Haemochromatosis and HFE gene

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Abstract

The discovery of the *HFE* gene has improved classification and diagnosis of iron overload. Most patients with a phenotypic diagnosis of haemochromatosis are homozygote for the C282Y mutation. Among those with other genotypes, only compound heterozygotes, who present the C282Y mutation on one chromosome and the H63D on the other, may present with haemochromatosis, but with a low penetrance and a mild expression. Other patients usually present with another cause of iron overload, such as insulin resistance, alcoholic liver disease or liver cirrhosis. The practical management of haemochromatosis has been greatly modified, since liver biopsy is no more necessary for diagnosis in C282Y homozygotes, and is only needed for exclusion of cirrhosis. Family screening has also greatly benefited from genotyping.
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Key words: chromosomes, human, pair 6; haemochromatosis/*diagnosis/*genetics; HFE gene; mutation.

Genetic haemochromatosis is characterised by increased iron absorption, leading to accumulation of iron in liver and other organs and ultimately to cirrhosis, diabetes, skin pigmentation, cardiac insufficiency and osteoarticular disease. The demonstration of the autosomal recessive transmission of the disease was done by Simon *et al.* in 1975 (1), and the responsible gene — named HFE — was discovered only in 1996 by Feder *et al.* (2).

1. Genetic

1.1. *HFE gene and haemochromatosis*

1.1.1. Link between HFE gene and iron overload (3)

The *HFE* gene is located on the short arm of chromosome 6, 4.5 megabases distant from *HLA-A* gene. The HFE protein has 343 amino acids, and its predicted structure has strong homologies with HLA class I proteins. Two mutations have been described: the C282Y mutation is characterised by a cysteine-to-tyrosine substitution at amino acid 282, and the H63D mutation by a histidine -to-aspartate substitution at amino acid 63. These two mutations are in complete linkage disequilibrium.

There are now good arguments in favour of the implication of the C282Y mutation of the *HFE* gene in haemochromatosis. (i) 83% of patients in the *princeps* study were homozygote for the C282Y mutation (2), and all series have confirmed the strong prevalence of C282Y homozygosity in haemochromatosis (Table I). (ii) The predicted structure of the protein gives an important functional role to C282Y mutation, which abolished the liaison of the HFE protein with beta-2-microglobulin (ß2M) (2); interestingly, the ß2M knocked-out mice present with spontaneous iron overload (4). (iii) *HFE* knocked-out mice have been obtained and present also with progressive iron overload (5).

Molecular studies had shown that the HFE protein is located in cryptic enterocytes, with a particular distribution compatible with a localisation in endosomes. The normal protein links to ß2M in Golgi apparatus (6). It has been demonstrated in placenta that wild HFE associates not only with ß2M, but also with transferrin receptor, and decreases the affinity of transferrin receptor to transferrin, which is the main transporter of iron in serum (7). These interactions are abolished by the C282Y mutation. Research is in progress to finally elucidate the links between these informations and increased iron absorption.

1.1.2. Prevalence of HFE gene mutations in general population

Table II showed prevalence of the C282Y and H63D mutations in European populations. C282Y mutation is not found in non-caucasoid populations (8-10). In Europe, there is a decreasing gradient from North to South: the highest frequencies are found in Brittany and in Ireland, which fits well with the hypothesis of a founder effect of Celtic origin, and the lowest in Basks. The H63D mutation is very prevalent in most populations. In Brittany, 25% of subjects in the general population are heterozygote and 2% homozygote. As a whole, 40% of a European descent population carry at least one HFE mutation.

1.1.3. Prevalence of HFE gene mutations in phenotypically defined hemochromatosis

1.1.3.1. Hemochromatosis phenotype

The diagnostic criteria for hemochromatosis are variable and have evolved with time. This could explain some discrepancies in results. These criteria are presented in Table III.

1.1.3.2. C282Y

In all caucasoid populations, a prevalence of C282Y homozygosity was found in 80 to 100% of hemo-

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Table I. — **Prevalence of C282Y homozygosity and compound heterozygosity in patients with phenotypic haemocromatosis**

Country	Number of patients	C282Y Homozygotes n (%)	compound n (%)	Juvenile Haemochromatosis	strict criteria (1)	reference
USA	178	148 (83.1)	8 (4.5)	?	no	(2)
Australia	112	112 (100.0)	0	0	yes (2)	(12)
France (South)	94	68 (72.3)	4 (4.3)	?	?	(60)
France (Brittany)	478	388 (81.2)	32 (6.7)	?	no	(61)
UK	115	105 (91.3)	3 (2.6)	2 (1.7)	no	(62)
USA (Rochester)	61	41 (67.2)	5 (8.2)	0	no	(63)
Austria	40	31 (77.5)	3 (7.5)	2 (5.0)	no	(64)
England (East)	18	18 (100.0)	0	0	no	(65)
USA (Alabama)	74	44 (59.5)	4 (5.4)	0	no (3)	(66)
France (Brittany)	132	122 (92.4)	3 (2.3)	0	yes	(67,68)
Germany	57	51 (89.4)	2 (3.5)	0	no	(69)
Italia	188	120 (63.8)	10 (5.3)	0	yes	(14,70)
Canada	128	122 (95.3)	0	2 (1.5)	no	(71)
Spain	31	27 (87.1)	2 (6.5)	0	yes	(72)
France	61	41 (67.2)	4 (6.6)	?	?	(73)
Ireland	30	27 (90.0)	0	0	no	(74)
Germany (North)	92	87 (94.6)	4 (4.3)	0	no	(75)
Sweden	87	80 (91.9)	3 (3.4)	0	yes	(76)
Ireland	78	70 (89.7)	3 (3.8)	1 (1.3)	no	(77)
France (Brittany)	217	209 (93.3)	4 (1.8)	0	yes	(11)

(1) Strict phenotypic criteria (as defined in table II).
(2) HLA linked familial transmission demonstrated in all patients.
(3) criteria : TS > 60% in men and 50% in women. 82% of patients are C282Y homozygote when HII is greater than 2.

Table II. — **Prevalence of C282Y and H63D mutations in European populations**

origin	C282Y genotype		allelic frequency	reference
	homozygote	heterozygote		
Brittany	0.50	12.00	6.5	(78)
Scandinavia	0.72	8.72	5.1	(79)
Brittany	0.79	17.46	9.4	(80)
Spain		7.41	3.7	(81)
Basks		3.92	1.9	(81)
		3.26	1.6	(73)
Hungary		11.19	5.6	(82)
USA	0.70	11.89	6.6	(83)
Ashkenazi		2.62	1.3	(84)
origin	H63D genotype		allelic frequency	reference
	homozygote	heterozygote		
Scandinavia	0.84	15.65	11	(79)
Brittany	2.40	29.13	16.9	(80)
Spain	1.85	27.78	15.7	(81)
Basks	7.84	39.22	27.4	(81)
Hungary	1.81	20.94	12.3	(82)
USA	1.70	26.77	15.1	(83)
Ashkenazi	1.57	16.27	9.7	(84)

Table III. — **Classic phenotypic criteria for the diagnosis of homozygous haemochromatosis**

1. Iron overloaded patients

Obligatory criteria

- Increased transferrin saturation (> 45%)
- Hepatic iron overload, found predominantly in hepatocytes, with a decreasing gradient from periportal to perivenular zones
- Liver iron concentration / age (HII) > 2 or iron removed by venesection greater than 5 g in men and 3 g in women
- exclusion of secondary iron overload

Supplementary criteria

- Demonstration of HLA-linked familial transmission

2. Siblings of a proband

HLA identity to the proband

chromatotic patients (Table I). However, this association is less strong in some studies, and decreases from North to South. This could be due in part to the heterogeneity of phenotypic criteria : indeed, in all but one studies based on strict diagnostic criteria, the prevalence of C282Y homozygosity was greater than 90%. Furthermore, when the demonstration of a familial transmission linked to HFE was required, all patients were C282Y homozygote (11,12). The decreased prevalence of C282Y homozygotes in some studies could be explained by the inclusion of patients with other causes of iron overload (13). On the other hand, in Italy, 36.2% of patients with strict phenotypic criteria were not C282Y homozygotes (14). In conclusion, most patients with a haemochromatotic phenotype are C282Y

homozygotes, in particular in populations of North European descent.

The penetrance of C282Y homozygosity is unknown, but is far from complete, as shown by the demonstration, in family studies or in systematic screening, of C282Y homozygote patients without any iron metabolism abnormalities (15,16).

A few patients with phenotypic haemochromatosis are heterozygotous for the C282Y mutation. On the other chromosome, some carry the H63D mutation (= compound heterozygotes), while most carry the wild allele (= C282Y heterozygotes). Before the discovery of the *HFE* gene, studies based on family transmission of HLA haplotypes had concluded that heterozygotes could present with biochemical abnormalities in 15-25% of cases (17). Recently, we have studied phenotype - genotype correlations in 531 patients explored for unexplained iron overload, whether they were classified as haemochromatotic on a phenotypic basis or not (16). C282Y homozygotes were strikingly different from other genotypes, while no difference was observed between C282Y heterozygotes and homozygotes for the wild allele. In conclusion, C282Y heterozygosity cannot explain iron overload by itself.

Compound heterozygotes may be somewhat different. There are some arguments to think that they may present haemochromatosis (11,18), but with low penetrance and mild expression (16) : (i) compound heterozygotes represent 2 to 8% of haemochromatotic patients, versus 1% in the general population (Table I). (ii) In our study (16), we observed an important prevalence of compound heterozygotes, who exhibited mildly increased iron stores when compared to other non C282Y homozygote genotypes. However, (i) most had also another cause of iron overload, namely insulin resistance syndrome, (ii) only patients with another toxic factor (alcohol, non alcoholic steatohepatitis) presented with liver damage, and (iii) familial transmission of iron overload associated with compound heterozygosity was not demonstrated.

1.1.3.3. H63D

H63D role remains controversial. Some results are not in favour of its significant role : (i) H63D was highly prevalent in general population, including ethnics in whom haemochromatosis is not present. (ii) In iron-overloaded patients, H63D homozygotes were far less frequent than H63D heterozygotes, and did not differ in terms of iron tests or clinical expression. This observation is in contradiction with the hypothesis of a putative recessive mutation. (iii) H63D heterozygotes were also not distinguishable from homozygote normal subjects (16). This contrasts, however, with the increased allelic frequency of the H63D mutation observed in haemochromatotic patients when C282Y chromosomes are excluded (18,19), and the — mildly — increased expression in compound heterozygotes. Therefore, we suggest that H63D mutation alone is not sufficient to cause significant iron overload, but is a co-factor of phenotypic expression in other situations susceptible to be associated with iron overload, such as alcoholism, insulin resistance syndrome, cirrhosis or C282Y heterozygosity.

1.1.4. Non-C282Y homozygote haemochromatosis phenotype

Two exceptional genetic diseases have been described. (i) Hereditary aceruloplasminemia is characterised by massive iron overload with neurological manifestations and diabetes mellitus, associated to a striking decrease in serum iron and transferrin saturation, and a complete lack of caeruloplasmin in serum. It is due to homozygous mutations in ceruloplasmin gene (20,21). (ii) Juvenile haemochromatosis is characterised by massive iron overload, with cardiac and gonadal deficiency and liver cirrhosis, before the age of 30. Its transmission is not linked to *HFE* (22-25), but has been recently linked to chromosome 1 (26).

Several studies have looked for other mutations, but only a few polymorphisms have been described (27,28). It remains a few atypic patients with iron overload characteristic of genetic haemochromatosis and lacking C282Y homozygosity, but familial transmission was not demonstrated (11,13). The discovery of *HFE* gene allowed then to classify the vast majority of haemochromatotic patients.

1.2. *HFE gene and other diseases*

1.2.1. Porphyria cutanea tarda

Mild, mixed liver siderosis is observed in up to 70% of patients with Porphyria Cutanea Tarda (PCT), and venesections are effective on cutaneous manifestations of the disease. An association between PCT and the gene of haemochromatosis was suspected on the basis of HLA typing (29). Roberts *et al.* (15) have confirmed, this association, showing that C282Y prevalence was increased in PCT by compared to the general population (44% versus 11%). This has been confirmed in the Netherlands (30) and Australia (31). However, Italian results are different, with no increase in C282Y prevalence, but an increase in H63D mutation prevalence in PCT patients (32). None of these studies demonstrated any relationship between iron stores and *HFE* mutations in PCT patients. Altogether, these results suggest that *HFE* mutations confer a susceptibility to PCT, but the mechanisms of this effect remain quite unclear.

1.2.2. Haematologic diseases

All haematologic diseases with ineffective erythropoiesis can lead to increased iron stores, due to increased iron absorption, even in the absence of transfusions. It has been suggested that compound heterozygosity could favour the increase in iron stores (33), but most results observed in the thalassaemia intermedia (34) or sideroblastic anaemia (35) are against the implication of *HFE* gene.

1.2.3. Insulin resistance-associated iron overload

This new iron overload syndrome (36) associates, mild to moderate iron overload and features of insulin resistance in middle-aged men (37). It includes some clinical situations described earlier, as iron overload with normal transferrin saturation (38), iron overload observed in non-alcoholic steatohepatitis (39), and hyperferritinemia described in the type 2 diabetes (40). Similar, albeit yet isolated, observations have been reported in Italy (41,42). The role of *HFE* mutations is not evident : there is an increased prevalence of compound heterozygotes (25% vs 1% in general population), leading to an increase in C282Y and H63D allelic frequencies, but other genotypes prevalence are not different from that of general population, and the extent of iron overload and liver damage is not linked to *HFE* genotype.

1.2.4. Liver diseases

Increased serum iron tests are frequent in chronic liver diseases, but are not always associated with increased iron stores (43). The discovery of the *HFE* gene will perhaps bring some lights on the pathogenesis of these abnormalities, but first results are not yet conclusive and contradictory.

1.2.4.1. Chronic hepatitis C

Serum iron tests are increased in up to one third of chronic hepatitis C (44) and these abnormalities are frequently associated with a mild, mesenchymal hepatic siderosis (45). The role of *HFE* mutations in the constitution of this siderosis remains unclear. Hézode *et al.* (46) found hepatosiderosis in 89/211 patients with chronic hip and the prevalence of C282Y was not different between patients with or without hepatosiderosis. Similar results were reported in Germany (47). In Italy, H63D, but not C282Y prevalence was increased in iron overloaded CAH patients (48). One study (49), on a limited number of patients, suggested that C282Y heterozygosity could increased the occurrence of liver fibrosis.

1.2.4.2. Alcoholic liver diseases

C282Y heterozygosity is not associated with liver damage in excessive alcohol consumption (50).

1.2.5. Diabetes mellitus

In type 2 diabetes, some studies found a moderate increase in *HFE* mutations prevalence (51,52), and others a similar prevalence to controls (53-55). Therefore, it is not advisable to perform genetic testing when discovering diabetes.

In conclusion, since the description of the *HFE* gene, mutations had been investigated in a variety of pathological conditions, with somewhat controversial results. To fully understand these studies, one must separate what is associated with genetic status or with iron stores or with factors associated with increased iron stores.

2. Practical implications : clinical management of haemochromatosis

2.1. *Diagnosis in an index patient or proband*

Haemomatosis should be suspected not only when there is a typical phenotypic presentation, such as cirrhosis with skin pigmentation and diabetes. Indeed, such patients already have significant end-stage organ damage and may have decreased life expectancy. Patients, therefore, need to be identified earlier during a treatable and reversible stage of injury. The diagnosis of haemochromatosis must be considered, in men as well in women, and at all adult ages, whenever the clinician is confronted with unexplained chronic fatigue, arthralgia, or unexplained elevation of serum aminotransferases (usually less than three times the upper normal limit).

Once diagnostic of haemochromatosis is suspected, the next step is to evaluate transferrin saturation (TS). It is indeed the most sensitive single test for phenotypic identification of homozygous haemochromatosis, since it is always increased when iron overload is present. Normal TS is 30-40%. Among asymptomatic patients, TS is usually $> 60\%$ in men and 50% in women. False positive results can be observed in excessive alcohol consumption, liver diseases (acute or chronic hepatitis, hepatocellular insufficiency) and oral iron intake.

The confirmation of the diagnosis of haemochromatosis is based on testing for the C282Y mutation. Homozygosity for the C282Y mutation confirms the diagnosis of homozygous haemochromatosis. The appropriate subsequent step in such a patient is to determine the extent of organ damage. It depends from clinical examination, degree of iron overload evaluated by serum ferritin, and association with concomitant factors, such as excessive alcohol consumption. Among patients who are C282Y homozygotes, liver biopsy is only needed for fibrosis assessment. We have shown that when serum ferritin is < 1000 µg/l and neither hepatomegaly nor increase in aspartate-amino-transferase are present, cirrhosis is never observed (56), and liver biopsy can be avoided.

When the patient is not C282Y homozygote, there is often another explanation for iron overload, such as insulin resistance (36), alcoholic liver disease (16) or cirrhosis (57). In the absence of known cause of iron overload in a patient with a phenotypic presentation of haemochromatosis who lack C282Y homozygosity, it should be emphasised that liver biopsy remains essential. The place of testing H63D mutation in such patients when they are C282Y heterozygote remains debated, (see above), since it does not modify practical management.

2.2. *Role of genotyping for C282Y in family screening*

Haemochromatosis is an asymptomatic disease during the first decades of life, and it is most important

to diagnose patients before organ damages occur. The opportunity of mass screening is still debated. However, there is no doubt that family screening is mandatory, since the prevalence of other patients in the family of a proband is much more important than in the general population.

The approach to family screening has been considerably modified and simplified by the genetic test. Starting from a C282Y homozygote proband, it is now possible to evaluate the risk of haemochromatosis among family members : those who are C282Y homozygote are homozygous for the haemochromatosis gene and either already express the disease, or are at high risk of developing it. C282Y heterozygote subjects are heterozygous for the haemochromatosis gene : they will not develop the disease (see above), but may transmit the gene to offspring. C282Y negative family members can be reassured that they (and their children) are not at risk for iron overload.

On a practical point of view, screening should be proposed to all first degree relatives of a C282Y homozygote proband. Several caveats are to be kept in mind. First, some C282Y homozygotes do not present with any iron metabolism abnormalities, and will require regular follow-up. The second issue applies to genetic testing in children. Significant iron overload requiring treatment does not occur in C282Y homozygosity associated haemochromatosis during early childhood. Therefore, screening should be postponed until age 18 years. An alternate strategy for family screening is to screen the spouse. If the spouse lacks the C282Y mutation, the maximal risk for the children is to be heterozygous for the C282Y mutation. This strategy is also cost-saving (58). Third, genetic testing can lead to some significant problems, as insurance or employment discrimination (59). An individual genetic counselling session should precede the genetic testing, and individuals who chose testing should give informed consent.

In conclusion, haemochromatosis management has immediately benefit from the discovery of its molecular basis, even if on a fundamental point of view, the link between the genetic abnormality and iron overload is not fully understood.

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