New *TFR2* mutations in young Italian patients with hemochromatosis

This work describes the identification of two subjects with young-age iron overload carrying new causative mutations in transferrin receptor-2 gene. One was compound heterozygous (Asn411del/Ala444Thr) and the second was homozygous for a mutation affecting RNA splicing (IVS17+5636G>A). Another mutation (His33Asn) and a polymorphism were found in a group of 50 controls.

Haematologica 2008 Feb; 93:(2)309-310. DOI: 10.3324/haematol.11942

Genetic analysis of hereditary hemochromatosis (HH) identified various genes whose mutations lead to iron overload.[1] HFE is responsible for the most common, adult onset form of HH, while HAMP and HJV genes are responsible for the juvenile types of HH, with fast and severe progression of iron overload.[1] Also transferrin receptor-2 (TfR2) is associated with HH in a few cases, often diagnosed at young age.[2] Furthermore, ferroportin is responsible for a different, dominant form of iron overload with variable iron deposition in hepatocytes or in reticuloendothelial cells.[1] Despite the improvements in genetic analyses of HH, identifying defects in large and complex genes like TfR2, which has 18 exons, remains difficult. So far only a limited number of subjects has been analysed for TfR2, with the identification of 17 different significant mutations.[2-10] In this study, we developed DHPLC conditions to detect all DNA variations in the 18 exons and flanking regions. They consisted in analysing 14 amplicons on a Transgenomic WAVE™ system at a minimum of 2 different temperatures. We initially studied the DNA from 50 Italian control subjects with normal transferrin saturation (TS) and serum ferritin (SF) values. We found an intronic polymorphism (IVS3+49C>A) and a c.DNA 97C>A transversion (Genebak access: NM_003227.3), causing the amino acid substitution His33→Asn. This modifies His33 in the cytosolic domain, which is conserved in mouse and dog TfR2, aspargine in rat, and it is not expected to have major structural consequences. The finding of two DNA variations in a small control group suggests that heterozygous TfR2 mutations may be rather common. In fact, 7 of the 21 mutations so far reported are at the heterozygous state (Figure 1) and their effects on TfR2 functionality are unknown. In a retrospective analysis of a small series of 33 patients with abnormally high SF and TS, and exclusion of HFE hemochromatosis or causes of secondary iron overload,[1] we identified two Italian cases with causative mutations (Table 1). Patient #1 was a 21-year-old woman who presented with symptoms of fatigue. She showed high values of TS (>100%) and of SF (~1,000 μg/L) and liver iron concentration (LIC) was relatively high for her age, but no signs of organ involvement. She was compound heterozygous for the c.1330G>A mutation in exon 10 and for the deletion of the triplet AAC (c.1231-3 del) in exon 9, which causes the substitution of Ala444→Thr and the deletion of Asn411 respectively. Asn411 is the first of a tandem of two asparagines and it is a serine in mouse, rat and dog TfR2. The equivalent residue in TfR1 (Ser388) is located in a beta-strand, and its deletion should have strong destabilizing effects. Ala444 is conserved in the TfR2 sequences, but the consequences of the substitution are unclear. Patient #2 was a 12-year-old boy with TS >100% and

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Table 1. Iron indices and TfR2 genotypes of the patients

Cases	*Sex*	*Age*	*Hb g/dL*	*Serum iron (μg/dL)*	*Transferrin saturation %*	*Serum Ferritin (μg /L)*	*ALT U/L*	*LIC[a] μg/g*	*TfR2 mutations*
1	F	21	13.1	275	>100	958	24	958	N411del / A444T
2	M	12	14	265	>100	158	66	464	IVS17+5636G>A[b]
3	F	13	13.1	266	64	19	31	202	IVS17+5636G>A[b]

[a]Evaluation of liver iron concentration (LIC) was performed non-invasively by superconducting quantum interference device (SQUID). Normal adult values: <400 μg/g tissue, wet weight bhomozygous mutation

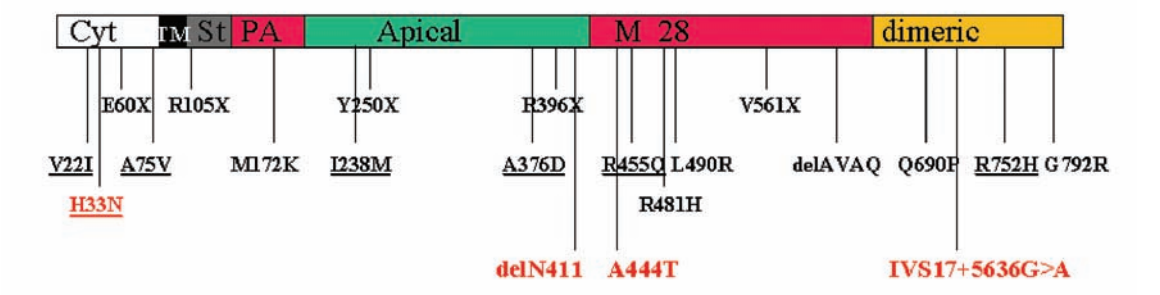

Figure 1. Amino acid mutations and predicted domain organization of TfR2. The domain organization of TfR2 was predicted by homology with the crystallographic structure of TfR1. The domains are indicated in the sequence: the cytosolic (Cyt), the transmembrane (TM), the stalk (St), the protease-like (PA), the apical (Apical), the peptidase M_28 like (M_28) and the dimerization (dimeric). The nonsense mutations are indicated in the upper line, the missense mutations are in the second line and those described in this work are in the third line. The mutants underlined are those identified only at the heterozygous state, the pathogenic role of which has not been established. Intronic DNA polymorphisms are not shown.

high SF and LIC values for his age. The 13-year-old sister (Patient 3) had high TS (64%) but normal SF and LIC (Table 1). They were both homozygous for the substitution IVS17+5636G>A, which modifies the last base of intron 17. It changes the GCA**G**/GTG sequence (where / indicates intron-exon boundary) into GCA**A**GTG. This is expected to affect intron splicing by altering the GU/AG rule and the consensus sequence of the 3'intron end, therefore causing an alteration of the C-terminal dimerization domain of the protein. This could not be experimentally verified, since TfR2 is not expressed in the circulating blood cells *obtained* from the patient. In conclusion, present data confirm that TFR2-related HH may occur at a young age and is characterized by high TS levels.[2-9] Therefore, TfR2 genotyping may be useful in selected patients with early-onset of iron overload to exclude those juvenile forms associated with HAMP or HJV mutations. This may important because iron loading and clinical course in TfR2-related HH are generally milder than in juvenile-HH,[3] although they may be more serious than in HFE-HH.

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Funding: this work was partially supported by MIUR-PRIN 06 grant to PA and by the EEC FP6 grant EU LSHM-CT-2006-037296 EuroIron1 to PA and CC.

Acknowledgments: we are grateful to Prof. A. Piga for Squid measurements.

Key words: hemochromatosis, iron overload, DHPLC.

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