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Iron uptake from plasma transferrin by a transferrin receptor 2 mutant mouse model of haemochromatosis

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Abstract

Background & Aims—Hereditary haemochromatosis type 3 is caused by mutations in transferrin receptor (TFR) 2. TFR2 has been shown to mediate iron transport *in vitro* and regulate iron homeostasis. The aim of this study was to determine the role of Tfr2 in iron transport *in vivo* using a *Tfr2* mutant mouse.

Methods—*Tfr2* mutant and wild-type mice were injected intravenously with ⁵⁹Fe-transferrin and tissue ⁵⁹Fe uptake was measured. Tfr1, Tfr2 and ferroportin expression was measured by real-time PCR and Western blot. Cellular localisation of ferroportin was determined by immunohistochemistry.

Results—Transferrin-bound iron uptake by the liver and spleen in *Tfr2* mutant mice was reduced by 20% and 65%, respectively, whilst duodenal and renal uptake was unchanged compared with iron-loaded wild-type mice. In *Tfr2* mutant mice, liver Tfr2 protein was absent, whilst ferroportin protein was increased in non-parenchymal cells and there was a low level of expression in hepatocytes. Tfr1 expression was unchanged compared with iron-loaded wild-type mice. Splenic Tfr2 protein expression was absent whilst Tfr1 and ferroportin protein expression was increased in *Tfr2* mutant mice compared with iron-loaded wild-type mice.

Conclusions—A small reduction in hepatic transferrin-bound iron uptake in *Tfr2* mutant mice suggests that Tfr2 plays a minor role in liver iron transport and its primary role is to regulate iron

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metabolism. Increased ferroportin expression due to decreased hepcidin mRNA levels is likely to be responsible for impaired splenic iron uptake in *Tfr2* mutant mice.

Keywords

Transferrin receptor 2; Iron transport; Haemochromatosis; Iron overload; Liver

Introduction

Iron is an essential trace metal required for cellular growth and metabolism. Iron is normally transported in the plasma bound to transferrin and binds to transferrin receptors (TFR) expressed at the cell surface. There are two types of TFRs; TFR1 is ubiquitously expressed whilst TFR2 is highly expressed in hepatocytes and to a lesser extent in erythrocytes and duodenum [1]. The main function of TFR1 is to deliver transferrin-bound iron to cells via receptor-mediated endocytosis [2]. TFR2 has a lower binding affinity for diferric transferrin than TFR1 [3,4]. When TFR2 is over-expressed in Chinese Hamster Ovary cells, it mediates the endocytosis and recycling of transferrin [2] and uptake of transferrin-bound iron [1,2]. Whether this is the case in the liver, erythrocytes and duodenum, where TFR2 is expressed is yet to be determined. Recent evidence suggests that TFR2, like TFR1, also binds to HFE. Conversely, HFE does not compete with transferrin for TFR2 binding and binds TFR2 at a site that is distinct from the site of HFE-TFR1 interaction [5]. HFE has also been shown to increase the affinity of TFR2 for transferrin and increase transferrin-bound iron uptake *in vitro* [6].

TFR1, but not TFR2, is inversely regulated by intracellular iron levels by a post-transcriptional mechanism involving iron responsive elements (IRE). Instead, TFR2 is regulated by extracellular diferric transferrin levels by a post-translational mechanism. Diferric transferrin binds to TFR2 and increases its stability by redirecting TFR2 from a degradative pathway to a recycling pathway inside the cell, thereby increasing the half-life of the protein [7]. The regulation of TFR2 by transferrin saturation controls the expression of the iron regulatory peptide, hepcidin, by an unknown mechanism. The interaction of HFE and TFR2 regulates hepcidin expression [8] which may involve haemojuvelin/bone morphogenetic protein (HJV/BMP) signalling in hepatocytes [9]. Hepcidin is highly expressed by hepatocytes and is secreted into the circulation to regulate systemic body iron levels. It binds to the iron export protein, ferroportin (FPN), which is highly expressed in macrophages and enterocytes and is also expressed in hepatocytes. Upon binding, hepcidin induces the internalisation and degradation of FPN resulting in decreased iron release [10].

Mutations in TFR2 results in the iron overload disorder, hereditary haemochromatosis (HH) type 3. A *Tfr2* mutant mouse model of HH type 3 has been generated with a Y245X mutation in the *Tfr2* gene which is orthologous to the Y250X mutation identified in humans [11]. These mice have similar characteristics of the iron overload observed in subjects with HH type 3 [11,12]. The Y245X mutation in *Tfr2* results in decreased hepcidin mRNA expression leading to increased iron absorption and the rapid deposition of the absorbed iron in the liver resulting in hepatic iron overload [12]. Iron overload that results from liver specific deletion of the *Tfr2* gene is comparable to the complete *Tfr2* knockout mice [13], indicating the central role of the liver in the regulation of iron metabolism.

In the present study, the role of *Tfr2* in transferrin-bound iron uptake *in vivo* was investigated using a *Tfr2* mutant mouse model of HH type 3. We provide evidence that TFR2 has a minor role in iron transport and hepatic iron loading *in vivo*. In the spleen, a decrease in iron uptake in *Tfr2* mutant mice is likely to be due to increased Fpn-mediated iron export as a result of a down-regulation of hepcidin expression.

Materials and methods

Animals

Tfr2(Y245X) mutant mice were generated on a C57Bl/6x129/SVJ hybrid strain background as described previously [11]. The mice were backcrossed for 5 generations onto an AKR background and homozygous mutant and wild-type mice were derived from *Tfr2* (Y245X) heterozygous mice (Animal Resource Centre, Australia). Female *Tfr2* mutant and wild-type mice were fed either a control diet (70 mg iron/kg) or an iron-supplemented diet (20 g carbonyl iron/kg; Specialty Feeds, Australia) for 3 weeks from 7–10 weeks of age. All mice were studied between 10–14 weeks of age. This study was approved by The University of Western Australia Animal Ethics Committee.

Non-haem Iron Measurements

Liver and spleen non-haem iron levels were measured using the method of Kaldor [14].

Plasma Iron Clearance

Tfr2 mutant and wild-type mice were injected with 150 µg of ⁵⁹Fe-¹²⁵I-transferrin and 150 µg ¹³¹I-albumin intravenously into the ventral tail vein. Blood samples were collected at 2, 30, 60, and 90 minutes after injection and blood, liver, spleen, kidney and duodenum were collected 120 minutes after injection and counted for radioactivity. Tissue uptake of transferrin-bound iron and the rate of plasma iron turnover were determined as described previously [15].

Western blot analysis

Tfr1, Tfr2, Fpn and actin protein expression were determined in liver and spleen tissue from *Tfr2* mutant and non-iron and iron-loaded wild-type mice as described previously [16,17]. Tfr1, Tfr2 and Fpn protein expression were normalised to actin expression and expressed relative to non-iron-loaded wild-type mice.

RNA expression

Total RNA was isolated from liver and spleen tissue and reverse transcribed as described previously [16,17]. *Tfr1*, *Tfr2*, *Fpn*, *Hamp1* and β -actin mRNA transcripts were measured by real-time polymerase chain reaction (PCR) in a Rotorgene (Corbett Research, Australia) using primers listed in Table 1 and quantified using standard curves generated from serial dilutions of known copy number of plasmids containing cDNA of the gene of interest. *Tfr1*, *Tfr2*, *Fpn* and *Hamp1* mRNA expression were normalised against β -actin mRNA expression.

Immunohistochemistry

Frozen liver tissue was fixed with methanol/acetone (1:1), permeabilised with 0.02% Tween[®] 20 in PBS and blocked with 5% goat serum in PBS. Tissue sections were incubated with primary antibodies, rabbit anti-Fpn (Alpha Diagnostic, USA) and/or rat anti-F4/80, a macrophage marker (kind gift from Professor Ruth Ganss, Western Australian Institute for Medical Research) at 1:150 overnight at 4°C in REAL[™] antibody diluent (DAKO, Denmark). Proteins were detected with secondary antibodies, goat anti-rabbit Alexa Fluor[®] 488 and/or goat anti-rat Alexa Fluor[®] 594 (Invitrogen Australia) at 1:200 in antibody diluent for 1 hour in the dark. Sections were washed in PBS and mounted with ProLong Gold[®] antifade reagent with DAPI (Invitrogen) to counterstain the nuclei.

Statistics

Results are expressed as mean $\pm$ SEM where $n = 4-8$ mice per group. Differences between group means were analysed using ANOVA with Tukey's multiple comparison tests (GraphPad PRISM, USA) and were statistically significant for $p < 0.05$.

Results

Non-haem Iron

Non-haem iron levels in the livers of *Tfr2* mutant, non-iron-loaded and iron-loaded wild-type mice were measured to confirm the iron status of the mice. Liver non-haem iron concentration in *Tfr2* mutant and iron-loaded wild-type mice was similar and 5-fold greater than non-iron-loaded wild-type mice (Fig. 1A). Similarly, plasma iron and transferrin saturation levels were increased in *Tfr2* mutant and iron-loaded wild-type mice compared to non-iron-loaded mice as shown previously [11,12]. In the spleen, non-haem iron concentration in *Tfr2* mutant mice was reduced by 70% and 80% compared with non-iron-loaded and iron-loaded wild-type mice, respectively (Fig. 1B). Liver and spleen ferritin protein expression correlated with non-haem iron concentrations (data not shown).

Transferrin-bound Iron Uptake

Liver uptake of transferrin-bound iron by *Tfr2* mutant mice was not significantly different from iron uptake by non-iron-loaded wild-type mice and was reduced by 20% compared with iron-loaded wild-type mice (Fig. 2A). When *Tfr2* mutant mice were fed an iron-supplemented diet, liver iron uptake was increased (17.7 ± 1.0 μmol iron/g tissue; $p < 0.001$) but was not significantly different to iron uptake by iron-loaded wild-type mice. Uptake of transferrin-bound iron by the spleen in *Tfr2* mutant mice was reduced by 50% and 65% compared with non-iron-loaded and iron-loaded wild-type mice, respectively (Fig. 2B). There was, however, no difference in transferrin-bound iron uptake by the duodenum and kidneys in *Tfr2* mutant mice compared with both non-iron-loaded and iron-loaded wild-type mice (Fig. 2C, D; $p > 0.05$). Plasma iron turnover, which was calculated from the rate of ^{59}Fe clearance from the plasma over 2 hours, was not significantly different between *Tfr2* mutant mice, iron-loaded and non-iron-loaded wild-type mice (Fig. 2E; $p > 0.1$).

Expression of iron metabolism genes

Liver *Tfr2* mRNA expression was low and protein expression was absent in *Tfr2* mutant mice. In contrast, *Tfr2* was highly expressed in wild-type mice and *Tfr2* protein expression increased with iron-loading. *Tfr2* protein expression in the spleen was not detected in all groups of mice (Table 2, Fig. 3A, B). Liver *Tfr1* mRNA and protein expression in *Tfr2* mutant mice was similar to iron-loaded wild-type mice and was decreased significantly compared with non-iron-loaded wild-type mice. Splenic *Tfr1* mRNA and protein expression in *Tfr2* mutant mice was increased significantly compared with non-iron-loaded mice and both were greater than iron-loaded wild-type mice. (Table 2, Fig. 4A, B).

Liver *Hamp1* mRNA expression was markedly reduced in *Tfr2* mutant mice compared with wild-type mice and was increased significantly in wild-type mice with iron-loading (Table 2). *Fpn* mRNA expression was similar in all types of mice in both the liver and spleen (Table 2). In the liver, *Fpn* protein was significantly increased in *Tfr2* mutant mice compared with wild-type mice with increased expression in non-parenchymal macrophages and low levels of expression in hepatocytes mainly in the periportal regions (Fig. 5A, 6B, D-F). In wild-type mice, *Fpn* protein was detected in non-parenchymal macrophages and expression was increased with iron-loading; however, not to the same extent seen in *Tfr2* mutant mice (Fig. 5A, 6A, C). In the spleen, *Fpn* protein was increased in *Tfr2* mutant mice compared with wild-

type mice. Iron-loading of wild-type mice resulted in significantly reduced Fpn levels (Fig. 5B).

Discussion

TFR2 has an important role in iron homeostasis since mutations in *TFR2* result in HH type 3 causing iron overload [18]. In addition to its role in iron regulation, TFR2 has been shown to transport transferrin-bound iron *in vitro* [1,2]. In the present study, the role of TFR2 in transferrin-bound iron uptake *in vivo* was investigated using *Tfr2* mutant mice. Here, we demonstrate that the absence of Tfr2 did not have a substantial impact on transferrin-bound iron uptake by the liver, spleen, duodenum and kidney indicating that the primary role of TFR2 is to regulate cellular iron metabolism with a minor role in iron transport.

As indicated by liver non-haem iron levels, iron-loaded mice achieved elevated hepatic iron stores to the same degree as the *Tfr2* mutant mice. In the liver, hepatocytes acquire iron from plasma transferrin by receptor-mediated endocytosis via TFRs. Liver Tfr1 mRNA and protein expression in *Tfr2* mutant and iron-loaded wild-type mice was similar and decreased compared to non-iron-loaded wild-type mice. Liver Tfr2 mRNA was similar and Tfr2 protein expression was up-regulated in iron-loaded wild-type mice compared with non-iron-loaded wild-type mice and the protein was absent in *Tfr2* mutant mice. The Y245X mutation introduces a stop codon in the *Tfr2* gene and stops the expression of the protein [11]. These results are consistent with the post-transcriptional regulation of Tfr1 by iron via the IRE-iron regulatory protein (IRP) mechanism [19] and post-translational regulation of Tfr2 by diferric transferrin [20,21] which is increased in iron overload. Iron-loading increased transferrin-bound iron uptake by the liver in wild-type mice. Since liver Tfr1 expression is relatively low and down-regulated by iron-loading, it is unlikely to contribute to hepatic iron loading in both iron-loaded wild-type and *Tfr2* mutant mice. Hence, the increase in liver iron uptake in iron-loaded wild-type mice may be attributable at least in part by an increase in Tfr2 protein levels. Despite high Tfr2 protein expression in the liver, the loss of functional Tfr2 in the mutant mice had little impact on transferrin-bound iron uptake. Iron uptake from plasma transferrin by the liver in *Tfr2* mutant mice was decreased by approximately 20% compared to uptake by iron-loaded wild-type mice. In contrast, *Tfr2* mutant mouse liver takes up over 10-fold more iron from the portal circulation compared with wild-type mice with approximately 90% of the absorbed iron deposited in the liver [12]. As plasma transferrin in *Tfr2* mutant mice is almost completely saturated, iron absorbed by the duodenum will most likely be in the form of non-transferrin-bound iron and the rapid deposition of iron in the liver suggests that this pathway contributes to liver iron-loading in TFR2-related haemochromatosis. These results highlight that Tfr2 plays a small role in liver iron transport and suggest the presence of another route of transferrin-bound iron uptake that is independent of Tfr1 and Tfr2. The observation that liver uptake of transferrin-bound iron by *Tfr2* mutant mice fed an iron-supplemented diet was increased rather than attenuated by the loss of functional Tfr2 corroborates this concept. Furthermore, this is supported by our previous findings in hepatocytes where up-regulation of Tfr2 protein expression was not accompanied by an increase in transferrin-bound iron uptake [17]. This raises the possibility that iron may be released from tissues in the form of non-transferrin bound iron, particularly in the iron-loaded mice, which is rapidly cleared from the plasma by the liver and contributes to iron-loading by a Tfr-independent pathway.

We and others have shown that hepcidin mRNA expression is down-regulated in *Tfr2* mutant mice and up-regulated in iron-loaded wild-type mice [12,22,23]. In the current work, liver Fpn protein expression in *Tfr2* mutant mice was increased compared with wild-type mice in agreement with the intracellular targeting of Fpn for degradation by hepcidin [10]. In *Tfr2* mutant mice, Fpn was expressed mainly in liver macrophages with low expression detected in hepatocytes. Given the role of Fpn in iron export, this suggests that iron export by liver

macrophages, and to a lesser degree by hepatocytes, may be increased in *Tfr2* mutant mice. Transferrin-bound iron is taken up mainly by hepatocytes. Hence, the reduction in liver iron uptake by *Tfr2* mutant mice compared to iron-loaded wild-type mice is likely to be due to a loss of Tfr2 which is highly expressed in hepatocytes and to a lesser degree due to an increase in Fpn-mediated iron export by hepatocytes and liver macrophages, since hepatic Fpn expression is relatively low [24]. Despite increased hepcidin expression [12], liver Fpn protein expression was increased in non-parenchymal macrophages in iron-loaded wild-type compared with non-iron-loaded wild-type mice suggesting there is also iron regulation of Fpn in liver macrophages. *Fpn* gene contains an IRE in its 5'-untranslated region that is involved in iron-dependent post-transcriptional gene regulation [24,25]; this process may contribute to the up-regulation of Fpn protein expression in macrophages of the iron-loaded wild-type mice.

Splenic non-haem iron levels in *Tfr2* mutant mice were decreased compared with non-iron-loaded wild-type mice consistent with previous reports [11,13] whilst levels in iron-loaded wild-type mice were increased. Similarly, iron uptake by *Tfr2* mutant mice was decreased compared with wild-type mice. These results suggest that the spleen was relatively iron-deficient in *Tfr2* mutant mice due to reduced net splenic transport of transferrin-bound iron resulting from two processes involving the uptake and release of iron by the spleen. Tfr2 protein was not expressed in the spleens from both *Tfr2* mutant and wild-type mice. Splenic Tfr1 expression was up-regulated in *Tfr2* mutant mice and down-regulated in iron-loaded wild-type mice compared with non-iron-loaded wild-type mice. This is as expected due to the iron-dependent regulation of Tfr1 by the IRE-IRP system. Tfr1 is expressed at relatively low levels in the spleen and an increase in Tfr1 expression in *Tfr2* mutant mice is likely to have only a small effect on splenic iron uptake. Fpn is highly expressed by reticulo-endothelial macrophages in the spleen [24] and Fpn protein expression was up-regulated in *Tfr2* mutant mice, reflecting the hepcidin-deficient state of *Tfr2* mutant mice. Furthermore, the overall trends in Fpn protein expression were consistent with iron deposition in the spleen suggesting that the reduction in splenic iron accumulation by *Tfr2* mutant mice is most likely to be due to more iron being exported by the highly expressed Fpn than imported by the relatively low levels of Tfr1 in the spleen.

Tfr2 is expressed at relatively low levels in the duodenum [26] and there was no difference in transferrin-bound iron uptake by the duodenum. Tfr2 is not expressed in the kidneys [1] and consistent with this, there was no change in transferrin-bound iron uptake observed between *Tfr2* mutant and wild-type mice. These results suggest that Tfr2 has a minor role, if any, in transferrin-bound iron transport by the duodenum and kidneys. The clearance of plasma transferrin-bound iron reflects the iron requirements of the bone marrow for erythropoiesis. Expression of Tfr2 by erythropoietic cells is contentious [27,28]. However, the lack of change in plasma iron turnover in *Tfr2* mutant mice suggests that Tfr2 does not mediate iron uptake by the erythroid cells in the bone marrow which may be due to the absence of Tfr2 expression by normal erythroid cells [28].

The hepatic peptide, hepcidin is regulated by iron levels and hepcidin levels increase when iron is present in excess. Patients and mouse models with TFR2- and HFE-related HH develop hepcidin deficiency despite high tissue iron stores [12,23,29–31] demonstrating the crucial role of both TFR2 and HFE in the iron regulation of hepcidin. Furthermore, iron overload in patients with TFR2-related HH has been shown to be more severe than HFE-related HH [32,33] indicating that the loss of functional TFR2 may be more detrimental than the loss of functional HFE. It has been postulated that TFR2 and HFE act as iron sensors to signal to hepatocytes the degree of transferrin saturation in the plasma modulating hepcidin synthesis accordingly. A current model of iron-dependent regulation of hepcidin proposes that at high plasma transferrin saturation, diferric transferrin binds to both TFR1 and TFR2 in hepatocytes. HFE is displaced from TFR1 and binds to TFR2 which is stabilised by the increased diferric

transferrin levels. The HFE-TFR2 complex signals to hepcidin directly or associates with HJV and promotes BMP signalling which leads to increased hepcidin synthesis [8,9,34].

In conclusion, iron uptake from plasma transferrin in mice lacking functional Tfr2 protein was decreased by only a small amount in the liver and was unchanged in the duodenum and kidney compared to wild-type mice with equivalent iron overload, indicating that Tfr2 has a lesser role in iron transport and a greater role in the regulation of iron metabolism to balance the demands of iron utilization with iron absorption, recycling, and storage in the liver. Furthermore, impaired splenic iron deposition in *Tfr2* mutant mice is likely to be a result of increased Fpn-mediated iron export as a direct consequence of down-regulation of liver hepcidin production.

Abbreviations

TFR	transferrin receptor 1
IRE	iron regulatory element
HJV	haemojuvelin
BMP	bone morphogenetic protein
FPN	ferroportin
HH	hereditary haemochromatosis
Hamp	hepcidin
IRP	iron regulatory protein.

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Figure 1. Non-haem iron concentrations in the liver (A) and spleen (B) of *Tfr2* mutant (*Tfr2* mut), non-iron-loaded (WT) and iron-loaded (WT + Fe) wild-type mice

Results are expressed as mean $\pm$ SEM, n = 6–8. Significant difference between mouse groups: * $p < 0.0001$, *Tfr2* mut vs WT, + $p < 0.0001$, *Tfr2* mut vs WT + Fe and WT; # $p < 0.002$ WT + Fe vs WT.

Figure 2. Plasma transferrin-bound iron uptake by the liver (A), spleen (B), duodenum (C) and kidneys (D) and plasma iron turnover (E) in *Tfr2* mutant (*Tfr2* mut), non-iron-loaded (WT) and iron-loaded (WT + Fe) wild-type mice

Results are expressed as mean $\pm$ SEM, n = 5–6. Significant difference between mouse groups: * p < 0.05, *Tfr2* mut vs WT + Fe; + p < 0.05, *Tfr2* mut vs WT+Fe and WT; # p < 0.01 WT + Fe vs WT.

Figure 3. Tfr2 protein expression in the liver (A) and spleen (B) in *Tfr2* mutant (*Tfr2* mut), non-iron-loaded (WT) and iron-loaded wild-type mice (WT + Fe)
 Results are expressed as mean $\pm$ SEM, $n = 5-6$. Representative blots of hepatic (A) and splenic (B) Tfr2 protein expression from 3 independent experiments are shown. Significant difference between mouse groups: $^+p < 0.001$, *Tfr2* mut vs WT + Fe and WT; $^{\#}p < 0.05$ WT + Fe vs WT.

Figure 4. Tfr1 protein expression in the liver (A) and spleen (B) in *Tfr2* mutant (*Tfr2* mut), non-iron-loaded (WT) and iron-loaded wild-type mice (WT + Fe)

Results are expressed as mean $\pm$ SEM, $n = 4-6$. Representative blots of hepatic and splenic Tfr1 protein expression from 3 independent experiments are shown. Significant difference between mouse groups: * $p < 0.001$, *Tfr2* mut vs WT; + $p < 0.025$, *Tfr2* mut vs WT + Fe and WT; # $p < 0.025$ WT + Fe vs WT.

Figure 5. Fpn protein expression in the liver (A) and spleen (B) in *Tfr2* mutant (*Tfr2* mut), non-iron-loaded (WT) and iron-loaded wild-type mice (WT + Fe)
 Results are expressed as mean $\pm$ SEM, $n = 4-7$. Representative blots of hepatic and splenic Fpn protein expression from 3 independent experiments are shown. Significant difference between mouse groups: ⁺ $p < 0.001$, *Tfr2* mut vs WT + Fe and WT; [#] $p < 0.001$ WT + Fe vs WT.

Figure 6. Cellular localisation of hepatic Fpn expression. Frozen liver sections from wild-type (WT), *Tfr2* mutant (Tfr2 mut) and iron-loaded wild-type mice (WT + Fe) were immunofluorescently stained with an antibody against Fpn (green) and DAPI (blue) for nuclear quantitation (A–C). Double staining of Tfr2 mut liver for F4/80 (red, D) and Fpn (green, E) demonstrated that the majority of cells that strongly express Fpn were F4/80-positive macrophages (Mac), while hepatocytes (Hep) only express the Fpn antigen at lower levels (F).

Table1

Primers for real-time PCR

Gene	sequence (5' - 3')	Genbank number
<i>Tfr2</i>	Fwd:TTCCTACATCATCTCGCTTAT	NM 015799
	Rev:TGGCGACACATACTGGGGACAG	
<i>Tfr1</i>	Fwd:TTCCTACATCATCTCGCTTAT	NM 011638
	Rev:CATAGTGTTTCATCTCGCCAGA	
<i>Fpn</i>	Fwd:TTGCAGGAGTCATTGCTGCTA	NM 016917
	Rev:TGGAGTTCTGCACACCATT	
<i>Hamp1</i>	Fwd:TTGCGATACCAATGCAGAAGA	NM 032541
	Rev:GATGTGGCTCTAGGCTATGTTTTG	
<i>β-actin</i>	Fwd:CTGGCACCCACCTTCTA	NM 007393
	Rev:GGGCACAGTGTGGGTGAC	

Tfr1, transferrin receptor 2; *Tfr1*, transferrin receptor 2; *Fpn*, ferroportin; *Hamp1*, hepcidin1

Table 2

mRNA expression of iron genes

Tissue	Gene	WT	Tfr2 mutant	Iron loaded WT
Liver	<i>Tfr2</i>	1.62 ± 0.25	0.30 ± 0.04 ^{*#}	1.49 ± 0.15
	<i>Tfr1</i>	0.0098 ± 0.0020	0.0043 ± 0.0006 [*]	0.0058 ± 0.0005 [*]
	<i>Fpn</i>	0.25 ± 0.02 (7)	0.31 ± 0.04	0.29 ± 0.05
	<i>Hamp1</i>	7.29 ± 1.42 (4)	2.93 ± 0.75 ^{*#}	11.98 ± 1.19 [*]
Spleen	<i>Tfr2</i>	0.0010 ± 0.0003	0.00010 ± 0.00002 ^{*#}	0.0010 ± 0.0001
	<i>Tfr1</i>	0.018 ± 0.002	0.037 ± 0.003 ^{*#}	0.012 ± 0.001 [*]
	<i>Fpn</i>	0.10 ± 0.02	0.08 ± 0.02	0.11 ± 0.01

Results are expressed as mean ± SEM, where n=4–8 for the gene interest relative to β-actin.

^{*} $p < 0.05$ versus wild-type mice and

[#] $p < 0.05$ versus iron loaded wild-type mice. *Tfr2*, transferrin receptor 2; *Tfr1*, transferrin receptor 1, *Fpn*, ferroportin; *Hamp1*, hepcidin1