*Genetics and Molecular Biology*, 31, 3, 602-620 (2008)

www.sbg.org.br

Review Article

# Polymorphism of human haptoglobin and its clinical importance

Vânia Peretti de Albuquerque Wobeto, Tânia Regina Zaccariotto and Maria de Fátima Sonati

*Departamento de Patologia Clínica, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Campinas, SP, Brazil.*

## Abstract

Haptoglobin (Hp) is a plasma glycoprotein, the main biological function of which is to bind free hemoglobin (Hb) and prevent the loss of iron and subsequent kidney damage following intravascular hemolysis. Haptoglobin is also a positive acute-phase protein with immunomodulatory properties. In humans, the HP locus is polymorphic, with two codominant alleles (HP1 and HP2) that yield three distinct genotypes/phenotypes (Hp1-1, Hp2-1 and Hp2-2). The corresponding proteins have structural and functional differences that may influence the susceptibility and/or outcome in several diseases. This article summarizes the available data on the structure and functions of Hp and the possible effects of Hp polymorphism in a number of important human disorders.

*Key words:* haptoglobin, hemoglobin, genetic polymorphisms.

Received: October 26, 2007; Accepted: January 24, 2008.

## Human Haptoglobin

Haptoglobin, first described by Polonovski and Jayle (1938), is an α2-sialoglycoprotein synthesized mainly in hepatocytes in response to the secretion of cytokines such as interleukin (IL)-6, IL-1 and tumour necrosis factor (TNF) (Raynes *et al.*, 1991). The intravascular destruction of erythrocytes, which accounts for ~10%-20% of the normal destruction of erythrocytes, releases free hemoglobin (Hb) into the general circulation. The primary function of Hp is to bind to this Hb, thereby preventing the renal excretion of iron and protecting blood vessels from the oxidative effects of this protein (Giblett, 1968). Even when the destruction is mainly extravascular, some erythrocytes still undergo lysis in the intravascular compartment, as shown by the reduced serum levels of Hp in sickle cell diseases and thalassemias (Hillman and Finch, 1992).

The serum concentration of Hp is influenced by age and is generally measurable from three months onwards, with a gradual increase until adult concentrations (30-200 mg $dL^{-1}$) are reached at 20 years of age (Jayle and Moretti, 1962; Shinton *et al.*, 1965). When not bound to Hb, Hp is cleared from the plasma in ~3.5-5 days, but when bound to Hb, the average time for removal of the complex (mainly by hepatocytes) is ~20 min (Noyes and Garby, 1967; Bissell *et al.*, 1972; Javid, 1978). Measurement of the circulating Hp concentration can be used to determine whether there have been recent episodes of hemolysis since increased Hp consumption during these episodes leads to reduced plasma levels of this protein (Silverman and Christenson, 1994). Because Hp is also a positive acute-phase protein with immunomodulatory properties that may inhibit or stimulate the immune response, the concentration of this protein is elevated in inflammatory and infectious processes and in malignancies (Braeckman *et al.*, 1999).

Send correspondence to Maria de Fátima Sonati. Departamento de Patologia Clínica, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Caixa Postal 6111, 13083-970 Campinas, SP, Brazil. E-mails: sonati@fcm.unicamp.br.

## Protein Structure

Haptoglobin is a tetrameric protein that structurally resembles certain immunoglobulins because it has two light chains (α) and two heavy chains (β) covalently bound to each other by disulphide bridges (S-S) (Malchy *et al.*, 1973; Raugei *et al.*, 1983). Although present in all vertebrates, in humans Hp is characterized by molecular heterogeneity caused by genetic polymorphism. Jayle and Judas (1946) were the first to suspect that there were differences in the structure of Hp molecules and Smithies (1955), using starch gel electrophoresis, identified three main phenotypes: Hp1-1, Hp2-1 and Hp2-2. Subsequently, Smithies and Walker (1956) showed that these phenotypes were controlled by two autosomal codominant alleles identified as HP1 and HP2.

The β-chain of Hp has a molecular mass of 40 kDa (245 amino acids) and is not polymorphic. Haptoglobin polymorphism reflects inherited variations in the α-chain (the smallest chain) of Hp that result from differences between the α1-chain (with 83 amino acids) and the α2-chain

(with 142 amino acids) (Kurosky *et al.*, 1980; Maeda, 1991). The α1-chain can be further classified into α1S (slow) or α1F (fast), depending on the electrophoretic mobility. The difference between these chains lies in the amino acids at positions 52 and 53, which are asparagine and glutamic acid in α1S and aspartic acid and lysine in α1F, respectively (Smithies *et al.*, 1962; Connell *et al.*, 1966). This polymorphism results in Hp with different molecular masses, *i.e.*, 86 kDa for Hp1-1, 86-300 kDa for Hp2-1 and 170-900 kDa for Hp2-2 (Lange, 1992; Langlois and Delanghe, 1996).

The polymeric composition of Hp is also type-dependent, with the protein product of the HP1 allele being monovalent and that of the HP2 allele being bivalent. Consequently, Hp occurs as a dimer in Hp1-1 homozygotes, as a linear polymer in Hp2-1 heterozygotes and as a cyclic polymer in Hp2-2 homozygous individuals (Javid, 1965; Langlois and Delanghe, 1996; Frank *et al.*, 2001). These variations in shape and size form the basis of the most commonly used method for phenotyping Hp subtypes (Santoro *et al.*, 1982).

## Haptoglobin Genes

The HP *locus* is located on the long arm of chromosome 16 (16q22) (Robson *et al.*, 1969). The *loci* corresponding to the α and β chains are linked to each other so that a single mRNA molecule generates a large polypeptide chain that is then cleaved to yield the two Hp chains (Raugei *et al.*, 1983; Koch *et al.*, 2003).

The HP1 allele has five exons while the HP2 allele has seven; the 5$^{th}$ and 7$^{th}$ exons in the HP1 and HP2 alleles, respectively, correspond to the β chain *locus* (Figure 1). The divergences between HP1S and HP1F are caused by base substitutions in codons 52 and 53 located in the 4$^{th}$ exon (Black and Dixon, 1968; Van der Straten *et al.*, 1984). In contrast, the HP2 allele is a partially duplicated gene derived from a rare unequal crossover between the HP1F and HP1S alleles. This gene is 1.7 kb longer than the HP1 alleles, and the region responsible for encoding from the 11$^{th}$ to the 69$^{th}$ residue (exons 3 and 4 of HP1) is duplicated

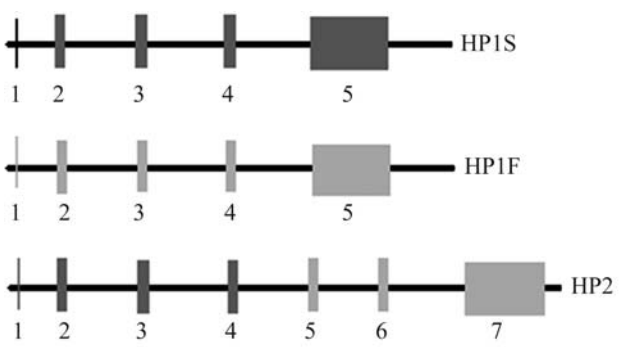


**Figure 1** - Schematic representation of the organization of the Hp gene. The boxes indicate exons (adapted from Yano *et al.*, 1998).

(Bearn and Franklin, 1958; Smithies *et al.*, 1962; Yang *et al.*, 1983). Combinations of these three alleles yield six distinct genotypes and their corresponding phenotypes, namely, Hp1S-1S, Hp1S-1F, Hp1F-1F, Hp2-1S, Hp2-1F and Hp2-2 (Maeda *et al.*, 1984).

The HP gene belongs to a small multigene family that originated from an ancestral single-copy gene probably after the separation of New World from Old World primates. New World primates have only one HP gene, whereas chimpanzees, gorillas and orangutans have three (haptoglobin, HP; haptoglobin-related, HPR; haptoglobin-primate, HPP) and humans have two genes (HP and HPR) (Maeda and Smithies, 1986; McEvoy and Maeda, 1988). Triplication of the HP locus is believed to have occurred before the separation of Old World monkeys from the family Hominidae (human-chimpanzee-gorilla-orangutan), 25-30 million years ago (mya), while the event that deleted one locus in humans and resulted in the two-gene cluster took place after divergence of the human and chimpanzee lineages, about 5 mya (Maeda and Smithies, 1986; McEvoy and Maeda, 1988). Insertions, deletions, recombinations and gene conversions have contributed to the evolution of the HP gene family (Erickson *et al.*, 1992). Homologous pairings facilitated by exon 5, the longest and most conserved exon of these genes, resulted in unequal crossovers that led to the duplication, triplication and deletion of HP genes (Erickson and Maeda, 1994). Since the HP2 allele apparently occurs only in humans, it is probable that this allele also originated after the separation of humans from other primates (Bowman and Kurosky, 1982).

## The Haptoglobin-Related Gene (Hpr)

As mentioned above, in humans the HP gene sequence is duplicated (2.2 kb downstream of the gene itself) on chromosome 16 (Maeda *et al.*, 1984; Muranjan *et al.*, 1998). This second gene is known as the Hp-related gene (HPR). In some individuals of African origin, multiple copies are present (Maeda *et al.*, 1984; Maeda, 1985). The HPR gene differs from the HP gene mainly in that it has a retrovirus-type sequence inserted into the first intron. The promoter region is active and encodes a protein called haptoglobin-related protein (Hpr) (Maeda, 1985), the serum concentration of which is ~5%-10% of that of Hp in healthy individuals.

The Hpr protein is believed to be part of the trypanosome lytic factor (TLF), a toxic subtype of high-density lipoproteins (HDLs) that provides humans with an innate defense against infection by *Trypanosoma brucei brucei* (Maeda, 1985; Smith *et al.*, 1995), the parasite responsible for Nagana disease in cattle. When Hpr binds to free Hb, it kills the trypanosome via oxidative damage initiated by its peroxidase activity (Smith *et al.*, 1995). Since Hp is the major serum inhibitor of the TLF, the balance between the serum concentrations of Hp and Hpr determines the degree of

protection against trypanocidal infection (Smith and Hajduk, 1995).

Muranjan *et al.* (1998) examined the Hp and Hpr concentrations in sera from patients with paroxysmal nocturnal hemoglobinuria. In this pathology, extensive intravascular hemolysis results in an excess of free Hb in the circulation. As expected, the Hp levels in these patients were much lower than in healthy individuals, but the Hpr levels did not differ significantly, suggesting that Hpr did not bind to Hb. More recently, Nielsen *et al.* (2006) used surface plasmon resonance to compare the binding affinities of Hp and Hpr recombinant proteins for Hb and found that Hpr competed in a similar manner to Hp for binding to Hb. However, the Hpr-Hb complex is not internalized via the CD163 receptor of macrophages.

## The Hp0 Phenotype

The Hp0 phenotype is characterized by the absence or reduced levels of Hp in plasma (referred to as ahaptoglobinemia and hypohaptoglobinemia, respectively) and shows that Hp is not essential for human survival (Koda *et al.*, 1998). This phenotype may be secondary to increased consumption or reduced production of Hp, as occurs during intravascular hemolysis and liver diseases, respectively, or may be genetically determined (Delanghe *et al.*, 1998b; Koda *et al.*, 2000). A study using Hp knockout mice showed that the lack of Hp did not impair the clearance of free plasma Hb, but the induction of severe hemolysis resulted in more serious tissue damage than in normal (control) mice (Lim *et al.*, 1998).

In East Asian populations, genetically determined hypohaptoglobinemia results from an ~28 kb deletion, referred to as $Hp^{Del}$, that extends from the HP gene promoter region to exon 5 of the HPR gene. This deletion was identified in ahaptoglobinemic patients who developed anaphylactic transfusion reactions caused by antihaptoglobin antibodies (Koda *et al.*, 1998, 2000). The homozygous genotype ($Hp^{Del}/Hp^{Del}$) corresponds to the complete absence of serum Hp whereas the two forms of hypohaptoglobinemia ($Hp2/Hp^{Del}$ genotype and $Hp1/Hp^{Del}$ genotype) are associated with extremely low levels of Hp and levels that are approximately 50% of those observed in normal genotypes, respectively. The $Hp^{Del}$ gene frequencies in Japanese, Chinese and Korean populations are between 0.15 and 0.30 (Koda *et al.*, 2000).

A more recent study that investigated several other Asian populations also detected $Hp^{Del}$ in Mongolians (frequency of 0.08), but could not in populations from Central, Southeast, South and West Asia (Soejima *et al.*, 2007). Possible explanations for the lack of detection in these population include the sample size of the studied populations, the extinction of the $Hp^{Del}$ allele in other Asian populations except in East Asia, or the expansion of this deletion to East Asia by chance. Based on the migratory movements that have occurred in that region, it is suggested that $Hp^{Del}$ originated in China and from there spread into Mongolia, Korea and Japan (Soejima *et al.*, 2007).

The $Hp^{Del}$ allele has not been found in European and African populations. Mutations in the promoter region of the HP gene appear to be the primary cause of congenital ahaptoglobinemia (Teye *et al.*, 2003). In Caucasians, the prevalence of the Hp0 phenotype is estimated to be 0.1% (Langlois and Delanghe, 1996), whereas in Africans it can be as high as 40% or more (Constans *et al.*, 1981; Teye *et al.*, 2004); the occurrence of this phenotype is influenced by acquired ahaptoglobinemia in areas where malaria is endemic and untreated (Boreham *et al.*, 1979). In North Americans of African descent, the frequency of the Hp0 phenotype is ~2.3% (Carter and Worwood, 2007).

## Structural Variants

Structural variants of Hp have been described (Carter and Worwood, 2007). For example, Haptoglobin Carlberg was identified in 1958 and is associated with reduced synthesis of the $\alpha$1S chain (Galatius-Jensen, 1958). Modified Hp2-1 (Hp2-1M) has a different electrophoretic pattern from that of the Hp2-1 phenotype because of its greater number of Hp1 bands, which are heavier and more intense (Connell and Smithies, 1959). This phenotype is generated by the polymorphism of a single nucleotide in the promoter region of the HP2 gene and is more frequent in African populations (Maeda, 1991; Quaye *et al.*, 2006). Giblett (1959) reported that the prevalence of this phenotype in North American Blacks in the Seattle region was 9.8%. Azevedo *et al.* (1969) studied a population of 541 Afro-descendants from northeastern Brazil and found an association between the $HP^{\alpha}2M$ allele and the presence of hypohaptoglobinemia. The rare Hp Johnson is the result of a crossover between the two Hp2 alleles and causes hypohaptoglobinemia or ahaptoglobinemia. The electrophoretic pattern of this phenotype consists of the Hp1 band and various polymers that migrate slowly (Smithies *et al.*, 1962; Bowman and Kurosky, 1982; Langlois and Delanghe, 1996).

## Geographic Distribution of Hp Alleles

There is marked variation in the frequency of HP genes with geographic region (Giblett, 1961; Schultze and Heremans, 1966). The HP2 allele originated in India ~2 mya and propagated around the world as a result of intense genetic pressure, gradually replacing the hegemony of the HP1 allele. This suggests that the HP2 allele may have a selective advantage over the HP1 allele (Schultze and Heremans, 1966). The frequency of the HP1 allele increases from Southeast Asia to Europe and Africa, and from Asia to America, by way of Alaska. In America, the highest frequencies are found in indigenous populations of Chile, Peru, Mexico, Venezuela and on the Brazilian-Venezuelan border, among the Yanomama Indians (Sutton *et al.*, 1960; Nagel and Etcheverry, 1963; Johnston *et*

*al.*, 1969; Nagel *et al.*, 1964; Shim and Bearn, 1964; Schultze and Heremans, 1966; Tanis *et al.*, 1973**;** Marini *et al.*, 1993).

The equilibrium of the HP1/HP2 polymorphism is broadly constant throughout the world. The allele frequencies in European populations are ~0.43 for the HP1 allele and 0.57 for the HP2 allele; in American populations, the corresponding figures are ~0.54 and 0.46 (Langlois and Delanghe, 1996). Recent studies of populations from southern and southeastern Brazil have revealed allele frequencies of ~0.53 and 0.46 for HP1 and 0.47 and 0.54 for HP2, respectively (Souza *et al.*, 2003; Zaccariotto *et al.*, 2006). Shreffler and Steinberg (1967) found frequencies of 0.48 and 0.47 for HP1 and 0.52 and 0.53 for HP2 among Xavante Indians living in the villages of Simões Lopes and São Marcos, respectively, in central-western Brazil. More recently, Simões *et al.* (1989) found very high frequencies of the HP1S allele and low frequencies or complete absence of the HP1F allele among Macushi and Içana River Indians in the Amazon region. Table 1 summarizes several studies that have examined the frequency of HP alleles in different populations around the world.

**Table 1** - HP1 gene frequencies in different populations.

| Continent | Country | Population studied | *n* | HP1 allele frequency | Reference |
|---|---|---|---|---|---|
| Africa | Algeria (Sahara) | Saoura Valley region | 373 | 0.64 | Constans *et al.*, 1981 |
| | | Hoggar Montains region | 260 | 0.66 | |
| | Central African Republic | Pygmies | 919 | 0.35 | |
| | | Sahara region | 280 | 0.53 | |
| | Ghana | General population | 123 | 0.52 | Teye *et al.*, 2006 |
| | Kenya | Luo tribe (north of Lake Victoria) | 227 | 0.57 | Herzog *et al.*, 1970 |
| | Mali | Menaka region | 285 | 0.44 | Constans *et al.*, 1981 |
| | Nigeria | Ibadan town | 63 | 0.73 | Shim and Bearn, 1964 |
| | | Zaria city | 56 | 0.38 | Mastana *et al.*, 1994 |
| | Sudan | General population | 208 | 0.54 | Elagib *et al.*, 1998 |
| | Senegal | Bedik group | 733 | 0.73 | Constans *et al.*, 1981 |
| | | Malinkes | 404 | 0.64 | |
| | | Peulhs | 169 | 0.70 | |
| America | Brazil | Two populations (Costa da Lagoa and São João do Rio Vermelho) from Santa Catarina Island, southern Brazil [descended from immigrants from the Azores Archipelago (Portuguese), with a minor contribution of sub-Saharan Africans and Amerindians] | 266 | 0.52 and 0.53, respectively | Souza *et al.*, 2003 |
| | | Caucasoids and Afro-descendants from southeastern Brazil | 142 | 0.46 | Zaccariotto *et al.*, 2006 |
| | | Cayapo Indians (States of Pará and Mato Grosso; Txukahamae, Kuben-Kran-Kegn, Xikrin and Gorotire tribes) | 596 | 0.58 | Salzano *et al.*, 1972 |
| | | Pacaás Novos Indians (Rondônia State, northern Brazil; Tanajura and Santo André communities) | 222 | 0.82 | Salzano *et al.*, 1985 |
| | | Baniwa Indians (Içana River, northern Amazon region; Cué-Cué, Boa Vista, Jauaçanã, Maracajá and Jandu Cachoeira communities) | 511 | from 0.27 to 0.52 | Salzano *et al.*, 1986 |
| | | Xavante Indians (Simões Lopes village - Midwestern region) | 171 | 0.48 | Shreffler and Steinberg, 1967 |
| | | Xavante Indians (São Marcos village - Midwestern region) | 272 | 0.47 | |
| | | Macushi Indians (northern Amazon region) | 151 | 0.61 | Simões *et al.*, 1989 |
| | | Içana River Indians (northern Amazon region) | 133 | 0.43 | |
| | | Galibi Amazonian Indians | 167 | 0.63 | Santos *et al.*, 1998 |
| | | Palikour Amazonian Indians | 55 | 0.87 | |
| | | Mundurucu Amazonian Indians | 109 | 0.78 | |
| | | Tenharim Amazonian Indians | 22 | 0.61 | |
| | | Kubenkokre Amazonian Indians | 49 | 0.49 | |
| | | Pukany Amazonian Indians | 53 | 0.59 | |
| | | Yanomama Indians (Brazilian-Venezuelan border) | 984 | 0.83 | Tanis *et al.,* 1973 |

**Table 1 (cont.)**

| Continent | Country | Population studied | *n* | HP1 allele frequency | Reference |
|---|---|---|---|---|---|
| | Canada | Eskimos from Baffin Island | 67 | 0.24 | Shim and Bearn, 1964 |
| | | Mixed (French-Canadians, Africans, Asians and others) | 358 | 0.39 | Mahmud *et al.*, 2007 |
| | Chile | Araucanian Indians | 31 | 0.77 | Shim and Bearn, 1964 |
| | Colombia | Spanish-origin population from Bogotá | 109 | 0.43 | Mastana *et al.*, 1994 |
| | USA | Indian (Alabama Coushatta) | 143 | 0.37 | Shim and Bearn, 1964 |
| | | Eskimos (northern and southern Alaska) | 220 | 0.32 | Scott *et al.*, 1966 |
| | | General population | 3,273 | 0.39 | Levy *et al.*, 2004 |
| | Mexico | Durango State (Urban Indians) | 119 | 0.63 | Delanghe *et al.*, 2000 |
| | | Durango State (Isolated Indians: Tepehuanos, Huicholes, Tarahumara and Mexicaneros) | 206 | 0.62 | |
| | | Maya Indians (Itza, Mam, Quiche, Chol, Tzotzil, Tzeltal and Cakchiquel tribes) | 418 | from 0.50 to 0.69 | Sutton *et al.*, 1960 |
| | | Maya (Lancandon tribe) | 31 | 0.93 | |
| | | Non-Maya Indians (Zapoteca, Chiapaneca, Totonaca, Mestizo tribes) | 189 | from 0.53 to 0.67 | |
| | Peru | Cashinahua Indians | 128 | 0.74 | Johnston *et al.*, 1969 |
| | Venezuela | Piaroa Indians | 121 | 0.82 | Marini *et al.,* 1993 |
| | | Makiritare Indians | 186 | 0.49 | Tanis *et al.,* 1973 |
| Asia | Afghanistan | Tajik group | 310 | 0.26 | Rahimi *et al.*, 1977 |
| | | Pushtoons | 210 | 0.26 | |
| | | Hazaras | 172 | 0.28 | |
| | | Usbeks | 124 | 0.25 | |
| | China | Shenyang and Guangzhou cities (northeastern and southern China, respectively) | 57 | 0.31 | Teye *et al.*, 2006 |
| | | General population | 667 | 0.33 | Ko *et al.*, 1980 |
| | | Mongolian group | 204 | 0.26 | Zhao *et al.*, 1993 |
| | | Owenke | 197 | 0.26 | |
| | | Manchu | 199 | 0.29 | |
| | | Korean | 301 | 0.29 | |
| | | Tahur | 210 | 0.16 | |
| | | Han | 287 | 0.27 | |
| | India | Bengalee Hindus (heterogeneous caste composition) | 140 | 0.19 | Bandopadhaya *et al*, 1992 |
| | | Punjab (northern India) - Jat Sikh group | 192 | 0.23 | Kaur *et al.*, 1981 |
| | | Punjab (northern India) - Khatri group | 105 | 0.19 | |
| | | Punjab (northern India) - Balmiki group | 108 | 0.14 | |
| | | Hindus from Hyderabad | 116 | 0.20 | Mastana *et al.*, 1994 |
| | Israel | General population | 757 | 0.33 | Lavie *et al.*, 2003 |
| | Japan | Mixed (two different Japanese populations) | 1,211 | 0.26 | Shindo, 1990 |
| | | General population | 372 | 0.27 | Nakada and Abe, 1987 |
| | Jordan | Jordanian Afro-descendants | 163 | 0.36 | Janaydeh *et al.*, 2004 |
| | | General population | 200 | 0.29 | Awadallah and Hamad, 2003 |
| | Korea | General population (male athletes) | 120 | 0.31 | Kang *et al.*, 2003 |
| | | General population | 316 | 0.32 | Yang *et al.*, 2000 |
| | Malaysia | General population | 231 | 0.30 | Saha and Ong, 1984 |
| | Russia | Chukotka Evens (northeastern Russia) | 314 | 0.39 | Solovenchuk and Glushenko, 1985 |
| | Thailand | North-central and northeastern areas (male members of the Royal Thai Army) | 682 | 0.24 | Blackwell and Thephusdin, 1963 |
| | | General population (blood donors) | 200 | 0.25 | Shimada *et al.* 2007 |
| | Turkey | General population | 200 | 0.27 | Erdem and Aksoy, 1975 |

**Table 1 (cont.)**

| Continent | Country | Population studied | *n* | HP1 allele frequency | Reference |
|---|---|---|---|---|---|
| | Ukraine | Mixed (from Dnepropetrovsk, Kharkov, Odessa, Kiev, Uzhgorod, Zhitomir) | 596 | 0.38 | Nikol'chenko *et al*, 1997 |
| Europe | Belgium | General population | 918 | 0.40 | Van Vlierberghe *et al.*, 2001 |
| | Germany | Hamburg (northern Germany) | 1,725 | 0.39 | Krüger and Püschel, 1993 |
| | Greece | Northern Greece | 212 | 0.35 | Stromatias *et al*., 1987 |
| | Hungary | Budapest | 343 | 0.35 | Hevér, 1976 |
| | | General population | 384 | 0.35 | Papp *et al.*, 2007 |
| | Italy | Continental Italy (northeastern, northwestern, central and southern regions) | 441 | 0.11 | Santoro *et al.*, 1983 |
| | | Sardinia | 165 | 0.18 | |
| | Moldavia | Gaugauz | 190 | 0.35 | Varsahr *et al.*, 2001 |
| | Norway | Oslo | 6,668 | 0.38 | Teige *et al.*, 1992 |
| | Spain | Barcelona | 317 | 0.38 | Moral and Panadero, 1983 |
| | United Kingdom | Northeastern England | 101 | 0.41 | Mastana *et al.*, 1994 |
| | | Southern Wales | 265 | 0.40 | Carter *et al.*, 2003 |
| Oceania | Australia | Caucasian population | 307 | 0.40 | Lai *et al.*, 1986 |
| | | Aborigenes from Arnhem Land | 50 | 0.29 | Shim and Bearn, 1964 |
| | | Aborigenes from western desert | 101 | 0.19 | |
| | Micronesia | Marshall Island | 364 | 0.52 | Neel *et al.*, 1976 |
| | Papua New Guinea | Southern Highlands | 99 | 0.80 | Hill *et al.*,1986 |
| | | Eastern Highlands | 59 | 0.53 | |
| | | Western Highlands | 24 | 0.87 | |
| | | North Coast | 22 | 0.64 | |
| | Vanuatu | Espiritu Santo | 79 | 0.73 | |
| | | Pentecost | 101 | 0.68 | |
| | | Emae | 60 | 0.63 | |
| | | Central Group | 70 | 0.61 | |
| | | Tanna | 47 | 0.71 | |
| | | Futuna | 32 | 0.69 | |
| | | Aneityum | 46 | 0.61 | |

## Biological Functions of Hp

The Hp phenotypes have different biochemical and biophysical characteristics and functional efficiencies that account for their distinct antioxidant and immunomodulatory capacities (Langlois and Delanghe, 1996; Frank *et al.*, 2001; Guetta *et al.*, 2007). In the following sections, we discuss the main biological functions of Hp.

## Binding of Hemoglobin and Prevention of Renal Damage

Hemoglobin released from erythrocytes is highly toxic and mediates iron-driven oxidative stress and inflammation (Tseng *et al.*, 2004). As indicated above, the main physiological role of Hp is to remove free Hb released by intravascular hemolysis. Haptoglobin and Hb bind to each other to form an essentially irreversible, non-covalently bound, soluble complex characterized by high stability and affinity ($1 \times 10^{-15}$ mol $L^{-1}$) (Okazaki *et al.*, 1997). Some studies suggest that the β chain of human Hb contains two specific Hp binding sites (residues β11-25 and β131-146) whereas the α-chain only has one Hp binding site (residues α121-127). The Hb αβ dimers bind stoichiometrically to the αβ subunits of Hp (McCormick and Atassi, 1990; Langlois and Delanghe, 1996). Haptoglobin in the circulation system reaches saturation at a free Hb concentration of 500-1500 mg $L^{-1}$ (Langlois and Delanghe, 1996; Van Vlierberghe *et al.*, 2004).

Free Hb from lysed erythrocytes is eliminated by glomerular filtration, and this can cause renal damage. Hp reduces the loss of Hb and iron because the Hp-Hb complex is not filtered through the glomeruli (Langlois and Delanghe, 1996; Devlin, 1997; Sadrzadeh and Bozorgmehr, 2004; Van Vlierberghe *et al.*, 2004). Once formed, the Hp-Hb complex is quickly removed from the circulation by hepatocytes (90%) and tissue monocytes/macrophages (10%).

The specific receptor for the Hp-Hb complex in hepatocytes has not yet been cloned or characterized, but has a high binding affinity for the complex. This receptor apparently recognizes the conformational change in Hp caused by formation of the complex with Hb (Kino *et al.*, 1980). The receptor for this complex in macrophages was recently identified as CD163 (Kristiansen *et al.*, 2001; Horn *et al.*, 2003). After endocytosis, the complex is broken down by lysosomes. Haptoglobin is not recycled, but the heme is degraded by heme-oxygenase (HO) to release iron, which is used to synthesize new proteins such as Hb, and biliverdin, which is subsequently converted into bilirubin (Wagener *et al.*, 2003; Van Vlierberghe *et al.*, 2004). Interleukin-6 plays a very important regulatory role in this process since, in addition to stimulating Hp production, it also increases the expression of CD163 in macrophages and increases the efficiency with which the Hb heme group is degraded (Dennis, 2001).

## Protection Against Toxic Radicals

In the Fenton reaction, free iron can react with oxygen to generate superoxide radicals and with $H_2O_2$ to generate hydroxyl radicals (Kaplan, 2002). Free iron can also catalyze the oxidation of low-density lipoproteins that can then damage vascular endothelial cells (Grinshtein *et al.*, 2003). The ability of Hp to reduce the damage caused by free radicals is phenotype-dependent (Gutteridge, 1987; Van Vlierberghe *et al.*, 2004). Experiments with purified Hp have shown that the Hp1-1 protein confers greater protection against oxidative damage *in vitro* (Koda *et al.*, 1998). As the three main Hp phenotypes have the same binding affinities for Hb (Frank *et al.*, 2001), variations in their ability to prevent the release of heme probably reflect the differences in the size of these proteins. Thus, the Hp2-2 protein removes iron to the extravascular space more slowly because it is a larger molecule. Consequently, in individuals with this phenotype (Hp2-2), free Hb remains in the circulation longer and causes greater oxidative stress (Frank *et al.*, 2001).

## Inhibition of Nitric Oxide

Nitric oxide (NO), originally referred to as endothelium-derived relaxing factor (EDRF), is a highly reactive gas produced by various types of cells, including vascular endothelial cells and cytokine-activated macrophages (Hibbs *et al.*, 1988). Nitric oxide is involved in the maintenance of vascular tone and also modulates neurotransmitter function in the central and peripheral nervous systems, platelet aggregation and cellular defense (Green, 1995; Sadrzadeh and Bozorgmehr, 2004; Moncada and Higgs, 2006). Free Hb and Hp bound to Hb inactivate NO/EDRF, whereas Hp does not. Consequently, an increase in the level of circulating Hp-Hb may inhibit NO formation and endothelium relaxation, thereby enhancing the risk of cardiovascular disease (Griffith *et al.*, 1984; Edwards *et al.*, 1986; Moncada and Higgs, 2006). The Hp1-1 phenotype may be advantageous in this respect because the Hp1-1:Hb complex is removed from circulation more rapidly than the other Hp complexes (Frank *et al.*, 2001).

## Immunomodulation

The enhanced production of Hp during the acute phase of inflammation and infection and tumor growth suggests that this protein has additional functions. Haptoglobin has immunoregulatory properties, with Hp2-2 individuals showing greater immunological reactivity (including greater production of antibodies after vaccination) than Hp1-1 and Hp2-1 individuals (Nevo and Sutton, 1968; Langlois and Delanghe, 1996). Haptoglobin also inhibits prostaglandin synthesis and consequently has important anti-inflammatory properties, although these are less pronounced in Hp2-2 individuals (Langlois and Delanghe, 1996; Braeckman *et al.*, 1999).

Haptoglobin is a powerful suppressor of lymphocyte function, as shown by its ability to inhibit the mitogenic response of lymphocytes to phytohemagglutinin and concanavalin A (Baseler and Burrell, 1983). Different T helper (Th) lymphocyte subtypes, known as Th1 and Th2 cells, are responsible for inducing and regulating the cellular and humoral immune response, respectively. T helper-1 cells produce IL-2 and interferon gamma (IFN-$\gamma$) and induce strong IgG responses, thus favouring the cellular immune response, whereas Th2 cells produce IL-4, IL-5, IL-6, IL-10 and IL-13 and increase IgE production, thereby mediating a predominantly humoral and eosinophilic response (Abbas and Lichtman, 2003). Arredouani *et al.* (2003) showed that Hp plays an important role in modulating the balance between Th1 and Th2 lymphocytes (Th1/Th2) by promoting a predominantly Th1 cell response. These cells are more effective in protecting against infections involving intracellular parasites and inhibit the release of Th2 cytokines responsible for defence against extracellular microorganisms. These authors subsequently reported (Arredouani *et al.*, 2005) that Hp selectively modulates the inflammatory response through its ability to suppress the synthesis of TNF-$\alpha$, IL-10 and IL-12 by monocytes stimulated with lipopolysaccharide (LPS). More recently, Guetta *et al.* (2007) showed that Hp1-1-Hb induces much greater IL-6 and IL-10 production than Hp2-2-Hb and that the release of these cytokines depends on the binding of these complexes to macrophage CD163 receptors and on casein kinase II (CKII) activity. The action of CKII was differentially regulated by the type of binding between the different Hp-Hb complexes and the CD163 receptor. Based on these findings, Guetta *et al.* (2007) suggested that Hp1-1 individuals have greater vascular protection than Hp2-2 individuals.

## Angiogenesis

Angiogenesis involves the formation of new blood vessels under normal (healthy) and pathological conditions (Cid *et al.*, 1993). Haptoglobin is considered one of the serum angiogenic factors and is necessary for endothelial cell proliferation and differentiation (Dobryszycka, 1997). In arteries, Hp is involved in cell migration and restructuring of the vessel (De Kleijn *et al.*, 2002). Haptoglobin is useful for the treatment of chronic inflammatory conditions and systemic vasculitis because of its ability to stimulate tissue repair and to compensate for ischemia by promoting the growth of collateral vessels. Surprisingly, Hp2-2 is more angiogenic than the other Hp phenotypes (Cid *et al.*, 1993).

## Quantification, Phenotyping and Genotyping of Hp

The Hp concentration in humans is generally stable but changes with age. Haptoglobin levels may be affected by the Hp phenotype, with circulating concentrations in the following order Hp1-1 > Hp2-1 > Hp2-2 (Langlois and Delanghe, 1996; Imrie *at al*., 2006). The level of Hp in fetuses, cord blood and neonates is usually very low (Galatius-Jensen, 1958). Azevedo *et al.* (1974), in an investigation of the factors that could influence ahaptoglobinemia in Brazilian neonates, detected Hp in only 8% of neonates, with a predominance of the Hp1-1 phenotype.

Haptoglobin plasma levels were initially estimated by measuring the ability of plasma to bind Hb. Radial immunodiffusion is the simplest technique for quantifying Hp (Mancini *et al.*, 1965), although immunonephelometric and immunoturbidimetric assays are now widely used to quantify Hp (Ramakers and Kreutzer, 1976; Fink *et al.*, 1989).

Haptoglobin phenotyping is generally based on electrophoretic separation of the different subtypes according to their molecular size in an appropriate gel medium (Santoro *et al.*, 1982). Smithies (1955) developed the first method for determining the three main Hp phenotypes based on starch-gel electrophoresis with Hb-supplemented serum followed by peroxidase staining. The Hp1-1 protein migrates as a single fast band, while the Hp2-2 protein shows a series of slow bands. The Hp2-1 protein displays an additional series of slow bands and a weak Hp1-1 band (Bowman and Kurosky, 1982; Langlois and Delanghe, 1996). Starch gels were subsequently replaced by polyacrylamide gels (Peacock *et al.*, 1965). Haptoglobin subtyping has also been done by isoelectric focusing and enzyme-linked immunosorbent assay (ELISA) (Leaback and Walker, 1971; Yokoi and Sagisaka, 1990; Levy and Levy, 2004), while molecular methods, such as the polymerase chain reaction (PCR), have been used to determine Hp genotypes (Yano *et al.*, 1998; Koch *et al.*, 2002, 2003; Levy and Levy, 2004). Genotyping methods have the distinct advantage of being useful even when the Hp levels are low (Delanghe and De Buyzere, 2004).

## Hp Polymorphism and Diseases

The functional differences arising from the genetic polymorphism of Hp have led to investigation of the influence of Hp subtypes in different human pathologies (Carter and Worwood, 2007). Table 2 summarizes some important studies that have investigated this association, with emphasis on those involving Brazilian populations.

**Table 2** - Studies on association between HP phenotypes/genotypes and human pathologies (Brazilian Populations in Bold)..

| Disease | Specific condition | Population studied | Conclusion | Reference |
|---|---|---|---|---|
| Diabetes mellitus | Diabetic nephropathy (DM1) and restenosis after percutaneous transluminal coronary angioplasty (DM2) | 53 DM1 and 45 DM2 patients from Israel | Hp1-1 appears to provide protection against the development of these vascular complications | Levy *et al*., 2000 |
| | Diabetic nephropathy (DM1 and DM2) | 110 patients from Israel | Hp2-2 is a major susceptibility gene for development of diabetic nephropathy | Nakhoul e*t al*., 2001 |
| | Diabetic nephropathy (DM2) | 60 Egyptian DM patients and 20 controls | Hp2-2 is a major susceptibility gene for development of diabetic nephropathy | Bessa *et al*., 2007 |
| | Diabetic nephropathy (DM1) | 224 Irish patients (from Northern Ireland and the Republic of Ireland) and 285 controls | HP2 allele may confer susceptibility to the development of nephropathy | Conway *et al*., 2007 |
| | Diabetic retinopathy (DM1) | 52 patients from Israel | Hp2-2 is over-represented | Nakhoul *et al*., 2000 |
| | Diabetic retinopathy (DM2) | 133 Japanese patients | Hp phenotype is not directly associated with increased risk for development of diabetic retinopathy | Koda *et al*., 2002 |
| | **Diabetic retinopathy (DM1 and DM2)** | **147 DM1 and 170 DM2 patients from southeastern Brazil (Caucasians and Afro-descendants) and 142 controls** | **No association between the presence of diabetic retinopathy and HP genotypes was observed** | Wobeto *et al*., 2007 |

**Table 2 (cont.)**

| Disease | Specific condition | Population studied | Conclusion | Reference |
|---|---|---|---|---|
| | Cardiovascular disease (DM2) | Amerindians with and without DM - 206 cases and 206 controls | Hp2-2 diabetic patients have a five-fold greater probability of macrovascular complications | Levy *et al*., 2002 |
| | Acute myocardial infarction | 1,437 patients from Israel (506 with DM) | In DM patients, Hp1-1 was associated with smaller infarct size and lower mortality rates at 30 days | Suleiman *et al*., 2005 |
| | Atherosclerosis (DM2) | 64 patients from Israel | Small and large-artery elasticity indexes were lower and systemic vascular resistance was higher in Hp2-2 patients | Shor *et al*., 2007 |
| Atheros-clerosis and cardiovascular disease | Myocardial infarction | 496 patients from Belgium | Hp2-2 patients have more severe myocardial infarctions | Chapelle *et al*., 1982 |
| | Refractory hypertension (RH) | 445 patients from Belgium (383 with non-refractory and 62 with refractory hypertension) | Hp2-2 hypertension patients are at higher risk of developing RH than those with other Hp phenotypes | Delanghe *et al*., 1995 |
| | Coronary artery bypass grafting (CABG) | 765 male patients of Flemish descent and 253 controls | Hp2-2 type over-represented among victims of a previous acute myocardial infarction and among patients with a lower (- years) age at infarction. Graft survival time was shortest in Hp2-2 patients who had previously undergone CABG. Hp2-2 patients more likely to develop atherosclerotic lesions despite comparable serum lipid concentrations | Delanghe *et al*., 1997 |
| | Lipids, lipoproteins and inflammatory variables | 741 male patients from Belgium | Higher serum total and free cholesterol concentrations in Hp2-2 patients | Braeckman *et al*., 1999 |
| | Restenosis after coronary artery stent implantation | 214 patients from Belgium | The risk of developing restenosis was greater in Hp2-2 (36%) than in Hp2-1 (31%) and Hp1-1 patients (21%) | Roguin *et al*., 2002 |
| Malignancies | Ovarian cancer and nonmalignant ovarian tumors | 132 patients Polish patients with ovarian cancer and 114 with non-malignant ovarian tumors | Significant increase in HP1 gene frequency in both groups | Dobryszycka and Warwas, 1983 |
| | Primary ovarian cancer | 182 Swedish female patients | Significant excess of Hp2-1 among patients with a family history of cancer | Fröhlander and Stendahl, 1988 |
| | Gynecologic neoplasms and breast tumors | 246 German female patients with benign and malignant tumors | Higher HP1 gene frequency in all patients caused by a significant increase of Hp 1-1 and a lower rate of Hp 2-2 | Bartel *et al*., 1985 |
| | Familial and non-familial breast cancer | 128 Jordanian female breast cancer patients (42 familial and 86 non-familial cases) and 200 controls | Over-representation of HP1 and HP2 allele frequencies in familial and non-familial breast cancer patients, respectively. Hp2-2 patients might be considered as a high-risk group for the development of non-familial breast cancer | Awadallah and Atoum, 2004 |
| | Primary lung cancer | 309 patients from Sweden | Low Hp2-2 frequency among patients with pulmonary adenocarcinoma, with corresponding increase in Hp1-1and Hp2-1 | Beckman *et al*., 1986 |
| | Bladder cancer | 264 North-German patients | Significant decrease in Hp2-2 phenotype | Benkmann *et al*., 1987 |
| | Esophageal and gastric cancer | 72 esophageal and 104 gastric cancer patients from India, and 100 controls | Significantly increased frequency of Hp 2-1 (59.7%) and Hp2-2 (91.3%) among patients with both types of cancer | Jayanthi *et al*., 1989 |
| Infection | **Hansen's disease** | **1,009 patients from southern Brazil (935 Caucasians and 74 Afro-descendants), and controls (500 Caucasians and 381 Afro-descendants)** | **No significant relationships could be established either between Hp and the type of Hansen's disease or between Hp and severity of the disease** | Schwantes *et al*., 1967 |
| | **Filariasis** | **605 patients from northern Brazil and 597 controls** | **No association between disease and HP phenotypes** | Ayres *et al*., 1976 |

**Table 2 (cont.)**

| Disease | Specific condition | Population studied | Conclusion | Reference |
|---|---|---|---|---|
| | HIV | 653 HIV-infected Caucasian patients (493 from Belgium and 160 from Luxembourg), and 204 controls | Higher mortality for the Hp2-2 group; median survival time of 11.0 years (Hp 1-1 and Hp 2-1) versus 7.33 years (Hp 2-2). Plasma HIV-1 RNA levels prior to antiviral therapy and their increase over one year were highest in Hp2-2 patients. Hp2-2 was associated with higher serum iron, transferrin saturation and ferritin levels, and low vitamin C concentrations | Delanghe *et al*., 1998a |
| | **HIV** | **387 patients from southeastern Brazil (Caucasians and Afro-descendants) and 142 controls** | **No association between Hp genotypes and either HIV status or indices of HIV progression** | Zaccariotto *et al*., 2006 |
| | Tuberculosis | 84 Russian patients | Hp2-2 patients had large cavities of tissue destruction and more advanced dissemination | Fedoseeva *et al*., 1993 |
| | Tuberculosis | 98 African patients from Zimbabwe and 98 controls | Equal susceptibility to clinical pulmonary tuberculosis disease among different Hp phenotypes. Hp2-2 patients had a higher risk of mortality | Kasvosve *et al*., 2000 |
| | Nephorotic tuberculosis | 152 Russian patients | Hp2-2 patients with more severe nephrotic tuberculosis | Ubaidullaev *et al*., 2002 |
| | Chronic hepatitis C | 239 patients from Belgium and 220 controls | Hp phenotype distributions and HP allele frequencies in the patient group differed significantly from those in the reference population. There were no significant differences between Hp phenotype distribution and hepatitis C virus types or response to interferon alpha therapy | Louagie *et al.*, 1996 |
| | Malaria (A) | 273 Sudanese patients and 208 controls | Hp1-1 associated with susceptibility to falciparum malaria and development of severe complications | Elagib *et al.,* 1998 |
| | Malaria (B) | 113 *P. falciparum* malaria children from coastal Ghana and 42 controls | Hp1-1 phenotype associated with susceptibility to *P. falciparum* malaria in general, and to the development of severe disease in particular | Quaye *et al.,* 2000b |
| | **Malaria (C)** | **182 individuals from the Brazilian western Amazon (Amerindians, Caucasoids and Afro-descendants)** | **No association between Hp phenotypes and susceptibility to malaria infection was found** | Beiguelman *et al*., 2003 |
| | Malaria | 119 Indigenous pregnant African women from western Cameroon | Hp1-1 phenotype may play a role in susceptibility to placental infection by *P. falciparum* during pregnancy. Hp1-1 women had higher parasite prevalence in peripheral blood and placentas. Significant difference in parasite density between Hp1-1 and Hp2-2 phenotypes for placental infection but not for maternal peripheral blood infection | Minang *et al.,* 2004 |
| | **American trypanosomiasis** | **80 patients from southeastern Brazil (Caucasian and Afro-descendants) and 197 controls** | **Hp2-2 phenotype much more frequent in patients with any form of American trypanosomiasis, in patients with the indeterminate form of the disease and in patients with the chronic combined form** | Calderoni *et al*., 2006 |
| Neurological diseases | Epilepsy | 92 patients from USA and 100 controls | Association of Hp2-2 with the presence of seizures in patients with epilepsy | Sadrzadeh *et al*., 2004 |
| | Idiopathic generalized epilepsy (IGE) | 121 patients from Italy (children) and 177 controls | HP1 allele less represented in epileptic children, suggesting that Hp1-1 confers more protection against IGE | Saccucci *et al*., 2004 |
| Hematological diseases | ABO incompatibility | 953 families from northeastern Brazil (1,906 parents and 4,618 children) | Significant excess of Hp1-1 children born to parents in ABO incompatible mating classes, suggesting that Hp1-1 children have a greater chance of survival when suffering from postnatal hemolytic disease caused by ABO incompatibility | Kirk *et al*., 1970 |

**Table 2 (cont.)**

| Disease | Specific condition | Population studied | Conclusion | Reference |
|---|---|---|---|---|
| | Acute lymphatic, chronic lymphatic, acute myeloid, and chronic myeloid leukemia | 100 Swedish patients and 2,331 controls | Association between leukemia and HP1 allele/Hp1-1 phenotype not confirmed | Fröhlander, 1984 |
| | Acute lymphatic, chronic lymphatic, acute myeloid, and chronic myeloid leukemia | 211 patients (most of them Ashkenazy Jews) | Elevation of Hp1-1 type among patients with acute lymphatic, acute myeloid and chronic myeloid leukemia, but not with chronic lymphatic leukemia | Nevo and Tatarsky, 1986 |
| | **Acute lymphatic, chronic lymphatic, acute myeloid, and chronic myeloid leukemia** | **188 patients from southeastern Brazil (Caucasians and Afro-descendants) and 197 controls** | **No significant differences in the frequencies of the Hp phenotypes among the four leukemia groups and the control group** | Campregher *et al*., 2004 |

## Diabetes Mellitus (Dm)

The increased oxidative stress in diabetic patients results in the oxidation of glucose and the modification of low-density lipoproteins (LDL). These changes may stimulate the production of inflammatory cytokines that have been implicated in the morphological and pathological changes found in macrovascular and microvascular complications (Giugliano and Ceriello, 1996; Levy, 2003).

Different degrees of susceptibility to the development of vascular problems have observed in studies of the antioxidant properties of Hp in diabetic patients, with Hp 1-1 individuals showing better protection against DM than Hp 2-1 and Hp 2-2 individuals. This variation in oxidative capacity were not attributable to differences in the affinity between Hp subtypes and the Hb molecule, but rather reflected the fact that Hp1, probably because of its smaller size and structure, passed more easily through the endothelial barrier to reach extravascular spaces. Consequently, Hp 1-1 individuals were better protected against oxidative stress than Hp 2-1 and 2-2 individuals (Levy *et al.*, 2000; Nakhoul *et al.*, 2001; Asleh *et al.*, 2003; Levy *et al.*, 2004).

Levy *et al.* (2002) compared the Hp phenotype in type 2 DM patients with macrovascular complications and normal individuals. Diabetic patients with an Hp2-2 phenotype were five times more likely to have cardiovascular complications than those with an Hp1-1 phenotype. An intermediate risk was associated with the Hp2-1 phenotype. In agreement with this, Hp1-1 apparently protects against restenosis after coronary stent implantation in diabetic patients (Roguin *et al.*, 2002). More recently, Suleiman *et al.* (2005) analyzed the Hp phenotypes in diabetic patients with acute myocardial infarction and demonstrated that the Hp1-1 phenotype was associated with smaller infarct size and lower mortality rates at 30 days. Shor *et al.* (2007) reported that large and small-artery elasticity indexes were significantly lower and the systemic vascular resistance significantly higher in Hp2-2 compared with Hp2-1 or Hp1-1 type 2 DM patients, indicating a major predisposition to the development of atherosclerosis in homozygotes for the HP2 allele.

The Hp2-2 phenotype is also associated with microvascular complications in both types of DM (Nakhoul *et al.*, 2007). Nakhoul *et al.* (2000) found that the Hp2-2 phenotype is overrepresented in Israeli type 1 DM patients with diabetic retinopathy, while type 1 and type 2 DM patients with the Hp1-1 phenotype had greater protection against diabetic nephropathy (Nakhoul *et al.*, 2001). Similar findings were reported by Bessa *et al.* (2007) for Egyptian patients with type 2 DM. Koda *et al.* (2002), however, failed to detect a protective effect that could be attributed to the HP1 allele in Japanese type 2 DM patients.

## Atherosclerosis and Cardiovascular Disorders

The association between Hp phenotypes and heart disease has been investigated for many years. A significant increase has been reported in the incidence of the Hp 2-2 phenotype in high-risk cardiac patients compared to healthy subjects (Gogishvili *et al.*, 1985). Chapelle *et al.* (1982) showed that the damage after myocardial infarction was more severe in patients with Hp2-2 than in those with Hp1-1 or Hp2-1 phenotypes. Additionally, the survival time in patients with the Hp2-2 phenotype who underwent a coronary artery bypass graft was shorter than for patients with other Hp phenotypes and has been associated with the accumulation of atherosclerotic lesions in essential hypertension (Delanghe *et al.*, 1997).

The Hp2-2 phenotype is a genetic risk factor for coronary atherosclerosis, independently of classic risk factors such as dyslipidemia, hyperhomocysteinemia, cigarette smoking, hypertension and DM (Stein and McBride, 1998; Van Vlierberghe *et al.*, 2004). This phenotype provides less protection against oxidative stress in arteries of patients with atherosclerotic plaques and is considered a risk factor for developing refractory hypertension; patients with this phenotype therefore require more complex antihypertensive drug combinations to control their blood pressure (Delanghe *et al.*, 1995). In addition, serum cholesterol levels in individuals with the Hp2-2 phenotype are higher than in individuals with the other Hp phenotypes (Braeckman *et al.*, 1999).

Studies of the association between Hp phenotypes and peripheral blood disorders suggest that the Hp2-2 phenotype is more common in peripheral occlusive disorders. Curiously, Hp2-2 patients with severe atherosclerotic lesions subjected to a treadmill stress test reported longer maximal walking distance than patients with other Hp phenotypes, a finding that could be explained by the fact that the Hp2-2 molecule is more angiogenic than the other Hp molecules (Delanghe *et al.*, 1999). These different functions and biological capacities of Hp may be used as a predictor of the susceptibility to cardiovascular disorders and patient prognosis.

## Cancer

Several studies have reported a correlation between Hp phenotypes and cancer. Bartel *et al.* (1985) showed that the prevalence of breast tumors was higher in women with the Hp1-1 phenotype, and Awadallah and Atoum (2004) concluded that the distribution of the Hp phenotype in breast-cancer patients depended on the family history, the HP1 and HP2 allele frequencies being higher in patients with familial and non-familial breast cancer, respectively. The HP1 gene is overrepresented in ovarian carcinoma (Dobryszycka and Warwas, 1983), and an association has been reported between the Hp2-1 phenotype and a family history of ovarian carcinoma (Fröhlander and Stendahl, 1988).

In some studies, the Hp2-2 frequency was significantly lower in patients with pulmonary adenocarcinoma and bladder carcinoma than in normal subjects (Beckman *et al.*, 1986; Benkmann *et al.*, 1987), whereas a significantly higher frequency of Hp2-1 and Hp2-2 phenotypes was found in patients with esophageal and gastric cancer (Jayanthi *et al.*, 1989).

Several authors have examined the correlation between Hp phenotypes and different types of leukemia. Nevo and Tatarsky (1986) investigated Hp phenotypes in patients with the four most common types of leukemia, namely, acute lymphatic (ALL), chronic lymphatic (CLL), acute myeloid (AML) and chronic myeloid (CML) leukemia. A significantly higher frequency of the Hp1-1 phenotype was observed among leukemia patients with ALL, AML and CML, but not among those with CLL. Fröhlander (1984) found no association between Hp phenotypes and leukemia but observed a significantly higher frequency of ahaptoglobinemia in these patients. A low Hp2-2 frequency has been observed in patients with IgA myeloma (Germenis *et al.*, 1983).

## Infectious Diseases

Several studies have demonstrated that Hp polymorphism may play a role in a number of infectious diseases. Haptoglobin can act as a natural bacteriostat by preventing the consumption of iron that is necessary for the growth of some pathogenic bacteria such as *Neisseria meningitides*, *Campylobacter jejuni* and *Bacteroides fragilis* (Eaton *et al.*, 1982; Pickett *et al.*, 1992; Otto *et al.*, 1994; Lewis and Dyer, 1995). Eaton *et al.* (1982) reported that the simultaneous administration of Hp in rats inoculated intraperitoneally with pathogenic *Escherichia coli* and Hb protected the animals against death.

Kasvosve *et al.* (2000) showed that in a logistic regression model the odds of patients with tuberculosis dying were 6.1 times greater in individuals with the Hp 2-2 phenotype than in those with the Hp 1-1 phenotype. Furthermore, the Hp2-2 phenotype was overrepresented among patients with large cavities created by tissue destruction, more advanced dissemination and the presence of nephrotic tuberculosis (Fedoseeva *et al.*, 1993; Ubaidullaev *et al.*, 2002).

Some families of integrin adhesion receptors such as CD11a-c and CD18 are involved in cell-to-cell viral transmission and contribute to variations in the survival rates after HIV infection. The identification of Hp as an alternative ligand for CD11b/CD18 suggests that this protein plays an important role in HIV infection (El Ghmati *et al.*, 1996; Quaye *et al.*, 2000a). In addition, the residual iron circulating in the plasma of HIV-seropositive patients could enhance Hb-driven oxidative stress, thereby favoring viral replication and transmission. Delanghe *et al.* (1998a) reported that HIV-seropositive patients with the Hp2-2 phenotype had a higher mortality and worse prognosis than patients with other phenotypes.

Louagie *et al.* (1996) reported an association between hepatitis C and Hp polymorphism (Hp1-1 was overrepresented) in patients with the chronic form of this disease. This finding suggests that the Hp phenotype may influence the clinical evolution of hepatitis C. Interestingly, Hp2-2 individuals develop lower levels of antibodies after vaccination against hepatitis B than those with Hp1-1 or Hp2-1 phenotypes (Louagie *et al.*, 1993).

Elagib *et al.* (1998) reported a significant increase in the incidence of Hp1-1 in Sudanese patients with uncomplicated and complicated (cerebral) falciparum malaria. The phenotypic frequency distribution among patients was 60.8% for Hp1-1, 29.7% for Hp2-1 and 9.5% for Hp2-2, while in healthy (control) individuals from the same region it was 26.0%, 55.8% and 18.3%, respectively. Quaye *et al.* (2000b) also found an association between Hp1-1 and severe *P. falciparum* malaria in patients from the coastal region of Ghana. Minang *et al.* (2004) examined the influence of Hp phenotypes on susceptibility to placental infection by *P. falciparum* in pregnant women at delivery in western Cameroon and found that Hp1-1 women had a higher prevalence of parasites in peripheral blood and in the placenta.

## Neurological Diseases

The functional differences in Hp that are related to genetic polymorphism may be linked to the severity and frequency of seizures in patients with epilepsy. The etiology of most seizures is unknown, and Hp is a promising

candidate to explain the differences in susceptibility and resistance to convulsive disease (Sadrzadeh *et al.*, 2004). Microhemorrhagic events may occur in the brain, leading to the accumulation of iron released from Hb. This iron can enhance the formation of reactive oxygen species, increase neuronal excitability, and stimulate membrane lipid peroxidation in the brain, with consequent seizures. Depending on the Hp phenotype, the clearance of intracerebral Hb may not be efficient, and the antioxidant capacity of the interstitial fluid may be compromised (Saccucci *et al.*, 2004). Panter *et al.* (1984) showed that hypohaptoglobinemia was associated with epileptiform seizures. Sadrzadeh *et al.* (2004) reported an association between the Hp2-2 phenotype and recurrent seizures in epileptic patients who had one or more idiopathic seizures per month. Saccucci *et al.* (2004) reported that the Hp1-1 genotype was underrepresented in children with idiopathic generalized epilepsy (IGE) compared with healthy children. Idiopathic generalized epilepsy is linked to a complex pattern of inheritance, and the generalized seizures are probably related to multiple gene-gene and gene-environment interactions. In IGE, the small size of Hp1-1 allows this protein to diffuse in the interstitial cerebral fluid more readily than the other Hp subtypes, thereby protecting its carriers against IGE.

## Association Studies In Brazilian Populations

A few studies have examined Hp polymorphism and its association with diseases and systems, *e.g.*, blood groups, in Brazil. Schwantes *et al.* (1967) found no significant association between Hp polymorphism and Hansen's disease in a large series of patients (~1000) compared with healthy individuals (control group) from southern Brazil.

Kirk *et al.* (1970) found a significantly higher frequency of the Hp1-1 phenotype in children from northeastern Brazil with ABO-incompatible parental combinations than in children with ABO-compatible parents. These authors concluded that the higher levels of Hp1-1 protein in Hp 1-1 children probably enhanced their chances of surviving postnatal hemolytic disease caused by ABO incompatibility since this protein could reduce iron loss and kidney damage during hemolysis.

Ayres *et al.* (1976) found no significant differences in the distribution of Hp phenotypes among filarial-positive and filarial-negative individuals. Similarly, Beiguelman *et al.* (2003) reported no significant differences in the Hp haplotype distribution among individuals infected by *Plasmodium* in the western Amazon compared with non-infected individuals.

In contrast to previous studies (Nevo and Tatarsky, 1986; Mitchell *et al.*, 1988), Campregher *et al.* (2004) found no association between leukemia (ALL, CLL, AML and CML) and the higher frequencies of the Hp1 allele in these patients in southeastern Brazil, although the p-value for patients with CLL tended towards significance. In contrast, ahaptoglobinemia was more frequent among these patients, in agreement with the findings of Fröhlander (1984).

Calderoni *et al.* (2006) investigated the frequency of Hp phenotypes in patients suffering from indeterminate, chronic cardiac, chronic digestive or chronic 'combined' (*i.e.* cardiac plus digestive) forms of Chagas' disease (American trypanosomiasis) and found that the Hp2-2 phenotype was more frequent in the overall group of patients, in patients with the indeterminate form of the disease and in patients with the chronic combined form.

Zaccariotto *et al.* (2006) found no significant influence of the Hp genotypes on the iron status, negative and positive acute-phase serum protein concentrations, T-CD4+ lymphocyte counts and viral loads in HIV-seropositive patients and healthy HIV-seronegative individuals in southeastern Brazil. Similarly, Wobeto *et al.* (2007) observed no significant relationship between the frequency of the Hp genotype in type 1 and type 2 DM patients with and without diabetic retinopathy.

## Concluding Remarks

Haptoglobin is a positive acute-phase protein with antioxidant and immunomodulatory properties. Although there is considerable evidence that the different Hp subtypes may be associated with the outcome of many important disorders, the findings for different populations are often divergent because of the large number of variables involved, including the ethnic composition and population homogeneity, the number of individuals analyzed, the methods used for genotyping and phenotyping, the complex physiopathology of the target disorder, and the therapeutic measures used in different health systems, particularly for treating infectious diseases. A better understanding of the functional importance of Hp molecules would be gained if these variables were well-controlled.

## Acknowledgments

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, grant no. 05/02383-8).

## References

Abbas AK and Lichtman AH (2003) Cellular and Molecular Immunology. 5th edition. Saunders, Philadelphia, pp 65-274.

Arredouani M, Kasran A, Vanoirbeek JA, Berger FG, Baumann H and Ceuppens JL (2005) Haptoglobin dampens endotoxin-induced inflammatory effects both *in vitro* and *in vivo*. Immunology 114:63-271.

Arredouani M, Matthijs P, Hoeyveld V, Kasran A, Baumann H and Ceuppens JL (2003) Haptoglobin directly affects cells and suppresses T helper cell type 2 cytokine release. Immunology 108:144-151.

Asleh R, Marsh S, Shilkrut M, Binah O, Guetta J, Lejbkowicz F, Enav B, Shehadeh N, Kanter Y, Lache O, *et al.* (2003) Ge-

netically determined heterogeneity in hemoglobin scavenging and susceptibility to diabetic cardiovascular disease. Circ Res 92:1193-1200.

Awadallah S and Hamad M (2003) A study of haptoglobin phenotypes in patients with chronic renal failure. Ann Clin Biochem 40:680-683.

Awadallah SM and Atoum MF (2004) Haptoglobin polymorphism in breast cancer patients from Jordan. Clin Chim Acta 341:17-21.

Ayres M, Salzano FM, Helena M, Franco LP and de Souza Barros RM (1976) The association of blood groups, ABH secretion, haptoglobins and hemoglobins with filariasis. Hum Hered 26:105-109.

Azevedo E, Krieger H and Morton NE (1969) Ahaptoglobinemia in northeastern Brazil. Hum Hered 19:603-612.

Azevedo TFS, Salzano FM and Azevedo ES (1974) Factors influencing anhaptoglobinemia in newborns from Salvador, Brazil. Hum Hered 24:300-305.

Bandopadhaya AR, Banerjee AR and Banerjee S (1992) Haptoglobin subtypes in a Bengalee population sample. Anthropol Anz 50:303-305.

Bartel U, Elling D and Geserick G (1985) Distribuition of haptoglobin phenotypes in gynecologic tumors. Zentralbl Gynäkol 107:1492-1495.

Baseler MW and Burrell R (1983) Purification of haptoglobin and its effects on lymphocyte and alveolar macrophage responses. Inflammmation 7:387-400.

Bearn AG and Franklin EC (1958) Some genetical implications of physical studies of human haptoglobins. Science 128:596-597.

Beckman G, Eklund A, Fröhlander N and Stjernberg N (1986) Haptoglobin groups and lung of cancer. Hum Hered 36:258-260.

Beiguelman B, Alves FP, Moura MM, Engracia V, Nunes ACS, Heckmann MI, Ferreira RG, da Silva LH, Camargo EP and Krieger H (2003) The association of genetic markers and malaria infection in the Brazilian western Amazonian region. Mem Inst Oswaldo Cruz 98:445-460.

Benkmann HG, Hanssen HP, Ovenback R and Goedde HW (1987) Distribution of alpha-1-antitrypsin and haptoglobin phenotypes in bladder cancer patients. Hum Hered 37:290-293.

Bessa SS, Hamdy SM and Ali EM (2007) Haptoglobin gene polymorphism in type 2 diabetic patients with and without nephropathy: An Egyptian study. Eur J Intern Med 18:489-495.

Bissell DM, Hammaker L and Schmid R (1972) Hemoglobin and erythrocyte catabolism in rat liver: The separate roles of parenchymal and sinusoidal cells. Blood 40:812-822.

Black JA and Dixon GH (1968) Amino-acid sequence of alpha chains of human haptoglobin. Nature 218:736-741.

Blackwell RQ and Thephusdin C (1963) Distribution of haptoglobin among Thais. Nature 197:503.

Boreham PF, Lenahan JK, Boulzaguet R, Storey J, Ashkar TS, Nambiar R and Matsushima T (1979) Studies on multiple feeding by *Anopheles gambiae* s.l. in a Sudan savanna area of north Nigeria. Trans R Soc Trop Med Hyg 73:418-423.

Bowman BH and Kurosky A (1982) Haptoglobin: The evolutionary product of duplication, unequal crossover, and point mutation. Adv Hum Genet 12:189-261.

Braeckman L, Bacquer DD, Delanghe J, Claeys L and Backer GD (1999) Association between haptoglobin polymorphism, lipids, lipoproteins and inflammatory variables. Atherosclerosis 143:383-388.

Calderoni DR, Andrade T dos S and Grotto HZ (2006) Haptoglobin phenotype appears to affect the pathogenesis of American trypanosomiais. Ann Trop Med Parasitol 100:213-214.

Campregher PV, Metze IL, Grotto HZW and Sonati MF (2004) Haptoglobin phenotypes in Brazilian patients with leukemia. J Bras Pat Med Lab 40:307-309.

Carter K and Worwood M (2007) Haptoglobin: A review of the major allele frequencies worldwide and their association with diseases. Int J Lab Hematol 29:92-110.

Carter K, Bowen DJ, McCunne A and Worwood M (2003) Haptoglobin type neither influences iron accumulation in normal subjects nor predicts clinical presentation in HFE C282Y haemochromatosis: Phenotype and genotype analysis. Br J Haematol 122:326-332.

Chapelle JP, Albert A, Smeets JP, Heusghem C and Kulbertus HE (1982) Effect of the haptoglobin phenotype on the size of a myocardial infarct. N Engl J Med 307:457-463.

Cid MC, Grant DS, Hoffman GS, Auerbach R, Fauci AS and Kleinman HK (1993) Identification of haptoglobin as an angiogenic factor in sera from patients with systemic vasculitis. J Clin Invest 91:977-985.

Connell GE and Smithies O (1959) Human haptoglobins: Estimation and purification. Biochem J 72:115-121.

Connell GE, Smithies O and Dixon GH (1966) Gene action in the human haptoglobins. II. Isolation and physical characterization of alpha polypeptide chains. J Mol Biol 21:225-229.

Constans J, Viau M, Gouaillard C and Clerc A (1981) Haptoglobin polymorphism among Sharian and West African groups. Haptoglobin phenotype determination by radioimmunoelectrophoresis on Hp O samples. Am J Hum Genet 33:606-616.

De Kleijn DP, Smeets MB, Kemmeren PP, Lim SK, Van Middelaar BJ, Velema E, Schoneveld A, Pasterkamp G and Borst C (2002) Acute-phase protein haptoglobin is a cell migration factor involved in arterial restructuring. FASEB J 16:1123-1125.

Delanghe J, Cambier B, Langlois M, De Buyzereache M, Neels H and De Bacquer D (1997) Haptoglobin polymorphism, a genetic risk factor in coronary artery bypass surgery. Atherosclerosis 132:215-219.

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

Delanghe J, Langlois M, Esquivel CA, De Haene H and De Buyzere M (2000) Haptoglobin 1F allele frequency is high among indigenous populations in the State of Durango, Mexico. Hum Hered 50:263-265.

Delanghe JR and De Buyzere ML (2004) Development of an ELISA for the determination of the major haptoglobin phenotype: An interesting technical development and its potential consequences. Clin Chem 50:1972-1973.

Delanghe JR, Duprez DA, De Buyzere ML, Bergez BM, Claeys LR, Leroux-Roels GG and Clement DL (1995) Refractory hypertension is associated with the haptoglobin 2-2 phenotype. J Cardiovasc Risk 2:131-136.

Delanghe JR, Langlois M and De Buyzereache M (1998b) Congenital anhaptoglobinemia *vs.* hypohaptoglobinemia. Blood 91:3524.

Delanghe JR, Langlois MR, Boelart JR, Van Acker J, Van Wanzeele F, van der Groen G, Hemmer R, Verhofstede C, De Buyzere M, De Bacquer D, *et al.* (1998a) Haptoglobin polymorphism, iron metabolism and mortality in HIV infection. AIDS 12:1027-1032.

Dennis C (2001) Haemoglobin scavenger. Nature 409:141-143.

Devlin TM (1997) Textbook of Biochemistry with Clinical Correlations. 4th edition. Wiley-Liss, New York, pp 1020-1021.

Dobryszycka W (1997) Biological functions of haptoglobin - New pieces to an old puzzle. Eur J Chem Clin Biochem 35:647-54.

Dobryszycka W and Warwas M (1983) Haptoglobin types in ovarian tumors. Neoplasma 30:169-172.

Eaton JW, Brandt P, Mahoney JR and Lee JT (1982) Haptoglobin: A natural bacteriostat. Science 215:691-693.

Edwards DH, Griffith TM, Ryley HC and Henderson AH (1986) Haptoglobin-haemoglobin complex in human plasma inhibits endothelium dependent relaxation: Evidence that endothelium derived relaxing factor acts as a local autacoid. Cardiovas Res 20:549-556.

El Ghmati SM, Van Hoeyveld EM, Van Strijp JAG, Ceuppers JL and Stevens EAM (1996) Identification of haptoglobin as an alternative ligand for CD11b/CD18. J Immunol 156:2542-2552.

Elagib AA, Kider AO, Akerstrom B and Elbashir MI (1998) Association of the haptoglobin phenotype (1-1) with falciparum malaria in Sudan. Trans R Soc Trop Med Hyg 92:309-311.

Erdem S and Aksoy M (1975) A note on the distribution of haptoglobin types in Turkey. Hum Hered 25:18-19.

Erickson LM and Maeda M (1994) Parallel evolutionary events in the haptoglobin gene clusters of rhesus monkey and human. Genomics 22:579-589.

Erickson LM, Kim HS and Maeda N (1992) Junctions between genes in the haptoglobin gene cluster of primates. Genomics 14:948-958.

Fedoseeva SV, Iusopova MM, Chukanova VP and Pospelov LE (1993) Course of infiltrating pulmonary tuberculosis depending on the patient's genotype. Probl Tuberk 2:8-10.

Fink PC, Romer M, Haeckel R, Fateh-Moghadam A, Delanghe J, Gressner AM and Dubs RW (1989) Measurement of proteins with the Behring nephelometer. A multicentre evaluation. J Clin Chem Clin Biochem 27:261-276.

Frank MM, Lache O, Enav BI, Szafranek T, Levy NS and Ricklis RM (2001) Structure-function analysis of antioxidant properties of haptoglobin. Blood 98:3693-3698.

Fröhlander N (1984) Haptoglobin groups and leukemia. Hum Hered 34:311-313.

Fröhlander N and Stendahl U (1988) Haptoglobin groups in ovarian carcinoma. Hum Hered 38:180-182.

Galatius-Jensen F (1958) Rare phenotypes in the Hp system. Acta Genet 8:248-255.

Germenis A, Babionitakis A, Kaloterakis A, Filiotou A and Fertakis A (1983) Group-specific component and haptoglobin phenotypes in multiple myeloma. Hum Hered 33:188-191.

Giblett ER (1959) Haptoglobin types in American Negroes. Nature 183:192-193.

Giblett ER (1961) Haptoglobin: A review. Vox Sang 6:513-524.

Giblett ER (1968) The haptoglobin system. Ser Haematol 1:3-20.

Giugliano D and Ceriello A (1996) Oxidative stress and diabetic vascular complications. Diabetes Care 19:247-263.

Gogishvili AV, Kavtaradze VG, Mamaladze GT, Arutiunova MS and Takadze GSh (1985) Haptoglobin phenotype distribution in patients at high risk of developing myocardial infarct. Kardiologiia 25:55-58.

Green SJ (1995) Nitric oxide in mucosal immunity. Nat Med 1:515-517.

Griffith TM, Edwards DH, Lewis MJ, Newby AC and Henderson AH (1984) The nature of endothelium-derived vascular relaxant factor. Nature 308:645-647.

Grinshtein N, Bamm VV, Tsemakhovich VA and Shaklai N (2003) Mechanism of low-density lipoprotein oxidation by hemoglobin-derived iron. Biochemistry 42:6977-6985.

Guetta J, Strauss M, Levy NS, Fahoum L and Levy AP (2007) Haptoglobin genotype modulates the balance of Th1/Th2 cytokines produced by macrophages exposed to free hemoglobin. Atherosclerosis 191:48-53.

Gutteridge JM (1987) The antioxidant activity of haptoglobin towards haemoglobin-stimulated lipid peroxidation. Biochim Biophys Acta 917:219-223.

Herzog P, Bohatová J and Drdová A (1970) Genetic polymorphisms in Kenya. Am J Hum Genet 22:287-291.

Hever Ö (1976) Haptoglobin subtypes in Hungary. Hum Hered 26:324-326.

Hibbs JB Jr, Taintor RR, Vavrim Z and Rachlin EM (1988) Nitric oxide: A cytotoxic activated macrophage effector molecule. Biochem Biophys Res Commun 157:87-94.

Hill AVS, Bowden DK, Flint J, Whitehouse DB, Hopkinson DA, Oppeheimer SJ, Serjeantson SW and Clegg JB (1986) A population genetic survey of the haptoglobina polymorphism in Melanesians by DNA analysis. Am J Hum Genet 38:382-389.

Hillman RS and Finch CA (1992) Red Cell Manual. 6th edition. F.A. Davis Company, Philadelphia, 147 pp.

Horn IR, Nielsen MJ, Madsen M, Jacobsen C, Graversen JH and Moestrup SK (2003) Generation of a haptoglobin-hemoglobin complex-specific Fab antibody blocking the binding of the complex to CD163. Eur J Haematol 71:289-293.

Imrie H, Fowkes FJ, Michon P, Tavul L, Hume JC, Piper KP, Reeder JC and Day KP (2006) Haptoglobin levels are associated with haptoglobin genotype and alpha +-Thalassemia in a malaria-endemic area. Am J Trop Hyg 74:965-971.

Janaydeh M, Hamad M and Awadallah S (2004) The relationship between haptoglobin polymorphism and serum ceruloplasmin ferroxidase activity. Clin Exp Med 3:219-223.

Javid J (1965) The effect of haptoglobin polymer size on hemoglobin binding capacity. Vox Sang 10:320-325.

Javid J (1978) Human haptoglobin. Curr Top Hematol 1:151-192.

Jayanthi M, Habibullah CM, Ishag M, Ali H, Babu OS and Ali MM (1989) Distribution of haptoglobin phenotypes in oesophageal and gastric cancer. J Med Genet 26:172-173.

Jayle MF and Judas O (1946) Formule glycoprotéidique du plasma sanguin. Helv Chim Acta 29:1310-1314.

Jayle MF and Moretti J (1962) Haptoglobin: Biochemical, genetic and pathophysiologic aspects. Prog Hematol 3:342-359.

Johnston FE, Blumberg AS, Kensinger KM, Jantz RL and Walker GF (1969) Serum protein polymorphisms among the Peruvian Cashinahua. Am J Hum Genet 21:376-383.

Kang BY, Kang CY, Oh SD, Bae JS, Kim KT and Lee KO (2003) The protein polymorphism of haptoglobin in Korean elite athletes. Med Princ Pract 12:151-155.

Kaplan J (2002) Strategy and tactics in the evolution of iron acquisition. Semin Hematol 39:219-266.

Kasvosve I, Gomo ZA, Mvundura E, Moyo VM, Saungweme T, Khumalo H, Gordeuk VR, Boelaert JR, Delanghe JR, De Bacquer D, *et al.* (2000) Haptoglobin polymorphism and mortality in patients with tuberculosis. Int J Tuberc Lung Dis 8:771-775.

Kaur H, Sehajpal PK and Shrivastava PK (1981) Distribution of some genetic traits in three populations from Punjab, North India. Anthropol Anz 39:66-69.

Kino K, Tsunoo H, Higa Y, Takami M, Hamaguchi H and Nakajima H (1980) Hemoglobin-haptoglobin receptor in rat liver plasma membrane. J Biol Chem 255:9616-9620.

Kirk RL, Kinns H and Morton NE (1970) Interaction between the ABO blood group and haptoglobin system. Am J Hum Genet 22:384-389.

Ko TM, Su BT, May SY, Huang SC, Wen HK and Ouyang PC (1980) Haptoglobin typing and quantitation in normal Chinese females and gynecologic cancer patients. Chin J Microbiol Immunol 13:149-157.

Koch W, Latz W, Eichinger M, Roguin A, Levy AP and Schomig A (2002) Genotyping of the common haptoglobin Hp1/2 polymorphism based on PCR. Clin Chem 48:1377-1382.

Koch W, Latz W, Eichinger M, Roguin A, Levy AP and Schomig A (2003) Haptoglobin gene subtyping by restriction enzyme analysis. Clin Chem 49:1937-1940.

Koda Y, Soejima M, Yamagishi S, Amano S, Okamoto T, Inagaki Y, Yamada K and Kimura H (2002) Haptoglobin genotype and diabetic microangiopathies in Japanese diabetic patients. Diabetologia 45:1039-1040.

Koda Y, Soejima M, Yoshioka N and Kimura H (1998) The haptoglobin-gene deletion responsible for anhaptoglobinemia. Am J Hum Genet 62:245-252.

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

Kristiansen M, Graversen JH, Jacobsen C, Sonne O, Hoffman HJ, Law SKA and Moestrup SK (2001) Identification of the haemoglobin scavenger receptor. Nature 409:198-201.

Krüger A and Püschel K (1993) Haptoglobin subtypes in northern Germany (Hamburg). Int J Legal Med 106:53-54.

Kurosky A, Barnett DR, Lee TH, Touchstone B, Hay RE, Arnott MS, Bowman BH and Fitch WM (1980) Covalent structure of human haptoglobina: A serine protease. Proc Natl Acad Sci USA 77:3388-3392.

Lai LY, Constans J and Archer GT (1986) Isoelectric focusing studies of transferring and haptoglobina subtypes in an Australian white population. Ann Hum Biol 13:555-561.

Lange V (1992) Haptoglobin polymorphism - Not only a genetic marker. Anthropol Anz 50:281-302.

Langlois MR and Delanghe JR (1996) Biological and clinical significance of haptoglobin polymorphism in humans. Clin Chem 42:1589-1600.

Lavie L, Lotan R, Hochberg I, Herer P, Lavie P and Levy AP (2003) Haptoglobin polymorphism is a risk factor for cardiovascular disease in patients with obstructive sleep apnea syndrome. Sleep 26:593-595.

Leaback DH and Walker PG (1971) Some applications of gel isoelectric focusing to the examination of human serum proteins. Proc R Soc Med 64:645-646.

Levy AP (2003) Genetics of diabetic cardiovascular disease: Identification of a major susceptibility gene. Acta Diabetol 40:S330-S333.

Levy AP, Hochberg I, Jablonski K, Resnick H, Lee ET, Best L and Howard BV (2002) Haptoglobin phenotype is an independent risk factor for cardiovascular disease in individuals with diabetes: The strong heart study. JACC 40:1984-1990.

Levy AP, Larson MG, Corey D, Lotan R, Vita JA and Benjamin BJ (2004) Haptoglobin phenotype and prevalent coronary heart disease in the Framingham offspring cohort. Atherosclerosis 172:361-365.

Levy AP, Roguin A, Hochberg I, Herer P, Marsh S, Nakhoul FM and Skorecki K (2000) Haptoglobin phenotype and vascular complications in patients with diabetes. N Engl J Med 343:969-970.

Levy NS and Levy AP (2004) ELISA for determination of the haptoglobin phenotype. Clin Chem 50:2148-2150.

Lewis LA and Dyer DW (1995) Identification of an iron-regulated outer membrane protein of *Neisseria meningitides* involved in the utilization of hemoglobin complexed to haptoglobin. J Bacteriol 177:1299-1306.

Lim SK, Kim H, Lim SK, bin Ali A, Lim YK, Wang Y, Chong SM, Costantini F and Baumman H (1998) Increased susceptibility in Hp knockout mice during acute hemolysis. Blood 92:1870-1877.

Louagie H, Delanghe J, Desombere I, De Buyzere M, Hauser P and Leroux-Roels G (1993) Haptoglobin polymorphism and the immune response after hepatitis B vaccination. Vaccine 11:1188-1190.

Louagie HK, Brouwer JT, Delanghe JR, De Buyzere ML and Leroux-Roels GG (1996) Haptoglobin polymorphism and chronic hepatitis C. J Hepatol 25:10-14.

Maeda N (1985) Nucleotide sequence of the haptoglobin and haptoglobin-related gene pair. The haptoglobin-related gene contains a retrovirus-like element. J Biol Chem 260:6698-6709.

Maeda N (1991) DNA polymorphisms in the controlling region of the human haptoglobin genes: A molecular explanation for the haptoglobin 2-1 modified phenotype. Am J Hum Genet 49:158-166.

Maeda N and Smithies O (1986) The evolution of multigene families: Human haptoglobin genes. Annu Rev Genet. 20:81-108.

Maeda N, Yang F, Barnett DR, Bowman BH and Smithies O (1984) Duplication within the haptoglobin Hp2 gene. Nature 309:131-135.

Mahmud SM, Koushik A, Duarte-Franco E, Costa J, Fontes G, Bicho M, Coutlée F and Franco EL (2007) Haptoglobin phenotype and risk of cervical neoplasia: A case-control study. Clin Chim Acta 385:67-72.

Malchy B, Rorstad O and Dixon GH (1973) The half-molecule of haptoglobin: Studies on the product obtained by the selective cleavage of a haptoglobin disulfide. Can J Biochem 51:265-273.

Mancini G, Carbonara AO and Heremans JF (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 2:235-254.

Marini E, Moral P, Petralanda I, Pacheco M, Sandiumenge T, Succa V, Vives S and Vona G (1993) Serum protein markers in the Piaroa Indians of Amazonia (Venezuela). Hum Hered 43:232-238.

Mastana SS, Bernal JE, Onyemelukwe GC and Papiha SS (1994) Haptoglobin subtypes among four different populations. Hum Hered 44:10-13.

McCormick DJ and Atassi MZ (1990) Hemoglobin binding with haptoglobin: Delineation of the haptoglobin binding site on the alpha-chain of human hemoglobin. J Protein Chem 9:735-742.

McEvoy SM and Maeda N (1988) Complex events in the evolution of the haptoglobin gene cluster in primates. J Biol Chem 263:15740-15747.

Minang JT, Gvan BA, Anchang JK, Troye-Blomberg M, Permann H and Achidi EA (2004) Haptoglobin phenotypes and malaria infection in pregnant women at delivery in western Cameroon. Acta Trop 90:107-114.

Mitchell RJ, Carzino R and Janardhana V (1988) Association between the two serum proteins haptoglobin and transferring and leukemia. Hum Hered 38:144-150.

Moncada S and Higgs EA (2006) The discovery of nitric oxide and its role in vascular biology. Br J Pharmacol 147:S193-S201.

Moral P and Panadero AM (1983) Haptoglobin subtypes in Barcelona (Spain). Hum Hered 33:192-194.

Muranjan M, Nussenzweig V and Tomlinson S (1998) Characterization of the human serum trypanosome toxin, haptoglobin related protein. J Biol Chem 273:3884-3887.

Nagel R and Etcheverry R (1963) Types of haptoglobin in Araucanian Indians of Chile. Nature 197:187-188.

Nagel R, Etcheverry R and Guzman C (1964) Haptoglobin types in inhabitants of Easter Island. Nature 201:216-217.

Nakada N and Abe K (1987) Genetic polymorphism of haptoglobin subtypes in a Japanese population 37:376-380.

Nakhoul FM, Marsh S, Hochberg I, Leibu R, Miller BP and Levy AP (2000) Haptoglobin genotype as a risk factor for diabetic retinopathy. JAMA 284:1244-1245.

Nakhoul FM, Miller-Lotan R, Awaad H, Asleh R and Levy AP (2007) Hypothesis -haptoglobin genotype and diabetic nephropathy. Nat Clin Pract Nephrol 3:339-344.

Nakhoul FM, Zoabi R, Kanter Y, Zoabi M, Skorecki K, Hochberg I, Leibu R, Miller B and Levy AP (2001) Haptoglobin phenotype and diabetic nephropathy. Diabetologia 44:602-604.

Neel JV, Ferrell RE and Conard RA (1976) The frequency of "rare" protein variants in Marshall Islanders and other Micronesians. Am J Hum Genet 28:262-269.

Nevo S and Tatarsky I (1986) Serum haptoglobin types and leukemia. Hum Hered 73:240-244.

Nevo SS and Sutton HE (1968) Association between response to typhoid vaccination and known genetic markers. Am J Hum Genet 20:461-469.

Nielsen MJ, Petersen SV, Jacobsen C, Oxvig C, Rees D, Moller HJ and Moestrup SK (2006) Haptoglobin-related protein is a high-affinity hemoglobin binding plasma protein. Blood 108:2846-2849.

Nikol'chenko AIU, Omel'chenko EA, Gulevskii AK and Moroz OP (1997) The distribution of haptoglobin phenotypes among the population of Ukraine. Tsitol Genet 31:62-69.

Noyes WD and Garby L (1967) Rate of haptoglobin in synthesis in normal man. Determinations by the return to normal levels following hemoglobin infusion. Scand J Clin Lab Invest 20:33-38.

Okazaki T, Yanagisawa Y and Nagai T (1997) Analysis of the affinity of each haptoglobin polymer for hemoglobin by two-dimensional affinity electrophoresis. Clin Chim Acta 258:137-144.

Otto BR, Sparrius M, Wors DJ and de Graaf FK (1994) Utilization of haem from the haptoglobin-haemoglobin complex by *Bacteroides fragilis*. Microb Pathog 17:137-147.

Panter SS, Sadrzadeh SM, Hallaway PE, Haines J, Anderson VE and Eaton JW (1984) Hypohaptoglobinemia: A possible predisposition to epilepsy. Trans Assoc Am Physicians 97:56-62.

Papp M, Lakatos PL, Hungarian IBD Study Group, Palatka K, Foldi I, Udvardy M, Harsfalvi J, Tornai I, Vitalis Z, Dinya T, *et al.* (2007) Haptoglobin polymorphisms are associated with Crohn's disease, disease behavior, and extraintestinal manifestations in Hungarian patients. Dig Dis Sci 52:1279-1284.

Peacock AC, Bunting LS and Queen KG (1965) Serum protein electrophoresis in acrylamide gel: Patterns from normal human subjects. Science 147:1451-1453.

Pickett CL, Auffenberg T, Pesci EC, Sheen VL and Jusuf SS (1992) Iron acquisition and hemolysin production by *Campylobacter jejuni*. Infect Immun 60:3872-3877.

Polonovski M and Jayle MF (1938) Existence dans le plasma sanguin d'une substance activant l'action peroxydasique, l'haptoglobine. C R Soc Biol 129:457-460.

Quaye IK, Ababio G and Amoah AG (2006) Haptoglobin 2-2 phenotype is a risk factor for type 2 diabetes in Ghana. J Atheroscler Thromb 13:90-94.

Quaye IK, Ekuban FA, Brandful JA, Gyan BA, Akanmori BD and Ankrah NA (2000a) Haptoglobin phenotypes in HIV-1 seropositive patients in Ghana: Decreased risk for Hp0 individuals. Hum Hered 50:382-383.

Quaye IK, Ekuban FA, Goka BQ, Adabayeri V, Kurtzhals JA, Gyan B, Ankrah NA, Hviid L and Akanmori BD (2000b) Haptoglobin 1-1 is associated with susceptibility to severe *Plasmodium falciparum* malaria. Trans R Soc Trop Med Hyg 94:216-219.

Rahimi Ag, Goedde HW, Flatz G, Kaifie S, Benkmann HG and Delbrük H (1977) Serum protein polymorphisms in four populations of Afghanistan. Am J Hum Genet 29:356-360.

Ramakers JM and Kreutzer HJ (1976) Turbidimetric determination of haptoglobin. J Clin Chem Biochem 14:407-410.

Raugei G, Bensi G, Colantuoni V, Romano V, Santoro C, Costanzo F and Cortese R (1983) Sequence of human haptoglobin cDNA: Evidence that the $\alpha$ and $\beta$ subunits are coded by the same mRNA. Nuc Acids Res 11:5811-5819.

Raynes JG, Eagling S and McAdam KP (1991) Acute-phase protein synthesis in human hepatoma cells: Differential regulation of serum amyloid A (SAA) and haptoglobin by interleukin-1 and interleukin-6. Clin Exp Immunol 83:488-491.

Robson EB, Polani PE, Dart SJ, Jacobs PA and Renwick JH (1969) Probable assignment of the alpha locus of haptoglobin to chromosome 16 in man. Nature 223:1163-1165.

Roguin A, Ribichini F, Ferrero V, Matullo G, Herer P, Wijns W and Levy AP (2002) Haptoglobin phenotype and the risk of restenosis after coronary artery stent implantation. Am J Cardiol 7:806-810.

Saccucci P, Verdecchia M, Piciullo A, Bottini N, Rizzo R, Gloria-Bottini F, Lucarelli P and Curatolo P (2004) Convulsive disorder and genetics polymorphism. Association of idiopathic generalized epilepsy with haptoglobin polymorphism. Neurogenetics 5:245-248.

Sadrzadeh SM and Bozorgmehr J (2004) Haptoglobin phenotypes in health and disorders. Am J Clin Pathol 121 Suppl:S97-104.

Sadrzadeh SM, Saffari Y and Bozorgmehr J (2004) Haptoglobin phenotypes in epilepsy. Clin Chem 50:1095-1097.

Saha N and Ong YW (1984) Distribution of haptoglobin in different dialect groups of Chinese, Malays and Indians in Singapore. Ann Acad Med Singapore 13:498-501.

Salzano FM, Gershowitz H, Mohrenweiser H, Neel JV, Smouse PE, Mestriner MA, Weimer TA, Franco MHLP, Simões AL, Constans J, *et al.* (1986) Gene flow across tribal barriers and its effect among the Amazonian Içana River Indians. Am J Phys Anthropol 69:3-14.

Salzano FM, Neel JV, Weitkamp LR and Woodall JP (1972) Serum proteins, hemoglobins and erythrocyte enzymes of Brazilian Cayapo Indians. Hum Biol 44:443-458.

Salzano FM, Weimer TA and Franco MHLP (1985) Population structure and blood genetics of the Pacaás Novos Indians of Brazil. Ann Hum Biol 12:241-249.

Santoro C, Boccazzi C and Carbonara AO (1982) A simplified procedure for haptoglobin subtyping. Hum Genet 60:287-288.

Santoro C, Olivetti E and Carbonara AO (1983) Distribution of haptoglobin subtypes in continental Italy and Sardinia. Hum Hered 33:195-198.

Santos SEB, Ribeiro-dos-Santos AKC, Guerreiro JF, Santos EJM, Weimer TA, Callegari-Jacquest SM, Mestriner MA, Franco MHLP, Hutz MH and Salzano FM (1998) New protein genetic studies in six Amazonian Indian populations. Ann Hum Biol 25:505-522.

Schultze HE and Heremans JF (1966) Molecular Biology of Human Proteins. Elsevier, Amsterdam, pp 384-402.

Schwantes AR, Salzano FM, De Castro IV and Tondo CV (1967) Haptoglobins and leprosy. Acta Genet Stat Med 17:127-136.

Scott EM, Duncan IW, Ekstrand V and Wright RC (1966) Frequency of polymorphic types of red cell enzymes and serum factors in Alaskan Eskimos and Indians. Am J Hum Genet 18:408-411.

Shim BS and Bearn AG (1964) The distribuiton of haptoglobin subtypes in various populations, including subtype patterns in some nonhuman primates. Am J Hum Genet 16:477-483.

Shimada E, Odagiri M, Chainwong K, Watanabe Y, Anazawa M, Mazda T, Okazaki H, Juji T, O'Charoen R and Tadokoro K (2007) Detection of $Hp^{del}$ among Thais, a deleted allele of the haptoglobin gene that causes congenital haptoglobin deficiency. Transfusion 47:2315-2321.

Shindo S (1990) Haptoglobin subtyping with anti-haptoglobin alpha chain antibodies. Electrophoresis 11:483-488.

Shinton NK, Richardson RW and Willians JD (1965) Diagnostic value of serum haptoglobin. J Clin Pathol 18:114-118.

Shor M, Boaz M, Gavish D, Wainshtein J, Matas Z and Shargorodsky M (2007) Relation of haptoglobin phenotype to early vascular changes in patients with diabetes mellitus. Am J Cardiol 100:1767-1770.

Shreffler DC and Steinberg AG (1967) Further studies on the Xavantes Indians. IV. Serum protein groups and the SC1 trait of saliva in the Simões Lopes and São Marcos. Am J Hum Genet 19:514-523.

Silverman LM and Christenson RH (1994) Amino acids and proteins. In: Burtis CA and Ashwood ER (eds) Tietz Textbook of Clinical Chemistry. 2nd edition. W.B. Saunders Co., Philadelphia, pp 707-708.

Simões AL, Kompf J, Ritter H, Luckenbach C, Zischler H and Salzano FM (1989) Electrophoretic and isoelectric focusing studies in Brazilian Indians: Data on four systems. Hum Biol 61:427-438.

Smith AB and Hajduk SL (1995) Identification of haptoglobin as a natural inhibitor of trypanocidal activity in human serum. Proc Natl Acad Sci USA 92:10262-10266.

Smith AB, Esko JD and Hajduk SL (1995) Killing of trypanosomes by the human haptoglobina-related protein. Science 268:284-286.

Smithies O (1955) Zone electrophoresis in starch gels: Group variations in the serum proteins of normal human adults. Biochemistry 61:629-641.

Smithies O and Walker NF (1956) Notation for serum-protein groups and the genes controlling their inheritance. Nature 178:694-695.

Smithies O, Connell GE and Dixon GH (1962) Chromosomal rearrangements and evolution of haptoglobin genes. Nature 196:232-236.

Soejima M, Koda Y, Fujibara J and Takeshita H (2007) The distribution of haptoglobin-gene deletion (Hp del) is restricted to East Asians. Transfusion 47:1948-1950.

Solovenchuk LL and Glushenko NA (1985) Genetic structure of the populations of native inhabitants in the northeastern USSR. V. The chukot events. Genetika 21:1557-1566.

Souza IR, Muniz YCN, Saldanha GM, Alves Junior L, Rosa FC, Maegawa FAB, Susin MF, Lipinski MS and Petzl-Erler ML (2003) Demographic and genetic structures of two partially isolated communities of Santa Catarina island, southern Brazil. Hum Biol 75:241-253.

Stein JH and McBride PE (1998) Hyperhomocysteinemia and atherosclerotic vascular disease: Pathophysiology, screening and treatment of. Arch Intern Med 158:1301-1306.

Stromatias D, Xomali R and Kouvatsi A (1987) Haptoglobin subtypes in Greece. Gene Geogr 1:131-134.

Suleiman M, Aronson D, Asleh R, Kapeliovich MR, Roguin A, Meisel SR, Shochat M, Sulieman A, Reisner SA, Markiewicz W, *et al.* (2005) Haptoglobin polymorphism predicts 30-day mortality and heart failure in patients with diabetes and acute myocardial infarction. Diabetes 54:2802-2806.

Sutton HE, Matson GA, Robinson AR and Koucky RW (1960) Distribution of haptoglobin, transferrin, and hemoglobin types among Indians of southern Mexico and Guatemala. Am J Hum Genet 12:338-347.

Tanis RJ, Neel JV, Dovey H and Morrow M (1973) The genetic structure of a tribal population, the Yanomama Indians. IX. Gene frequencies for 18 serum protein and erythrocyte enzyme systems in the Yanomama and five neighboring tribes: Nine new variants. Am J Hum Genet 25:655-676.

Teige B, Olaisen B and Teisberg P (1992) Haptoglobin subtypes in Norway and a review of HP subtypes in various populations. Hum Hered 42:93-106.

Teye K, Quaye IKE, Koda Y, Soejima M, Pang H, Tsuneoka M, Amoah AG, Adjei A and Kimura H (2004) A novel 1247T

missense mutation in the haptoglobin 2 β-chain decreases the expression of the protein and is associated with ahaptoglobinaenia. Hum Genet 114:499-502.

Teye K, Quaye IKE, Koda Y, Soejima M, Tsuneoka M, Pang H, Ekem I, Amoah AG, Adjei A and Kimura H (2003) A-61C and C-101G Hp gene promoter polymorphism are, respectively, associated with ahaptoglobinaemia and hypohaptoglobinaemia in Ghana. Clin Genet 64:439-443.

Teye K, Soejima M, Quaye IKE, Pang H, Tsuneoka M, Koda Y and Kimura H (2006) Haptoglobin gene promoter polymorphism and haplotypes are unique in different populations. Hum Biol 78:121-126.

Tseng CF, Lin CC, Huang HY, Liu HC and Mao SJT (2004) Antioxidant role of human haptoglobin. Proteomics 4:2221-2228.

Ubaidullaev AM, Kazakov KS and Khakimov MA (2002) Genetics variants of haptoglobin in patients with nephrotuberculosis. Probl Tuberk 7:42-43.

Van der Straten A, Herzog A, Cabezon T and Bollen A (1984) Characterization of human haptoglobin cDNAs coding for alpha 2FS beta and alpha 1S beta Variants. FEBS Lett 168:103-107.

Van Vlierberghe H, Langlois M and Delanghe J (2004) Haptoglobin polymorphism and iron homeostasis in health and in disease. Clin Chim Acta 345:35-42.

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

Varsahr AM, Spitsyn VA, Bychcovscaya LS and Kravchuk OI (2001) To the research of the gene pool of the Gagauz population of Moldavia. Anthropol Anz 59:11-17.

Wagener FADT, Volk HD, Willis D, Abraham NG, Soares MP, Adema GA and Figdor CG (2003) Different faces of the heme-heme oxygenase system in inflammation. Pharmacol Rev 55:551-571.

Wobeto VPA, Rosim ET, Melo MB, Calliari LEP and Sonati MF (2007) Haptoglobin polymorphism and diabetic retinopathy in Brazilian patients. Diabetes Res Clin Pract 77:385-388.

Yang F, Brune JL, Baldwin WD, Barnett DR and Bowman BH (1983) Identification and characterization of human haptoglobin cDNA. Proc Natl Acad Sci USA 80:5875-5879.

Yang SE, Min WK, Park H, Chun S, Nah J and Kim JQ (2000) Distribution of haptoglobin phenotypes in a Korean population, using the semi-automated PhastSystem. Ann Clin Biochem 37:205-209.

Yano A, Yamamoto Y, Miyaishi S and Ishizu H (1998) Haptoglobin genotyping by allele-specific polymerase chain reaction amplification. Acta Med Okayama 52:173-181.

Yokoi T and Sagisaka K (1990) Haptoglobin typing of human bloodstains using a specific DNA probe. Forensic Sci Int 45:39-46.

Zaccariotto TR, Rosim ET, Melo D, Garcia PMD, Munhoz RR, Aoki FH and Sonati MF (2006) Haptoglobin polymorphism in a HIV-1-seropositive Brazilian population. J Clin Pathol 59:550-553.

Zhao H, Zhang G, Duan Y and Yu S (1993) Haptoglobin types in Chinese ethnic groups. Hum Hered 43:131-133.

*Associate Editor: Francisco Mauro Salzano*