Early-onset haemochromatosis caused by a novel combination of TFR2 mutations(p.R396X/c.1538-2 A>G) in a woman of Italian descent

Haemochromatosis (HC) refers to a group of inherited disorders of iron metabolism characterized by progressive iron accumulation in parenchymal cells. If not recognized and treated, iron loading impairs the function of target organs and damages their structure. HC includes the historical and predominant HFE-related condition, and rarer conditions which have more recently been associated with mutations in the hemojuvelin (*HJV*), hepcidin (*HAMP*) and transferrin receptor 2 (*TFR2*) genes. HC also involves certain mutations of the ferroportin gene (*SLC40A1*), albeit most of the *SLC40A1*-related patients display a distinct iron overload syndrome that is recognized as *the ferroportin disease*.[1]

In the present study, we aimed to identify the genetic basis of iron overload in a 28-year-old woman of Italian descent. The patient was referred to us, in the context of a premarital medical evaluation, because of high levels of serum iron (381 µg/dL; Normal range: 50-160), transferrin saturation (determined twice: at 80 and 93%, respectively) and serum ferritin (900 µg/L; N: 10-291). She did not present clinical manifestations of HC, except of astheny. Secondary causes of iron overload were excluded. The patient denied any history of alcohol abuse or blood transfusion, serologies for hepatitis A and C were negative, vaccination against hepatitis B had been performed, and hematological constants were in the normal range (hemoglobin: 13 g/dL, white blood cells: 7.15 10^9/L, platelets: 355x10^9/L). In addition, the patient had normal levels of liver enzymes (ASAT, ALAT, γ-GT) and normal blood sugar parameters, while moderate increases in C reactive protein concentration (8 mg/L, N<5) and Erythrocyte Sedimentation Rate (36, N<15) were observed. Because of the clearly elevated serum ferritin concentration, the hepatic iron overload was investigated by magnetic resonance imaging (MRI). It was estimated to be quite high (with a Hepatic Iron Content of 310±50 µg/L, N <36; the calculation being performed with the algorithm developed by Gandon Y. and collaborators,[2] for a magnetic field of 1.5 Tesla). Imaging further showed that iron deposits were homogeneously distributed. No liver biopsy was performed. At present, the patient is included in a phlebotomy program. Although 2.3g of iron had been removed, both the serum ferritin concentration (279 µg/L) and the transferrin saturation level (53%) remain elevated. Of note, the C reactive protein concentration had been normalized (1 mg/L).

After obtaining written informed consent, we searched for the p.C282Y, p.H63D and p.S65C HFE variations. As this specific screening was negative, we scanned the entire coding regions and splicing junctions of the five haemochromatosis genes. This allowed us to only detect two mutations in the *TFR2* gene (Genbank # NM_003227.3): a heterozygous C>T transition at nucleotide c.1186 in exon 9 (Figure 1A), and a heterozygous A>G transition at nucleotide c.1538-2, in intron 12 (Figure 1B). The c.1186 C>T substitution results in a premature stop codon at amino-acid 396 (p.R396X). This mutation is not new, since it has been previously reported in an haemochromatosis patient of Scottish descent.[3] The novel c.1538-2 A>G substitution abrogates the intron 12 acceptor splicing site as it changes the critical AG sequence into GG. This splicing mutation is expected to dramatically modify the *TFR2* reading frame, which encompasses 18 exons and ends with nucleotide triplets encodings the *TFR2* dimerization domain. This was not experimentally verified because of the paucity of TFR2 mRNA in circulating blood cells.

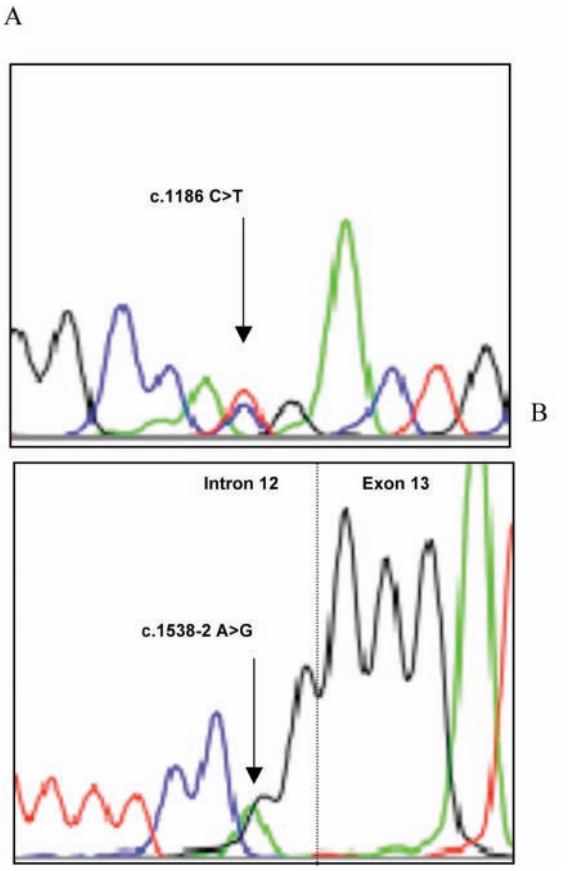

Figure 1. Sequencing electrophoregrams showing the mutated *TFR2* exon 9 (A) and intron 12 (B) forward heterozygous sequences (position of the c.1186 A>G and c.1538-2 A>G nucleotide change are indicated by arrows and the intron 12/exon 13 junction is delimited by dashes).

It is well recognized that iron overload phenotypes observed in HC are highly variable. Taking into account the influence of environmental factors and of potential modifier genes, one may consider that these phenotypes actually constitute a continuum. Nevertheless, different grades of severity can be attributed to the genetic heterogeneity of the disease, in particular when considering the respective roles of HC proteins in iron homeostasis. These grades range from the *HJV* and *HAMP*-related juvenile conditions to the typical adult-onset *HFE*-related condition, which is usually diagnosed in the fourth decade for men and the fifth for women. The iron overload course in *TFR2*-related patients is distinct from that

of the juvenile conditions, but it can be more rapid and impressive than usually observed in *HFE*-related patients. Indeed, based on the review of the 23 *TFR2*-related cases reported in the literature until 2004 and with considering a cohort of 421 *HFE* p.C282Y/p.C282Y homozygous patients, we previously showed that age at diagnosis in *TFR2*-related patients was significantly lower than in *HFE*-related patients. We also highlighted the descriptions of clinical features (including arthralgy, hypogonadism, skin pigmentation and cirrhosis) in *TFR2*-related patients before the age of 30 years.[4] Our observations have been reinforced by some recent reports, in which the identification of *TFR2* mutations was correlated with the description of either high serum iron parameters in young patients or severe clinical pictures in older patients[3,5-9] In a retrospective analysis of 33 cautiously selected Italian cases, Biasiotto and co-workers have more especially identified two children with new *TFR2* causative mutations.[9] The authors argued for a *TFR2* genotyping in patients with early-onset of iron overload, and they further stressed that the *TFR2*-related HC is characterized by high transferrin saturation levels. Furthermore, mice homozygous for the p.Y245X *TFR2* mutation (the murine ortholog of the human p.Y250X mutation 5 identified in the first *TFR2*-related pedigrees of HC) have proved to develop a more rapid hepatic iron overload than *HFE* knock-out mice of the same genetic background.[10]

In conclusion, it becomes more and more evident that *TFR2*-related HC is an intermediate syndrome between the typical adult-onset *HFE*-related HC and the two juvenile conditions. Thus, the search for *TFR2* mutations should be particularly considered in young patients with haemochromatosis which do not exhibit a juvenile condition.

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Fundings: this work was supported by grants from the Centre Hospitalier Universitaire de Brest (Programme Hospitalier de Recherche Clinique) and the conseil scientifique de l'Etablissement Français du Sang (projet 2003.19).

Acknowledgments: we thank Ms. Isabelle Gourlaouen for technical assistance, and Pr. Pierre Lehn for preparation of this manuscript.

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References

1. Pietrangelo A. Hemochromatosis: an endocrine liver disease. Hepatology 2007;46:1291-301.
2. Gandon Y, Olivié D, Guyader D, Aubé C, Oberti F, Sebille V, Deugnier Y. Non-invasive assessment of hepatic iron stores by MRI. Lancet 2004;363:357-62.
3. Lee PL, Barton JC. Hemochromatosis and severe iron overload associated with compound heterozygosity for TFR2 R455Q and two novel mutations TFR2 R396X and G792R. Acta Haematol 2006;115:102-5.
4. Le Gac G, Mons F, Jacolot S, Scotet V, Férec C, Frébourg 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.
5. 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.
6. Koyama C, Wakusawa S, Hayashi H, Suzuki R, Yano M, Yoshioka K, et al. Two novel mutations, L490R and V561X, of the transferrin receptor 2 gene in Japanese patients with hemochromatosis. Haematologica 2005;90:302-7.
7. Majore S, Milano F, Binni F, Stuppia L, Cerrone A, Tafuri A, et al. Homozygous p.M172K mutation of the TFR2 gene in an Italian family with type 3 hereditary hemochromatosis and early onset iron overload. Haematologica 2006;91:92-3.
8. Hsiao PJ, Tsai KB, Shin SJ, Wang CL, Lee ST, Lee JF, Kuo KK. A novel mutation of transferrin receptor 2 in a Taiwanese woman with type 3 hemochromatosis. J Hepatol 2007;47:303-6.
9. Biasiotto G, Camaschella C, Forni GL, Polotti A, Zecchina G, Arosio P. New TFR2 mutations in young Italian patients with hemochromatosis. Haematologica 2008;93:309-10.
10. 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.

Citation: Gérolami V, Le Gac G, Mercier L, Nezri M, Bergé-Lefranc J-L, Férec C. Early-onset haemochromatosis caused by a novel combination of TFR2 mutations(p.R396X/c.1538-2 A>G) in a woman of Italian descent. Haematologica 2008; 93:e45-e46 DOI: 10.3324/haematol.12884