Why do humans need two types of transferrin receptor? Lessons from a rare genetic disorder

The notion that humans have two types of transferrin receptor is rather recent. The classical transferrin receptor (TFR1) is a key molecule, essential for iron uptake through its endosomal cycle.[1] The second transferrin receptor (TFR2) was only recently cloned, independently by two groups. Glockner *et al*. cloned the whole region of chromosome 7q22 which contains *TFR2* as well as other important genes, such as erythropoietin in 1998.[2] One year later Kawabata *et al*. serendipitously cloned the gene during an effort to identify new transcriptional factors involved in cancer.[3]

At a protein level the two receptors share moderate homology. Like TFR1, the predicted TFR2 protein has short cytoplasmic and transmembrane domains and a large extracellular domain. The amino acid identity between the two receptors reaches 45% in the extracellular region.[3] However, compared with TFR1, TFR2 has distinct structural features (Table 1). The most significant are the tissue specific expression, which is restricted to the liver, and the lack of iron-regulation. At variance with TFR1, TFR2 has no iron responsive elements (IRE) in its RNA untranslated regions. As IRE are able to interact with iron regulatory proteins (IRP1 and IRP2), it follows that TFR2 is not iron-regulated at a post-transcriptional level.[3] Another distinctive feature of TFR2 is its inability to bind HFE, while HFE-binding is a constitutive feature of TFR1.[4] Since, if hyperexpressed in cell cultures, TFR2 is able to bind transferrin and to internalize iron,[5] it was initially interpreted as a subsidiary tool for cellular iron uptake. Experiments in iron-loaded and in Hfe -/- mice led to the hypothesis that iron uptake through TFR2 might be one mechanism leading to liver iron accumulation.[6] This interpretation was hardly convincing in the light of the observation that TFR2 did not compensate for the lack of TFR1 in TFR1-deficient mice, which died before embryonic day 12.5 of severe iron-deficient anemia and central nervous system abnormalities.[7] These observations underlined the essential role of TFR1 in iron uptake and suggested a different function for TFR2.

The role of TFR2 in iron metabolism remained unclear until we identified mutations of *TFR2* in patients with HFE-unrelated hemochromatosis (now called type 3 hemochromatosis, OMIM # 604250). Using a positional candidate cloning approach we showed that hemochromatosis in 2 Sicilian families was linked to chromosome 7q22 and that all the affected subjects had *TFR2* inactivating mutations in the homozygous state.[8] The phenotype of iron loading following its inactivation was inconsistent with the idea of TFR2 as an iron uptaker, but rather pointed to it having a regulatory function on iron homeostasis. A mouse model homozygous for *Y245X,* a mutation orthologous to the first mutation reported in humans (*Y250X*), developed the same features of iron loading observed in patients, confirming the regulatory role of TFR2 in rodents.[9] Studies on the tissue distribution of TFR2 protein using specific monoclonal antibodies and immunohistochemical techniques revealed major staining in the human liver and duodenal cells.[10] It was subsequently shown that TFR2 co-localizes with HFE in duodenal crypts,[11] although a direct binding between the two proteins has been excluded *in vitro*.[12] A puzzling features of TFR2 remains its expression in K562[3] and in primary leukemic blasts, especially M6 type,[13] whereas in normal erythroid cells the TFR2 protein is not expressed at any stage of differentiation.[14] Exposure of K562 cells to transferrin-bound iron induced a significant up-regulation and relocalization of membrane TFR2,[10] whereas after addition of apo-transferrin the expression of TFR2 was unmodified. Whether this has some physiological relevance in early erythropoiesis is presently unknown.

The discovery of TFR2 and of type 3 hemochromatosis has spread some excitement in the scientific community, since at that time it was recognized that a proportion of hemochromatosis patients had no *HFE* mutations. However, the expectation that TFR2 might account for all these cases was not met by the finding that type 3 hemochromatosis is rare. Worldwide screening of non-HFE patients for *Y250X* (the first identified mutation of *TFR2*) did not reveal a single positive case.[15-17] However, the finding of other rare *TFR2* mutations in non-HFE hemochromatosis patients both in Italy[18-21] and elsewhere[22-23] confirmed the association of TFR2 with hemochromatosis. All the mutations so far reported are private and, as shown in Figure 1, spread along the entire gene sequence.

Preliminary data suggested that TFR2-hemochromatosis might be restricted to Italy and Europe. Surprisingly, an identical (AVAQ 594-597) deletion, originally described in Italians,[19] was found in a Japanese family.[24] In this issue of the Journal (page 302) Koyama *et al*. enrich the number of *TFR2* mutations. They report the molecular study of nine Japanese patients with a clinical diagnosis of hemochromatosis. None had *HFE* mutations, but two had novel *TFR2* mutations. The first, a 41-year old patient with cirrhosis and diabetes, was homozygous for a 1469T→G nucleotide change, which causes the substitution of arginine for leucine at position 490 of the protein (*L490R*). This mutation was found in association with a previously described polymorphism 714C→G (*I1238M*). The second, a 58-year old patient with cirrhosis, diabetes and skin pigmentation, was homozygous for 1665delC leading to a premature stop at valine 561 (*V561X*). The latter mutation produces a truncated protein, as occurs in the cases of *E60X, C130X, Y250X* (Figure 1). *L490R* is a missense change with a substitution of a neutral with a charged amino acid in a conserved residue. As in the cases of AVAQ deletion in the AVAQ motif and of *Q690P,* the novel *L490R* missense mutation targets a conserved residue of the *TFR2* extracellular domain, which must have an important role in the protein.

Hemochromatosis is rare among Orientals and in particular Japanese, among whom another genetic form of iron overload is due to aceruloplasminemia.[25] Since only one case of *C282Y*, a typical Caucasian mutation, has been reported,[26] the present evidence is that the most common form of hemochromatosis in Japan is TFR2-related. How has our understanding of TFR2 function progressed since its discovery at the end of the last century? The major recent advance in elucidating iron regulation has been the discovery of the liver hepcidin peptide as the central effector of iron homeostasis.[27] Hepcidin is also central to the pathogenesis of hemochromatosis, both of the severe juvenile type[28-29] and of the HFE-classic type.[30-31] Thus the question becomes: is TFR2 related to hepcidin? Data from mice[32] and humans[33] suggest that *TFR2* contributes to modulating hepcidin production. Indeed hepcidin liver RNA is downregulated in TFR2-

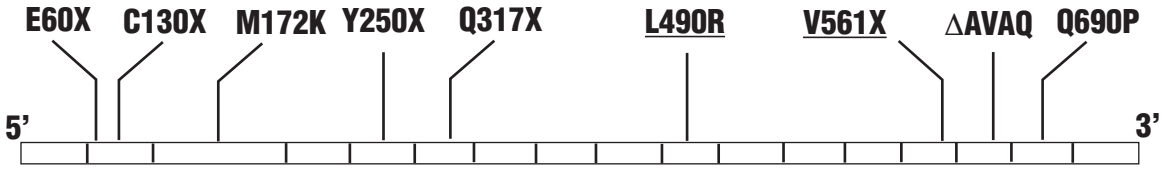

Figure 1. Schematic representation of the *TFR2* gene codifying exons. The position of all reported mutations is shown in the corresponding exon. The mutations characterized in the paper by Koyama *et al.* are underlined.

Table 1. Main differences between TFR1 and TFR2.

Features	*TFR1*	*TFR2*
Protein family	TFRC	TFRC
Expression	ubiquitous	liver, duodenum
High expression	erythroblasts	hepatocytes
IRE elements	3'UTR (several)	none
Affinity for transferrin	high	low
HFE binding	yes	no
Knockout mouse	iron-deficient anemia lethal	iron overload
Mutations in humans	not reported	iron overload

TRFC: transferrin receptor; IRE: iron responsive elements; 3'UTR: 3' untranslated region.

deficient mouse[32] and urinary hepcidin is low/absent in patients with *TFR2* mutations.[33] Recent findings in a HEPG2 cell line indicate that TFR2 might be a sensor of transferrin saturation, a function compatible with its low affinity for transferrin,[4] as the TFR2 protein is stabilized in vitro in the presence of diferric transferrin.[34-35] Thus, if TFR2 modulates hepcidin, this effect would likely occur in response to transferrin saturation. TFR2 forms heterodimers with TFR1; these heterodimers might have a role in the sensing mechanism.[36] However, if both HFE and TFR2 modulate hepcidin, their activities must be distinct, since a normal TFR2 does not compensate for HFE inactivation in *C282Y* patients. Conversely, in TFR2-deficient subjects increased transferrin saturation does not induce hepcidin production through HFE. Interestingly digenic inheritance of mutations of *TFR2* and *HFE* may cause juvenile hemochromatosis.[37] The future challenge is to understand the mechanisms of hepcidin regulation by HFE and TFR2. As for now we are beginning to understand why we have two distinct transferrin receptors.

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References

1. Hentze MW, Muckenthaler MU, Andrews NC. Balancing acts: molecular control of mammalian iron metabolism. Cell 2004; 117:285-97.
2. Glockner G, Scherer S, Schattevoy R, Boright A, Weber J, Tsui LC, et al. Large-scale sequencing of two regions in human chromosome 7q22: analysis of 650 kb of genomic sequence around the EPO and CUTL1 loci reveals 17 genes. Genome Res 1998;8:1060-73.
3. Kawabata H, Yang R, Hirama T, Vuong PT, Kawano S, Gombart AF, et al. Molecular cloning of transferrin receptor 2. A new member of the transferrin receptor-like family. J Biol Chem 1999;274:20826-35.
4. Feder JN, Penny DM, Irrinki A, Lee VK, Lebron JA, Watson N, et al. The hemochromatosis gene product complexes with the transferrin receptor and lowers its affinity for ligand binding. Proc Natl Acad Sci USA 1998;95:1472-7.
5. Kawabata H, Germain RS, Vuong PT, Nakamaki T, Said JW, Koeffler HP. Transferrin receptor 2-α supports cell growth both in iron-chelated cultured cells and in vivo. J Biol Chem 2000;275:16618-25.
6. Fleming RE, Migas MC, Holden CC, Waheed A, Britton RS, Tomatsu S, et al. Transferrin receptor 2: continued expression in mouse liver in the face of iron overload and in hereditary hemochromatosis. Proc Natl Acad Sci USA. 2000;97:2214-9.
7. Levy JE, Jin O, Fujiwara Y, Kuo F, Andrews NC. Transferrin receptor is necessary for development of erythrocytes and the nervous system. Nat Genet 1999;21:396-9.
8. Camaschella C, Roetto A, Cali A, De Gobbi M, Garozzo G, Carella M, et al. The gene TFR2 is mutated in a new type of haemochromatosis mapping to 7q22. Nat Genet 2000;25:14-5.
9. Fleming RE, Ahmann JR, Migas MC, Waheed A, Koeffler HP, Kawabata H, et al. Targeted mutagenesis of the murine transferrin receptor-2 gene produces hemochromatosis. Proc Natl Acad Sci USA 2002;99:10653-8.
10. Deaglio S, Capobianco A, Cali A, Bellora F, Alberti F, Righi L, et al. Structural, functional, and tissue distribution analysis of human transferrin receptor-2 by murine monoclonal antibodies and a polyclonal antiserum. Blood 2002;100:3782-9.
11. Griffiths WJ, Cox TM. Co-localization of the mammalian hemochromatosis gene product (HFE) and a newly identified transferrin receptor (TfR2) in intestinal tissue and cells. J Histochem Cytochem 2003;51:613-24.
12. West AP Jr, Bennett MJ, Sellers VM, Andrews NC, Enns CA, Bjorkman PJ. Comparison of the interactions of transferrin receptor and transferrin receptor 2 with transferrin and the hereditary hemochromatosis protein HFE. J Biol Chem 2000; 275:38135-8.
13. Kollia P, Samara M, Stamatopoulos K, Belessi C, Stavroyanni N, Tsompanakou A, et al. Molecular evidence for transferrin receptor 2 expression in all FAB subtypes of acute myeloid leukemia. Leuk Res 2003;27:1101–3.
14. Calzolari A, Deaglio S, Sposi NM, Petrucci E, Morsilli O, Gabbianelli M, et al. Transferrin receptor 2 protein is not expressed in normal erythroid cells. Biochem J 2004;381:629-34.

15. Aguilar-Martinez P, Esculie-Coste C, Bismuth M, Giansily-Blaizot M, Larrey D, Schved JF. Transferrin receptor-2 gene and non-C282Y homozygous patients with hemochromatosis. Blood Cells Mol Dis 2001;27:290-3.
16. Barton EH, West PA, Rivers CA, Barton JC, Acton RT. Transferrin receptor-2 (TFR2) mutation Y250X in Alabama Caucasian and African American subjects with and without primary iron overload. Blood Cells Mol Dis 2001;27:279-84.
17. Lee PL, Halloran C, West C, Beutler E. Mutation analysis in the transferring receptor 2 gene in patients with iron overload. Blood Cell Mol Dis 2001;27:285-9.
18. Roetto A, Totaro A, Piperno A, Piga A, Longo F, Garozzo G, et al. New mutations inactivating transferrin receptor 2 in hemochromatosis type 3. Blood 2001;97:2555-60.
19. Girelli D, Bozzini C, Roetto A, Alberti F, Daraio F, Colombari R, et al. Clinical and pathologic findings in hemochromatosis type 3 due to a novel mutation in transferrin receptor 2 gene. Gastroenterology 2002;122: 1295-302.
20. Piperno A, Roetto A, Mariani R, Pelucchi S, Corengia C, Daraio F, et al. Homozygosity for transferrin receptor-2 Y250X mutation induces early iron overload. Haematologica 2004; 89:359-60.
21. Biasiotto G, Belloli S, Ruggeri G, Zanella I, Gerardi G, Corrado M, et al. Identification of new mutations of the HFE, hepcidin, and transferrin receptor 2 genes by denaturing HPLC analysis of individuals with biochemical indications of iron overload. Clin Chem 2003;49:1981-8.
22. Mattman A, Huntsman D, Lockitch G, Langlois S, Buskard N, Ralston D, et al.Transferrin receptor 2 (TfR2) and HFE mutational analysis in non-C282Y iron overload: identification of a novel TfR2 mutation. Blood 2002;100:1075-7.
23. Le Gac G, Mons F, Jacolot S, Scotet V, Ferec C, Frebourg T. Early onset hereditary hemochromatosis resulting from a novel TFR2 gene nonsense mutation (R105X) in two siblings of north French descent. Br J Haematol 2004;125:674-8.
24. Hattori A, Wakusawa S, Hayashi H, Harashima A, Sanae F, Kawanaka M, et al. AVAQ 594-597 deletion of the TfR2 gene in a Japanese family with hemochromatosis. Hepatol Res 2003;26:154-6.
25. Harris ZL, Takahashi Y, Miyajima H, Serizawa M, MacGillivray RT, Gitlin JD. Aceruloplasminemia: molecular characterization of this disorder of iron metabolism. Proc Natl Acad Sci USA 1995;92:2539-43.
26. Sohda T, Okubo R, Kamimura S, Ohkawara T. Hemochromatosis with HFE gene mutation in a Japanese patient. Am J Gastroenterol 2001;96:2487-8.
27. Ganz T. Hepcidin, a key regulator of iron metabolism and mediator of anemia of inflammation. Blood 2003;102:783-8.
28. Roetto A, Papanikolaou G, Politou M, Alberti F, Girelli D, Christakis J, et al. Mutant antimicrobial peptide hepcidin is associated with severe juvenile hemochromatosis. Nat Genet 2003;33:21-2.
29. Papanikolaou G, Samuels ME, Ludwig EH, MacDonald ML, Franchini PL, Dube MP, et al. Mutations in HFE2 cause iron overload in chromosome 1q-linked juvenile hemochromatosis. Nat Genet 2004;36:77-82.
30. Bridle KR, Frazer DM, Wilkins SJ, Dixon JL, Purdie DM, Crawford DH, et al. Disrupted hepcidin regulation in HFE-associated haemochromatosis and the liver as a regulator of body iron homoeostasis. Lancet 2003; 361:669-73.
31. Gehrke SG, Kulaksiz H, Herrmann T, Riedel HD, Bents K, Veltkamp C, et al. Expression of hepcidin in hereditary hemochromatosis: evidence for a regulation in response to the serum transferrin saturation and to non-transferrin-bound iron. Blood 2003;102:371-6.
32. Kawabata H, Fleming RE, Gui D, Moon SY, Saitoh T, O'kelly J, et al. Expression of hepcidin is down-regulated in TfR2 mutant mice manifesting a phenotype of hereditary hemochromatosis. Blood 2005;105:376-81.
33. Nemeth E, Roetto A, Garozzo G, Ganz T, Camaschella C. Hepcidin is decreased in TFR2-Hemochromatosis. Blood 2005; 105:1803-6.
34. Johnson MB, Enns CA. Regulation of transferrin receptor 2 by transferrin: diferric transferrin regulates transferrin receptor 2 protein stability. Blood 2004;104:4287-93.
35. Robb A, Wessling-Resnick M.Regulation of transferrin receptor 2 protein levels by transferrin. Blood 2004;104:4294-9.
36. Vogt TM, Blackwell AD, Giannetti AM, Bjorkman PJ, Enns CA. Heterotypic interactions between transferrin receptor and transferrin receptor 2. Blood 2003;101:2008–14.
37. Pietrangelo A, Caleffi A, Henrion J, Ferrara F, Corradini E, Kulaksiz H, et al. Juvenile haemochromatosis associated with pathogenic mutations of adult hemochromatosis genes. Gastroenterology 2005;128:470-9.

The future of anticoagulation clinics: a journey to thrombosis centers?

Coumarins were discovered in the late 1930s as a result of decades of research spent identifying the cause of a hemorrhagic disease in cattle. At first they were used as rat poison, but from the mid 1950s they began to have some clinical impact.[1] Since their efficacy was proved in several clinical studies,[2] the use of coumarins, in particular warfarin, has increased progressively in many countries. Concomitantly with their clinical use, there was a need for more precise laboratory control, since bleeding can at times be fatal. Over the years the prothrombin time, as a monitoring test to tailor the dosage of oral anticoagulants in the single patient, underwent a process of standardization, which was started in 1962 by Leon Poller.[3] In 1983 Kirkwood[4] proposed the international normalized ratio (INR) system, approved by the World Health Organisation. Despite a few limitations, the INR, recently reviewed by Poller,[5] is currently the standard way to express the result of a prothrombin time test, and has served to validate the efficacy of oral anticoagulants in a number of clinical studies.

The organization of anticoagulation clinics in Italy

The need for periodic monitoring and the complexity of the therapy have given rise to a whole culture on this topic and led to the creation of Centers for the surveillance of anticoagulation drugs in Europe and the US: the so-called anticoagulation clinics.

In Italy the Federation for the Surveillance of Anticoagulated Patients (FCSA)[6] was founded in 1989 with the aim of improving standardization in oral anticoagulation therapy in the country. From the initial 8 founding institutions, the Federation has grown into a network of more than 300 anticoagulation clinics spread over the country. From a survey performed by the FCSA in 2003 it emerged that these clinics were located in general laboratories (39%), trasfusion services (15%), or departments of internal medicine (10%), hematology (9%), cardiology (8%), and angiology (4%). A minority (15%) have declared that they are thrombosis services. A more detailed survey will be necessary to know exactly how Anticoagulation Clinics actually work in terms of activities other than the surveillance of oral anticoagulation. Each year national congresses, courses, and workshops are held for physicians, technicians, and nurses. Many studies have been conducted and published by FCSA Centers in the past few years. These have dealt with several aspects of oral anticoagulant therapy, such as hemorrhagic[7] and thrombotic complications,[8] atrial fibrillation,[9] different degrees of anticoagulation in prosthetic heart valves,[10] malignancy,[11] the elderly,[12] computerized therapy management,[13] and the patient's own point of view.[14]

A guide to oral anticoagulant therapy has recently been published.[15] Moreover, studies on portable coagulometers and on the management of therapy using these devices[16] have led to the publication of a consensus document by the FCSA on this topic.[17]

Nevertheless, for some time now a new concept has been taking shape: new anti-thrombotic drugs will replace Anticoagulation Clinics and perhaps cause their downfall, since it has been shown in clinical trials that these new drugs will probably render laboratory monitoring unnecessary in clinical practice. If we consider that