Homozygous p.M172K mutation of the TFR2 gene in an Italian family with type 3 hereditary hemochromatosis and early onset iron overload

The p.M172K TFR2 mutation was identified in two Italian siblings aged 32 and 40 years old with primary iron overload. The two patients showed a severe increase in serum iron indices. From the age of 25, the male sib also revealed abnormal levels of hepatic enzymes, presumably in relation to iron induced liver damage. Clinical findings seem to evidence that type 3 hemochromatosis can be more serious than classic hemochromatosis. This report adds two more type 3 hereditary hemochromatosis cases which suggest that TFR2 mutations could be more frequently involved in non-HFE hemochromatosis than has been actually thought.

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Hereditary hemocromatosis (HH), previously regarded as a single gene disorder, has been recognised to be clinically and genetically heterogeneous. Five distinct genes, encoding proteins with different functions in iron metabolism, have been characterized to date. HFE[1] is responsible for the autosomal recessive classic hemochromatosis (HFE, MIM:235200). Both HJV[2] and HAMP[3] cause juvenile hemochromatosis (type 2A, MIM:602390 and type 2B, MIM: 606464), which is also an autosomal recessive disorder. Homozygous mutations in the transferrin receptor 2 (TFR2)[4] gene are associated with type 3 hemochromatosis (MIM# 604250), while the SLC40A1[5,6] gene, which encodes ferroportin 1, is related to an atypical autosomal dominant form of the disease (hemochromatosis type 4, MIM: 606069). TFR2 seems to account for a limited number of HH cases worldwide.[7] To our knowledge, only 11 different causative TFR2 mutations in a total of 14 Italian, Scottish, Japanese, Portuguese and French families have been detected to date 4,[8-16] (Table 1). Here we report on two siblings with Central Italian descent in whom we have identified, in the homozygous condition, the TFR2 p.M172K mutation, originally described in a family originated from the same Italian geographical area.[8] Patient N°1 is a 32 year old pregnant woman, born from apparently non-consanguineous parents both originating from the same rural village in Abruzzo, Central Italy. She was referred to us, in the course of her second pregnancy, because of high levels of transferrin saturation (106%), serum iron (247 μg/mL) and serum ferritin (737 ng/mL). The patient's laboratory findings are summarised in table 2. One of her brothers (patient N°2), aged 40, successively enrolled for clinical investigation and molecular analysis. He had shown augmented liver enzyme serum concentration since the age of 25. As reported in Table 2, in this subject, serum iron indexes were severely increased. Secondary causes of iron overload, such as haematological diseases, aceruloplasminemia, dysmetabolic iron overload syndrome and a history of chronic iron supplementation were all excluded in the proband and in her brother. In the male sib, we also ruled out abuse of alcohol consumption and other causes of liver damage. Due to the fact that the same patient declined liver biopsy and nuclear magnetic resonance (NMR), it was not possible to assess his amount of hepatic iron overload and tissue damage. After

Table 1. Previously reported and present case TFR2 causative

DNA variation	*Protein change*	*N° families*	*Type*	*Country*	*Exon*	*References*
c.84-88insC°	E60X	1	Frameshift	Southern Italy	2	Roetto et al, 20018
c.313C>T°	R105X	1	Nonsense	Northern France	2	Le Gac et al, 200412
c.515T>A°	M172K	1 1	Missense	Central Italy Central Italy	4	Roetto et al, 20018 Present report
c.750C>G°	Y250X	2 2	Nonsense	Sicily Sicily	6	Camaschella et al, 20004 Piperno et al, 200413
not described°	Q317X	1	Nonsense	Southern Italy	8	Pietrangelo et al, 200515
c.1186C>T*	R396X	1*	Nonsense	Scotland^	10	Lee and Barton, 200616
c.1469T>G°	L490R	1	Missense	Japan	11	Koyama et al, 200514
c.16665delC°	V561X	1	Deletion	Japan	14	Koyama et al, 200514
c.1780-1791del°	AVAQ594-597del	1 1	Deletion	Northern Italy Japan	16	Girelli et al, 20029 Hattori et al, 200310
c.2069A>C°	Q690P	1	Missense	Portugal	17	Mattman et al, 200211
c.2374G>A*	C792R	1*	Missense	Scotland^	18	Lee and Barton, 200616

^The patient was reported as being of Scottish descent; ° Mutation detected in the homozygous condition; * TFR2 p.R396X and p.C792R were detected in the heterozygous condition in the same patient. The described individual was shown to carry also the heterozygous p.R455Q variant, previously described as a probable polymorphism.

Table 2. *Main biochemical data of our patients*

Patient	*Age (YR)*	*Sex*	*Iron (NV: 50-150 μg/dL)*	*TS° (NV:20-45%)*	*Ferritin (NV:15-200;300 ng/mL)*	*RBC (NV:4.6-5.6x10⁶/mm³)*	*Hb (NV:12-16 g/L)*	*MCV (NV: 80.7-95.5fl)*	*ALT (NV :5-35 U/L)*	*AST (NV :0-35 IU/L U/l)*	*γGT (NV: 5-49U/l)*
N.1	32§	F	239	>100	737	3,99	13,4	101,3	21	32	17
N.2	40	M	257	>100	3415	4,35	14,5	100	93	112	117

°TS= transferrin saturation; §Blood sample was taken from the patient during her second pregnancy.

obtaining written, informed consent, analysis of hereditary hemochromatosis-associated mutations was performed as previously described,[17] using a non radioactive reverse hybridization assay, able to identify the following mutations: p.C282Y, p.H63D, p.S65C, p.V53M, p.V59M, p.Q127H, p.E168Q, p.E168X, p.W169X, p.Q283P, p.P160fs (HFE gene); p.Y250X, p.E60X, p.M172K, p.A594_Q597del (TFR2 gene); p.N144H, p.V162del (SLC40A1 gene) (Haemochromatosis Strip Assay, Nuclear Laser Medicine, Settala, Milan, Italy). The molecular study showed the homozygous condition for the TFR2 p.M172K mutation in both the brother and sister. We did not find this TFR2 missense variant in 200 healthy control subjects of Central Italian descent. Although this data indicates the rarity of the p.M172K TFR2 mutation in the investigated population, it was retrieved by us for the second time in a geographical area in which primary iron overload is often not related to HFE p.C282Y mutation.[18,19] This suggests a possible role of the TFR2 gene, at least in some clustered areas. Therefore, the analysis of the entire coding region and exon/intron boundaries of TFR2 gene could lead to the identification of new type 3 hemochromatosis cases. Patients that could be studied are those HFE negative, with increased serum iron indices and evidence of the presence of hepatic iron overload assessed by liver biopsy or by hepatic NMR. In line with some recent reports,[12,13,16] which indicate that type 3 hemochromatosis can be more rapid and severe in comparison to HFE-related hemochromatosis, both our patients showed a marked increase of iron overload indexes (Table 2). In addition, the male sib developed hepatic damage, presumably iron-related, at an early age. Both sibs revealed a remarkably high transferrin saturation, a finding already recognised as a common feature in humans and mice lacking TFR2.[20,21] These and other recent data suggest that some TFR2 mutations are able to cause *per se* a severe phenotype, although it is possible that, at least in some cases, the unusual course of type 3 hemochromatosis could be due to the presence of undetected variants in other genes or to environmental factors.[3,9,20,22] We conclude that, although HH type 3 is a rare hereditary disorder, systematic investigations of the whole TFR2 gene in patients with unexplained iron overload could lead to the identification of the faulty gene in a number of patients. Further studies are required to verify the presence of TFR2 mutations in defined populations worldwide and to provide better genotype-phenotype correlations.

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