Disorders of Iron Metabolism

Homozygosity for transferrin receptor-2 Y250X mutation induces early iron overload

Two Italian subjects, aged three and sixteen years, presented with early iron overload as shown by increased serum iron indices and hepatic iron concentration. They both carried the Y250X mutation of the *TFR2* gene in the homozygous state. We suggest that transferrin receptor-2 is important in maintaining iron balance in the first decades of life.

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Hereditary hemochromatosis (HH) is a genetically heterogeneous disorder.[1] Type 3 HH is a rare recessive disorder due to mutations of the transferrin receptor 2 (*TFR2*) gene.[2] Y250X was the first *TFR2* mutation originally described in two Sicilian families.[2] Here we describe two unrelated homozygotes for Y250X who had early iron overload.

The first subject came to our observation when he was 3 years old. One year earlier he had been found to have a high serum iron level. The second subject is a 16-year old boy who presented with fatigue. The hematologic and iron indices of both patients are reported in Table 1 and Figure 1. The parents of both probands, apparently unrelated, originated from Sicily, were symptomless and had normal iron indices (Figure 1). Analysis of HH-associated mutations was performed using a reverse hybridization assay that detects 11 *HFE* mutations and one *TFR2* (Y250X) mutation (Haemochromatosis Strip Assay, Nuclear Laser Medicine, Settala, Milan, Italy). A *TFR2* Y250X homozygous mutation and H63D heterozygous substitution were identified in the probands. Figure 1 shows the two pedigrees and the results of molecular analyses.

The important finding of this study is that patients lacking TFR2 develop early iron overload. It is unlikely that the heterozygous H63D mutation had influenced the probands' phenotype, since this genotype has only minimal, if any, effects on iron metabolism.[3] Two siblings (female and male), with 594-597 AVAQ homozygous deletion of *TFR2* showed findings similar to those in our cases, when they were aged 14 and 16 years, respectively.[4] These data suggest that iron rapidly accumulates in type 3 HH, whereas iron overload in HFE-related HH is rare before the age of 20, usually developing in the fourth to fifth decade in males and even later in females.[3] Accordingly, mice homozygous for Y245X *TFR2* mutation (the murine ortholog of the human Y250X), developed hepatic iron loading by 4 weeks of age even on a standard diet,[5] whereas HFE knock-out mice in the same genetic background developed comparable iron overload only at 10 weeks.[6] Juvenile hemochromatosis presents in early childhood, but progression of iron overload is more severe than in TFR2-related HH.[7] Overall, the absence of functional *TFR2* protein induces a higher rate of iron absorption than that determined by a non-functional HFE, but lower than that caused by mutations in hepcidin or in the still uncloned juvenile hemochromatosis gene. Mutations inactivating HFE, *TFR2*, and hepcidin lead to a similar pattern of iron overload,[4-6,8] suggesting that these proteins are components of the same regulatory pathway.

Our results and the variable severity of iron overload among different types of HH, do, however, indicate a distinct role for these proteins in iron homeostasis. A common feature of the Y250X homozygotes and of their orthologous *TFR2* mice is remarkably high transferrin saturation.[3-5] We have previously suggested a sensor function for *TFR2*;[9] we speculate that

Table 1. Baseline and follow-up hematologic and iron indices of the two young patients homozygous for the Y250X TFR2 mutation.

	Serum iron (μg/dL)	*Transferrin saturation (%)*	*Serum ferritin (μg/L)*	*Hepatic iron concentration (μg Fe/g wet weight)*
Case #1				
Baseline	230	80	58	–
Time of our evaluation	297	90	56	524
Case #2				
Baseline	307	100	963	933
After 16 venesections	208	80	70	240
After 20 venesections	33	–	29	–

Hb: hemoglobin; MCV: mean corpuscular volume; TS: transferrin saturation; HIC: hepatic iron concentration. Hepatic iron concentration was determined by SQUID and was compared to a normal reference value for adults (< 400 μg Fe/g wet weight) since there are no reference data available for children and teenagers. SQUID analysis was performed using a Biosusceptometer 5700 3-Channel (TRISTAN Technologies Inc., San Diego, CA, USA).

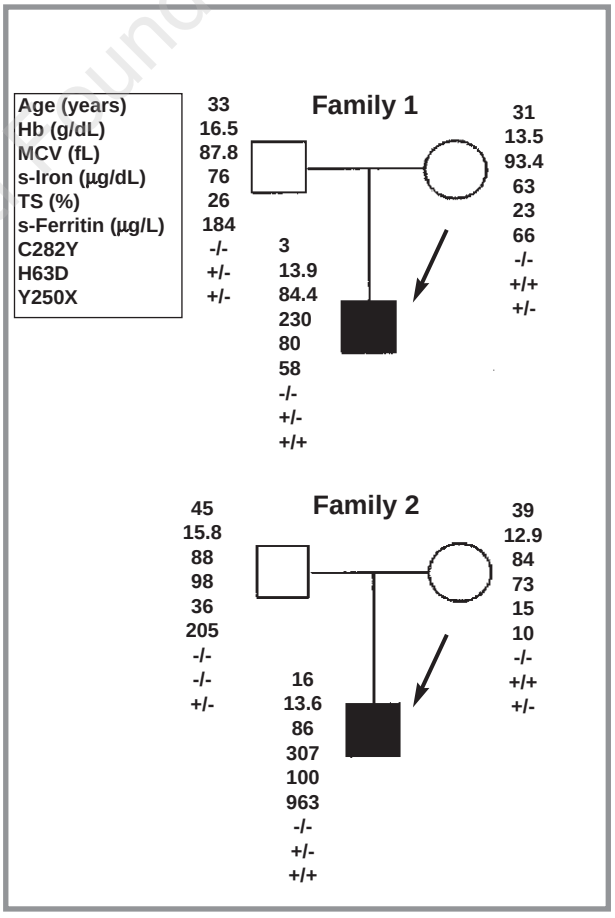

Figure 1. Pedigrees of the two patients homozygous for the TFR2 Y250X mutation. (Arrows indicate the two probands).

this protein is important in maintaining normal transferrin saturation, possibly by regulating hepcidin, as recently proposed.[10] Indeed the inactivation of both proteins causes early iron overload, indicating that *TFR2*, like hepcidin, is crucial for maintaining iron balance in the first decades of life.

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Key words: transferrin receptor-2, iron overload, Y250X mutation

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Sickle Cell Anemia

Circulating endothelin-3 levels in patients with sickle cell disease during hydroxyurea treatment

Abnormal adhesion of red blood cells to the endothelium and the production of cytokines and vasoactive substances, such as endothelin-1 contribute to the pathogenesis of microvascular occlusion in sickle cell disease (SCD), even during the steady state. Endothelin-3 (ET-3) is a vasoconstrictive agent, which has not yet been studied in SCD.

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Sicke cell disease (SCD) is a heritable hemoglobinopathy characterized by chronic hemolysis, frequent infections and recurrent occlusion of the microcirculation that cause painful crises. Subclinical vaso-occlusion occurs even during the steady state and results in chronic organ damage.[1] Vaso-occlusion involves not only the polymerization of hemoglobin S but also abnormal adhesion of young deformable red blood cells to the endothelium and the production of cytokines and vasoactive substances, such as endothelin-1 (ET-1) by the activated endothelial cells.[1,2] ET-1 is a potent vasoconstrictor and pro-inflammatory agonist whose levels have been shown to be elevated in SCD both during crises and in steady state.[3] ET-1 production is regulated by cytokines, growth factors and other vasoactive substances, such as endothelin-3 (ET-3).[4] ET-3 is principally produced by the endothelium and functions not only as a vasoconstrictor, but also as a mediator of inflammation by inducing interleukin-6 (IL-6) production by endothelial cells.[5]

ET-3 has not yet been studied in SCD. Since the actions of ET-3 could be involved in endothelial dysfunction and the pathophysiology of vaso-occlusion in SCD, we decided to measure plasma ET-3 levels in steady state SCD and to investigate their relationship with hydroxyurea (HU) treatment. We studied 12 patients with sickle/β^0 thalassemia, 5 with sickle/β^+ thalassemia and 2 with homozygous SCD. These 19 patients were aged from 24 to 60 years; there were 8 males and 11 females; HU (25 mg/kg of body weight daily) was administered over a 5-month period. All the patients had experienced at least three painful crises necessitating hospitalization in the preceding year. None of the patients had had a painful crisis in the 4 weeks prior to starting HU treatment, nor had they received a transfusion within the 3 months prior to the start of the study. HbF and ET-3 levels were determined before the start of HU treatment after 1 month and 5 months of treatment. HbF was quantified by cation exchange high-performance liquid chromatography. ET-3 was assayed using an ELISA technique according to the manufacturer's guidelines (ET-3: Assay Designs, Inc.). The cross reactivity with ET-1 (1-21) and ET-1 (1-31) was 1.8% and less than 0.1%, respectively. Forty healthy volunteers served as controls. Data were analyzed by Wilcoxon's signed rank test and the Mann-Whitney U test.

The study results are summarized in Table 1. Hematologic changes occurring during HU treatment included a non-significant increase in hemoglobin concentration and a non-significant reduction in white blood cell and reticulocyte counts. HbF levels did not increase significantly by one month after the start of the treatment although they did increase significantly after 5 months. None of the patients had painful crises, required transfusion, or developed serious myelotoxicity. Steady state ET-3 levels were found to be significantly higher in patients than in healthy controls and dropped significantly during HU treatment, even after one month.

To our knowledge this is the first report of high ET-3 levels in SCD. One possible source of ET-3 production is the activated and injured endothelium due to its abnormal interactions