



Polymorphism of human haptoglobin and its clinical importance

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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.

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Human Haptoglobin

Haptoglobin, first described by Polonovski and Jayle (1938), is an α 2-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).

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 α 1-chain (with 83 amino acids) and the α 2-chain

(with 142 amino acids) (Kurosky *et al.*, 1980; Maeda, 1991). The α 1-chain can be further classified into α 1S (slow) or α 1F (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 α 1S and aspartic acid and lysine in α 1F, 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 5th and 7th 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 4th 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 11th to the 69th residue (exons 3 and 4 of HP1) is duplicated

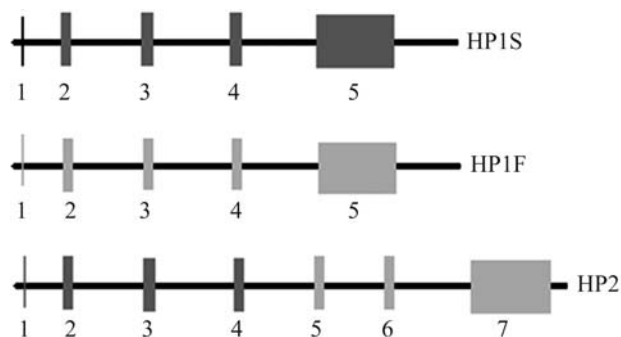


Figure 1 - Schematic representation of the organization of the Hp gene. The boxes indicate exons (adapted from Yano *et al.*, 1998).

(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 anti-haptoglobin 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 populations 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} origi-

nated 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 α 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 ^{α} 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.*

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	<i>n</i>	HP1 allele frequency	Reference
Africa	Algeria (Sahara)	Saoura Valley region	373	0.64	Constans <i>et al.</i> , 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 <i>et al.</i> , 2006
	Kenya	Luo tribe (north of Lake Victoria)	227	0.57	Herzog <i>et al.</i> , 1970
	Mali	Menaka region	285	0.44	Constans <i>et al.</i> , 1981
	Nigeria	Ibadan town	63	0.73	Shim and Bearn, 1964
		Zaria city	56	0.38	Mastana <i>et al.</i> , 1994
	Sudan	General population	208	0.54	Elagib <i>et al.</i> , 1998
	Senegal	Bedik group	733	0.73	Constans <i>et al.</i> , 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 <i>et al.</i> , 2003
		Caucasoids and Afro-descendants from southeastern Brazil	142	0.46	Zaccariotto <i>et al.</i> , 2006
		Cayapo Indians (States of Pará and Mato Grosso; Txukahamae, Kuben-Kran-Kegn, Xikrin and Gorotire tribes)	596	0.58	Salzano <i>et al.</i> , 1972
		Pacaás Novos Indians (Rondônia State, northern Brazil; Tanajura and Santo André communities)	222	0.82	Salzano <i>et al.</i> , 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 <i>et al.</i> , 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 <i>et al.</i> , 1989
		Içana River Indians (northern Amazon region)	133	0.43	
		Galibi Amazonian Indians	167	0.63	Santos <i>et al.</i> , 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 <i>et al.</i> , 1973		

Table 1 (cont.)

Continent	Country	Population studied	<i>n</i>	HPI 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 <i>et al.</i> , 2007
	Chile	Araucanian Indians	31	0.77	Shim and Bearn, 1964
	Colombia	Spanish-origin population from Bogotá	109	0.43	Mastana <i>et al.</i> , 1994
	USA	Indian (Alabama Coushatta)	143	0.37	Shim and Bearn, 1964
		Eskimos (northern and southern Alaska)	220	0.32	Scott <i>et al.</i> , 1966
		General population	3,273	0.39	Levy <i>et al.</i> , 2004
	Mexico	Durango State (Urban Indians)	119	0.63	Delanghe <i>et al.</i> , 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 <i>et al.</i> , 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 <i>et al.</i> , 1969
	Venezuela	Piaroa Indians	121	0.82	Marini <i>et al.</i> , 1993
		Makiritare Indians	186	0.49	Tanis <i>et al.</i> , 1973
Asia	Afghanistan	Tajik group	310	0.26	Rahimi <i>et al.</i> , 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 <i>et al.</i> , 2006
		General population	667	0.33	Ko <i>et al.</i> , 1980
		Mongolian group	204	0.26	Zhao <i>et al.</i> , 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 <i>et al.</i> , 1992
		Punjab (northern India) - Jat Sikh group	192	0.23	Kaur <i>et al.</i> , 1981
		Punjab (northern India) - Khatri group	105	0.19	
		Punjab (northern India) - Balmiki group	108	0.14	
		Hindus from Hyderabad	116	0.20	Mastana <i>et al.</i> , 1994
	Israel	General population	757	0.33	Lavie <i>et al.</i> , 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 <i>et al.</i> , 2004
		General population	200	0.29	Awadallah and Hamad, 2003
	Korea	General population (male athletes)	120	0.31	Kang <i>et al.</i> , 2003
		General population	316	0.32	Yang <i>et al.</i> , 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 <i>et al.</i> , 2007
	Turkey	General population	200	0.27	Erdem and Aksoy, 1975

Table 1 (cont.)

Continent	Country	Population studied	<i>n</i>	HPI allele frequency	Reference
	Ukraine	Mixed (from Dnepropetrovsk, Kharkov, Odessa, Kiev, Uzhgorod, Zhitomir)	596	0.38	Nikol'chenko <i>et al.</i> , 1997
Europe	Belgium	General population	918	0.40	Van Vlierberghe <i>et al.</i> , 2001
	Germany	Hamburg (northern Germany)	1,725	0.39	Krüger and Püschel, 1993
	Greece	Northern Greece	212	0.35	Stromatias <i>et al.</i> , 1987
	Hungary	Budapest	343	0.35	Hevér, 1976
		General population	384	0.35	Papp <i>et al.</i> , 2007
	Italy	Continental Italy (northeastern, northwestern, central and southern regions)	441	0.11	Santoro <i>et al.</i> , 1983
		Sardinia	165	0.18	
	Moldavia	Gaugauz	190	0.35	Varsahr <i>et al.</i> , 2001
	Norway	Oslo	6,668	0.38	Teige <i>et al.</i> , 1992
	Spain	Barcelona	317	0.38	Moral and Panadero, 1983
	United Kingdom	Northeastern England	101	0.41	Mastana <i>et al.</i> , 1994
		Southern Wales	265	0.40	Carter <i>et al.</i> , 2003
Oceania	Australia	Caucasian population	307	0.40	Lai <i>et al.</i> , 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 <i>et al.</i> , 1976
	Papua New Guinea	Southern Highlands	99	0.80	Hill <i>et al.</i> , 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 ($1 \times 10^{15} \text{ mol L}^{-1}$) (Okazaki *et al.*, 1997). Some studies suggest that the β chain of human Hb contains two specific Hp binding sites (residues $\beta 11-25$ and $\beta 131-146$) whereas the α -chain only has one Hp binding site (residues $\alpha 121-127$). The Hb $\alpha\beta$ dimers bind stoichiometrically to the $\alpha\beta$ subunits of Hp (McCormick and Atassi, 1990; Langlois and Delanghe, 1996). Haptoglobin in the circulation system reaches saturation at a free Hb concentration of $500-1500 \text{ mg L}^{-1}$ (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 H₂O₂ 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 endo-

thelium 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- γ) 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- α , 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 (Dobryszczyka, 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 *et 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 <i>et al.</i> , 2000
	Diabetic nephropathy (DM1 and DM2)	110 patients from Israel	Hp2-2 is a major susceptibility gene for development of diabetic nephropathy	Nakhoul <i>et al.</i> , 2001
	Diabetic nephropathy (DM2)	60 Egyptian DM patients and 20 controls	Hp2-2 is a major susceptibility gene for development of diabetic nephropathy	Bessa <i>et al.</i> , 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 <i>et al.</i> , 2007
	Diabetic retinopathy (DM1)	52 patients from Israel	Hp2-2 is over-represented	Nakhoul <i>et al.</i> , 2000
	Diabetic retinopathy (DM2)	133 Japanese patients	Hp phenotype is not directly associated with increased risk for development of diabetic retinopathy	Koda <i>et al.</i> , 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 <i>et al.</i> , 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 <i>et al.</i> , 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 <i>et al.</i> , 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 <i>et al.</i> , 2007
Atheros-clerosis and cardiovascular disease	Myocardial infarction	496 patients from Belgium	Hp2-2 patients have more severe myocardial infarctions	Chapelle <i>et al.</i> , 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 <i>et al.</i> , 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 <i>et al.</i> , 1997
	Lipids, lipoproteins and inflammatory variables	741 male patients from Belgium	Higher serum total and free cholesterol concentrations in Hp2-2 patients	Braeckman <i>et al.</i> , 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 <i>et al.</i> , 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	Dobryszczyka 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 <i>et al.</i> , 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 Hp1-1 and Hp2-1	Beckman <i>et al.</i> , 1986
	Bladder cancer	264 North-German patients	Significant decrease in Hp2-2 phenotype	Benkmann <i>et al.</i> , 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 <i>et al.</i> , 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 <i>et al.</i> , 1967
	Filariasis	605 patients from northern Brazil and 597 controls	No association between disease and HP phenotypes	Ayres <i>et al.</i> , 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 <i>et al.</i> , 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 <i>et al.</i> , 2006
	Tuberculosis	84 Russian patients	Hp2-2 patients had large cavities of tissue destruction and more advanced dissemination	Fedoseeva <i>et al.</i> , 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 <i>et al.</i> , 2000
	Nephrotic tuberculosis	152 Russian patients	Hp2-2 patients with more severe nephrotic tuberculosis	Ubaidullaev <i>et al.</i> , 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 <i>et al.</i> , 1996
	Malaria (A)	273 Sudanese patients and 208 controls	Hp1-1 associated with susceptibility to falciparum malaria and development of severe complications	Elagib <i>et al.</i> , 1998
	Malaria (B)	113 <i>P. falciparum</i> malaria children from coastal Ghana and 42 controls	Hp1-1 phenotype associated with susceptibility to <i>P. falciparum</i> malaria in general, and to the development of severe disease in particular	Quaye <i>et al.</i> , 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 <i>et al.</i> , 2003
	Malaria	119 Indigenous pregnant African women from western Cameroon	Hp1-1 phenotype may play a role in susceptibility to placental infection by <i>P. falciparum</i> 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 <i>et al.</i> , 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 <i>et al.</i> , 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 <i>et al.</i> , 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 <i>et al.</i> , 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 <i>et al.</i> , 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 south-eastern 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 <i>et al.</i> , 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 been 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 (Dobryszczyka 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 (Germetis *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 meningitidis*,

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 con-

trast, 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.

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