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HAMP promoter mutation nc.-153C>T in non p.C282Y homozygous patients with iron overload

In a recent paper, Island and colleagues[1] described a heterozygous hepcidin (*HAMP*) promoter mutation, nc.-153C>T, which in association with *HFE* p.C282Y homozygosity appeared to lead to very severe iron overload (IO). They demonstrated *in vitro* that this *HAMP* mutation decreases transcriptional activity of the hepcidin promoter, alters IL6 total responsiveness and impairs binding of the SMAD protein complex to the BPM-RE.

HFE genotypes other than the homozygous p.C282Y (YY) or the compound heterozygous state for the p.C282Y and p.H63D mutations are not considered responsible for causing symptomatic forms of IO. This led us to re-evaluate 30 unrelated patients with non-YY *HFE* genotype associated with the diagnostic criteria of hemochromatosis who were referred to our laboratory. Among them, 12 were previously reported H63D homozygotes.[2] The diagnosis of IO was based on either liver biopsy and/or therapeutic criteria (phlebotomies). Informed written consent was obtained from all patients according to the French regulation.

Table 1. Characteristics of 3 iron loaded male patients with the *HAMP* nc.-153 C>T.

Patient #	HG224	HG707	HG118
Age at diagnosis (years)	54	75	69
HFE genotype			
• p.C282Y	absent	absent	absent
• p.H63D	homozygote	homozygote	absent
• p.S65C	absent	absent	heterozygote
• other	none	none	none
Serum ferritin (µg/L)	1270	2220	2330
Transferrin Saturation (%)	51	95	90
Liver biopsy	yes	yes	yes
• Cirrhosis	no	no	yes
• Fibrosis	yes	no	
• LIC (µmole/g)	145	193	565
• LIC/age	2.7	2.6	8.3
Clinical manifestations possibly related to IO	dark skin joint pain hepatomegaly	fatigue joint pain hepatomegaly	dark skin hypertrophic cardiomyopathy
Other			
• Alcohol	yes	stopped 2 years ago	NA
• Viral hepatitis	no	no	NA
• Overweight	BMI: 26	BMI: 30.5	NA
Family history of IO or hemochromatosis	yes	no	yes
Phlebotomies	>5 g iron removed	>5 g iron removed	regularly over 4 years

LIC: liver iron concentration; BMI: body mass index; NA: not available.

DNA sequencing of the *HAMP* gene found no mutation in the coding sequence or in the exon-intron junctions of the 30 patients. However, two gene substitutions, a C to T replacement at position -153 upstream of the ATG and a C to T substitution in intron 1 at position -66, 5' to exon 2 (c.91-66C>T, rs#2293689) were identified in 3 patients. Both sequence alterations were found in all 3 patients but we could not confirm whether they were in linkage disequilibrium or whether they were inherited on different chromosomes, as family segregation could not be performed. The patients, 3 white men living in different cities of the Southern part of France, were referred separately by three different physicians. To the best of our knowledge the men are not related. They all had ferritin levels over 1000 µg/L and transferrin saturation over 50%. They had been regularly phlebotomized for more than one year. Two of them, a 75-year old and a 54-year old male respectively, were p.H63D homozygotes (Table 1). The third patient, diagnosed at 69 years of age, was simply heterozygote for the p.S65C substitution on the *HFE* gene. His medical record indicated that he had myocardiopathy, cirrhosis and a liver biopsy compatible with the diagnosis of hemochromatosis (Table 1). Two patients had a family history of iron overload. Both substitutions, nc.-153C>T and c.91-66T>C, were also found in one out of 224 unrelated control chromosomes from white individuals of the same region. The iron status of this anonymous control was not available. The *HAMP* nc.-153C>T mutation was thus present at a high allele frequency (0.05) in this series of non-YY iron loaded patients. It was also found in one control subject, with a low allele frequency of 0.004 in this group (P=0.008).[3]

Barton and colleagues[4] did not detect *HAMP* nc.-153C>T in any of 191 *HFE* p.C282Y homozygotes from the HEIRS (Hemochromatosis and Iron Overload Screening) Study. They also screened a control group with various ethnic backgrounds, including non-Hispanic whites, Hispanics, blacks and Asian subjects, and found this mutation in only one Hispanic woman with apparently no IO at a control sample. They concluded that routine testing to detect *HAMP* nc.-153C>T is not indicated in population-based hemochromatosis and IO screening programs in North America. However, the data presented here indicate that in a selected sample of iron loaded individuals, especially those with *HFE* non-YY genotypes, the search for this mutation could be of interest as it can possibly explain IO in certain patients. Indeed, we found that 2 out of 12 iron loaded H63D homozygotes[2] had the *HAMP* nc.-153 C>T as a potential genetic factor of IO. Screening for this mutant may be, therefore, indicated in this particular population, or for individuals with high transferrin saturation and unexplained IO, as suggested by Loréal.[4]

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A novel *XIAP* mutation in a Japanese boy with recurrent pancytopenia and splenomegaly

X-linked lymphoproliferative syndrome (XLP) is a rare inherited primary immunodeficiency. It is clinically characterized by hemophagocytic lymphohistiocytosis (HLH), which usually develops in response to an Epstein-Barr virus (EBV) infection, dysgammaglobulinemia and malignant lymphoma. Most cases of XLP are caused by mutation in the SLAM-associated protein (SAP) or *SH2D1A*[1-3]. The X-linked inhibitor of apoptosis (XIAP) is also reported to cause XLP,[4] and at least 9 families with XIAP deficiency have been reported in Europe and the United States.[4,5] This report describes a Japanese boy with recurrent HLH and splenomegaly, and identifies a novel mutation in the *XIAP* gene.

A 3-year old boy was admitted to Kyoto Prefectural University Hospital because of fever and pancytopenia. The patient presented with splenomegaly, lymphadenopathy and pancytopenia at the age of 20 months. Epstein-Barr virus (EBV) serological tests disclosed a primary EBV infection. He was clinically diagnosed with EBV-associated HLH, and was treated with prednisolone and thereafter showed some improvement. The patient again presented with pancytopenia at the age of 23 months followed by a measles-rubella vaccination. He had frequently experienced mild pancytopenia and splenomegaly followed by infections. A physical examination at the time of admission revealed a temperature of 38.2°C, a right cervical lymphadenopathy and splenomegaly, but neither hepatomegaly nor skin eruptions. Laboratory tests showed a white blood cell count of 3.1×10^9/L with 44% neutrophils, 52% lymphocytes, 2% monocytes and 2% atypical lymphocytes, hemoglobin 10.0 g/dL, platelets of 111×10^9/L, lactate dehydrogenase 1,261 IU/L, ferritin 2,240 ng/mL (normal: <480), triglyceride 220 mg/dL (normal: <149), and soluble interleukin-2 receptor 1,700 U/mL (normal: <466). The serum immunoglobulin levels were within normal ranges (IgG; 1,362 mg/dL, IgA; 129 mg/dL, IgM; 175 mg/dL). EBV-DNA was detected in whole blood but showed low copies (48 copies/μgDNA).

The patient was clinically diagnosed with mild HLH, and he was first treated with prednisolone alone, but had only a partial response. Treatment with cyclosporine A and dexamethasone improved his condition and resulted in a decreased spleen size. The patient is scheduled to receive hematopoietic stem cell transplantation to achieve a complete remission in the near future.

Although the patient had no family history of HLH, his recurrent episodes of HLH implied that this might be caused by a genetic defect. No mutations were identified in the causative genes for familial HLH including perforin, Munc13-4 and syntaxin 11. Another possible genetic disease was XLP. The flow cytometric detection of SAP and XIAP was used to screen for XLP, as previously described.[5,6,7] The patient showed normal expression of SAP in lymphocytes, but clearly had deficient expression of XIAP, suggesting XIAP deficiency (Figure 1). Intriguingly, his mother showed bimodal expression of XIAP protein in lymphocytes, suggesting an obligate carrier. A gene analysis of *XIAP* was performed with parental informed consent, which disclosed a novel nonsense mutation (840C>T, R238X) in the patient (*data not shown*). The mother showed heterozygous alleles in this position.

The causative gene for XLP was *SAP* or *SH2D1A* (type 1)[1-3] and *XIAP* (type 2).[4] Both types of XLP can be identified by genetic analysis even in a sporadic case. However, the genetic analysis is labor-intensive and time-consuming. A flow cytometric screening for XIAP deficiency has been recently reported.[5] The present case was first screened by flow cytometry and was later confirmed by genetic analysis. The patient had 840C>T, thus resulting in R238X. Nine mutations have been identified in the *XIAP* gene (2 missense mutations, 3 nonsense mutations, one small deletion and 3 large deletions).[4,5] The nonsense mutations include Q104X, E118X and Q333X and also R238X which is a novel mutation.

Both SAP deficiency and XIAP deficiency result in XLP, but they are described as clinically indistinguishable. However, lymphoma has so far not been described and only about 50% had EBV-HLH in XIAP deficient patients.[4] In addition, patients with XIAP deficiency often have splenomegaly, unlike patients with SAP deficiency. Clinical manifestations of splenomegaly may be typical signs of XIAP deficiency, as demonstrated in the present case. EBV-HLH in SAP deficiency usually results in a fatal course, but the present case showed a mild course of EBV-HLH. EBV-HLH in XIAP deficiency may, therefore, show a milder presentation than that of SAP deficiency. To clarify the differences in the clinical picture of SAP deficiency and XIAP deficiency, a greater number of patients with XLP should be surveyed.

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