Haptoglobin: a review of the major allele frequencies worldwide and their association with diseases

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SUMMARY

Haptoglobin (Hp) is a plasma α_2-glycoprotein which binds free haemoglobin, thus preventing oxidative damage. The complex is rapidly removed from the circulation by a specific receptor (CD163) found on macrophages. Three major subtypes, Hp1-1, Hp2-1 and Hp2-2 are the product of two closely related genes HP^1 and HP^2. The frequency of the HP^1 and HP^2 genes varies worldwide depending on racial origin: the HP^1frequency varying from about 0.07 in parts of India to over 0.7 in parts of West Africa and South America. Both HP^1 and HP^2 have been linked to susceptibility to various diseases. Such associations may be explained by functional differences between the subtypes in the binding of Hb and its rate of clearance from the plasma. However, there are also corresponding negative reports for disease associations. The conflicting evidence on disease association and the lack of association between disease and particular populations, despite the wide range of HP^1 and HP^2 gene frequencies across the world, may indicate that any associations are marginal.

HAPTOGLOBIN STRUCTURE AND FUNCTION

Polonovski and Jayle (1940) first described a protein with haemoglobin binding properties, which they later designated 'haptoglobin', from the Greek word *haptein* – to bind.

Haptoglobin (Hp) is a plasma α_2-glycoprotein synthesized primarily by hepatocytes (Putnam, 1975). Synthesis is stimulated by infection or inflammation (the acute phase response). After release into the circulation it has a half-life of 2–4 days (Garby & Noyes, 1959). Haptoglobin binds oxygenated, free haemoglobin with a very high affinity of approximately 1×10^{-15} mol/l (Okazaki, Yanagisawa & Nagai, 1997). Free haemoglobin can be harmful to the body, promoting the accumulation of hydroxyl radicals via the Fenton reaction ($H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + OH$), resulting in oxidative tissue damage (Sadrzadeh *et al.*, 1984). Once irreversibly bound to haptoglobin, free haemoglobin loses its oxidizing ability (Sadrzadeh *et al.*, 1984; Gutteridge, 1987). The haptoglobin–haemoglobin (Hp–Hb) complex is removed from the

circulation by binding to a receptor (CD163) found on the cell surface of monocytes and macrophages (Kristiansen *et al.*, 2001). Binding instigates the endocytosis of the Hp–Hb complex into the macrophage. Here the iron is released by haem oxygenase and transported back to the bone marrow via plasma transferrin, for synthesis of new haemoglobin. Haptoglobin is not recycled and when haptoglobin is completely saturated with free haemoglobin, levels can take 5–7 days to recover, because synthesis is not increased by low haptoglobin levels (Putnam, 1975).

THE HAPTOGLOBIN GENE (HP)

Haptoglobin was first described as polymorphic by Smithies who used starch gel electrophoresis to separate the various types (Smithies, 1955) and the existence of two autosomal genes with incomplete penetrance was proposed by Smithies and Walker (1955). These give rise to three major phenotypes in man (Smithies & Walker, 1956) still commonly referred to as Hp1-1, Hp2-1 and Hp2-2. They can be distinguished using starch gel or polyacrylamide gel electrophoresis according to their different sizes and band patterns (Hp1-1 = *ca* 100 kDa; Hp2-1 = 120–220 kDa; Hp2-2 = 160–500 kDa). Haptoglobin is composed of two α-chains and two identical β-chains. The α-chains are linked together by a disulphide bond, and each β-chain is similarly bonded to an α-chain giving the simple chain formula of $(\alpha\beta)_2$ for the monomeric form. Haptoglobin shows a high degree of homology to the proteins in the chymotrypsinogen family of serine proteases. The molecular weight of the Hp^1 α-chains is 8900 ± 400, while that for the Hp^2 α-chain is 17 300 ± 1400 (Smithies, Connell & Dixon, 1962). The β-chain is identical for both types, with a molecular weight of approximately 40 000 (Cheftel & Moretti, 1966).

The identification of haptoglobin phenotype based on its molecular size using electrophoresis has led to the suggestion that the complex patterns of Hp2-1 and Hp2-2 result from a series of polymers composed of two kinds of subunits. The product of the $HP\alpha^1$ forms only a dimer, while the product of $HP\alpha^2$ forms higher polymers, including polymers with the $HP\alpha^1$ product (Allison, 1959; Smithies, 1959). The series of Hp2-2 polymers ($\alpha_3\beta_3$, $\alpha_4\beta_4$,…) differ in molecular weight by an average increment of 54 500 (Fuller *et al.*, 1973).

In the Chimp, Gorilla and Old World monkeys, there are three genes in the haptoglobin family, HP, HPR and HPP. The evolution of these genes in primates is discussed by Maeda and Smithies (1986) and McEvoy and Maeda (1988). Humans have HP and HPR, and New World monkeys have only HP. There is no evidence of multiple band patterns in any species but man, suggesting that the $HP\alpha^2$ allele arose subsequent to the separation of the human evolutionary line from other primates, approximately 6 million years ago. Although the HPR gene was thought to be expressed only in primates (Maeda & Smithies, 1986) the haptoglobin-related protein (Hpr) has been identified in human serum at 5–10% of Hp levels. This protein binds Hb with high affinity but the Hpr–Hb complex is not recognized by the CD163 receptor (Nielsen *et al.*, 2006). Hpr is a component of trypanosome lytic factor-1, a subclass of HDL (Smith & Hajduk, 1995).

The HP^1 gene consists of five exons that encode a protein of 347 amino acids with a molecular mass of 38 kDa. The first four exons encode the α-subunit (α1) consisting of 42 amino acids while the fifth encodes the β-subunit (Maeda, 1991). The HP^1 allele can be further subdivided into the HP^{1F} and HP^{1S} alleles, which differ in their α-chains (see Table 1). This leads to a difference in electrophoretic mobilities, as HP^{1F} migrates faster than HP^{1S} – hence the names fast (F) and slow (S) (Connell, Smithies & Dixon, 1966).

The HP^2 gene consists of seven exons encoding a protein of 406 amino acids with a molecular mass of 45 kDa. The first six exons encode a larger α-subunit (α2) consisting of 83 amino acids and the seventh encodes the β-subunit (Maeda, 1991).

Smithies, Connell and Dixon (1962) and Bearn and Franklin (1958) proposed that HP^2 was a partially duplicated gene formed by a rare non-homologous crossover event that fused HP^{1F} and HP^{1S} genes (see later). This was confirmed by sequence analysis which

Table 1. Differences between the HP^{1F} and HP^{1S} variants of the HP^1 allele

	HP^{1F}	HP^{1S}
Amino acid 47	Valine (GTA)	Valine (GTG)
Amino acid 51	Asparagine (AAT)	Asparagine (AAC)
Amino acid 52	Aspartic acid	Asparagine
Amino acid 53	Lysine	Glutamic acid

Table 2. Haptoglobin α- and β-chain variants detected by electrophoresis listed in order of discovery. Deletions and other mutations associated with ahaptoglobinaemia and anhaptoglobinaemia are listed at http://www.hgmd.cf.ac.uk/. There are population specific base substitutions at the promoter region (Teye *et al.*, 2006)

Name	Reported by	Notes
Hp Ca (Carlberg)	Galatius-Jensen (1958)	An α-chain variant with a mixture of Hp2-2 and Hp2-1 bands
Hp2-1M (Modified)	Connell and Smithies (1959)	An α-chain variant encoded by a $Hp\alpha^{2M}$ allele (Giblett & Steinberg, 1960). Maeda found 3 other promoter sequences to explain the variability of the modified phenotype (Maeda, 1991)
Hp2-1 Haw	Giblett, Hickman and Smithies (1964)	An α-chain variant whose pattern shows a high proportion of α^1-chains compared with α^2
Hp Johnson	Oliviero *et al.*, (1985)	A variant with a larger α-chain due to a threefold tandem repeat of a 1.7 kbp DNA segment
Hp1-P & Hp2-P	Robson *et al.* (1964)	A β-chain variant identified in the presence of haemoglobin by the faster migration of bands, some of which were doubled
Hp1-H & Hp2-H	Robson *et al.* (1964)	A β-chain variant resembling Hp2-1 and Hp2-2 but with an extra band in the presence of haemoglobin
Hp Mb (Marburg)	Cleve and Deicher (1965)	A β-chain variant resulting from a mutational event causing one or two mutant β-chains
Hp2-1D (Dashing)	Renwick and Marshall (1966)	An α-chain variant from the $Hp\alpha^{1D}$ allele, only distinguishable in the absence of haemoglobin
Hp Ba	Giblett, Uchida and Brooks (1966)	An α-chain variant. Designated 1-B and 2-B when the $Hp\alpha^{B}$ allele is combined with $Hp\alpha^{1}$ or $Hp\alpha^{2}$ respectively
Hp2-1 Bellevue	Javid (1967)	A β-chain variant identified by an additional fast moving band in the β-chain region

indicated a 1.7 kb intragenic duplication that encoded an additional two exons identical to exons 3 and 4 of HP^1 (Yang *et al.*, 1983). Unequal crossing over between HP^{1F} and HP^{1S} in a HP^{1F}/HP^{1S} heterozygote produced an HP^2 allele described as HP^{2FS}. Crossing over between HP^2 (HP^{2FS}) and either HP^{1F} or HP^{1S} in HP^2/HP^1 heterozygotes produced the HP^{2FF}, HP^{2SS} and the HP^{2SF} alleles. The product of the latter is indistinguishable on electrophoresis from that of HP^{2FS}.

Since the discovery of the three major phenotypes of haptoglobin, other haptoglobin α- and β-chain have been identified on electrophoresis (see Table 2). Most are not commonly screened for since they are rare and do not constitute a significant source of error in estimating the major gene frequencies.

IDENTIFICATION OF HAPTOGLOBIN PHENOTYPES

Most methods of haptoglobin phenotyping are based upon electrophoretic separation in a gel medium according to size. Larger molecules are impeded more than small molecules in their migration through the gel. Hp1-1 has a single, fast moving band, whilst Hp2-1 and Hp2-2 each have multiple bands, which differ in size and electrophoretic mobility (see Figure 1). This is because the HP^2 product is capable of producing homopolymers (Hp2-2) and heteropolymers (Hp2-1) (Bearn & Franklin, 1958; Allison, 1959; Smithies, 1959). The bands present in Hp2-1 differ from those of Hp2-2, and the Hp1-1 band can be seen in the Hp2-1 series (Allison & ap Rees, 1957).

Assessing haptoglobin phenotype population frequencies using electrophoretic techniques is not always accurate. In many populations, a proportion of subjects have low or even undetectable haptoglobin levels due to severe haemolysis. These people are termed anhaptoglobinaemic (no detectable haptoglobin) or hypohaptoglobinaemic (extremely low levels of haptoglobin) and are classified as 'Hp0' in most screening studies. The real phenotype of 'Hp0' subjects may not be detectable so where a significant proportion of the population are Hp0 the frequencies of Hp1-1, 2-1 and 2-2 may not be accurate.

PCR-based methods have been developed enabling the identification of haptoglobin allele types in all

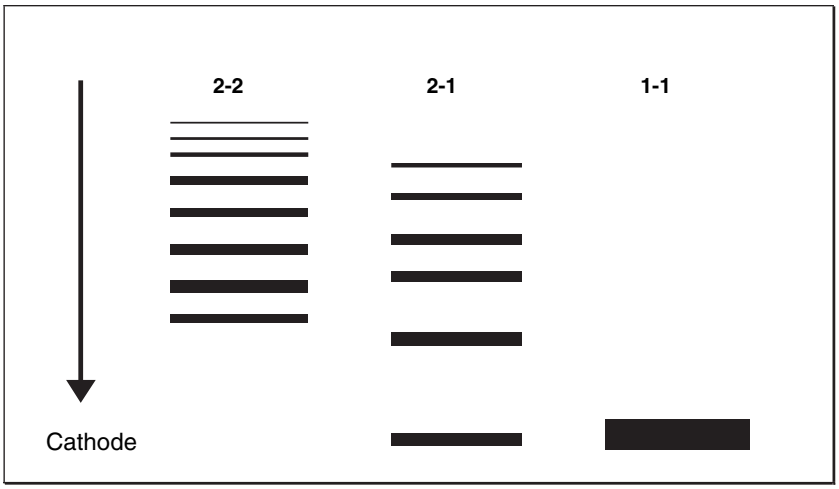


Figure 1. Band distributions of Hp1-1, Hp2-1 and Hp2-2 using starch gel electrophoresis.

subjects, including those designated Hp0 (Yano *et al.*, 1998; Beutler, Gelbart & Lee, 2002; Koch *et al.*, 2002; Carter *et al.*, 2003). Koda *et al.* (1998) discovered a congenital cause of anhaptoglobinaemia, designated HP^{del}. Sufferers are homozygous or heterozygous for a deletion that occurs from at least the promoter region of the HP gene to the 5′ flanking region of the β region of the HPR gene. A PCR has been developed to determine whether a person has congenital or acquired anhaptoglobinaemia by testing for the gene deletion (Koda *et al.*, 2000).

HAPTOGLOBIN CONCENTRATIONS AND PHENOTYPE

Total concentrations vary with phenotype, being lower for Hp2-2 subjects than for Hp1-1 and 2-1 (Vanrijn *et al.*, 1987; Langlois & Delanghe, 1996; Kasvosve *et al.*, 2000a). Values are lowest for the Hp2-1M phenotype (found in African populations). Here fewer Hp2 polypeptides are synthesized than Hp1 polypeptides (Maeda, 1991). However, in Chinese subjects, Hp concentrations were highest for male subjects with Hp2-2 (Na *et al.*, 2006b).

HAPTOGLOBIN ALLELE FREQUENCIES WORLDWIDE

In this review, we have collected haptoglobin allele frequencies from phenotyping surveys and from the control groups of reports linking haptoglobin to disease and have tabulated them according to continent (see Supplementary Material, Tables S1–S5). Rarer Hp phenotype variants such as Hp2-1M are not tabulated, and these numbers have been included as the Hp2-1 phenotype. Hp0 data in all surveys refer to subjects with acquired anhaptoglobinaemia. Hp0 has been excluded for HP^1 and HP^2 allele frequency estimations.

The frequencies of HP^1 and HP^2 in populations vary worldwide depending upon racial origin but both have been found in every population examined to date. There is also a significant difference in the distribution of the HP^{1F} and HP^{1S} alleles in populations worldwide. Before the development of highly specific DNA fingerprinting techniques for forensic work, Hp polymorphisms were used, with other markers, for the purpose of identification of subjects (Gaensslen, Bell & Lee, 1987).

AFRICA (FIGURE 2A AND TABLE S1)

In the last few hundred years, Africa has seen an influx of Caucasian, Mongoloid and Arab populations. Since the 16th century, Europeans here migrated to South Africa, while countries in the north have populations with strong historical, cultural and ethnic ties with the Middle East. There is a strong Arab influence in North African countries such as Algeria, whose inhabitants are a mix of Berber and Arab origin. The Berbers were the original people of that country until the Arabs arrived in the seventh century. Arabs now constitute 80% of the population.

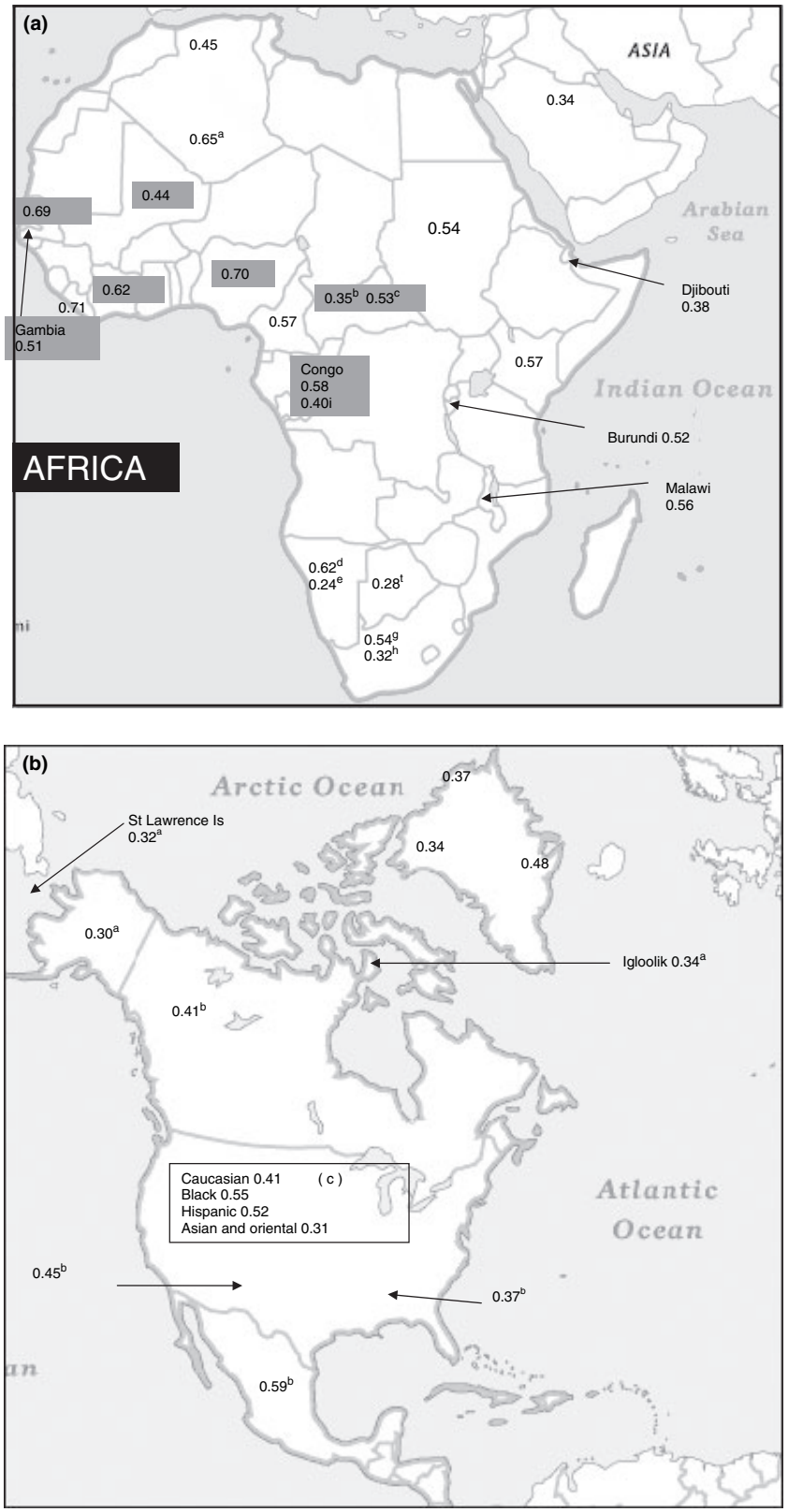
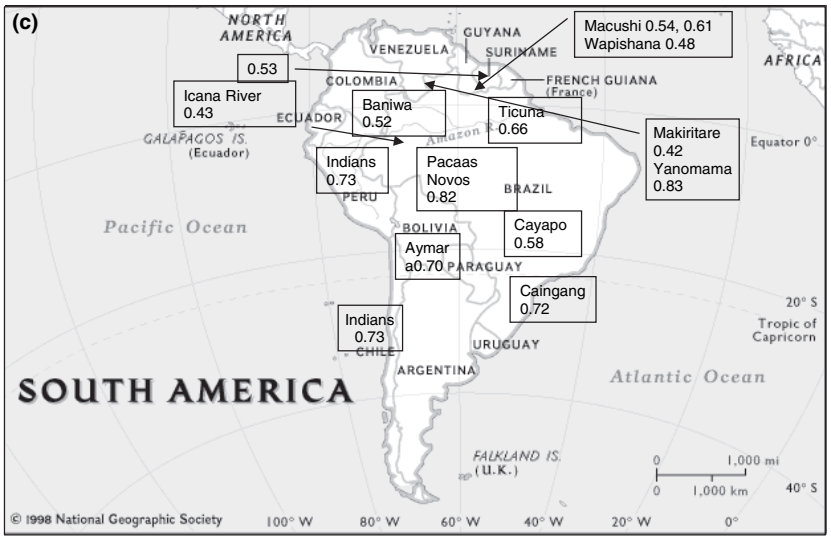


Figure 2. HP[1] frequencies throughout the world. Data are taken from Roychoudhury and Nei (1988) supplemented with later studies (see Supplementary Material). In general population samples of <100 have not been included, except where a number of populations are combined for a small area. In almost all case Hp1-1, 2-1 and 2-2 frequencies were determined by electrophoresis. The maps were downloaded from the National Geographic Society Website (http://www.nationalgeographic.com/xpeditions/atlas/). (a) Africa: [a]Algerian Sahara; [b]Central Africa Republic, Pygmies; [c]Sara; [d]Namibia, Kavango; [e]Kung San; [f]Botswana, Bushmen; [g]S Africa, Zulu, Xhosa, Msutu; [h]Bushmen !Kung, Dobe; [I]Pygmies; Frequencies in grey boxes are studies where the Hp0 frequency was >20%. (b) North America: [a]Eskimo; [b]Indian; [c]Data from Gaensslen, Bell and Lee (1987). (c) South America: These studies are for the Indian populations of S America only. (d) Asia: (e) Europe: The mean frequency of HP[1] is 0.386 95% CI: 0.384–0.388. In total the European study includes 163 087 subjects. *Significant difference from the mean frequency for Europe. Note that frequencies tend to be lower in Southern and Eastern Europe, as well as in the far North. (f) Oceania: [a]Aboriginal populations; [b]Combined frequency from the study of Hill *et al.* (1986); [c] Caucasian population. Maps are used with permission of the National Geographic Society.



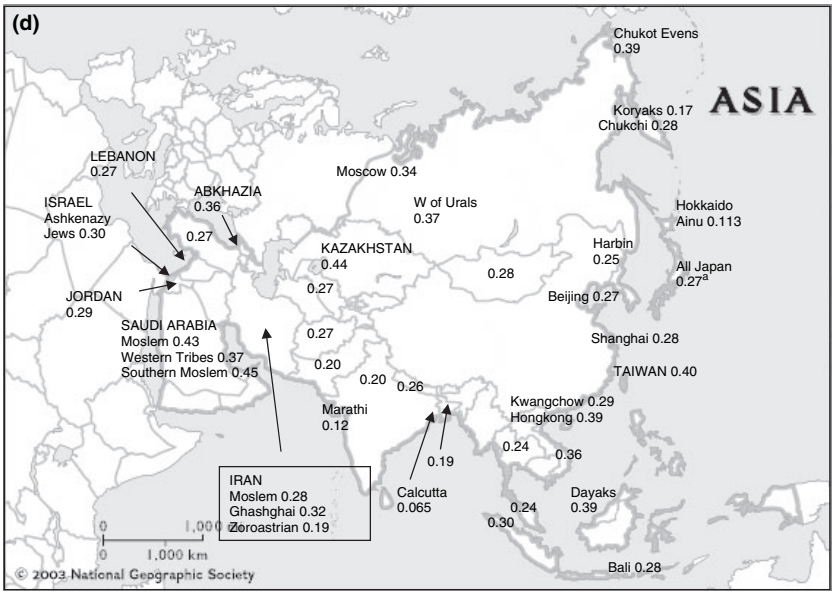
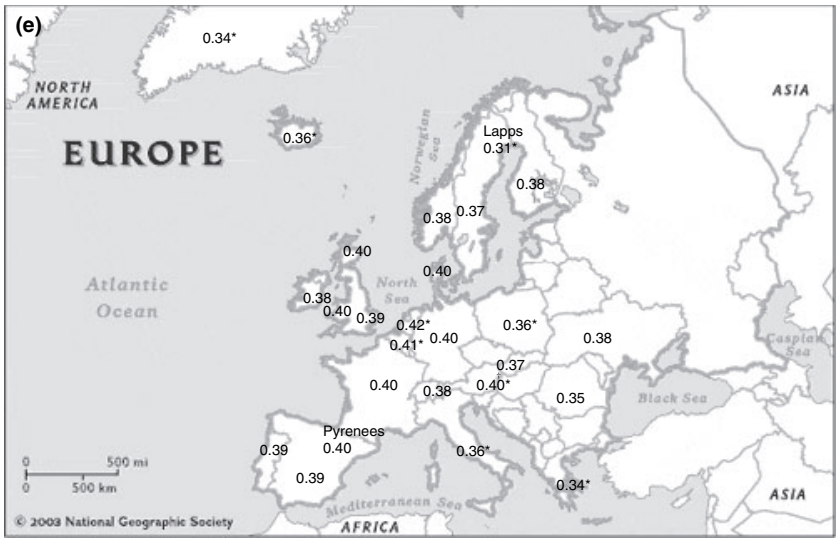
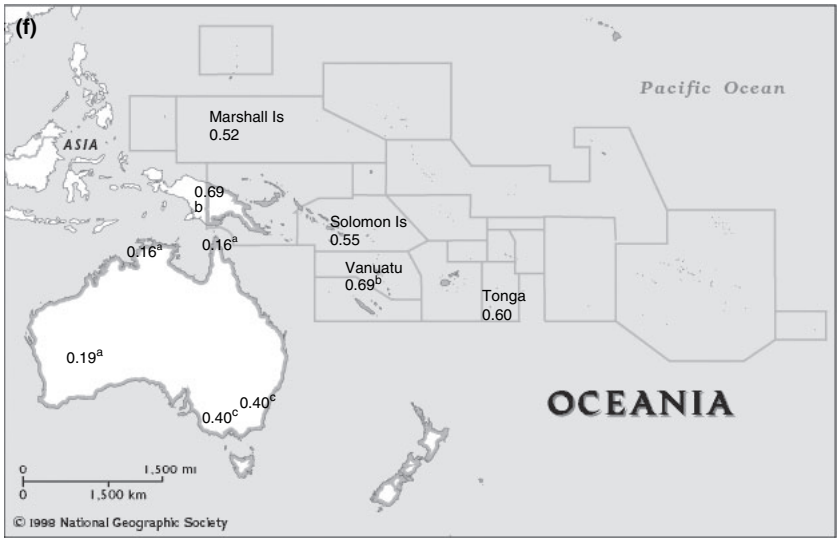


Figure 2. Continued.

Many tribes remain isolated, either by geography or by culture. Studies of these tribes should indicate the true Hp allele frequency for the original Black inhabitants of the continent. Table S1 shows the studies performed in Africa. The continent as a whole has a HP^1 allele frequency of 0.56 (95% CI: 0.46–0.66) with up to 47% of people tested being designated Hp0. However, the individual countries show marked differences, with surveys in Ghana and Namibia having HP^1 frequencies as low as 0.24 whilst in Nigeria HP^1 frequencies were as high as 0.87. The higher HP^1 frequencies seem to be linked to a higher HP^{1F} frequency for those studies with that data available. Figure 2 illustrates the geography of the average HP^1 and Hp0 frequency for the countries listed in Table S1.

High Hp0 frequencies are concentrated in the Western part of the continent. High numbers of Hp0 subjects are attributed to haemolytic diseases and are found in areas where malaria is endemic. Malaria is caused by infection of the single cell parasite, *Plasmodium*, during a bite from the *Anopheles* mosquito. Human malaria is most severe when caused by *Plasmodium falciparum*, which infects red blood cells and causes them to rupture in order to release more parasites into the blood. When left untreated, this results





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in severe haemolysis and anaemia. The infected subject is usually left anhaptoglobinaemic, as haptoglobin complexed with the free haemoglobin released by the rupture of the cell is rapidly removed from the circulation and is therefore undetectable by conventional electrophoresis methods. The prevalence of Hp0 is correlated with the level of malaria endemicity in several districts of the Congo (Trape & Fribourgblanc, 1988). However, both gene promoter polymorphisms (Teye *et al.*, 2003) and point mutations (Teye *et al.*, 2004) may also cause the Hp0 phenotype (ahaptoglobinaemia) in African populations.

For some countries there are large differences in the frequencies reported for different surveys. Some differences may be explained by tribal and cultural differences in each population surveyed. A good example of this are the Pygmy populations of the Congo and the Central African Republic, both of which show a reduced HP^1 allele frequency when compared with the other surveys of the country where there has been non-Negroid migration from other continents. Not all differences are this easily accounted for. Studies in Namibia and Nigeria show marked differences in HP^1 allele frequency between ethnically similar populations.

THE AMERICAS (TABLE S2)

Native Indians populated the Americas until the 1500s, when Europeans conquered much of the

continent. Today, Caucasians dominate the population of North America and Hispanics South America.

North America (Figure 2b)

Over the last century, North America has become a truly mixed nation in terms of its ethnic content. While the majority of the population is Caucasian, there are also significant numbers of Black, Hispanic and Oriental subjects who vastly outnumber the original Indian societies. Table S2 shows the HP allele frequencies for the Americas. The only significant Hp0 frequencies recorded were from surveys of purely Black people. The surveys of Black communities give a similar HP^1 result (0.55) to that of the continental average in Africa (0.562), although the Hp0 frequency is much lower (2.3%). Orientals have a HP^1 frequency of 0.31, higher than the average for SE Asia (0.27), probably because of inter-racial marriages.

The native Indians and Eskimos can be considered the closest ethnic groups to the original inhabitants of this continent. We would expect both of these groups to be culturally and, in the case of the Eskimos, geographically isolated. Only the Indian groups show an increased HP^1 allele frequency. The Eskimos have lower HP^1 allele frequencies ranging from 0.24 to 0.32. The Mexican Indians have an average HP^{1F} frequency of 0.38, while the only study to incorporate HP^{1F} screening for Eskimos detected none (Shim & Bearn, 1964). Their Hp1-1 frequency was only 3%, the lowest for the Americas and similar to that seen in some Asian subcontinents (see later). However, there were only 67 subjects in the population sample.

South America (Figure 2c)

Indians inhabited the continent of South America before Spanish conquest. Since then, there has been an influx of immigrants of Hispanic background. Most haptoglobin type studies performed in South America have targeted the native Indians. HP^1 frequencies are high.

ASIA (FIGURE 2D AND TABLE S3)

Asia is the largest of the continents with environmental extremes ranging from the frozen lands in the north to the arid deserts in the south. As a rule, those populations of Caucasian origin are based in the South and South Western Asia, whilst those in the East and South East are Mongoloid in origin.

The majority of Asians have very low HP^{1F} values compared with any other continent, with Indians averaging about 0.05 and its virtual absence in Japanese, Koreans and Taiwanese. There is also a low but significant occurrence of the Hp0 type in the South Eastern part of the continent, which can be related to the geographical distribution of malaria. For the purpose of this review, we have split the continent into five subcontinents, listed in alphabetical order as follows:

East Asia including Japan, Korea, China and Taiwan

HP^1 frequencies are approximately 0.3. The highest HP^1 value of 0.387 for Hong Kong may reflect intermixing of genes from other races, although it was a small sample. The HP^{1F} allele is virtually absent in East Asia, while HP^{1S} averages 0.273. The frequency of Hp0 varies from 0% to 3.8%.

Eurasia: Russia, Kazakhstan, Uzbekistan, Ukraine and Turkey

Although relatively few studies were available for this subcontinent, there is an interesting pattern emerging illustrated by a study of Russian populations west of the Urals (Balanovskaya *et al.*, 2001). HP^1 frequencies tend to increase from northeast (approximately 0.30) to southwest (approximately 0.46). The HP^1 frequency is also high in the Ukraine and in Kazakhstan where the Russians and the Kazaks have a similar allele frequency (0.455 and 0.429, respectively) despite differing racial origins. The subjects studied from Uzbekistan gave a lower HP^1 allele frequency (approximately 0.27) than their northern neighbours.

One study from Turkey suggests a difference between the HP^1 values of the east and west of the country. Western Turkey (European) has an HP^1 allele frequency of 0.326, whilst Eastern Turkey (Asian) has 0.200.

South Asia: India, Afghanistan, Nepal

Of the countries in this subcontinent, India is by far the most studied. The study of 533 subjects in Cal-

cutta provides the lowest recorded HP^1 allele frequency in this review at 0.065 (lower than the overall value for South Asia of 0.167, $P < 0.001$). As well as the low HP^{1F} frequency, approximately 0.05, South Asians have an HP^{1S} allele frequency of approximately 0.12.

South East Asia: Malaysia, Singapore, Thailand

The countries of this subcontinent are geographically isolated from one another, but are occupied by ethnically similar people. HP^1 frequencies (approximately 0.294) are similar to those for East Asia (approximately 0.273). Singapore has a mixture of different ethnic groups and the study by Saha and Ong (1984) illustrates the HP^1 differences between the majority of the Singapore population (0.3–0.33) and that of the Tamil Indians (0.167) who have remained a separate ethnic group.

South West Asia: Iran, Israel, Jordan, Lebanon, Yemen

This subcontinent shows no significant difference between each country's average HP^1 allele frequency and the subcontinental HP^1 average of 0.287. However, Iran is a country with cultural divisions leading to some significant differences in haptoglobin frequency. The Zoroastrian sect is believed to originate from the first people of Iran, and 'outsiders' are not accepted into their group – not even other Iranians. Since the Islamic conquest, Arabs, Turks, Mongols and Afghans settled in Iran and have contributed to the other religious sects commonly found in the country. The Ghashghai are thought to be descended from the Shah Abbas, who settled in the 1700s from what is now the USSR. The three distinct groups reported for Iran show the effect of remaining religiously distinct (and presumably genetically distinct). The Zoroastrian sect have a lower HP^1 allele frequency than was seen in the Moslem or Ghashaghai groups, both of whom have mixed with other races.

EUROPE (FIGURE 2E AND TABLE S4)

Numerous studies of haptoglobin type frequency have been reported for Europe. Table S4 summarises studies with a total of 53 893 subjects. The mean HP^1 frequency is 0.381 (95% CI: 0.377–0.385).

The only minor deviations from this come from Greece and Sardinia and Greenland. The studies of Greece give an average HP^1 frequency of 0.346. This may be attributed to the lower HP^1 allele frequency of the immigrating people of Western Turkey. Immigration from North Africa may also explain the higher than expected average HP^1 frequency of 0.43 for Sardinia.

Greenland has a low HP^1 value (0.34). The country's original HP^1 frequency was probably comparable to its North American neighbours until the more recent immigration from Denmark, increasing the HP^1 allele frequency to what it is today.

There is also an interesting result from a survey in Norway, which reports a significant decrease in the HP^1 allele frequency of the race of Saamis (0.26), which is also reflected in a decreased HP^{1F} frequency (0.065; Teige, Olaisen & Teisberg, 1992). This ethnic group are one of the aboriginal peoples of the Fennoscandian area, and are the oldest known Scandinavian culture, living more or less isolated. The Hp0 frequency was less than 1% in every survey except that of Allison, Blumberg and ap Rees (1958) who reported a figure of 2.7% in 218 English subjects.

OCEANIA (FIGURE 2F AND TABLE S5)

This continent is made up of numerous islands that extend over a vast distance. The HP^1 allele frequency for the continent is 0.571, the highest of all the continents. Aborigines originally inhabited the largest of these islands, Australia, until the colonization by Europeans. Today it holds a mixture of Europeans (most of the population) with some Orientals in the more densely populated areas as well as Aboriginals. This is reflected by two Caucasian surveys that report a HP^1 allele frequency of 0.4. The studies performed on Australian aborigines show a large range of allele frequencies (0.18–0.63). Two of the surveys showed a low frequency of HP^{1F} in the aboriginal people (0–0.025) compared with the Caucasian study (0.168).

Most of the smaller islands that make up Oceania are geographically and culturally isolated and there has been little movement people from other races. The detailed survey performed in Vanuatu and Papua New Guinea revealed similar, high HP^1 allele frequencies for regions across the island (Hill *et al.*, 1986). The study provided evidence of anhaptoglobinaemia

present in the Maewo island of Vanuatu, as well as finding three cases out of the 165 tested that were Hp Johnson. In the three countries surveyed by Hill *et al.* (1986) HP^{1F} was tested for, but it was not detected.

CONCLUSION

Sutton *et al.* (1956) made the first comparison of HP frequencies between populations, when they compared African Blacks and Caucasians. They discovered a highly significant difference between the two racial groups, which led to further studies being carried out on populations worldwide by many authors. These surveys have found that the frequencies of HP^1 and HP^2 vary considerably among people of different racial origin, but both have always been found in every population examined to date.

Mongoloids, the Melanesian islanders and most Australian aborigines are monomorphic for the HP^{1S} subtype. The HP^2 allele is thought to have originated soon after the divergence of man (Bowman & Kurosky, 1982). If so, Mongoloids must have once had both HP^{1F} and HP^{1S} subtypes, and subsequently lost HP^{1F}, since HP^2 was originally formed during the non-homologous chromosomal breakage and reunion between HP^{1F} and HP^{1S}.

The Hp2-1 Modified (Hp2-1M) allele is found almost exclusively in populations of Black ancestry, with a frequency ranging from 1% to 10% (Giblett, 1959; Constans *et al.*, 1981; Kasvosve *et al.*, 2002), although the Hp2-1M allele has also been reported as 1.4% in a Lebanese population (Lefranc *et al.*, 1981). This can be accounted for, since Lebanese have an African admixture as demonstrated by the frequency of Gm haplotypes. Differences in Hp allele frequencies may be the result of both genetic drift and natural selection. The functional differences between Hp1-1, 2-1 and 2-2 discussed below provide mechanisms for selection through protection against particular diseases.

DISEASE ASSOCIATIONS

Changes in total haptoglobin levels in plasma

Hp is an acute phase protein and plasma levels increase by 200–500% during the 7 days after the onset of inflammation and only slowly return to normal during the 2–4 weeks after the removal of the pathological insult (Gabay & Kushner, 1999).

Levels decrease during haemolysis, ineffective erythropoiesis, liver disease and late pregnancy. Levels are decreased in people with allergy reactions (Piessens, Marien & Stevens, 1984). Very low levels have been reported in familial idiopathic epilepsy (Panter *et al.*, 1985). The association of Hp2-2 with protein losing nephropathy may be due to the preferential loss of the smaller Hp1-1 (Nakhoul *et al.*, 2001).

Haptoglobin types and disease – mechanisms

Free haemoglobin can damage renal tissues and so any reduction in Hp concentration or Hb binding ability may result in increased renal damage. Free Hb may also be a source of iron for pathogenic bacteria, and reduction in Hp concentration or Hb binding ability may aggravate bacterial infection (Eaton *et al.*, 1982). Haptoglobin is an integral part of the acute phase response – its synthesis being stimulated by IL-6 (Figure 3). The resulting increase in Hp levels may also generate a feedback dampening the severity of the initial acute phase reaction (Arredouani *et al.*, 2005). Such downregulation may also relate to the binding of the Hp–Hb complex by macrophage CD163 which leads to secretion of the anti-inflammatory cytokine IL-10 and the breakdown products of haem which also have potent anti-inflammatory activity (Moestrup & Moller, 2004). Release of IL-10 induces CD163 synthesis and also haem oxygenase-1 via an autocrine mechanism. Thus there is a co-ordinated regulation of Hb uptake and breakdown (Philippidis *et al.*, 2004).

There are differences in the binding of haptoglobin types by the CD163 receptor. The Hp(2-2)–Hb complex has a 10-fold higher functional affinity for CD163 than the Hp(1-1)–Hb complex, probably due to the clustering effect of several binding sites in the multimeric ligand complex (Graversen, Madsen & Moestrup, 2002). This may increase the efficiency of the macrophage in clearing the Hp(2-2)–Hb complexes from the plasma when compared with that of the Hp(1-1)–Hb complex binding to CD163 (Graversen, Madsen & Moestrup, 2002). However, studies in cultured CHO cells transfected with CD163 have demonstrated more rapid uptake of the Hp(1-1)–Hb complex than the Hp(2-2)–Hb complex (Asleh *et al.*, 2003).

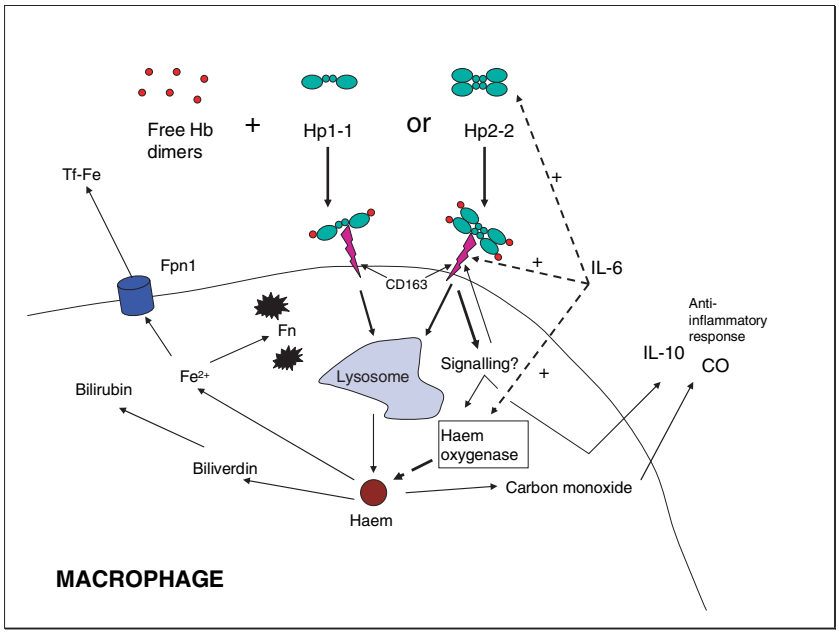

Figure 3. Uptake of the Haptoglobin–haemoglobin complex by macrophages. In the plasma Hb tetramers dissociate into dimers that bind tightly to the β-chain of Haptoglobin. The complex binds to CD163 and is taken up by endocytosis after which both Hp and Hb are degraded in the lysosome. The CD163 receptor is recycled. Haem is degraded to biliverdin and bilirubin. Iron and carbon monoxide are released. Iron is transported to the plasma by ferroportin-1 or retained within the cell as ferritin. Synthesis of the proteins of the haem degradation pathway is co-ordinated as IL-6 increases synthesis of Hp, CD163 and haem oxygenease. Binding of the Hp–Hb complex to CD163 induces intra-cellular signalling leading to increased haem oxygenease activity and release of anti-inflammatory cytokines (IL-10). Carbon monoxide release augments the anti-inflammatory response.

Rates of breakdown of Hb within the cell were similar. *In vitro* studies have also demonstrated the protective effect of Hp against oxidative damage to erythrocytes by extracellular Hb. Again protection was greatest for Hp1-1 and least for Hp2-2 (Gueye *et al.*, 2006).

The difference in size of each haptoglobin phenotype may affect both access to tissues and the rate of clearance of the haptoglobin–haemoglobin complex. Hp2-2 may provide less protection against haemoglobin iron driven peroxidation leading to lower concentrations of ascorbic acid in the plasma of subjects carrying this Hp type compared with those carrying Hp1-1 and 2-1(Langlois *et al.*, 1997b; Na *et al.*, 2006a). Healthy men carrying Hp2-2 had higher levels of serum iron, serum ferritin and transferrin saturation, and lower levels of serum transferrin receptor, than men carrying Hp2-1 and 1-1. L-ferritin concentrations were also higher in monocytes from Hp2-2 subjects (Langlois *et al.*, 2000). Van Vlierberghe *et al.* (2001b)) proposed that Hp2-2 was a potential modifier of iron status in hereditary haemochromatosis. However, two later studies showed no correlation between haptoglobin type and ferritin or transferrin saturation in C282Y homozygous, hereditary haemochromatosis patients, asymptomatic, C282Y homozygous blood donors and random blood donors (Beutler, Gelbart & Lee, 2002; Carter *et al.*, 2003). Furthermore, Langlois *et al.* (2004) did not observe any relationship between serum ferritin and Hp type in men and women over 50 years old. In men, but not women, Hp2-2 was associated with increased levels of oxidized low-density lipoprotein compared with Hp1-1 and Hp2-1. This association was independent of the increase in oxidized low-density lipoprotein with increasing ferritin concentration (Brouwers *et al.*, 2004).

Hp2-2 may also be associated with higher serum total cholesterol levels (Saha *et al.*, 1992; Braeckman

et al., 1999). Neither Borresen *et al.* (1987) nor Frohlander (1987) noted this but Borresen *et al.* noted a significantly higher frequency of Hp2-2 among those with HDL cholesterol values in the upper quartile.

Hp may have a direct regulatory role in the control of blood flow. Nitric oxide (NO) is a free radical that plays a principal role in basal blood flow regulation and vascular homeostasis. As an NO scavenger the Hp–Hb complex has a role in regulating NO bioavailability and vascular homeostasis (Wang *et al.*, 2004). Haptoglobin, by limiting the availability of haem compounds to catalyze the oxidation of arachidonic acid by prostaglandin synthetase, inhibits prostaglandin synthesis and so has an anti-inflammatory action (Jue, Shim & Kamg, 1983).

There is also direct interaction with other cells of the immune system as Hp suppresses the cytokine response of T helper cells type 2 (Arredouani *et al.*, 2003). Hp prevents epidermal LC from spontaneously undergoing functional maturation in the skin. This novel property of Hp may be important in ameliorating or preventing certain T cell-dependent inflammatory skin diseases (Xie *et al.*, 2000). Haptoglobin binds to monocytes, granulocytes, natural killer cells and a subset of CD8 T cells through the CD11b/CD18 (MAC1) receptor. Haptoglobin may regulate MAC-1 dependent cell function in vivo. Haptoglobin also binds to mature B lymphocytes through the CD22 surface receptor (Hanasaki, Powell & Varki, 1995). Peripheral blood B-cell and $CD4^{+}$T-lymphocyte counts are higher in people with Hp2-2 than in 1-1 (Langlois *et al.*, 1997a). Hp is an angiogenic factor promoting endothelial cell growth and differentiation of new blood vessels (Cid *et al.*, 1993; De Kleijn *et al.*, 2002). Hp2-2 has more angiogenic activity than Hp1-1 or Hp2-1 (Cid *et al.*, 1993).

Haptoglobin types and disease

Table 3 shows many disease associations linked to haptoglobin type. Hp1-1 seems to be a susceptibility factor for infection and liver disease. There are also negative reports for almost all these associations.

The essential role of Hp in removing free haemoglobin from the circulation and the existence of several Hp types of differing molecular mass suggest the possibility of associations between Hp types and disease. Furthermore, the wide differences in frequency of the Hp types in differing ethnic groups provides the possibility that particular populations may have a susceptibility to particular diseases.

Biocore studies indicate that both Hp1-1 and 2-2 bind Hb with similar affinity (Asleh *et al.*, 2005). The macrophage CD163 receptor has an approximately eight times greater affinity for Hp2-2 than Hp1-1 yet studies in cultured CHO cells transfected with CD163 demonstrated more rapid uptake of the Hp(1-1)–Hb complex (see above). Rates of breakdown of Hb within the cell were similar. The ability of Hp1-1 to penetrate intracellular spaces is likely to be greater than the much larger Hp2-2 molecule. All three factors indicate that Hp1-1 will be the more effective molecule in preventing oxidative damage by free Hb. Normally, there is an excess of Hp compared with free Hb and differences in the function of Hp types will not be significant. In the case of diabetes such differences may be important. Glycosylated Hb is a more potent source of redox active iron and Hp2-2 has less ability to inhibit the release of such iron from glycosylated Hb (Asleh *et al.*, 2005). The lack of simple associations between Hp type and disease may reflect the complex interactions between Hp and the immune system as well as anti-oxidant functions (Figure 3).

CONCLUSION

The population distribution of Hp subtypes throughout the world and disease associations have been studied since 1955. The heyday of such studies was in the 1960s when reports of subtype frequencies in particular racial groups were published regularly in the journal *Nature*. Hp types were used not only to study the origin of particular populations but also in forensic science. In both applications, haptoglobin has been replaced by use of highly polymorphic DNA sequences. There are remarkable differences in the frequency of the HP^{1} allele between racial groups with frequencies varying from about 0.07 in Calcutta to over 0.7 in parts of West Africa and South America. Both HP^{1} and HP^{2} have been linked to susceptibility to various diseases including heart disease and infection. Such associations may be explained by functional differences between Hp1-1, Hp2-1 and Hp2-2 in the clearance of haemoglobin from the plasma and in modulation of immune function. However, there are many reports drawing the conclusion that there is

Table 3. Associations between haptoglobin type and disease. These are case control studies with similar or greater numbers of samples in the control group. Studies with fewer than 50 subjects have not been included. Increased frequency is indicated by ↑, decreased frequency by ↓ and no change by –

		Hp type or HP[1] frequency				
Disease type	Specific condition (no. cases)	1-1	2-1	2-2	HP[1]	Reference
Cancer	Cancer of the ovaries (132) and non-malignant tumours (114)	–	↑	↓	↑	Dobryszycha and Warwas (1983)
	Primary ovarian cancer (175)	–	–	–	–	Frohlander and Stendahl (1988)
	Oesophageal cancer (72)	–	↑	–		Jayanthi *et al.* (1989)
	Gastric cancer (100)	–	–	↑		Jayanthi *et al.* (1989)
	Lung cancer (318)	–	–	↑	↓	Bettendorf *et al.* (1980)
	Lung cancer (309)	–	–	–	–	Beckman *et al.* (1986)
	Bladder cancer (264)	–	–	↓	–	Benkmann *et al.* (1987)
	Breast cancer (264)	–	–	–	–	Hudson *et al.* (1982)
	Acute myeloid, lymphoid and chronic myeloid leukaemia (multiple studies)	↑	–	–	–	Nevo and Tatarsky (1986)
	Chronic lymphoid leukaemia (140)	–	–	–	–	Nevo and Tatarsky (1986)
	Multiple myeloma (177)	–	–	–	–	Germenis *et al.* (1983)
Infection	Chronic hepatitis C (239)	↑	–	↓		Louagie *et al.* (1996)
	Chronic hepatitis C (132)	↑	–	–	↑	Van Vlierberghe *et al.* (2001a)
	HIV seropositive Ghanaians (58)	Hp0 ↓				Quaye *et al.* (2000a)
	HIV infected subjects (653)	–	–	–	–	Delanghe *et al.* (1998)
	HIV infected subjects (387)	–	–	–	–	Zaccariotto *et al.* (2006)
	Falciparum malaria (273)	↑	↓	↓		Elagib *et al.* (1998)
	Children with severe falciparum malaria (113)	↑	↓	↓		Quaye *et al.* (2000b)
	Severe malaria (473)	–	–	–	–	Aucan *et al.* (2002)
	Pulmonary tuberculosis (98)	–	–	–	–	Kasvosve *et al.* (2000b)
	American trypanasomiasis	–	–	–	↑	Calderoni, Andrade and Grotto (2006)
Liver disease	Cirrhosis (107)	↑	↓	–	↑	Zhao and Zhang (1993)
	Chronic non-alcoholic liver disease (100)	↑	–	–	–	Zipprich *et al.* (1986)
	Cirrhosis (174)	↑	–	↓	↑	Blenk and Junge (1978)
Diabetes	Type II diabetes (265)	–	–	–	–	Awadallah and Hamad (2000)
	Type II diabetes [anglo 97 and hispanic 191)]	–	–	–	–	Iyengar *et al.* (1989)
	Insulin dependent diabetes (144)	–	–	–	–	Ratzmann *et al.* (1984)
	Type II diabetes in Ghana (129)	–	–	↑	–	Quaye, Ababio and Amoah (2006)
Cardiovascular disease	Cardiovascular disease (565)	–	–	–	–	Hong *et al.* (1997)
	Coronary heart disease (200)	–	–	–	↑	Golabi, Kshatriya and Kapoor (1999)
	Coronary heart disease (297 of 3273)	–	–	–	–	Levy *et al.* (2004)
	Coronary heart disease mortality (107)	↑	–	–	–	De Bacquer *et al.* (2001)
	Peripheral arterial occlusive disease (141)	–	–	↑	↓	Delanghe *et al.* (1999)
	Essential arterial hypertension (302)	–	–	–	–	Delanghe *et al.* (1993)
	Essential hypertension (257)	–	–	↑		Surya Prabha, Padma and Ramaswamy (1987)

Table 3. Continued

Disease type	Specific condition (no. cases)	Hp type or HP[1] frequency				Reference
		1-1	2-1	2-2	HP[1]	
Cardiovascular disease	Myocardial infarction (121)	–	–	–	–	Frohlander and Johnson (1989)
	Myocardial infarction (496)	–	–	–	–	Chapelle *et al.* (1982)
	Preeclampsia (60)	↑	–	–	–	Depypere *et al.* (2006)
Mental illness and neurological disorders	Unipolar major depression (72)	↑	↑	↓		Maes *et al.* (1994)
	Unipolar depression in elderly patients (65)	–	–	–	–	Matsuyama and Joseph (1984)
	Schizophrenia (98)	↓	↓	↑	↓	Maes *et al.* (2001)
	Alzheimer disease (several studies < 100 samples each)	–	–	–	–	See Matsuyama, Cripe and Joseph (1986)
Inflammation	Rheumatoid arthritis (200)	–	–	–	–	Dahlqvist and Frohlander (1985) and references therein
	Family history of polyarthritis (86)				↓	Dahlqvist and Frohlander (1985)
Miscellaneous	Haemochromatosis (167)	–	–	↑	–	Van Vlierberghe *et al.* (2001b)
	Haemochromatosis (115)	–	–	–	–	Beutler, Gelbart and Lee (2002)
	Haemochromatosis (173)	–	–	–	–	Carter *et al.* (2003)
	Sarcoidosis (226)				↑	Fan *et al.* (1995)
	Postmenopausal osteoporosis (135)	↓	–	↑		Pescarmona *et al.* (2001)

no association between Hp subtype and a particular disease.

Clearly many studies are of limited value due to small sample numbers. Nevertheless, the conflicting reports of disease associations and the lack of association between disease and particular populations despite the huge range of Hp type proportions across the world suggests that any associations are marginal. A true appreciation of the significance of Hp types in disease awaits a better understanding of the functional differences between the major Hp types and studies of large numbers of subjects. So far it is not apparent that particular populations are at risk from common diseases because of the prevalence of either HP^1 or HP^2.

REFERENCES

Allison A.C., Blumberg B.S. & ap Rees W. (1958) Haptoglobin types in British, Spanish, Basque and Nigerian African populations. Nature 181, 824–825.

Allison A.C. (1959) Genetic control of human haptoglobin synthesis. Nature 183, 1312–1314.

Allison A.C. & ap Rees W. (1957) The binding of haemoglobin by plasma proteins (haptoglobins). British Medical Journal 2, 1137–1143.

Arredouani M., Matthijs P., Van Hoeyveld E., Kasran A., Baumann H., Ceuppens J.L. & Stevens E. (2003) Haptoglobin directly affects T cells and suppresses T helper cell type 2 cytokine release. Immunology 108, 144–151.

Arredouani M.S., Kasran A., Vanoirbeek J.A., Berger F.G., Baumann H. & Ceuppens J.L. (2005) Haptoglobin dampens endotoxin-induced inflammatory effects both *in vitro* and *in vivo*. Immunology 114, 263–271.

Asleh R., Marsh S., Shilkrut M., Binah O., Guetta J., Lejbkowicz F., Enav B., Shehadeh N., Kanter Y., Lache O., Cohen O., Levy N.S. & Levy A.P. (2003) Genetically determined heterogeneity in hemoglobin scavenging and susceptibility to diabetic cardiovascular disease. Circulation Research 92, 1193–1200.

Asleh R., Guetta J., Kalet-Litman S., Miller-Lotan R. & Levy A.P. (2005) Haptoglobin genotype- and diabetes-dependent differences in iron-mediated oxidative stress *in vitro* and *in vivo*. Circulation Research 96, 435–441.

Aucan C., Walley A.J., Greenwood B.M. & Hill A.V.S. (2002) Haptoglobin genotypes are not associated with resistance to severe malaria in The Gambia. Transactions of the Royal Society of Tropical Medicine and Hygiene 96, 327–328.

Awadallah S. & Hamad M. (2000) The prevalence of type II diabetes mellitus is haptoglobin phenotype-independent. Cytobios 101, 145–150.

Balanovskaya E.V., Balanovsky O.P., Spitsyn V.A., Bychkovskaya L.S., Makarov S.V., Pai G.V., Rusakov A.E. & Subbota D.S. (2001) The Russian gene pool: gene geography of serum gene markers (HP, GC, PI, and TF). Russian Journal of Genetics 37, 939–950.

Bearn A.G. & Franklin E.C. (1958) Some genetical implications of physical studies of human haptoglobins. Science 128, 596–597.

Beckman G., Eklund A., Frohlander N. & Stjernberg N. (1986) Haptoglobin groups and lung cancer. Human Heredity 36, 258–260.

Benkmann H.G., Hansenn H.P., Ovenbeck R. & Goedde H.W. (1987) Distribution of alpha-1-antitrypsin and haptoglobin phenotypes in bladder cancer patients. Human Heredity 37, 290–293.

Bettendorf A., Colonna J., Kleisbauer J.P. & Laval P. (1980) Groupes haptoglobine au cours des cancers bronchiques primitifs. Nouvelle Presse Medicale 9, 3359–3360.

Beutler E., Gelbart T. & Lee P. (2002) Haptoglobin polymorphism and iron homeostasis. Clinical Chemistry 48, 2232–2235.

Blenk H. & Junge W. (1978) Haptoglobin phenotypes and liver cirrhosis. I. Klinische Wochenschrift 56, 973–976.

Borresen A.L., Leren T., Berg K. & Solaas M.H. (1987) Effect of haptoglobin subtypes on serum-lipid levels. Human Heredity 37, 150–156.

Bowman B.H. & Kurosky A. (1982) Haptoglobin: the evolutionary product of duplication, unequal crossing over, and point mutation. Advances in Human Genetics 12, 189–261.

Braeckman L., De Bacquer D., Delanghe J., Claeys L. & De Backer G. (1999) Associations between haptoglobin polymorphism, lipids, lipoproteins and inflammatory variables. Atherosclerosis 143, 383–388.

Brouwers A., Langlois M., Delanghe J., Billiet J., De Buyzere M., Vercaemst R., Rietzschel E., Bernard D. & Blaton V. (2004) Oxidized low-density lipoprotein, iron stores, and haptoglobin polymorphism. Atherosclerosis 176, 189–195.

Calderoni D.R., Andrade T.D. & Grotto H.Z.W. (2006) Haptoglobin phenotype appears to affect the pathogenesis of American trypanosomiasis. Annals of Tropical Medicine and Parasitology 100, 213–221.

Carter K., Bowen D.J., McCune C.A. & Worwood M. (2003) Haptoglobin type neither influences iron accumulation in normal subjects nor predicts clinical presentation in HFE C282Y haemochromatosis: phenotype and genotype analysis. British Journal of Haematology 122, 1–7.

Chapelle J.P., Albert A., Smeets J.P., Heusghem C. & Kulbertus H.E. (1982) Effect of the haptoglobin phenotype on the size of a myocardial infarct. New England Journal of Medicine 307, 457–463.

Cheftel R.I. & Moretti J. (1966) Sur la structure des haptoglobines humaines. Comptes Rendues des Séances de la Société de Biologie 262, 1982–1984.

Cid M.C., Grant D.S., Hoffman G.S., Auerbach R., Fauci A.S. & Kleinman H.K. (1993) Identification of haptoglobin as an angiogenic factor in sera from patients with systemic vasculitis. Journal of Clinical Investigation 91, 977–985.

Cleve H. & Deicher H. (1965) Haptoglobin ''Marburg'': Untersuchungen ueber eine seltene erbliche Haptoglobin-variante mit zwei verschiedenen Phaenotypen innerhalb einer Familie. Humangenetik 1, 537–550.

Connell G.E. & Smithies O. (1959) Human haptoglobins: estimation and purification. Biochemical Journal 72, 115–121.

Connell G.E., Smithies O. & Dixon G.H. (1966) Gene action in the human haptoglobins. II. Isolation and physical characterization of alpha polypeptide chains. Journal of Molecular Biology 21, 225–229.

Constans J., Viau M., Gouaillard C. & Clerc A. (1981) Haptoglobin polymorphism among Saharian and West African groups. Haptoglobin phenotype determination by radioimmunoelectrophoresis on Hp0 samples. American Journal of Human Genetics 33, 606–616.

Dahlqvist S.R. & Frohlander N. (1985) Haptoglobin groups and rheumatoid arthritis. Human Heredity 35, 207–211.

De Bacquer D., De Backer G., Langlois M., Delanghe J., Kesteloot H. & Kornitzer M. (2001) Haptoglobin polymorphism as a risk factor for coronary heart disease mortality. Atherosclerosis 157, 161–166.

De Kleijn D.P.V., Smeets M.B., Kemmeren P.P.C.W., Lim S.K., Van Middelaar B.J., Velema E., Schoneveld A., Pasterkamp G. & Borst C. (2002) Acute phase protein haptoglobin is a cell migration factor involved in arterial restructuring. Faseb Journal 16, 1123–1125.

Delanghe J.R., Duprez D.A., DeBuyzere M.L., Bergez B.M., Callens B.Y., LerouxRoels G.G. & Clement D.L. (1993) Haptoglobin polymorphism and complications in established essential arterial hypertension. Journal of Hypertension 11, 861–867.

Delanghe J.R., Langlois M.R., Boelaert J.R., Van Acker J., Van Wanzeele F., van der Groen G., Hemmer R., Verhofstede C., De Buyzere M., De Bacquer D., Arendt V. & Plum J. (1998) Haptoglobin polymorphism, iron metabolism and mortality in HIV infection. AIDS 12, 1027–1032.

Delanghe J.R., Langlois M.R., Duprez D., De Buyzere M. & Clement D. (1999) Haptoglobin polymorphism and peripheral arterial occlusive disease. Atherosclerosis 145, 287–292.

Depypere H.T., Langlois M.R., Delanghe J.R., Temmerman M. & Dhont M. (2006) Haptoglobin polymorphism in patients with preeclampsia. Clinical Chemistry and Laboratory Medicine 44, 924–928.

Dobryszycha W. & Warwas M. (1983) Haptoglobin types in ovarian tumors. Neoplasma 30, 169–172.

Eaton J.W., Brandt P., Mahoney J.R. & Lee J.T. (1982) Haptoglobin: a natural bacteriostat. Science 215, 691–693.

Elagib A.A., Kider A.O., Akerstrom B. & Elbashir M.I. (1998) Association of the haptoglobin phenotype (1-1) with falciparum malaria in Sudan. Transactions of the Royal Society of Tropical Medicine and Hygiene 92, 309–311.

Fan C.H., Nylander P.O., Sikstrom C. & Thunell M. (1995) Orosomucoid and haptoglobin types in patients with sarcoidosis. Experimental and Clinical Immunogenetics 12, 31–35.

Frohlander N. (1987) Haptoglobin groups and serum cholesterol levels. Human Heredity 37, 323–325.

Frohlander N. & Johnson O. (1989) Haptoglobin groups in acute myocardial infarction. Human Heredity 39, 345–350.

Frohlander N. & Stendahl U. (1988) Haptoglobin groups in ovarian carcinoma. Human Heredity 38, 180–182.

Fuller G.M., Rasco M.A., McCombs M.L., Barnett D.R. & Bowman B.H. (1973) Subunit composition of haptoglobin 2-2 polymers. Biochemistry 12, 253–258.

Gabay C. & Kushner I. (1999) Mechanisms of disease: acute-phase proteins and other systemic responses to inflammation. New England Journal of Medicine 340, 448–454.

Gaensslen R.E., Bell S.C. & Lee H.C. (1987) Distributions of genetic-markers in United-States populations.3. Serum group systems and hemoglobin-variants. Journal of Forensic Sciences 32, 1754–1774.

Galatius-Jensen F. (1958) Rare phenotypes in the Hp system. Acta Genetica et Statistica Medica 8, 248–255.

Garby L. & Noyes W.D. (1959) Studies on hemoglobin metabolism.1. The kinetic properties of the plasma hemoglobin pool in normal man. Journal of Clinical Investigation 38, 1479–1483.

Germenis A., Babionitakis A., Kaloterakis A., Filiotou A. & Fertakis A. (1983) Group-specific component and haptoglobin phenotypes in multiple myeloma. Human Heredity 33, 188–191.

Giblett E.R. (1959) Haptoglobin types in American negroes. Nature 183, 192–193.

Giblett E.R. & Steinberg A.G. (1960) The inheritance of serum haptoglobin types in American Negroes: evidence for a third allele Hp^{2M}. American Journal of Human Genetics 12, 160–169.

Giblett E.R., Hickman G.C. & Smithies O. (1964) Variant haptoglobin phenotypes. Cold Spring Harbor Symposia on Quantitative Biology 29, 321–326.

Giblett E.R., Uchida I. & Brooks L.E. (1966) Two rare haptoglobin phenotypes, 1-B and 2-B, containing a previously undescribed alpha-polypeptide chain. American Journal of Human Genetics 18, 448–453.

Golabi P., Kshatriya G.K. & Kapoor A.K. (1999) Association of genetic markers with coronary heart disease (myocardial infarction) – a case control study. Journal of Indian Medical Association 97, 6–7.

Graversen J.H., Madsen M. & Moestrup S.K. (2002) CD163: a signal receptor scavenging haptoglobin-hemoglobin complexes from plasma. International Journal of Biochemistry & Cell Biology 34, 309–314.

Gueye P.M., Glasser N., Ferard G. & Lessinger J.M. (2006) Influence of human haptoglobin polymorphism on oxidative stress induced by free hemoglobin on red blood cells. Clinical Chemistry and Laboratory Medicine 44, 542–547.

Gutteridge J.M.C. (1987) The antioxidant activity of haptoglobin towards hemoglobin-stimulated lipid-peroxidation. Biochimica et Biophysica Acta 917, 219–223.

Hanasaki K., Powell L.D. & Varki A. (1995) Binding of human plasma sialoglycoproteins by the B-cell_specific lectin CD22 – selective recognition of immunoglobulin-M and haptoglobin. Journal of Biological Chemistry 270, 7543–7550.

Hill A.V., Bowden D.K., Flint J., Whitehouse D.B., Hopkinson D.A., Oppenheimer S.J., Serjeantson S.W. & Clegg J.B. (1986) A population genetic survey of the haptoglobin polymorphism in Melanesians by DNA analysis. American Journal of Human Genetics 38, 382–389.

Hong S.H., Kang B.Y., Lim J.H., Namkoong Y., Oh M.Y., Kim J.Q. & Lee C.C. (1997) Haptoglobin polymorphism in Korean patients with cardiovascular diseases. Human Heredity 47, 283–287.

Hudson B.L., Sunderland E., Cartwright R.A., Benson E.A., Smiddy F.G. & Cartwright S.C. (1982) Haptoglobin phenotypes in two series of breast cancer patients. Human Heredity 32, 219–221.

Iyengar S., Hamman R.F., Marshall J.A., Baxter J., Majumder P.P. & Ferrell R.E. (1989) Genetic-studies of type-2 (non-insulin-dependent) diabetes-mellitus – lack of association with 7 genetic-markers. Diabetologia 32, 690–693.

Javid J. (1967) Haptoglobin 2-1 Bellevue, a haptoglobin beta-chain mutant. Proceedings of the National Academy of Sciences of the United States of America 57, 920–924.

Jayanthi M., Habibullah C.M., Ishaq M., Ali H., Babu P.S. & Ali M.M. (1989) Distribution of haptoglobin phenotypes in oesophageal and gastric cancer. Journal of Medical Genetics 26, 172–173.

Jue D.M., Shim B.S. & Kamg Y.S. (1983) Inhibition of prostaglandin synthase activity of sheep seminal vesicular gland by human-serum haptoglobin. Molecular and Cellular Biochemistry 51, 141–147.

Kasvosve I., Gomo Z.A., Gangaidzo I.T., Mvundura E., Saungweme T., Moyo V.M., Khumalo H., Boelaert J.R., Gordeuk V.R. & Delanghe J.R. (2000a) Reference range of serum haptoglobin is haptoglobin phenotype-dependent in blacks. Clinica Chimica Acta 296, 163–170.

Kasvosve I., Gomo Z.A., Mvundura E., Moyo V.M., Saungweme T., Khumalo H., Gordeuk V.R., Boelaert J.R., Delanghe J.R., De Bacquer D. & Gangaidzo I.T. (2000b) Haptoglobin polymorphism and mortality in patients with tuberculosis. International Journal of Tuberculosis and Lung Disease 4, 771–775.

Kasvosve I., Gordeuk V.R., Delanghe J.R., Gomo Z.A., Gangaidzo I.T., Khumalo H., Moyo V.M., Saungweme T., Mvundura E. & Boelaert J.R. (2002) Iron status in black persons is not influenced by haptoglobin polymorphism. Clinical Chemistry and Laboratory Medicine 40, 810–813.

Koch W., Latz W., Eichinger M., Roguin A., Levy A.P., Schömig A. & Kastrati A. (2002) Genotyping of the common haptoglobin Hp1/2 polymorphism based on PCR. Clinical Chemistry 48, 1377–1382.

Koda Y., Soejima M., Yoshioka N. & Kimura H. (1998) The haptoglobin-gene

deletion responsible for anhaptoglobinemia. American Journal of Human Genetics 62, 245–252.

Koda Y., Watanabe Y., Soejima M., Shimada E., Nishimura M., Morishita K., Moriya S., Mitsunaga S., Tadokoro K. & Kimura H. (2000) Simple PCR detection of haptoglobin gene deletion in anhaptoglobinemic patients with anti-haptoglobin antibody that causes anaphylactic transfusion reactions. Blood 95, 1138–1143.

Kristiansen M., Graversen J.H., Jacobsen C., Sonne O., Hoffman H.J., Law S.K.A. & Moestrup S.K. (2001) Identification of the haemoglobin scavenger receptor. Nature 409, 198–201.

Langlois M.R. & Delanghe J.R. (1996) Biological and clinical significance of haptoglobin polymorphism in humans. Clinical Chemistry 42, 1589–1600.

Langlois M., Delanghe J., Philippe J., Ouyang J., Bernard D., DeBuyzere M., VanNooten G. & LerouxRoels G. (1997a) Distribution of lymphocyte subsets in bone marrow and peripheral blood is associated with haptoglobin type – binding of haptoglobin to the B-cell lectin CD22. European Journal of Clinical Chemistry and Clinical Biochemistry 35, 199–205.

Langlois M.R., Delanghe J.R., DeBuyzere M.L., Bernard D.R. & Ouyang J. (1997b) Effect of haptoglobin on the metabolism of vitamin C. American Journal of Clinical Nutrition 66, 606–610.

Langlois M.R., Martin M.E., Boelaert J.R., Beaumont C., Taes Y.E., De Buyzere D.R., Neels H.M. & Delanghe J.R. (2000) The haptoglobin 2-2 phenotype affects serum markers of iron status in healthy males. Clinical Chemistry 46, 1619–1625.

Langlois M.R., De Buyzere M.L., Vam Vlierberghe H. & Delanghe J.R. (2004) Haptoglobin polymorphism and serum ferritin concentration in ageing subjects. British Journal of Haematology 124, 555–556.

Lefranc G., Lefranc M.P., Seger J., Salier J.P., Chakhachiro L. & Loiselet J. (1981) Sex limited ahaptoglobinaemia. Human Genetics 58, 294–297.

Levy A.P., Larson M.G., Corey D., Lotan R., Vita J.A. & Benjamin E.J. (2004) Haptoglobin phenotype and prevalent coronary heart disease in the Framingham offspring cohort. Atherosclerosis 172, 361–365.

Louagie H.K., Brouwer J.T., Delanghe J.R., DeBuyzere M.L. & LerouxRoels G.G. (1996) Haptoglobin polymorphism and chronic hepatitis C. Journal of Hepatology 25, 10–14.

Maeda N. (1991) DNA polymorphisms in the controlling region of the human haptoglobin genes: a molecular explanation for the haptoglobin 2-1 modified phenotype. American Journal of Human Genetics 49, 158–166.

Maeda N. & Smithies O. (1986) The evolution of multigene families: human haptoglobin genes. Annual Review of Genetics 20, 81–108.

Maes M., Delanghe J., Scharpe S., Meltzer H.Y., Cosyns P., Suy E. & Bosmans E. (1994) Haptoglobin phenotypes and gene-frequencies in unipolar major depression. American Journal of Psychiatry 151, 112–116.

Maes M., Delanghe J., Chiavetto L.B., Bignotti S., Tura G.B., Pioli R., Zanardini R. & Altamura C.A. (2001) Haptoglobin polymorphism and schizophrenia: Genetic variation on chromosome 16. Psychiatry Research 104, 1–9.

Matsuyama S.S. & Joseph J. (1984) Haptoglobin types and unipolar depression. Human Heredity 34, 65–68.

Matsuyama S.S., Cripe A.T. & Joseph J. (1986) Haptoglobin phenotypes in dementia of the Alzheimers type. Human Heredity 36, 93–96.

McEvoy S.M. & Maeda N. (1988) Complex events in the evolution of the haptoglobin gene cluster in primates. Journal of Biological Chemistry 263, 15740–15747.

Moestrup S.K. & Moller H.J. (2004) CD163: a regulated hemoglobin scavenger receptor with a role in the anti-inflammatory response. Annals of Medicine 36, 347–354.

Na N., Delanghe J.R., Taes Y.E.C., Torck M., Baeyens W.R.G. & Jin O.Y. (2006a) Serum vitamin C concentration is influenced by haptoglobin polymorphism and iron status in Chinese. Clinica Chimica Acta 365, 319–324.

Na N., Delanghe J.R., Taes Y.E.C., Torck M., Baeyens W.R.G. & Jin O.Y. (2006b) Serum vitamin C concentration is influenced by haptoglobin polymorphism and iron status in Chinese. Clinica Chimica Acta 365, 319–324.

Nakhoul F.M., Zoabi R., Kanter Y., Zoabi M., Skorecki K., Hochberg I., Leibu R., Miller B.P. & Levy A.P. (2001) Haptoglobin phenotype and diabetic nephropathy. Diabetologia 44, 602–604.

Nevo S. & Tatarsky I. (1986) Serum haptoglobin types and leukemia. Human Genetics 73, 240–244.

Nielsen M.J., Petersen S.V., Jacobsen C., Oxvig C., Rees D., Moller H.J. & Moestrup S.K. (2006) Haptoglobin-related protein is a high-affinity hemoglobin-binding plasma protein. Blood 108, 2846–2849.

Okazaki T., Yanagisawa Y. & Nagai T. (1997) Analysis of the affinity of each haptoglobin polymer for hemoglobin by two-dimensional affinity electrophoresis. Clinica Chimica Acta 258, 137–144.

Oliviero S., DeMarchi M., Carbonara A.O., Bernini L.F., Bensi G. & Raugei G. (1985) Molecular evidence of triplication in the haptoglobin Johnson variant gene. Human Genetics 71, 49–52.

Panter S.S., Sadrzadeh S.M., Hallaway P.E., Haines J.L., Anderson V.E. & Eaton J.W. (1985) Hypohaptoglobinemia associated with familial epilepsy. Journal of Experimental Medicine 161, 748–754.

Pescarmona G.P., D'Amelio P., Morra E. & Isaia G.C. (2001) Haptoglobin genotype as a risk factor for menopausal osteoporosis. Journal of Medical Genetics 38, 636–638.

Philippidis P., Mason J.C., Evans B.J., Nadra I., Taylor K.M., Haskard D.O. & Landis R.C. (2004) Hemoglobin scavenger receptor CD163 mediates interleukin-10 release and heme oxygenase-1 synthesis – antiinflammatory monocyte-macrophage responses *in vitro*, in resolving skin blisters *in vivo*, and after cardiopulmonary bypass surgery. Circulation Research 94, 119–126.

Piessens M.F., Marien G. & Stevens E. (1984) Decreased haptoglobin levels in respiratory allergy. Clinical Allergy 14, 287–293.

Polonovski M. & Jayle M.F. (1940) Sur la préparation d'une nouvelle fraction des protéines plasmatiques, l'haptoglobine. Comptes Rendues des Séances de la Société de Biologie 211, 517–519.

Putnam F.W. (1975) Haptoglobin. In: The Plasma Proteins: Structure, Function and Genetic Control (ed. by F. W. Put-

nam), pp. 2–50. Academic Press, New York.

Quaye I.K., Brandful J., Ekuban F.A., Gyan B. & Ankrah N.A. (2000a) Haptoglobin polymorphism in human immunodeficiency virus infection: Hp0 phenotype limits depletion of CD4 cell counts in HIV-1-seropositive individuals. Journal of Infectious Diseases 181, 1483–1485.

Quaye I.K.E., Ekuban F.A., Goka B.Q., Adabayeri V., Kurtzhals J.A.L., Gyan B., Ankrah N.A., Hviid L. & Akanmori B.D. (2000b) Haptoglobin 1-1 is associated with susceptibility to severe *Plasmodium falciparum* malaria. Transactions of the Royal Society of Tropical Medicine and Hygiene 94, 216–219.

Quaye I.K., Ababio G. & Amoah A.G. (2006) Haptoglobin 2-2 phenotype is a risk factor for type 2 diabetes in Ghana. Journal of Atherosclerosis and Thrombosis 13, 90–94.

Ratzmann K.P., Strese J., Keilacker H., Giebelmann R., Scheibe F. & Witt S. (1984) Is there a relationship between genetically determined haptoglobin phenotype and insulin-dependent diabetes mellitus (IDDM)? Experimental and Clinical Endocrinology 83, 207–215.

Renwick J.H. & Marshall H. (1966) A new type of human haptoglobin, Hp2-1D. Annals of Human Genetics 29, 389–390.

Robson E.B., Glen-Bott A.M., Cleghorn T.E. & Harris H. (1964) Some rare haptoglobin types. Annals of Human Genetics 28, 77–86.

Roychoudhury A.K. & Nei M. (1988) Human Polymorphic Genes: World Distribution. Oxford University Press, New York.

Sadrzadeh S.M., Graf E., Panter S.S., Hallaway P.E. & Eaton J.W. (1984) Hemoglobin: A biologic Fenton reagent. Journal of Biological Chemistry 259, 14354–14356.

Saha N. & Ong Y.W. (1984) Distribution of haptoglobins in different dialect groups of Chinese, Malays and Indians in Singapore. Annals of the Academy of Medicine Singapore 13, 498–501.

Saha N., Liu Y., Tay J.S.H., Basair J. & Ho C.H. (1992) Association of haptoglobin types with serum-lipids and apolipoproteins in a Chinese population. Clinical Genetics 42, 57–61.

Shim B.S. & Bearn A.G. (1964) The distribution of haptoglobin subtypes in various populations, including subtype patterns in some nonhuman primates. American Journal of Human Genetics 16, 477–483.

Smith A.B. & Hajduk S.L. (1995) Identification of haptoglobin as a natural inhibitor of trypanocidal activity in human serum. Proceedings of the National Academy of Sciences of the United States of America 92, 10262–10266.

Smithies O. (1955) Zone electrophoresis in starch gels: group variations in the serum proteins of normal human adults. Biochemical Journal 61, 629–641.

Smithies O. (1959) An improved procedure for starch-gel electrophoresis: further variations in the serum proteins of normal individuals. Biochemical Journal 71, 585–587.

Smithies O. & Walker N.F. (1955) Genetic control of some serum proteins in normal humans. Nature 176, 1265–1266.

Smithies O. & Walker N.F. (1956) Notation for serum protein groups and the genes controlling their inheritance. Nature 178, 694–695.

Smithies O., Connell G.E. & Dixon G.H. (1962) Inheritance of haptoglobin subtypes. American Journal of Human Genetics 14, 14–21.

Surya Prabha P., Padma T. & Ramaswamy M. (1987) Haptoglobin patterns in essential hypertension and associated conditions – increased risk for Hp2-2. Human Heredity 37, 345–348.

Sutton H.E., Neel J.V., Binson G. & Zuelzer W.W. (1956) Serum protein differences between Africans and Caucasians. Nature 178, 1287.

Teige B., Olaisen B. & Teisberg P. (1992) Haptoglobin subtypes in Norway and a review of HP subtypes in various populations. Human Heredity 42, 93–106.

Teye K., Quaye I.K.E., Koda Y., Soejima M., Tsuneoka M., Pang H., Ekem I., Amoah A.G.B., Adjei A. & Kimura H. (2003) A-61C and C-101G Hp gene promoter polymorphisms are, respectively, associated with ahaptoglobinaemia and hypohaptoglobinaemia in Ghana. Clinical Genetics 64, 439–443.

Teye K., Quaye I.K.E., Koda Y., Soejima M., Pang H., Tsuneoka M., Amoah A.G.B., Adjei A. & Kimura H. (2004) A novel I247T missense mutation in the haptoglobin 2 beta-chain decreases the expression of the protein and is associated with ahaptoglobinemia. Human Genetics 114, 499–502.

Teye K., Soejima M., Quaye I.K.E., Pang H., Tsuneoka M., Koda Y. & Kimura H. (2006) Haptoglobin gene promoter polymorphism and haplotypes are unique in different populations. Human Biology 78, 121–126.

Trape J.F. & Fribourgblanc A. (1988) Ahaptoglobinemia in African populations and its relation to malaria endemicity. American Journal of Epidemiology 127, 1282–1288.

Van Vlierberghe H., Delanghe J.R., De Bie S., Praet M., De Paepe A., Messiaen L., De Vos M. & Leroux-Roels G. (2001a) Association between Cys282Tyr missense mutation and haptoglobin phenotype polymorphism in patients with chronic hepatitis C. European Journal of Gastroenterology and Hepatology 13, 1977–1981.

Van Vlierberghe H., Langlois M.R., Delanghe J.R., Horsmans Y., Michielsen P., Henrion J., Cartuyvels R., Billiet J., De Vos M. & Leroux-Roels G. (2001b) Haptoglobin phenotype 2-2 overrepresentation in Cys282Tyr hemochromatotic patients. Journal of Hepatology 35, 707–711.

Vanrijn H.J.M., Vanderwilt W., Stroes J.W. & Schriver J. (1987) Is the turbidimetric immunoassay of haptoglobin phenotype dependent. Clinical Biochemistry 20, 245–248.

Wang X.D., Tanus-Santos J.E., Reiter C.D., Dejam A., Shiva S., Smith R.D., Hogg N. & Gladwin M.T. (2004) Biological activity of nitric oxide in the plasmatic compartment. Proceedings of the National Academy of Sciences of the United States of America 101, 11477–11482.

Xie Y., Li Y., Zhang Q., Stiller M.J., Wang C.L. & Streilein J.W. (2000) Haptoglobin is a natural regulator of Langerhans cell function in the skin. Journal of Dermatological Sciences 24, 25–37.

Yang F., Brune J.L., Baldwin W.D., Barnett D.R. & Bowman B.H. (1983) Identification and characterization of human haptoglobin cDNA. Proceedings of the National Academy of Sciences of the United States of America 80, 5875–5879.

Yano A., Yamamoto Y., Miyaishi S. & Ishizu H. (1998) Haptoglobin genotyping by allele-specific polymerase chain reac-

SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article online:

Appendix to Carter and Worwood Summary of population surveys of Hp types throughout the world.

Table S1. Haptoglobin allele frequencies in Africa.

Table S2. Haptoglobin allele frequencies in the Americas.

Table S3. Haptoglobin allele frequencies in Asia.

Table S4. Haptoglobin allele frequencies in Europe.

Table S5. Haptoglobin allele frequencies in Oceania.

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