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SHORT REPORT

Prevalence of the C282Y and H63D mutations in the HFE gene in patients with hereditary haemochromatosis and in control subjects from Northern Germany

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Summary. Mutation analysis was performed for two HFE mutations (C282Y, H63D) in unrelated patients with hereditary haemochromatosis ($n=92$), family members of patients ($n=34$), and unrelated controls ($n=157$) from Northern Germany. 87/92 patients (94·6%) revealed the C282Y mutation in homozygous form, five were heterozygous. No H63D mutation was found in 174 chromosomes of patients homozygous for C282Y, whereas four of the heterozygote patients also carried the H63D mutation. Among the control group, 9·6% were heterozygotes for C282Y. 2/157 subjects were homozygous, 37/157 were heterozygous for the H63D mutation, but showed no signs of iron overload.

Keywords: haemochromatosis, C282Y, H63D, German patients, liver iron.

Hereditary haemochromatosis (HH) is the most common genetic disease in Caucasian populations. The affected gene, HFE, is located on chromosome 6, telomeric to the HLA region. A single mutation, resulting in a cysteine to tyrosine substitution at amino acid 282 (C282Y), was found on both chromosomes in 83% of patients with clinically classified HH (Feder *et al*, 1996). The C282Y variant disrupts the interaction of the HFE chain with β2-microglobulin (Feder *et al*, 1997) and prevents the association of the HFE protein with transferrin receptor (Feder *et al*, 1998). 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 chromosomes carrying the C282Y mutation.

The frequency of the H63D mutation is increased in HH patients heterozygous for the C282Y mutation (Feder *et al*, 1996). Several reports on the frequency of the two HFE mutations in patients with HH from different countries have already been published (Feder *et al*, 1996; Jazwinska *et al*, 1996; Jouanolle *et al*, 1997; Borot *et al*, 1997; Piperno *et al*. 1998).

A report from a different part of Germany has been published recently (Gottschalk *et al*, 1998). In the present study we analysed the frequency of the two HFE mutations in patients and controls from North Germany.

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MATERIALS AND METHODS

In the time period between 1993 and 1997 about 600 subjects with a suspected iron overload were studied in our department. Most of them had a history of increased values of serum iron or serum ferritin or had a family member affected with HH. Among these patients, the diagnosis of a haemochromatosis was established in 92 unrelated subjects by the presence of at least three of the following criteria: (a) transferrin iron saturation > 62%, serum ferritin > 300 μg/l; (b) liver iron concentration (LIC) of > 2000 μg Fe/g wet weight; (c) hepatic iron index (HI[μg/y] = (LIC/age)) > 30; (d) grade 3 or 4 stainable iron in the liver, (e) > 4 g of iron removed by phlebotomy. Starting from each identified patient, a family study was performed in all relatives available. In addition, 157 unrelated healthy volunteers were also studied. All patients and controls were of German ancestry (at least three generations) located in Hamburg or in neighbouring North-German federal states.

Genomic DNA from patients or volunteers was isolated from EDTA blood samples using the QIAamp Blood Kit (QIAGEN, Hilden, Germany). The PCR was performed using primers for the C282Y mutation (forward, 5′-GTC ACC TCT

TCA GTG ACC; reverse, 5′-AAT GAG GGG CTG ATC CAG) and the H63D mutation (forward 5′-ATG GGT GCC TCA GAG CAG; reverse 5′-AGT CCA GAA GTC AAC AGT). The amplified products were digested with *Sna*Bl (MBI Fermentas, St Leon-Rot, Germany) for the C282Y mutation or with *Bcl*I (MBI Fermentas) for the H63D mutation according to the manufacturer's recommendations.

Serum-iron, TIBC and serum ferritin were measured using routine methods. Liver iron concentrations were measured non-invasively using a SQUID biomagnetometer (Ferritometer, BTi, San Diego, U.S.A.) as described elsewhere (Fischer *et al*, 1992).

RESULTS

Patients

The parameters of iron metabolism were clearly different between the group of patients and controls (Table I). Of the patients, 94·6% were homozygous and 4·3% were heterozygous for the C282Y mutation. In the patient group there was no obvious difference in the range of iron loading between homozygous ($n = 87$) and heterozygous ($n = 5$) carriers. In 174 chromosomes of patients who were homozygous for the C282Y mutation, no H63D mutation was found. However, four out of five chromosomes of risk in the patients with heterozygous C282Y mutation also revealed the H63D mutation.

Family members

Among family members, nine homozygous and 21 heterozygous carriers for the C282Y mutation were found. 4/26 chromosomes at risk carried the H63D mutation (gene frequency 0·15), including three subjects with compound heterozygosity. No differences in transferrin saturation and serum ferritin were found between C282Y heterozygotes from the family group and the control group.

Controls

No control subject was found homozygous for the C282Y mutation. 15/314 chromosomes studied carried the C282Y

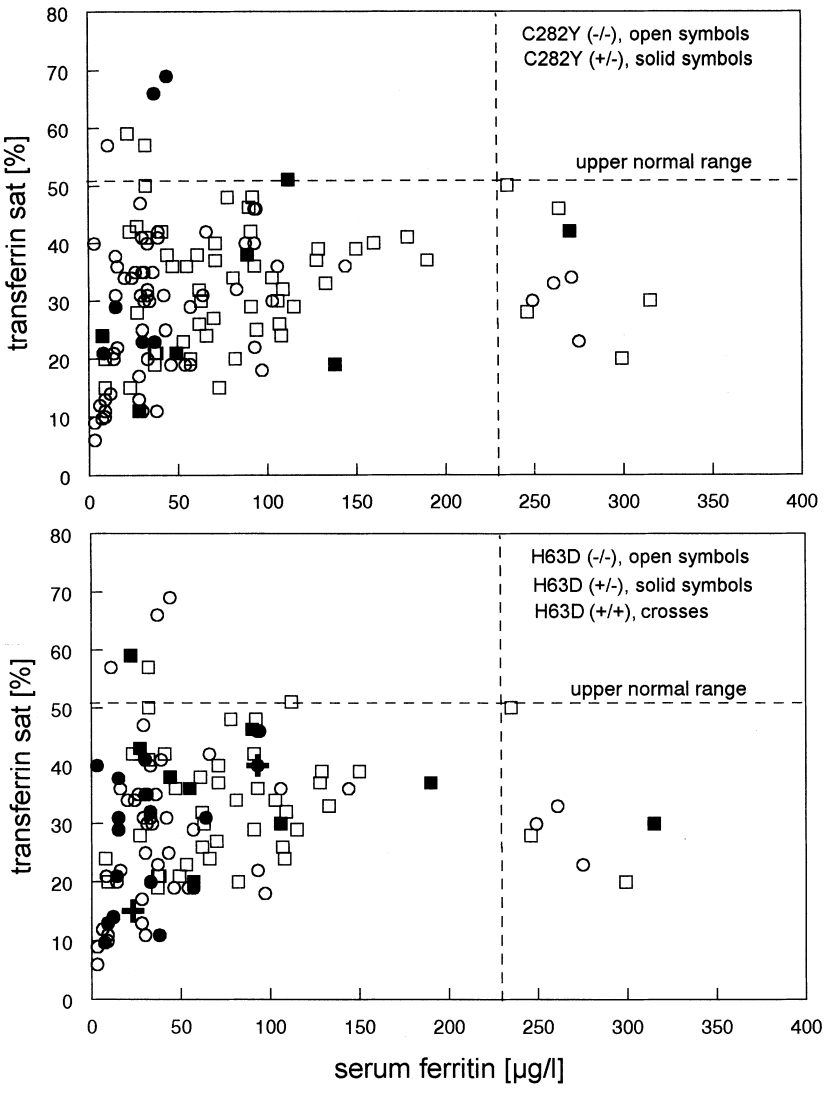

Fig 1. Transferrin saturation and serum ferritin in control subjects with 'wild-type' HFE gene, C282Y (top) or H63D mutations (bottom). Circles, females; squares, males.

Table I. Parameters of iron metabolism in clinically classified unrelated patients with hereditary haemochromatosis, family members, and controls (mean values ±standard deviation).

Subjects	*n*	Age	Serum ferritin* (μg/l)	Transferritin saturation (%)	Liver Fe* (μg/g)
Patients†	92	49·1 ± 12·5	1·087(2·858, 413)	90 ± 11	2·16 (3·51, 1·33)
Females	33	49·6 ± 12·6	701 (1·721, 286)	88 ± 10	2·08 (3·21, 1·35)
Males	59	48·7 ± 12·6	1451 (3·471, 607)	96 ± 11	2·22 (3·73, 1·32)
Family members‡	34	40·2 ± 21·9	120 (381, 38)	49 ± 22	0·44 (0·96, 0·20)
Homozygotes	9	49·8 ± 19·4	144 (341, 61)	79 ± 16	0·69 (1·29, 0·37)
Heterozygotes	21	35·2 ± 22·6	79 (235, 27)	40 ± 13	0·37 (0·79, 0·17)
'Wild type'	4	46·4 ± 14·4	113 (173, 73)	34 ± 4	0·41 (0·87, 0·19)
Controls	157	42·3 ± 17·1	49 (138, 17)	31 ± 13	n.d.
Females	77	41·8 ± 17·8	33 (90, 12)	29 ± 14	n.d.
Males	80	42·8 ± 16·3	75 (183, 31)	33 ± 11	n.d.
C282Y (+/−)	15	48·1 ± 21·8	39 (121, 13)	35 ± 18	n.d.
H63D (+/−)	34	39·5 ± 17·8	38 (119, 12)	31 ± 14	n.d.

* Geometric mean and asymmetric widths.
† Values given before treatment.
‡ Classified according to the C282Y mutation.

mutation, giving a gene frequency of 0·048. 40/299 chromosomes at risk carried the H63D mutation, giving a frequency of 0·13 in controls. Two subjects (1·3%) were homozygous and 23·6% were heterozygous for the H63D mutation.

Blood values characterizing the individual iron status such as serum ferritin and transferrin saturation showed no significant differences between the group of 'wild-type' gene carriers and heterozygous carriers of both the C282Y mutation and the H63D mutation (Fig 1, Table I).

DISCUSSION

The high frequency (94·6%) of the C282Y mutation in homozygous form in patients from North Germany is in good agreement with previous studies in other Caucasian populations (Feder *et al*, 1996; Jazwinska *et al*, 1996; Jouanolle *et al*, 1997). The C282Y homozygosity seems to be lower (about 70%) in iron-loaded patients from Southern European countries such as France and Italy (Borot *et al*, 1997; Piperno *et al*, 1998).

Also similar to other studies, a high number of compound heterozygotes (4/5) was found in the iron-loaded patients with heterozygous C282Y (Feder *et al*, 1996). The reason for the complete linkage disequilibrium between the C282Y and the H63D mutation is still unclear at the moment.

In the control group of 157 unrelated subjects, no case with iron overload and no homozygous C282Y mutation was found. 15/314 chromosomes carried the C282Y mutation, giving an allelic frequency of 4·8%. Assuming that this mutation is the only cause for haemochromatosis, one can calculate for the prevalence of homozygous HH to be 1:440. This is in perfect agreement with a screening study in 2812 prospective blood donors from Hamburg, in which increased values of serum iron and/or serum ferritin were used as filter parameters. A relevant iron overload was found in seven subjects, giving a prevalence of 1:402 (Nielsen *et al*, 1995).

Comparing the values for transferrin saturation and serum ferritin in heterozygote carriers from the family group and the control group, no differences between both groups were found (Table I, Fig 1). This indicates that the 'approved' haemochromatosis genes in the family group are not different from 'by-chance C282Y' genes in the normal population. This would support the argument that the C282Y mutation is the only cause which is sufficient for haemochromatosis disease.

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