Fellowship from the NHMRC.

References

2. C. L. Keen, J. L. Ensunsa and M. S. Clegg, Manganese metabolism in animals and humans including the toxicity of manganese, Met Ions Biol Syst, 2000, 37, 89-121.


Knockout mice with constitutively low ferroportin show that ferroportin does not make a major contribution to manganese homeostasis \textit{in vivo}. 

\begin{align*}
\text{Normal hepcidin} & \quad \text{Normal hepcidin} \\
\text{High hepcidin} & \quad \text{High hepcidin}
\end{align*}
Author/s:
Jin, L; Frazer, DM; Lu, Y; Wilkins, SJ; Ayton, S; Bush, A; Anderson, GJ

Title:
Mice overexpressing hepcidin suggest ferroportin does not play a major role in Mn homeostasis

Date:
2019-05-01

Citation:

Persistent Link:
http://hdl.handle.net/11343/230696

File Description:
Accepted version