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HAPTOGLOBIN TYPE NEITHER INFLUENCES IRON ACCUMULATION IN MEN NOR PREDICTS CLINICAL PRESENTATION IN HFE C282Y HAEMOCHROMATOSIS: PHENOTYPE AND GENOTYPE ANALYSIS

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ABSTRACT

In the UK 90% of patients with hereditary haemochromatosis (HH) are homozygous for HFE C282Y as are 1 in 150 people in the general population. However only a minority of these will develop clinical haemochromatosis. Iron loss modifies iron accumulation but so may other genetic factors. Haptoglobin (Hp) exists in three major types (Hp 1-1,Hp 2-1 or Hp 2-2) and binds free plasma haemoglobin. Hp 2-2 in men has been shown to be associated with increased macrophage iron accumulation and serum ferritin concentration. Furthermore, the frequency of Hp 2-2 has been shown to be increased in patients with HH. We determined Hp types by polyacrylamide gel electrophoresis and genotyping in 265 blood donor controls and 173 subjects homozygous for HFE C282Y. The latter included 66 blood donors lacking clinical features suggestive of haemochromatosis and without a family history and 68 patients presenting clinically with haemochromatosis. To investigate the relationship between iron accumulation and haptoglobin type we determined transferrin saturation and serum ferritin concentration in 192 male, first-time blood donors aged 20-40y who lacked both HFE C282Y and H63D. Hp frequencies did not differ between the groups. Transferrin saturation and serum ferritin concentrations did not vary with Hp type. Hp 2-2 does not appear to be a risk factor for iron accumulation in normal men or for the development of disease in subjects homozygous for HFE C282Y.

INTRODUCTION

Hereditary haemochromatosis (HH) is an autosomal recessive disorder in which there is iron accumulation as a result of increased dietary absorption. In 1996, the HFE gene was located on the short arm of chromosome 6 about 5Mb telomeric to the HLA Class 1 region (Feder *et al*, 1996). It codes for an HLA Class 1 protein and 90% of chromosomes from patients with HH were found to have a single mutation of this gene (C282Y). In the UK over 90% of patients are homozygous for this mutation (The UK Haemochromatosis Consortium, 1997) as are about 1 in 150 people in the general population (Jackson *et al*, 2001).

The replacement of cysteine by tyrosine at position 282 in the HFE gene (C282Y) causes the loss of a disulphide bridge essential for the protein's ability to bind to β_2-microglobulin (Feder *et al*, 1996). Unlike the wildtype protein, the mutant protein is not expressed at the cell surface. Ordinarily, the HFE protein binds to the transferrin receptor to inhibit the binding of iron-loaded transferrin, thereby regulating the amount of iron imported into cells. How this causes the enhanced iron absorption in haemochromatosis remains a matter for debate (Roy & Andrews, 2001;Townsend & Drakesmith, 2002).

Once diagnosed the excess iron is readily removable by regular venesection and if treatment is started before complications have arisen, life expectancy is not reduced (Niederau *et al*, 1996). The frequency of the mutation, the availability of a genetic test and the effective treatment by phlebotomy have led to pressure to implement population screening (Burke *et al*, 1998). However the clinical significance of *HFE* mutations remains uncertain.

Iron accumulation is influenced by blood loss and diet but studies of twins (Whitfield *et al*, 2000) and in several strains of HFE knock-out mice suggest that genetic factors other than HFE are also important (Levy *et al*, 2000b) (Fleming *et al*, 2001;Dupic *et al*, 2002). A recent study has suggested that haptoglobin 2-2 may cause increased iron accumulation in macrophages and serum ferritin concentration in men (Langlois *et al*, 2000). Furthermore, patients with haemochromatosis homozygous for C282Y have been shown to have an increased frequency of Hp 2-2 compared with the general population (Van Vlierberghe *et al*, 2001).

Haptoglobin (OMIM No 140100) is an α_2 plasma glycoprotein (Polonovski & Jayle, 1938). It is produced mainly by hepatocytes and released into the plasma where it remains for up to 10 days. It binds with high affinity in a 1:1 ratio to free oxygenated haemoglobin. This binding prevents loss of free haemoglobin in the urine and protects against oxidative tissue damage. After removal from the circulation by hepatocytes, Kupffer cells and other macrophages, the complex is endocytosed (Kristiansen *et al*, 2001) but haptoglobin is not recycled. The haptoglobin-haemoglobin complex is rapidly removed from the plasma with a T½ of 10-30 min (Garby & Noyes, 1959). However, the essential role of haptoglobin appears to relate more to reduction in oxidative damage than to the rate of clearance of haemoglobin from the circulation (Lim *et al*, 1998).

Haptoglobin is made up of 2 α chains and 2 β chains linked by disulphide bonds. Smithies first proposed that the haptoglobin protein was polymorphic (Smithies, 1955). There are three major types of haptoglobin, Hp 1-1 (~86kDa), Hp 2-1 (86-300kDa) and Hp 2-2 (170-1000kDa). In the UK, the allele frequency for Hp^1 is approximately 0.4 (Mastana *et al*, 1994;Hudson *et al*, 1982). Each phenotype is determined by a pair of autosomal, codominant allelic genes found on chromosome 16q22, designated Hp^1 and Hp^2. The Hp^1 allele can be further subdivided into Hp^{1F}, which has aspartic acid and lysine at positions 52 and 53 respectively, and Hp^{1S}, which has asparagine and glutamic acid (Bowman & Yang, 1987). Genetic crossover between Hp^1 alleles has resulted in a partial deletion and duplication, producing a new, elongated Hp^2 allele. This duplication has resulted in an additional exon 3 and 4 being translated in the Hp^2 product. The Hp^1 gene encodes the α1 subunit (approx 8.86kDa), and the Hp^2 gene encodes the α2 subunit (approx 17.3kDa).

Many studies implicate haptoglobin phenotype as a possible contributor towards the risk of developing disease (Langlois & Delanghe, 1996). Haptoglobin has also been implicated as a factor modifying the phenotype in hereditary haemochromatosis as Hp 2-2 was over-represented in haemochromatosis patients homozygous for the C282Y mutation, and these patients had

higher serum iron and ferritin concentrations than patients with Hp2-1 and 1-1(Van Vlierberghe *et al*, 2001).

In the present study, we have determined the haptoglobin types of subjects homozygous for HFE C282Y, a control group of blood donors not homozygous for HFE C282Y and male first time blood donors aged 20-40 y who lacked HFE C282Y and H63D mutations. We have compared the transferrin saturation and serum ferritin concentrations between haptoglobin types within each group.

MATERIALS AND METHODS

Sample Selection

Whole blood and serum samples were available from 10,500 consenting blood donors from South Wales who had participated in a research-based genetic screening programme (Jackson *et al*, 2001). Among these donors, 72 were found to be homozygous for HFE C282Y. Initially, both blood and serum samples were available from these donors. The control group consisted of 265 randomly selected samples from blood donors (Jackson *et al*, 2001), none of whom were homozygous for C282Y. Samples were available from unrelated patients homozygous for HFE C282Y who presented clinically with haemochromatosis in South Wales (McCune *et al*, 2002). A total number of 173 subjects homozygous for C282Y included 68 of these index patients, 66 blood donors and 39 individuals discovered through family screening of both groups. In addition, 192 samples were tested from male first time blood donors aged 20-40y who lacked the C282Y and H63D mutations of the HFE gene. When available, serum was used to determine Hp phenotypes but for many blood donors only frozen whole blood was available and a method of genotyping was developed for this study. The blood donor study and the study of families of C282Y homozygous individuals received approval from the local ethical committee.

Measurement Of Transferrin Saturation And Serum Ferritin Concentration

For blood donor serum iron concentration, unsaturated iron binding capacity, transferrin

saturation and serum ferritin concentration were determined as described by Worwood

(Worwood, 2001). For the patients, only presentation values were included. The assays were

carried out in several hospitals in South Wales.

HFE Genotyping

HFE genotypes of all blood donor samples included in this study were determined by PCR using

the method of Guttridge et al (Guttridge *et al*, 1998). For patients, HFE genotypes were

determined by heteroduplex analyses (Jackson *et al*, 1997;Worwood *et al*, 1999).

Haptoglobin genotyping

Frozen whole blood (500 μL) was added to an equal volume of cell lysis buffer (Tris-HCl 10

mmolL^{-1} pH 8.0, sucrose 11% w/v, MgCl$_2$ 5 mmolL^{-1}, Triton X-100 1% v/v). After vortexing briefly

and incubating at room temperature (RT) for 2 min, the nuclei were harvested by centrifugation

(Heraeus microfuge 6,000 rpm, 2 min, RT). The supernatant was discarded, and the nuclei were

resuspended by briefly vortexing in cell lysis buffer (500 μL) and again centrifuged. The

supernatant was discarded, and the nuclei gently resuspended in 300 μL nuclei lysis buffer (Tris-

HCl pH 8.0 10 mmolL^{-1}, EDTA 10 mmolL^{-1}, sodium citrate 10 mmolL^{-1}, SDS 1% w/v). NaCl (6

molL^{-1}, 100 μL) and then chloroform (500 μL) were added. The mixture was inverted gently until

a uniform emulsion formed and this was centrifuged at 6,000 rpm, 5 min, RT. A portion of the

upper aqueous layer (300 μL) was decanted into absolute ethanol (600 μL) and was then mixed

by gentle inversion until the high molecular weight DNA had precipitated as a small, fibrous ball.

The DNA was transferred to sterile water (25 μL), dissolved at 4°C for 3 h or at RT for 1 h, and

the concentration was then determined by dilution of an aliquot (10μL) in water (1mL) followed by

UV spectrometry.

The Hp^2 cDNA (Genbank Entry: NT_010494. Homo sapiens chromosome 16) starts at position 1152337 (preceding Exon 1) and ends at position 1146076 (the last amino acid of Exon 5). The Hp^2 allele runs in the 3' to 5' direction on the reverse complement strand of chromosome 16. Figure 1 is a diagrammatic representation of the Hp^2 allele indicating the positions of exons and introns. Using this diagram, we estimated the approximate sizes of the bands produced by the primers annealing at Exons 2 and 5. Since Hp^2 is completely documented, we were confident of our prediction of a 4.37Kb band. However, for Hp^1 only the cDNA sequence was available. We estimated the size of the predicted band by eliminating any combination of exon 3 (i and ii) and 4 (i and ii) and their introns in turn, all of which gave an approximate size of 3.0kb.

Primers Hp-F (5' CTGCTCTGGGGACAGCTTTTTGCAGTGG 3') and Hp-R (5' TGGTCAGTAAATTTAAAATTGGCATTTC 3') exploit the size difference due to the extra two exons of the Hp^2 allele. Primer design took into consideration the haptoglobin-related gene (HpR), which is highly homologous with the haptoglobin gene. Long primers were designed with 3' ends that were mismatched against HpR.

Approximately 250ng of high molecular weight DNA was amplified using the Hp-F and Hp-R primers in a 15μL reaction mix consisting of 1x PCR Buffer II and 2.25mM MgCl$_2$ (GeneAmp®, Perkin Elmer), 0.5mM dNTPs (GeneAmp®, Applied Biosystems), 0.6 μM of each primer (Oswel DNA Service, Southampton) and 1 unit of Amplitaq DNA Polymerase (Applied Biosystems). After a "hot start" at 92ºC for 2 min, there were 28 cycles of denaturing at 92ºC for 30 s, annealing at 62ºC for 30 s and extension at 68ºC for 10 min. There was a 20 s increment in the extension step of each cycle. The PCR was performed on a Phoenix thermocycler (Helena Biosciences). PCR product (4μL) was added to 1.5μL loading buffer containing bromophenol blue and loaded on a 0.8% (w/v) agarose gel (8cmx10cmx1cm) in 0.5X TBE (Tris-borate-EDTA) buffer pH 8.0 containing ethidium bromide for electrophoresis at 60V for 3 h. The size marker was a 1Kb DNA ladder marker (Gibco BRL).

Haptoglobin Phenotyping

A red cell lysate containing free haemoglobin was prepared by adding 500μL of whole blood to 500μL of water and centrifuging (Heraeus microfuge 13,000rpm, 5 min). The supernatant was removed, added to 500μL of water and re-centrifuged. The supernatant was removed and 1.5μL was added to 15μL of serum and left for 10 min for the haptoglobin-haemoglobin complexes to form. The 15μL sample was added to 1.5μL loading dye and loaded on to a 2mm (16cmx16cm) 5% polyacrylamide gel (37.5:1 polyacrylamide gel, Appligene) for electrophoresis in 1xTBE buffer pH 8.0 at 200V for 3 h. The gel was removed and incubated for 1 h with a solution of 15mL 1xTBE in which was dissolved 1 tablet of 3,3'-diaminobenzidine (Sigma) and 15μL hydrogen peroxide (Sigma), in order to detect haem.

Analysis Of Results

The distributions of haptoglobin frequencies in groups of subjects were compared using the Chi^2 test. Transferrin saturations are given as mean $\pm$ SD and mean values are compared using the two sample t-Test assuming unequal variance. Serum ferritin concentrations were not normally distributed so the median and the ranges are given. Median values were compared using the Mann-Whitney U Test. A probability of <0.05 was taken to indicate a significant difference.

RESULTS

Genotyping

We developed a new method of haptoglobin genotyping using frozen whole blood and a single PCR that exploits the 1.7Kb size difference between the product of the Hp^1 and Hp^2 alleles. The PCR method was validated by testing 86 samples that had been previously typed using PAGE. There was 100% agreement. Fig. 2a shows the PCR products for 6 subjects. The product present in samples 1 & 2 is approximately 4.7kb in size while the product present in samples 5 & 6 is approximately 3.0kb in size. The larger product was less intense, since it was less favourably amplified during the long distance PCR.

The Hp0 type has a gene deletion from the Hp promoter to the 5' end of HpRβ (Koda *et al*, 1998) and would be misinterpreted as a failure of the PCR in the very rare case of a homozygous deletion. Sometimes cases of hypo-haptoglobinaemia may be compound heterozygotes (Hp^2/Hp0) (Koda *et al*, 1998). These would be considered to be Hp 2-2 type after PCR. However, the frequency of Hp0 is very low in European Caucasians (Langlois & Delanghe, 1996). Furthermore, the observed haptoglobin frequencies did not differ from the expected Hardy-Weinberg distribution calculated by assuming that there were two genotypes, Hp^1 and Hp^2. The haptoglobin frequencies in controls are those expected for a Northern European population (Langlois & Delanghe, 1996).

Phenotyping

Fig. 2b shows the PAGE results for the serum of the same subjects tested in Fig 2a. Haptoglobin phenotypes were easily distinguishable by the number and position of the bands stained. The Hp 1-1 phenotype has a single band that rapidly migrates towards the cathode. The Hp 2-2 phenotype has a series of slower bands, while the Hp 2-1 phenotype has a mixture of both types. The Hp 1-1 band has a similar mobility to that of free haemoglobin (which must always be present to ensure staining of all haptoglobin bands). Subjects carrying Hp0 may have anhaptoglobinaemia or may have very low plasma concentrations so only the free haemoglobin will electrophorese on the gel and be stained. Subjects with Hp0 may therefore be reported as Hp 1-1 but the frequency of Hp0 is very low in European populations (see above).

Haptoglobin Types In Blood Donors And Subjects Homozygous For C282Y

Table 1 shows the haptoglobin types for the control subjects (blood donors), first time male blood donors, all subjects homozygous for C282Y, blood donors homozygous for C282Y and unrelated patients homozygous for C282Y. There were no significant differences for haptoglobin distribution between any pair of groups. Haptoglobin frequencies for males and females within each group were not significantly different. When male and female subjects were compared

between groups there were no significant differences. The greatest % differences between groups were for Hp 1-1. When frequencies were compared using a 2x2 table (Hp 1-1 and others) there were no significant differences in frequency for all subjects or for males and females separately. There were no significant differences in haptoglobin frequency for subjects who were "wildtype" HFE, heterozygotes for H63D or heterozygotes for C282Y in the control blood donor group (data not shown).

Transferrin Saturation, Serum Ferritin Concentrations and Haptoglobin Type

Table 2 shows the mean transferrin saturation and the median serum ferritin concentration for each group according to haptoglobin type and sex. Information about iron status was available for almost all controls and for the blood donors homozygous for C282Y but values were not available for all patients at presentation. There were no differences in iron status for each haptoglobin type within the groups of subjects, even when divided into males and females. We also examined the variation of transferrin saturation and serum ferritin concentration in male, first time blood donors aged 20-40y who lacked both the C282Y and H63D mutations of the HFE gene (Table 3). There were no significant differences in either transferrin saturation or serum ferritin concentration between the haptoglobin types.

DISCUSSION

It was necessary to develop a method to determine haptoglobin types by genotyping, because many of the subjects had only frozen whole blood available. Recently a method of genotyping based on similar principles has been described (Koch *et al*, 2002). The authors confirmed their results using alternative PCR protocols. Both genotyping and the PAGE methods are suitable for healthy subjects of European origin where the frequency of Hp0 is very low.

Jackson et al (Jackson *et al*, 2001) tested 10,500 blood donors from South Wales and identified 72 who were homozygous for HFE C282Y. None were aware of any family history of iron overload and none had clinical features suggestive of haemochromatosis. Most of the men, but

only 45% of the women, had a transferrin saturation > 50%. Only 5 had both transferrin

saturations and serum ferritin concentrations raised above the clinical threshold for iron overload

(> 200 μg/L for pre-menopausal women, > 300 μg/L for men and post-menopausal women).

These findings suggest a low penetrance for homozygosity for C282Y for both iron overload and

disease. However, the mean age of these blood donors was 37y (range 17-66), below the mean

age of 50y for diagnosis of haemochromatosis (Mcdonnell *et al*, 1999). In order to assess the

life-long impact of haemochromatosis on morbidity a survey of hereditary haemochromatosis as a

clinical condition was carried out in South Wales. It was calculated that only 1.2% of adult C282Y

homozygotes have received a confirmed diagnosis (McCune *et al*, 2002), suggesting that the

morbidity associated with homozygosity of C282Y in our region was low. Restricting the

calculation to men over 45y, the figure rose to 2.8%. Similar conclusions about morbidity in

haemochromatosis were reached by Beutler et al in a study of 152 C282Y homozygotes from

41,038 subjects presenting to a Health Appraisal Clinic in California (Beutler *et al*, 2002a).

In healthy men Hp 2-2 was found to be associated with a higher transferrin saturation, lower

transferrin receptor concentrations and increased serum ferritin and macrophage ferritin

concentrations when compared to men with Hp 1-1 or Hp 1-2 (Langlois *et al*, 2000). The present

study does not support this finding. However, neither blood donors nor patients with

haemochromatosis provide a suitable group to examine a possible relationship between iron

status and Hp type, since blood donation reduces iron stores and HH patients will have varying

degrees of iron overload depending upon the age at diagnosis, among other factors. We have

therefore examined the variation of transferrin saturation and serum ferritin concentration in male,

first time blood donors aged 20-40 y who lacked both the C282Y and H63D mutations of the HFE

gene so that the HFE genotype did not influence measures of iron status (Jackson *et al*, 2001).

An estimated 3.9% will carry HFE S65C but this mutation is not associated with changes in

transferrin saturation or serum ferritin compared to HFE "wildtype" blood donors (Carter et al

unpublished). There would be little influence of blood loss, either pathological or as a result of

blood donation in these healthy young men. There were no significant differences in either

transferrin saturation or serum ferritin concentration between the Hp types.

There have been numerous reports of associations between Hp phenotypes and diseases (Langlois & Delanghe, 1996). There are no reports suggesting that the possession of a particular Hp type is a risk factor for arthritis or diabetes. However, the Hp 1-1 phenotype has been associated with protection against vascular complications in both Type I (Levy *et al*, 2000a) and Type II (Nakhoul *et al*, 2001) diabetes. Zhao and Zhang found that Hp1-1 was over-represented in 107 patients with cirrhosis of the liver compared with 552 normal adults (Relative risk 3.3, p = $2x10^{-4}$)(Zhao & Zhang, 1993). Zipprich et al found an increased frequency of Hp1-1 in 100 German patients with chronic, non-alcoholic liver disease but not in 90 patients with cirrhosis when compared with 1726 controls (Zipprich *et al*, 1986). There appear to be no clear associations between Hp type and risk of the major clinical manifestations of HH. Differences in turnover and haemoglobin binding may explain both changes in iron storage and in disease risk for Hp 2-2. The Hp 1-1 phenotype binds free haemoglobin more efficiently than either Hp 2-1 or Hp 2-2 (Langlois & Delanghe, 1996). It also has unrestricted access to tissues, possibly due to its smaller size and consequent ability to migrate across the endothelial cell barrier. These advantages may contribute to a more effective clearance of free haemoglobin from the plasma than is achieved by the other two phenotypes. However, it has been reported (Kristiansen *et al*, 2001) that Hp 2-2 complexed with haemoglobin exhibits a higher affinity for the CD163 receptor than the other complexed haptoglobin phenotypes, which would suggest a preferential delivery of haemoglobin complex to the macrophage. Subjects with the Hp 2-2 phenotype may therefore have a tendency to accumulate more iron in macrophages (Langlois *et al*, 2000). Plasma vitamin C concentrations were lowest in the Hp 2-2 phenotype (Langlois *et al*, 1997). These authors suggested that this may be a consequence of the weaker binding of haemoglobin by Hp 2-2 and the weaker anti-oxidative capacity.

The above findings provide a basis for differences in the handling of haemoglobin iron by the major haptoglobin types. However, our studies of normal men who had not donated blood and

lacked the HFE mutation, and subjects homozygous for HFE C282Y do not suggest that haptoglobin is a "disease-modifying" gene in haemochromatosis.

These findings confirm two recent reports. A recent study reported no differences in the frequency of Hp 2-2 between subjects homozygous for HFE C282Y and controls lacking the HFE mutations (Beutler *et al*, 2002b). Among subjects of African origin iron status did not vary with Hp type (Kasvosve *et al*, 2002). Beutler et al (2002) suggest that conflicting conclusions about the frequency of Hp 2-2 in haemochromatosis may reflect differences in ascertainment. However in our study we did not find significant differences in frequency between cases "discovered" by genetic testing to be homozygous for HFE C282Y and the patients presenting clinically with signs and symptoms of iron overload who were homozygous for HFE C282Y.
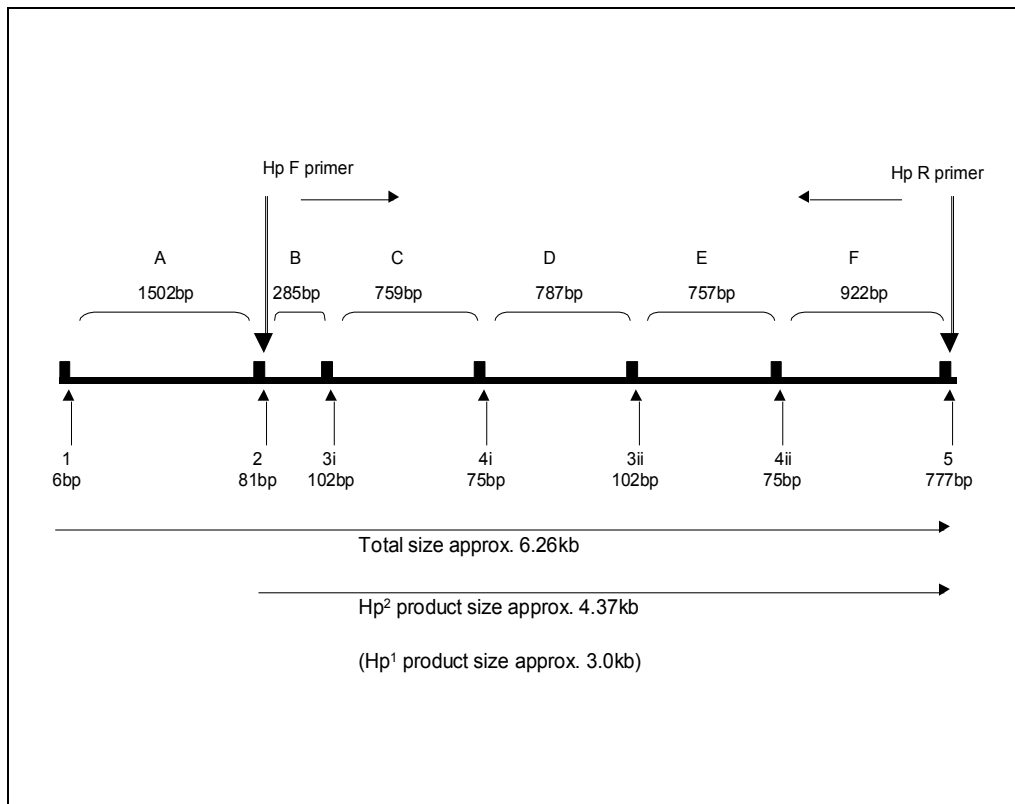
ACKNOWLEDGEMENTS

Figure 1

Model of the structure of Hp^2 cDNA based upon Genbank entry NT_010494.

Exons are numbered 1 to 5 with their approximate sizes below. Introns are labelled A to F along with approximate intron sizes.

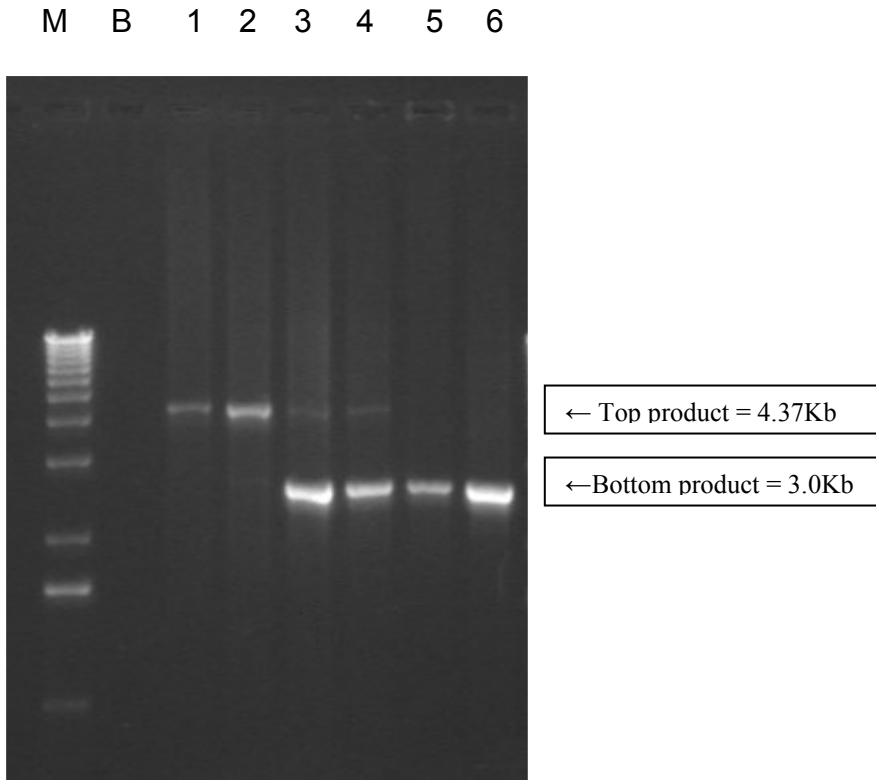PCR primers extend in the direction indicated by arrows.

Figure 2a: Haptoglobin genotyping using long distance PCR

Samples 1 & 2 are homozygous for the Hp^2 allele, samples 3 & 4 are heterozygous for the Hp^1

and Hp^2 alleles and samples 5 & 6 are homozygous for the Hp^1 allele.

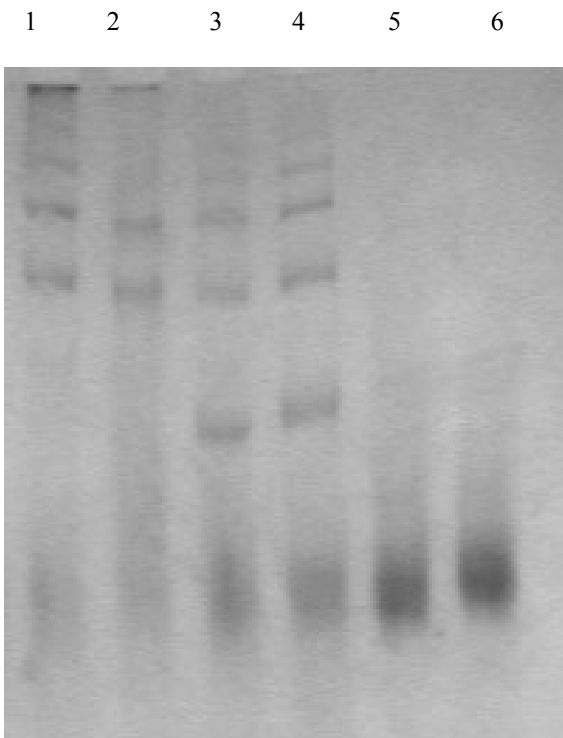M = 1kb DNA ladder ; B = water blank.

Figure 2b: Haptoglobin typing using polyacrylamide gel electrophoresis

The samples are the same as those depicted in Figure 2a.

GROUP	No. OF SUBJECTS	FEMALE / MALE	HP 1-1	HP 2-1	HP 2-2
Blood donor controls	265	140 / 125	43 (16.2%)	124 (46.8%)	98 (37%)
Male first time blood donors	192	N/A	27 (14.1%)	87 (45.3%)	78 (40.6%)
All homozygous for C282Y	173	76 / 97	26 (15%)	76 (43.9%)	71 (41%)
Blood donors homozygous for C282Y	66	39 / 27	14 (21.2%)	32 (48.5%)	20 (30.3%)
Patients homozygous for C282Y	68	22 / 46	8 (11.8%)	34 (50%)	26 (38.2%)

Table 1

Haptoglobin types in blood donors and patients with haemochromatosis.

GROUP	SEX	Hp Type	n	TS (%) mean ± SD	n	s Fn (µg/L) median	Range
Blood Donors	Male	1-1	24	31± 11	24	104	34 – 183
		2-1	59	31 ± 15	61	96	6 – 299
		2-2	40	31 ± 11	40	87	24 – 207
	Female	1-1	19	25 ± 12	19	57	9 – 116
		2-1	63	24 ± 11	63	37	7 – 301
		2-2	57	25± 11	58	41	9 – 137
Blood Donors – C282Y homozygous	Male	1-1	5	56 ± 15	5	141	51 – 374
		2-1	14	63 ± 16	14	221	14 – 650
		2-2	8	69 ± 23	8	135	85 – 410
	Female	1-1	9	56 ± 25	9	58	11 – 238
		2-1	18	49 ± 20	18	68	13 – 418
		2-2	12	50 ± 19	10	96	8 – 195
Patients – C282Y homozygous	Male	1-1	4	69 ± 13	4	1215	620 – 3675
		2-1	12	78 ± 13	15	1585	494 – 3422
		2-2	10	83 ± 9	11	1790	540 – 5738
	Female	1-1	1	89	2	980	655 – 1305
		2-1	4	81 ± 15	4	991	376 – 3002
		2-2	7	74 ± 15	8	845	263 – 2720

Table 2

Transferrin saturation and serum ferritin concentration according to Hp type.

The numbers tested are samples for which either transferrin saturation or serum ferritin

concentrations were available.

Hp type	Number	Transferrin Saturation (%) mean $\pm$ SD	Serum ferritin (μg/L) median and range
1-1	27	28 $\pm$ 10	112 (25 – 193)
2-1	87	30 $\pm$ 11	100 (34 – 263)
2-2	78	29 $\pm$ 9	108 (26 – 223)

Table 3

Transferrin saturation and serum ferritin concentration in male first time blood donors lacking HFE

C282Y and H63D and aged 20-40 y.

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