

Screening for hemochromatosis in a population with abnormal iron status

Hereditary hemochromatosis (HH) is a common autosomal recessive disorder of iron metabolism. The aim of this study was to screen a population of 339 subjects with abnormal iron status for the most common HH gene mutations in order to assess the incidence of each type of molecular alteration. A high frequency of HH gene mutations (55.5%) was found in our population.

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Iron overload is frequently associated with hereditary or secondary alterations of iron metabolism.^{1,2} Hereditary hemochromatosis (HH), the most common genetic disease among northern European populations, is an autosomal recessive disorder characterized by an enhanced gastrointestinal absorption of iron that leads to progressive increase of iron stores and, eventually, to multiple organ dysfunction. In 1996 a gene involved in HH pathogenesis, called HFE, was identified on the short arm of chromosome 6. Two missense mutations in the HFE gene, C282Y and H63D, were found to be responsible for most cases of HH.^{2,3} More recently other HFE and non-HFE mutations were discovered, allowing the number of cases of hemochromatosis defined as idiopathic to be reduced further.^{4,5} However, the etiology of iron overload is still unclear in many cases.⁶

In this study we analyzed the prevalence of C282Y and H63D mutations in a population with abnormal iron status. In addition, we evaluated the impact of 10 further mutations in

the HFE and Tfr2 genes on iron overload. Between October 2000 and December 2002, we tested 339 individuals with increased serum ferritin levels and/or transferrin saturation for HH gene mutations. Of these subjects 237 were male and 102 female; M/F ratio 2.3; their median age was 50.4 years, range 10-85 years; 164 were inpatients and 175 outpatients. Their mean serum ferritin level was 808.1 µg/L (range 9-5250) [normal range: 15-200 µg/L for women and 20-300 µg/L for men]. Their mean transferrin saturation was 48.9% (range 2.8-99%) [normal range 16-45%]. One hundred and forty-eight subjects (43.7%), analyzed between October 2000 and September 2001, were screened for the C282Y and H63D mutations in the HFE gene only. From October 2001 to December 2002, 191 individuals (56.3%) were tested for the presence of 12 mutations (C282Y, V53M, V59M, H63D, H63H, S65C, Q127H, E168Q, E168X, W169X, Q283P mutations in the HFE gene, and Y250X in the Tfr2 gene). Seventy-one subjects were related and members of 23 families, the remaining 268 were unrelated.

Regardless of the presence or the type of HH mutations, abnormal iron status was more frequent in men than in women (M/F ratio 2.3) and serum ferritin levels and transferrin saturation were significantly lower in women than in men (669.6 [range 11-3,823 µg/L] vs 862.5 [range 9-5,250 µg/L] µg/L for serum ferritin, $p = 0.03$ and 44.8 [range 2.8-90%] vs 50.0% [range 5.8-99%] for transferrin saturation, $p = 0.01$). One hundred and eighty-eight of the 339 subjects (55.5%) had at least one of the analyzed HH gene mutations. One hundred and three of the 188 cases (57.5%) with a documented HH mutation also had another disease (Table 1). Among the 188 HH positive subjects, 48 (14.2%) were heterozygous for C282Y, 14 (4.1%) homozygous for C282Y, 97 (28.6%) heterozygous for H63D, 14 (4.1%) homozygous for H63D, 11 (3.3%) compound heterozygous for C282Y/H63D, 2 (0.6%) heterozygous for S65C and 2 (0.6%) homozygous for Y250X.

Table 1. Relationship between diseases associated with secondary iron loading and hemochromatosis genotypes in 339 individuals enrolled in the study.

Associated diseases	No mutations n (%)	C ⁺ /H ⁻ n (%)	C ⁻ /H ⁻ n (%)	C ⁻ /H ⁺ n (%)	C ⁻ /H ⁻ n (%)	C ⁺ /H ⁺ n (%)	S65C ⁺ / n (%)	Tfr2 ⁺ / n (%)	Total n (%)
No diseases	20 (13.2)	12 (85.8)	26 (54.3)	7 (50.0)	29 (29.9)	9 (81.8)	0	2 (100.0)	105 (30.9)
Oncohematologic diseases*	41 (27.2)	1 (7.1)	5 (10.4)	4 (28.6)	9 (9.3)	1 (9.1)	2 (100.0)	0	63 (18.6)
Other hematologic diseases*	8 (5.3)	0	0	0	8 (8.2)	0	0	0	16 (4.7)
Oncologic diseases ^o	9 (6.0)	0	3 (6.2)	0	9 (9.3)	1 (9.1)	0	0	22 (6.5)
Alcoholic liver disease	20 (13.2)	0	3 (6.2)	1 (7.1)	12 (12.4)	0	0	0	36 (10.6)
Hepatitis C virus infection	35 (23.2)	1 (7.1)	7 (14.6)	2 (14.3)	19 (19.6)	0	0	0	64 (18.9)
Inflammatory diseases ^s	13 (8.6)	0	4 (8.3)	0	11 (11.3)	0	0	0	28 (8.3)
Dysmetabolic iron overload disease	5 (3.3)	0	0	0	0	0	0	0	5 (1.5)
Total	151 (100.0)	14 (100.0)	48 (100.0)	14 (100.0)	97 (100.0)	11 (100.0)	2 (100.0)	2 (100.0)	339 (100.0)

*Oncohematologic diseases (63 cases): acute leukemia (43 cases), myelodysplastic syndrome (4 cases), non-Hodgkin's lymphoma (6 cases), Hodgkin's lymphoma (2 cases), chronic myeloid leukemia (2 cases), multiple myeloma (2 cases), chronic lymphocytic leukemia (2 cases), aplastic anemia (1 case), and polycythemia vera (1 case). *Other hematologic diseases (16 cases): autoimmune hemolytic anemia (1 case), intermediate β -thalassemia (1 case), β -thalassemic trait (11 cases), sickle cell anemia (1 case), and cutaneous porphyria (2 cases). ^oOncologic diseases (22 cases): liver cancer (17 cases), pancreatic cancer (1 case), gastric cancer (2 cases), and prostate cancer (2 cases). All cases of liver cancer were associated with chronic hepatitis C virus infection or alcoholism. ^sInflammatory diseases (28 cases): collagen disease (14 cases), inflammatory bowel disease (6 cases), and inflammatory neuropathy (8 cases).

Subjects homozygous for C282Y or Y250X were significantly younger (36.5 and 34.0 years vs 47.5 and 54.0, respectively, $p < 0.001$) and had significantly higher ferritin levels and transferrin saturation (1134.5 $\mu\text{g/L}$ [range 68–3823 $\mu\text{g/L}$], 70.4% [range 12.6–96%] for individuals homozygous for C282Y and 900.0 $\mu\text{g/L}$ [range 649–1151 $\mu\text{g/L}$], 90.0% [range 81.3–98.6%] for individuals homozygous for Y250X vs 597.6 $\mu\text{g/L}$ [range 9–2737 $\mu\text{g/L}$], 46.2% [range 2.8–94.0%] for individuals with other mutations, $p < 0.001$) than those showing other mutations. We did not find any difference in the frequency of HH gene mutations in the two groups of patients who were screened for 2 (82/148, 55.4%) or 12 (102/191, 53.4%) mutations; in fact, the analysis of 12 HH mutations identified only 4 HH gene mutations (C282Y, H63D, S65C in the HFE gene and Y250X in TFR2 gene) in our population, selected for having an abnormal iron status. This evidence raises the question about the utility and costs of this kind of screening in the general population. As regards the 151 subjects lacking HH mutations, in most cases (86.8%) iron overload was associated with other diseases, such as hepatitis C virus infection, alcoholism or systemic diseases which are known to have a role in iron accumulation. Although we did not find any correlation between the presence of liver diseases or solid cancers and HH gene mutations, we observed a high frequency of β -thalassemic trait in heterozygous subjects for H63D mutation (7/97, 7.2%). Increased iron turnover can be easily explained in patients with intermediate thalassemia because of the presence of chronic hemolysis, but is more difficult to explain in β -thalassemia gene carriers, unless it is assumed that H63D mutation may have a modulating effect on iron absorption in these subjects.^{7,8} In conclusion, our data confirm the high frequency (55.5%) of HH gene mutations in subjects with abnormal iron status.

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Possible association between reactive oxygen metabolites and karyotypic abnormalities in myelodysplastic syndromes

We demonstrated a significant increase in reactive oxygen metabolites (ROMs), the direct mediators of oxidative damage, in a panel of 50 patients with myelodysplastic syndrome (MDS). Interestingly, increased ROMs were associated with karyotype abnormalities. ROM concentrations may, therefore, represent an easily assessable indicator of potential oxidation in MDS.

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Oxidative damage is caused by an imbalance in pro- and anti-oxidant ratio. Oxygen metabolites (ROMs) represent direct mediators of oxidation. The anti-oxidant system, defined by total antioxidant activity (TAT), comprises antioxidants and repair enzymes, which limit tissue damage.^{1–3} Myelodysplastic syndromes (MDS), clonal disorders with peripheral blood cytopenias and normal or hypercellular dysplastic bone marrow (BM), may progress to acute leukemia. Oxidative stress may play a pathogenic role in MDS,^{4–6} particularly in the genesis of cytogenetic lesions.⁷ We investigated the association of MDS and oxidative status by measuring ROMs, homocysteine (acting through ROM production),³ and TAT in a series of 50 MDS patients.

Thirty males, 20 females (M:F=1.5; aged 26–100 years, median age 75), were observed. According to French-American-British (FAB) classification and the International Prognostic Score System (IPSS), patients were distributed as follows: 16 had refractory anemia (RA), 8 had RA with ringed sideroblasts (RARS), 18 had RA with excess blasts (RAEB), 5 had RAEB in transformation (RAEB-t) and 2 had chronic myelomonocytic leukemia (CMML), 1 hypoplastic MDS; 15 LOW, 19 INT-1, 8 INT-2 and 6 HIGH risk (in 2 patients IPSS was not applicable because of unsuccessful karyotyping). Karyotype was normal in 31 patients and complex in 6; in the remaining cases single chromosome abnormalities were present (chromosomes 5, 7, 8, 20 or Y; Table 1). No significant co-morbidity was present. Fourteen healthy volunteers (M:F=1.3), aged 30–96 years, median 72, represented controls. Plasma total homocysteine (cut-off 10 $\mu\text{mol/L}$), serum vitamins B12, folate (plus erythrocyte), ROM concentrations, measuring hydroperoxides, and TAT, measuring antioxidant species, including GSH, GSH peroxidase, thiol groups, superoxide dismutase, and catalase, were determined as previously described.^{8–10} The ROM cut-off (300 U.Carr) and TAT reference interval (1.5–2 nmol/L) were calculated in controls. Vitamin B12 and folate concentrations were within the relevant reference intervals both in patients and controls (*data not shown*). MDS patients had higher mean plasma homocysteine levels than did controls (10.12 $\mu\text{mol/L}$ vs 8.4 $\mu\text{mol/L}$; $p=0.016$; t-test) (Table 1). Increased ROM concentrations were present in 44% (22/50) of the MDS patients and in 21% (3/14) of the controls. MDS patients showed significantly higher mean serum ROM levels than controls (335.54 U.Carr. vs 259.57 U.Carr.;