HFE gene mutation (C282Y) and phenotypic expression among a hospitalised population in a high prevalence area of haemochromatosis

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Abstract

Background—Previous studies have shown that up to 0.5% of the Caucasian population is homozygous for the HFE gene C282Y mutation. High prevalence values have been reported in Northern Europe. To what extent the presence of this mutation is associated with overt clinical haemochromatosis is unclear.

Aim—To determine the prevalence of the C282Y allele in a hospitalised population of an acute medical department, and study the phenotypic expression in the homozygotes.

Methods—Blood samples were obtained from 2027 hospitalised patients; 1900 Caucasians and 127 non-Caucasians. Serum iron, transferrin, and ferritin were measured at admission. The presence of the HFE gene mutation was determined by polymerase chain reaction based analysis. Follow up fasting blood samples were obtained from patients homozygous for the mutation.

Results—Fourteen of the 1900 Caucasian subjects (0.74%) were homozygous and 224 (11.8%) were heterozygous for the C282Y mutation, including 32 subjects (1.7%) who were compound heterozygous for the C282Y and H63D mutations. Ten of 14 (71%) homozygous patients displayed mild to moderate biochemical expression of haemochromatosis with a serum ferritin level <550 µg/l, two (14%) patients were “non expressing”, and two of five in whom liver biopsies were carried out had cirrhosis, including one with advanced hepatocellular carcinoma.

Conclusions—The prevalence of C282Y homozygosity in a hospitalised population was 0.74%. However, the majority of homozygous patients displayed mild to moderate biochemical expression. C282Y mutation screening may detect individuals that do not develop haemochromatosis. Transferrin saturation and ferritin, which are used as first line screening in haemochromatosis, may be highly unreliable in the presence of an inflammatory process.

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Keywords: haemochromatosis; HFE gene mutation; inherited disorders; screening; ferritin; transferrin saturation

Hereditary haemochromatosis (HH) is the most common genetic disorder in the Cau-

sian population. Affected individuals display inappropriately high iron absorption from the small intestine with progressive iron deposition which causes injury to the liver, joints, heart, and other organs.^{1 2}

A single mutation resulting in a cysteine to tyrosine substitution at amino acid position 282 (C282Y) was found on both HFE alleles in 83% of patients with clinical HH.³ Lebron and colleagues⁴ suggested that the protein product of the normal HFE gene binds to the transferrin receptor and reduces its affinity for iron loaded transferrin. The C282Y mutation may prevent the association of the HFE protein with the transferrin receptor.^{5 6} A second mutation, representing a histidine to aspartate substitution at position 63 (H63D), was found in the HFE gene of the normal population, but not on alleles carrying the C282Y mutation.

Previous haemochromatosis screening studies based on transferrin saturation as a screening parameter have suggested a lower prevalence of haemochromatosis in hospitalised populations with respect to the general population.^{7 8} This relationship has not been addressed since the identification of the C282Y mutation.

Phenotypic expression of C282Y homozygosity is variable and its presence is not always associated with iron overload and overt clinical disease,^{9 10} suggesting that as yet unidentified genetic and/or environmental factors play an important role in the pathogenesis of haemochromatosis. The aim of this study was (1) to determine the prevalence of homozygosity and heterozygosity for the C282Y mutation in a hospitalised population and (2) to observe the phenotypic expression of homozygous individuals. Studying this group gave the opportunity to observe the effect of an acute phase reaction¹¹ on iron metabolism.

Methods

SUBJECTS

We recruited 2337 patients, admitted to an acute medical ward at Aker University Hospital, Oslo, from November 1998 to May 1999. The hospital covers the eastern part of the city of Oslo which has approximately 130 000 inhabitants, with a predominance of working,

Abbreviations used in this paper: HH, hereditary haemochromatosis; PCR, polymerase chain reaction; HII, hepatic iron index; HIC, hepatic iron concentration; CRP, C reactive protein; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; GGT, gamma glutamyl transferase; ALP, alkaline phosphatase; TIBC, total iron binding capacity.

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lower middle class individuals, students, and immigrants. Only 5% of hospital admissions are elective. Acute admissions are most often as a result of cardiovascular disease, respiratory disease, malignancy, cerebrovascular disease, infectious disease, gastrointestinal, and alcohol related diseases.

Patients were given an information sheet and asked to join the study at admission. Those who were critically ill were asked to participate in the study after their condition had stabilised. Aker University Hospital is a regional referral centre for haemochromatosis. Eight patients referred for a liver biopsy with a suspicion of haemochromatosis were not included. Patients readmitted to the hospital at least once during the study inclusion time (n=228, 9.6% of study population) and three Caucasian patients who refused consent were excluded. Seventy nine randomly admitted patients whose blood samples were later found to be inadequate for DNA extraction were also excluded. In total, 2027 were included in this study.

Informed consent was obtained from each patient. The study was approved by the National Ethics Committee and Department of Social Affairs, and was performed according to the guidelines of the Helsinki declaration.

Of the 2027 patients included in the study, 1900 were Caucasians of Scandinavian origin, except for 15 patients who were East Europeans, and 127 patients (6.3%) were non-Caucasians, predominantly of Indian, Asian, or African origin. As none of the non-Caucasians displayed the C282Y mutation, iron indexes from this group were excluded. The median age of the Caucasian population was 71 years (range 15–102; 25th and 75th percentiles 54 and 79). There were 1019 (54%) women and 881 (46%) men; median age was 74 years (range 16–102) and 68 years (15–97), respectively.

Non-fasting blood samples were obtained at admission (that is, at any time of the day). Samples were analysed for serum iron, transferrin, and ferritin within 48 hours of collection. A 5 ml EDTA whole blood sample was collected and frozen for genetic analysis.

BIOCHEMICAL MEASUREMENTS

Serum iron (Fe)¹² and transferrin¹³ were measured on an Hitachi 917 with reagents from Boehringer Mannheim (Mannheim, Germany). Within run precision coefficients of variation were 0.5% and 2%, respectively. Transferrin was converted to total iron binding capacity (TIBC) based on the transferrin molecular weight of 79 570 Da and that each transferrin molecule binds two atoms of iron. Transferrin saturation was calculated as: Fe/TIBC×100%. Transferrin saturation ≥50 was defined as increased.

Serum ferritin was measured with an ACS 180 (Chiron Diagnostics, Walpole, Massachusetts, USA) using an immunochemiluminometric assay.¹⁴ Within run precision coefficient of variation was 3%. Serum ferritin concentrations ≥110 µg/l in women and ≥200 µg/l in men were defined as increased according to the

criteria of our department based on previous studies in first time blood donors.¹⁵

DNA ISOLATION AND AMPLIFICATION

Genomic DNA was extracted from venous EDTA blood stored at –20°C. DNA fragments were amplified by polymerase chain reaction (PCR) using primers and reaction conditions as described by Feder and colleagues.³ The PCR products were treated with the restriction enzyme *RsaI* to identify the C282Y mutation in all samples and with *BclI* to detect the H63D polymorphism among the C282Y heterozygotes and homozygotes.

It has recently been reported that a G to A polymorphism at position 5569 in intron 4 of the HFE gene may interfere with the function of the antisense primer used for C282Y genotyping.¹⁶ As a result, C282Y heterozygotes may have been misclassified as C282Y homozygotes. The C282Y homozygotes of the present study were therefore reanalysed using the same sense primer and reaction conditions as Feder and colleagues³ but with a new antisense primer (5'-TACCTCCTCAGG CACTCCTC-3') which is 3' to the G5569A polymorphism. No false positive C282Y homozygotes were detected with the new antisense primer.

FOLLOW UP OF HOMOZYGOUS PATIENTS

Patients homozygous for the C282Y mutation were reviewed six weeks after discharge from hospital. Fasting blood samples were obtained and analysed for serum iron, transferrin, ferritin, C reactive protein (CRP), alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), and bilirubin.

Liver biopsy was offered to patients in accordance with the criteria of our department (follow up serum ferritin ≥200 µg/l in men and >110 µg/l in women and exclusion of contraindications). However, we did not perform liver biopsy in patients aged more than 70 years unless there was a suspicion of malignancy or cirrhosis. Therefore, a biopsy was not performed in patients Nos 2, 4, and 11–13 because of age >70 years, in Nos 8 and 9 because of contraindications, and in patient Nos 3 and 7 because of normal ferritin concentrations (table 2). Liver biopsy specimens were stained with Perls' Prussian blue, and stainable iron was graded from 0 to 4+ according to Bassett and colleagues.¹⁷ Total iron content of the liver biopsy specimens was measured by inductively coupled plasma atomic emission spectrometry. The biopsy specimens were digested in ultrapure nitric acid and diluted as previously described.¹⁵ The hepatic iron index (HII) was defined as hepatic iron concentration (HIC) (µmol/g dry tissue)/age (years) of the patient.¹⁷ A diagnosis of haemochromatosis among C282Y homozygous subjects was based on the results of follow up, fasting blood samples, and liver biopsy. When liver biopsy was not available in homozygotes, we decided that serum ferritin concentration ≥110 µg/l in women and ≥200

Table 1 Distribution of the HFE gene C282Y mutation among 1900 hospitalised Caucasian patients. Median (range) values for serum ferritin and transferrin saturation at admission are shown. Only genotypes C282Y +/- and +/- were tested for the H63D mutation. None of the 127 non-Caucasian patients had the C282Y mutation

Genotypes		No (%) (n=1900)	Serum ferritin (µg/l)	Transferrin saturation (%)
C282Y	H63D			
-/-	-/-	1662 (87.0)	121 (2-28620)	15 (1-90)
+/-	-/-	192 (10.1)	151 (3-4453)†	17 (2-92)†
+/-	+/-	32 (1.7)	99 (14-1275)‡	21 (4-76)‡
+/+	-/-	14 (0.74)	370 (29-2203)*	27 (3-51)*

*C282Y homozygotes had significantly higher serum ferritin and transferrin saturation with respect to wild-type patients ($p=0.01$ and $p=0.02$, respectively). Iron indexes from one homozygous patient who had received treatment were excluded from the calculations. C282Y homozygotes also had significantly higher serum ferritin than C282Y heterozygotes ($p=0.03$) but there was no significant difference in transferrin saturation ($p=0.08$).

†All C282Y heterozygotes ($n=224$) had significantly higher serum ferritin concentrations and transferrin saturation with respect to wild-type patients ($p=0.02$ and $p=0.02$, respectively).

‡There were no significant differences in serum ferritin and transferrin saturation between C282Y heterozygotes and compound heterozygotes ($p=0.14$ and $p=0.40$, respectively).

µg/l in men together with transferrin saturation $\geq 50\%$ indicated "biochemical expression" of haemochromatosis.

STATISTICAL ANALYSIS

Statistical significance of the data was determined using the computer program Statistical Package for the Social Sciences (SPSS). Mann-Whitney and Fisher's exact tests were used. p values <0.05 were considered statistically significant.

Results

Fourteen of 1900 Caucasian hospital patients were homozygous for the C282Y mutation (0.74% (95% confidence interval 0.38)). There were 224 C282Y heterozygotes (11.8% (1.2)), including 32 (1.7%) C282Y and H63D compound heterozygotes (table 1). The C282Y allele fraction was 0.066 with no significant difference between males and females (0.058 v 0.073; $p=0.08$, Fisher's exact tests).

At admission only one homozygous woman had increased transferrin saturation $\geq 50\%$ but serum ferritin concentration was increased in 11 of 13 patients (84%) (table 2).

Twelve patients were offered follow up six weeks after hospital discharge (table 2). Patient No 14 had been diagnosed as having advanced haemochromatosis with cirrhosis six years prior to this admission. She had been treated with regular phlebotomies with removal of 12 g iron and normalisation of iron indexes. Her iron indexes were excluded from the calculations. Control blood samples showed that transferrin saturation was increased in nine of 12 (75%) patients and serum ferritin concentration in 10 of 12 patients (83%) (table 2).

Liver enzymes and serum bilirubin were normal in the homozygous patients who were offered follow up (except for patient No 13, data not shown).

In patient Nos 1, 6, and 10, liver biopsies revealed haemosiderin deposits with a typical periportal pattern of grade 2+ (Perls' staging) but no signs of fibrosis; HII was 1.8, 1.9, and 2.2, respectively (table 2). Postmortem examination in patient No 5 showed cirrhosis and hepatocellular carcinoma (table 2).

A liver biopsy performed six years previously in patient No 14 showed typical periportal haemosiderin deposits of grade 4+ (Perls' staging) and cirrhosis. Nine patients were not offered liver biopsy (see methods and table 2).

C282Y homozygotes had significantly higher serum ferritin concentrations compared with wild-type individuals and all C282Y heterozygotes (table 1). Transferrin saturation values at admission were also significantly higher in C282Y homozygotes compared with wild-type individuals (table 1).

Serum ferritin and transferrin saturation values at the time of admission according to HFE gene mutations are shown in table 3. Seventeen of 60 patients (28%) with serum ferritin concentration ≥ 1000 µg/l had malignant disease, 17 (28%) had suffered a cardiovascular or cerebrovascular accident, 16 (27%) had suffered an infectious disease, and 10 (17%) had alcohol related liver disease.

Thirty two (1.7%) patients (11 men and 21 women) were compound heterozygous for the

Table 2 Characteristics of the C282Y homozygous patients

Patient No	Age (y)	Sex	Acute phase samples		Follow up samples		Pregnancies	Liver biopsy		Discharge diagnosis
			Serum ferritin (µg/l)	Transferrin saturation (%)	Serum ferritin (µg/l)	Transferrin saturation (%)		HII	Perls' staging (0-4)	
1	36	M	428	3	286	80		1.8	2+	Pneumonia
2	74	M	377	27	547	68		-	-	Congestive heart failure
3	78	M	29	26	118‡	73		-	-	Gastrointestinal bleeding
4	79	M	2203	10	478	33		-	-	Urosepsis
5*	79*	M	947	41	-	-		0.4	0-3+	*Cirrhosis, hepatocellular carcinoma
6	31	F	152	51	140	83	1	1.9	2+	Intoxication by overdose
7	47	F	52	19	52	38	0	-	-	Diabetes
8	56	F	1200	49	1001	90	1	-	-	Emphysema, α_1 antitrypsin deficiency
9	57	F	332	8	231	58	3	-	-	Pneumococcal sepsis/emphysema
10	64	F	370	38	645	78	2	2.2	2+	Chest pain/angina
11	72	F	294	43	151	79	1	-	-	Pneumonia
12	77	F	1550	49	142	63	3	-	-	Pneumonia
13	80	F	120	21	207	42	5	-	-	Myocardial infarction
14†	82†	F	58†	45†	-	-	5	8.0	4+	†Congestive heart failure, cirrhosis
Median	73		370	27	219	71				
(range)	(31-82)		(29-2203)	(3-51)	(52-1001)	(33-90)				

HII, hepatic iron index.

*Patient died one week after admission; postmortem histology of the liver showed Perls' staging varying from 0 in malignant cells to 3+ in normal hepatocytes. Uneven haemosiderin deposits might have been the cause of a low HII.

†This patient was diagnosed six years prior to this admission; serum ferritin at presentation was 3160 µg/l and transferrin saturation was 100%. Original biopsy results are shown. The iron indexes measured at admission are after treatment and therefore excluded from calculations.

‡This patient received oral iron after hospital discharge.

Table 3 Serum ferritin and transferrin saturation values among 1900 hospitalised Caucasian patients at the time of admission (acute phase values) and according to HFE gene mutations. Percentages are given in parentheses

	C282Y				Total n=1864†
	+/+ n=14	+/- n=189	+/-* n=32	-/- n=1629	
Ferritin in (µg/l)					
≤12	0	2	0	33	35 (2)
13–199	5	113	22	1086	1226 (66)
200–999	6	67	8	462	543 (29)
≥1000	3	7	2	48	60 (3)
	n=14	n=187	n=32	n=1627	n=1860††
Transferrin saturation (%)					
≥50	1	8	1	40	50 (3)
<50	13	179	31	1587	1810 (97)

*Compound heterozygotes for C282Y and H63D.

†There were 36 missing values.

††There were 40 missing values.

C282Y and H63D mutations. Their median serum ferritin and transferrin saturation values were not significantly different from C282Y heterozygotes (table 1). Six of 10 compound heterozygotes with serum ferritin concentration ≥ 200 µg/l had suffered an infectious disease, three had suffered a cardiovascular or cerebrovascular accident, and one had a malignant disease.

Discussion

The prevalence of C282Y homozygosity among hospitalised individuals was 0.74%. The allele frequency was 6.6%. This is within the 95% confidence interval of our previously reported value of $7.8 \pm 1.6\%$ among healthy individuals in the same city.¹⁸ These results, together with data from a recent newborn screening pilot study,¹⁹ confirm that the prevalence of C282Y in Scandinavia is higher than the average allele frequency in Europe (3.8% (95% confidence interval 0.7)).²⁰ The prevalence of C282Y homozygosity was also higher than the prevalence of haemochromatosis observed in the same city in Norway (0.34%) where serum ferritin was used to screen 3500 first time blood donors.¹⁵ This discrepancy suggests that C282Y homozygosity can occur without biochemical expression or clinical disease.

C282Y mutation screening studies among healthy individuals^{18, 20–22} and newborns^{19, 23} have been reported recently. To our knowledge, ours is the first study of both the prevalence and phenotypic expression of C282Y mutation in a hospitalised population. We are aware that estimation of the prevalence of the C282Y mutation in a population of sick and elderly patients (median age 71 years) cannot be applied to the general population. Patients with haemochromatosis may have greater morbidity and are more likely to be admitted to hospital; hence our prevalence rates may be overestimated. In contrast, if homozygous individuals that fully express clinical disease have already died, the observed prevalence of 0.74% might be an underestimation. In a period of six months only two of 14 homozygotes were hospitalised because of diseases related to haemochromatosis. A recent study suggested that

C282Y homozygosity was not underrepresented in an elderly male population.²⁴

The results of our study indicate that not all patients who are homozygous for the C282Y mutation invariably develop iron overload and clinical disease. Phenotypic expression in our 14 homozygous patients was variable. Two patients had biopsy proven cirrhosis, including one with hepatocellular carcinoma, three patients had no fibrosis with a HII of 1.8–2.2, and two patients were non-expressing. The remaining seven homozygous patients all had moderately increased serum ferritin concentrations below 550 µg/l, except for one female with a ferritin level of 1000 µg/l. With the exception of one, it is highly unlikely that any had cirrhosis. Only one of 14 homozygous patients had a history of previous blood loss.

There is increasing evidence to support our observations of low levels of phenotypic expression among C282Y homozygotes. In Australia, 22 of 127 (17.3%) C282Y homozygous siblings of affected individuals had “non expressing” or “partially expressing” iron overload.⁹ In a C282Y homozygosity penetrance study, 18 (0.6%) homozygous individuals were found among 3017 adults. Only five (28%) had increased serum ferritin concentration (Jouanolle AM, *et al*, abstract No 254, Bioiron 1999). Similarly, in a screening study of 5211 blood donors in Canada, 16 (0.3%) C282Y homozygotes were identified and only four had increased serum ferritin concentration (Adams PC, *et al*, abstract No 73, Bioiron 1999). Another group in Australia identified 16 (0.5%) homozygous individuals from 3011 healthy subjects; eight were fully “expressing” whereas five were completely “non-expressing” (Olynyk JK, *et al*, abstract No 59, Bioiron 1999).

With regard to iron indexes, non-fasting blood samples should not have greatly affected our results in homozygous subjects. Edwards and colleagues²⁵ showed that there was no clear diurnal variation in transferrin saturation in patients with haemochromatosis. Transferrin saturation, however, can be decreased in inflammatory states because of decreased serum iron¹¹ and also in patients with haemochromatosis.²⁶ A low prevalence of haemochromatosis among hospitalised patients in Sweden was attributed to transferrin desaturation induced by inflammatory conditions.²⁷ Median transferrin saturation values among the homozygotes of our study were considerably lower during the acute phase. Release of ferritin from damaged tissues in acute inflammation and malignancy can markedly increase serum ferritin concentration.¹¹ There is evidence that cells of the macrophage lineage in haemochromatosis patients have an abnormal response to inflammation.^{28, 29} However, our findings suggest that C282Y homozygous individuals respond to inflammation by desaturation of transferrin and an increase in ferritin similar to normal individuals. Results from this study suggest that screening for haemochromatosis using DNA analysis would detect individuals who may not develop the disease. Moreover,

genetic testing for C282Y alone would fail to detect those haemochromatosis patients who do not have this HFE gene mutation.³⁰⁻³⁵ Until the cost effectiveness of genotyping can be better assessed, transferrin saturation appears to be the screening test of choice.^{1, 36-39} However, the presence of an inflammatory process should be excluded when measuring transferrin saturation.

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