Prevalence of the Cys282Tyr and His63Asp mutation in flemish patients with hereditary hemochromatosis

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Abstract

Recently Feder *et al.* have identified the gene responsible for hereditary hemochromatosis ; it is located 3 Mbp telomeric of the MHC region on chromosome 6p and is called the HFE gene. The majority of the patients with hemochromatosis harbour the same missense mutation, Cys282Tyr. A second missense mutation (His63Asp) of which the significance is less clear, has also been described. To our knowledge the percentage of these two missense mutations in Flemish hemochromatosis patients is not known.

***Materials and methods :* Forty nine (49) unrelated patients with the clinical diagnosis of hemochromatosis were screened for the two missense mutations. The missense mutations were diagnosed with a PCR technique.**

***Results :* Of the 49 patients, 46 patients were homozygous for the Cys282Tyr mutation (94%), 2 were heterozygous (4%) and 1 carried two normal alleles (2%). Of the 3 patients not homozygous for the Cys282Tyr mutation, 3 were heterozygous for the His63Asp mutation (2 patients were 'compound heterozygotes').**

***Discussion :* The percentage of homozygotes (Cys282Tyr) in a Flemish hemochromatotic population is comparable with the figures published in the literature. The second missense mutation (His63Asp) could be of importance in association with the Cys282Tyr missense mutation.** (Acta gastroenterol. belg., **2000**, 63, **250-253**).

Introduction

Hereditary hemochromatosis (HH) is an autosomal recessive genetic disorder of iron metabolism, which predominantly affects Caucasians. The disease has a prevalence of 2-5 per 1000 (1). HH is characterized by an increased intestinal iron absorption, resulting in progressive iron overload of parenchymal organs, leading in midlife to the onset of clinical complications such as cirrhosis of the liver, diabetes mellitus, cardiopathy, endocrine dysfunctions and arthropathy. If iron excess is detected at a very early stage, these complications can be prevented.

On the basis of an increased frequency of the HLA-A3 allele in hemochromatosis patients, Simon *et al.* were able to map a putative hemochromatosis gene on the short arm of chromosome 6 (2). Further refinement of its localization resulted in the discovery by Feder *et al.* in 1996 (3) of a candidate gene for hemochromatosis.

The authors identified a missense mutation in 85% of the patients with hemochromatosis. The missense mutation is located in a HLA-like protein ; on amino acid position 282 of that protein the missense mutation results in replacement of the amino acid cysteine by tyrosine (Cys282Tyr). Eighty-two % of the patients were homozygous for this Cys282Tyr mutation. Thereafter other authors confirmed this high assocation (4,5,6) (table 1).

In the study of Feder, a second missense mutation was found on the remaining allele in 8 of the 9 heterozygotes. That missense mutation results in a replacement of histidine by aspartate on position 63 (His63Asp). The association of this second missense mutation with HH is less clear than the assocation with the Cys282Tyr mutation, since the prevalence of the His63Asp mutation is not different between controls and patients with hemochromatosis (9).

We studied the frequency of these two missense mutations in Flemish patients with HH.

Materials and methods

Patients

Forty-nine unrelated Flemish patients with unequivocal HH were examined for the Cys282Tyr mutation. The clinical diagnosis of HH was made between 1985-1997. Patients carrying the Cys282Tyr mutation only on one allele or patients not carrying this mutation on both alleles were further examined for the His63Asp mutation.

Table 1

	Cys282Tyr+/+	Cys282Tyr+/–	Cys282Tyr–/–
Feder *et al.* (3) (n = 178)	83%	4.5%	12.5%
Beutler *et al.* (4) (n = 147)	82%	6%	12%
Jouanolle *et al.* (5) (n = 65)	91%	5%	4%
Jaxwinska *et al.* (6) (n = 112)	100%	0%	0%
Our group (n = 49)	94%	4%	2%

Prevalence of the Cys282Tyr mutation in patients with hereditary hemochromatosis.

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Flandres is located in the northern part of Belgium. Although in the past this region was colonized by different cultures (Spain, France, Austria), the majority of the people are from Celtic ancestry.

The diagnosis of HH was made using the following criteria :

1) iron saturation of more than 52% in female patients, more than 62% in male patients ; 2) a ferritin level above 750 ng/ml (normal : < 250 ng /ml), with the majority of our patients with a level above 1500 ng/ml ; 3) histochemical staining of iron on liver biopsy was performed semi-quantitatively by a Perl's stain. At least 75% of the hepatocytes had stainable iron ; 4) more than 3 g iron removed by phlebotomy.

The control group consisted of 96 unrelated normal persons. Iron status in these patients was not known. In these persons the frequency of both missense mutations was examined.

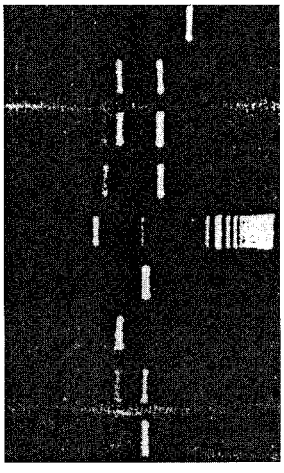
Methods

After extraction of genomic DNA from peripheral lymphocytes (2-3 ml EDTA blood) or from buccal cells, DNA was amplified by PCR using primers as described by Feder *et al.* (3). The amplified framents were further examined by restriction enzyme digestion followed by agarosegel electrophoresis.

The missense mutation Cys282Tyr results in an additional *Rsa*I restriction site. The missense mutation His63Asp results in a loss of a *Mbo*I restriction site.

The DNA profiles obtained in the homozygous and heterozygous individuals and in the normal subjects are readily distinguishable (fig. 1).

When a patient was found to be homozygous for the Cys282Tyr mutation, his partner was screened for this missense mutation.



Full PCR Fragment

CYS282TYR N N
CYS282TYR N M
CYS282TYR M M

Full PCR Fragment

HIS63ASP N N
HIS63ASP N M
HIS63ASP M M

Fig. 1. — Visualisation of the PCR fragments after 2% agarosegel electrophoresis and digestion with *Rsa*I (Cys282Tyr) or *Mbo*I (His63Asp). N means a normal sequence ; M means that the mutation is present.

Stastistical analysis

Chi-square test was used to determine differences between control group and patient group. P value of less than 0.05 was considered significant.

Results

In the control group (table 2) 87.5% of the subjects were homozygous normal for the Cys282Tyr missense mutation ; 10.5% were heterozygous and 2% were Cys282Tyr+/+. These results are in accordance with the Hardy-Weinberg equilibrium.

Forty-six out of 49 patients (94%) were homozygous for the Cys282Tyr mutation (table 2). Two patients (4%) were heterozygous and one (2%) did not carry the missense mutation on either of both alleles (Chi-square versus control population : $p < 0.001$). In the latter 3 patients the His63Asp mutation was additionally examined (table 3). All were heterozygous for the His63Asp missense mutation, implying that two of these three patients were compound heterozygotes (Cys282Tyr+/–, His63Asp+/–).

The characteristics of these 3 patients are presented in table 4.

Table 2

Cys282Tyr	Patients	Controls
+ / –	2% (1/49)	87.5%(84/96)
+ / –	4% (2/49)	10.5%(10/96)
+ / +	94%(46/49)	2%(2/96)

Table 3

His63Asp	Patients	Controls
– / –	94% (46/49)	68% (65/96)
+ / –	6% (3/49)	31% (30/96)
+ / +	0% (0/49)	1% (1/96)

Table 4. — Clinical details of the patients not homozygous for the Cys282Tyr mutation

Patient	Cys282Tyr	His63Asp	Known family history	Age at onset
1	– / –	+ / –	no	32
2	+ / –	+ / –	yes	55
3	+ / –	+ / –	no	55

Patient	Iron removed	Liver histology	Iron saturation	Serum ferritin (ng/ml)
1	15 g	siderosis	65%	1200
2	3.5 g	siderosis	62%	795
3	7 g	cirrhosis	67%	4220

Patient 1 did not carry the Cys282Tyr mutation and is heterozygous for the His63Asp mutation. This male patients complained about diffuse arthritic pains. The diagnosis of hemochromatosis was made on iron studies and liver biopsy (Perl's staining was highly positive in hepatocytes and Kupffer cells). He had no risk factors for secondary iron overload : alcohol abuse, hemolytic anaemia, blood transfusion, chronic viral liver disease. Phlebotomy was started and the iron donation was 15 g before normalisation of the ferritin level could be achieved. These findings are very suggestive for hemochromatosis. However there was no family history of hereditary hemochromatosis and there was no HLA pattern as found in hereditary hemochromatosis (A1 A2 B8 B18).

Patient 2 consulted because two brothers had hereditary hemochromatosis. This male patient is a compound heterozygote for both mutations, but he has only mild iron overload (ferritin : 795 ng/ml, moderate iron staining on liver biopsy, and an iron donation of 3.5 g before normalisation of the ferritin level was achieved). He had no risk factors for secondary iron overload.

Patient 3 consulted for chronic fatigue. The diagnosis of hemochromatosis was made on iron studies and liver biopsy (highly positive Perl's staining in hepatocytes and Kupffer cells in a cirrhotic liver). The iron donation before normalisation of ferritin was 7 g. There was no family history and the patient had no risk factors for secondary iron overload.

This male patient was compound heterozygote.

Concerning the His63Asp missense mutation, 68% of our control population did not carry the missense mutation, 31% were heterozygous and 1% was homozygous for this missense mutation (table 3). These results are in accordance with the Hardy-Weinberg equilibrium.

Family screening was extremely easy using the cheek brush technique. In 11 patients the partner was checked for the Cys282Tyr mutation. The partner was heterozygous in 8 out of 11 marriages (72%), which is high when compared with our control population (10.5%).

Discussion

The present results are in accordance with the previously reached conclusion that mutations in the HFE gene are present in a high percentage of patients with HH. Our results are comparable with those of other authors (4,5,6) (table 1). Patients were homozygous for the Cys282Tyr mutation in 94% of the cases, whereas in the control group homozygosity for this mutation was found in only 2%(results in accordance with the Hardy-Weinberg equilibrium, HFE allele frequency in the control group of 0.07 which is comparable with data from other centers in the Western World, cfr. table 1).

The finding of this high percentage of the Cys282Tyr missense mutation in our and other groups of patients demonstrates that this missense mutation really contributes to the phenotypic presentation of the disease. Crawford et al could demonstrate a very high penetrance of this missense mutation (10).

However, a recent publication by Adams et al reported about homozygous individuals with no evidence of clinical iron overload (11). Determination of the iron status of the homozygous people in our control group, could give some additional information about the genotype/phenotype correlation. However we were not able yet to compare the homozygous patients and the control group with respect to clinical symptoms, biochemical signs of iron overload or pathological iron overload.

Whether this gene is really responsible for the disease or is only associated at a high frequency in the patients with HH as an 'innocent bystander', is unclear at present. There is however some evidence for the former hypothesis. In the presence of the Cys282Tyr mutation, a disulfide bridge is broken which alters the presentation of the HLA-H protein outside the cell. This disulfide bridge is necessary for the binding with beta2-microglobulin. Animal studies show that beta2-microglobulin knock-out mice have an iron accumulation pattern similar to patients with HH (7,12).

Feder *et al.* could demonstrate that in cell culture, the binding between the transferrin receptor and the wild-type HFE gene, decreases the affinity between the transferrin receptor and transferrin. Whereas in the presence of the mutant HFE gene this binding is completely inhibited. These data show that the HFE gene could be directly involved in the iron metabolism (13).

The significance of the His63Asp mutation is less clear. It is suggested that it is only of importance in the compound heterozygote setting (9). In our study two patients heterozygous for the Cys282Tyr missense mutation were also heterozygous for the His63Asp missense mutation (compound heterozygotes).

Patient 2 had a family history of hereditary hemochromatosis. Since the iron overload was only moderate in this patient (table 4) and since the age at presentation was 55 years, he had only a mild form of hereditary hemochromatosis. Determining the missense mutations and the degree of iron overload in the other affected family members, would give us the opportunity to investigate whether the presence of the His63Asp missense mutation modifies the clinical presentation of HH.

Patient 3 had substantial iron overload and already advanced liver disease. On biochemical and pathological grounds there was no doubt about the diagnosis of hemochromatosis. The iron donation was 7 g before ferritin normalisation could be achieved. Although the patient had no family history, the clinical picture is highly suggestive for hemochromatosis. In this patient it seems that being compound heterozygous, could be a risk factor for developing hemochromatosis. However in the study of Jouanolle (5) the prevalence of compound heterozygotes was not different in the control group than in the group of patients with HH. This frequency was about 2%.

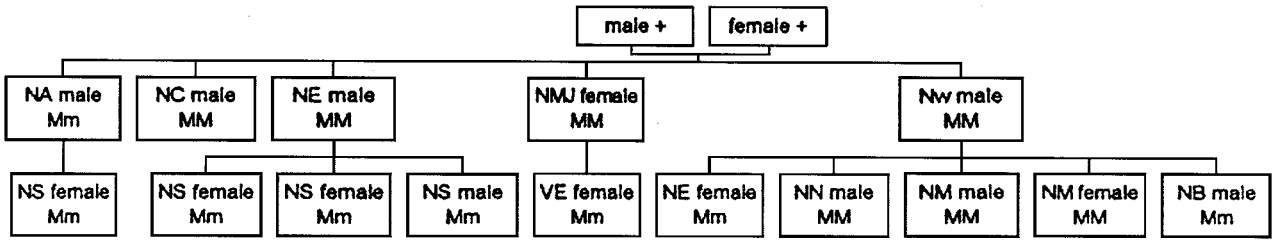


Fig. 2. — Family tree of an index patient with HH. MM means homozygous for the Cys282Tyr missense mutation. Mm means heterozygous for the Cys282Tyr missense mutation. + means deceased.

We found a frequency of about 30% of heterozygotes for the His63Asp mutation in our control population. As such marriages between Cys282Tyr homozygotes and His63Asp heterozygotes would have a 50% occurence risk of compound heterozygote offspring. If the His63Asp missense mutation is involved in the pathogenesis of HH, these children should develop hemochromatosis

This figure is higher than seen in clinical practice. These are indirect arguments that the His63Asp mutation is of only minor importance. However more longitudinal follow-up of compound heterozygous people and additional family tree studies are necessary to explain the real importance of the His63Asp missense mutation. However Rish could demonstrate that being compound heterozygote results only in a minor risk to develop HH (15).

Patient 1 did not carry the Cys282Tyr or the His63Asp missense mutation. And although there was no family history of HH, the clinical presentation was very typical for HH. Another still not established mutation could be the explanation of this finding. So recently, Wallace et al demonstrated the presence of a new gene responsible for HH (14).

Screening the family of an index patient with HH becomes extremely easy using the cheek brush technique. Buccal cells prelevated by this technique can be examined in the same way as lymphocytes prelevated by blood sampling. Prelevation can be done at home by the patient and his family. In our experience this makes the screening method more acceptable.

It is more cost-effective to screen the partner of the patient for the Cys282Tys mutation first and to screen only the children if the partner is heterozygous for this mutation (8). Since the His63Asp missense mutation is of minor importance(15), one can restrict the screening to determining only the Cys282Tyr missense mutation.

We were able to screen 11 partners of patients with HH. Eight out of 11 were heterozygotes. Although this prevalence is higher than in the control population, it seems to us to be purely coincidental. However this high frequency illustrates the importance of family screening (fig. 2.)

We conclude that in our group of Flemish patients with HH, a high number (94%) of patients is homozygous for the Cys282Tyr mutation. The His63Asp mutation seems to be of minor importance, however in some patients who are compound heterozygotes, clinical expression of hereditary hemochromatosis can be present.

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