LETTER TO JMG

Haptoglobin and its association with the HELLP syndrome

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Haptoglobin (Hp) is an acute phase α_2-sialoglycoprotein, which is characterised by molecular heterogeneity.[1] Owing to a genetic polymorphism, different Hp phenotypes exist of which Hp1-1, Hp1-2, and Hp2-2 are the three major isoforms in humans. Hp consists of two different polypeptide chains, the heavy β chain, which is identical in all haptoglobins, and the light α chain, consisting of two α^1 chains and a α^2 chain, modifications of which result in the different Hp phenotypes.[2]

The most important function of Hp is capturing haemoglobin, thereby preventing iron loss and subsequent oxidative damage generated by free iron in the vascular system of the kidneys. Binding of haemoglobin to Hp is beneficial for the human body in several other ways. Hp is protective against cell damage by scavenging free radicals, such as the hydroxyl radical, the formation of which is promoted by the presence of free haemoglobin. Furthermore, the Hp-haemoglobin complex inhibits the vasodilatory effect of nitric oxide and provides a non-specific defence against bacterial invasion, since free haem iron is necessary for bacterial growth. Furthermore, Hp itself was identified as a serum angiogenic factor and plays a role in proliferation and differentiation of vascular endothelium. Hp2-2 has stronger angiogenic functionality than Hp1-1, whereas Hp1-1 has the highest affinity for haemoglobin and is therefore associated with the antioxidant capacity of Hp.[1]

Pre-eclampsia, which is characterised by pregnancy induced hypertension and concurrent proteinuria, can be complicated by the haemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome, which may also occur alone.[3] The pathogenesis of pre-eclampsia and HELLP is largely unknown, although it is postulated that maladaptation of trophoblast invasion may result in poor placental perfusion and local oxidative stress,[4] which could subsequently affect maternal circulation. Systemic maternal oxidative stress may result in the clinical manifestations seen in women with pre-eclampsia, including dysfunction of the vascular endothelium.[5]

A previous study associated a higher incidence of the Hp2-2 phenotype with the occurrence of pregnancy induced hypertension.[6] Since Hp2-2 has poor affinity for haemoglobin and may therefore be less capable of preventing oxidative damage induced by free haemoglobin present after haemolysis, we hypothesised that occurrence of the *Hp2-2* genotype may be associated with the HELLP syndrome. Therefore, we investigated the prevalence of the *Hp2-2* genotype in patients with a history of severe pre-eclampsia with or without HELLP syndrome as compared to women with uncomplicated pregnancies only.

Key points

- Haptoglobin (Hp) consists of three phenotypes, Hp1-1, Hp1-2, and Hp2-2, and is protective against oxidative damage by its capacity to bind free haemoglobin for which Hp2-2 has the lowest affinity.
- In the pathogenesis of pre-eclampsia and the haemolysis, elevated liver enzymes, low platelets (HELLP) syndrome, oxidative stress may play an important role, which might be exaggerated by haemolysis.
- We conclude that presence of the *Hp2-2* genotype is not a risk factor for the development of either pre-eclampsia or the HELLP syndrome.

MATERIALS AND METHODS

The Institutional Review Board approved the study protocol. After informed consent was obtained whole blood was collected from 109 women, who had experienced severe pre-eclampsia (n=25) or pre-eclampsia with the HELLP syndrome (n=84).[7] Characteristics of the total patient group and both subgroups are shown in table 1. Pre-eclampsia was defined as a diastolic blood pressure >90 mm Hg on two or more occasions each more than four hours apart, with proteinuria (protein/creatinine ratio >0.30 g/10 mmol) according to the standard of the International Society for the Study of Hypertension in Pregnancy. HELLP was defined as haemolysis (lactic dehydrogenase level >600 IU/l), raised liver enzymes (both aspartate and alanine aminotransferase activity >70 IU/l), and low platelets (platelet count $<100 \times 10^9$/l). As a control group 166 women, who experienced uncomplicated pregnancies only, were recruited.

DNA was isolated using the Puregene genomic DNA isolation kit (Gentra Systems, Minneapolis, USA), according to the instructions of the manufacturer. Genomic DNA was analysed for the presence of the three different main genotypes encoding for haptoglobin using the polymerase chain reaction with primer sets and conditions exactly as described before by Yano *et al*.[2] In short, to detect the two different Hp1 and Hp2 alleles three different PCR reactions were performed using allele specific primer sets 5′-GCA ATG ATG TCA CGG ATA TC-3′, 5′-TTA TCC ACT GCT TCT CAT TG-3′ and 5′-CAG GAG TAT ACA CCT TAA ATG-3′, 5′-AAT TTA AAA TTG GCA TTT CGC C-3′ for Hp1 and primer set 5′-CAG GAG TAT ACA CCT TAA ATG-3′, 5′-TTA CAC TGG TAG CGA ACC GA-3′ for Hp2. Hp2 and Hp1 alleles of each DNA sample were amplified simultaneously in different reaction tubes resulting in fragments of 1200 bp, 1400 bp, and 935 bp, respectively. The PCR products of each DNA sample were put onto an agarose gel in three consecutive lanes and with ethidium bromide after electrophoresis. Since the DNA of each subject was simultaneously tested for the presence of the three alleles and 31 subjects were analysed at the same time, this provides an internal quality control. However, when a subject was homozygous or failed to produce a DNA fragment in all three reactions, the genotype analysis was repeated.

Odds ratios (OR) with 95% confidence interval (95% CI) were calculated for the *Hp2-2* genotype versus the other genotypes.

RESULTS

The distribution of the different Hp genotypes is summarised in table 2. In one woman who had pre-eclampsia with the

Table 1 Characteristics of women with severe pre-eclampsia and the subset of women with HELLP syndrome

	Total (n=109)	Pre-eclampsia (n=25)	HELLP syndrome (n=84)
GA at delivery (weeks+days)	31+3 (29+3–35+3)	34+0 (31+1–35+6)	30+5 (28+5–33+4)
Nulliparous, No (%)	84 (77%)	17 (69%)	67 (80%)
Systolic BP (mm Hg)	160 (150–175)	160 (150–170)	160 (150–180)
Diastolic BP (mm Hg)	110 (100–115)	110 (100–115)	110 (100–115)
Protein/creatinine ratio (g/10 mmol)	3.5 (0.4–6.0)	6.0 (3.0–7.9)	1.4 (0.3–4.6)
LDH (IU/l)	735 (506–1295)	373 (290–593)	845 (620–1658)
ASAT (IU/l)	143 (55–320)	29 (19–52)	201 (102–390)
ALAT (IU/l)	140 (51–270)	32 (16–51)	193 (88–354)
Platelet count (*10⁹/l)	78 (52–142)	157 (117–184)	65 (50–95)
Haemoglobin (g/dl)	7.6 (6.8–8.0)	8.0 (7.6–8.9)	7.4 (6.7–7.9)
Haematocrit	0.35 (0.32–0.38)	0.38 (0.36–0.42)	0.35 (0.31–0.37)
Serum creatinine (μmol/l)	75 (68–83)	77 (71–85)	74 (68–82)
Serum uric acid (μmol/l)	0.40 (0.34–0.46)	0.41 (0.37–0.45)	0.41 (0.34–0.46)

Data are given as medians (25th–75th centiles).
GA, gestational age; BP, blood pressure; LDH, lactic dehydrogenase; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase.

Table 2 Distribution of haptoglobin genotypes

Genotype	Total (n=109)	Pre-eclampsia (n=25)	PE + HELLP (n=84)	Controls (n=166)
Hp2-2	30 (28%)	6 (24%)	24 (29%)	58 (35%)
Hp2-1	53 (49%)	12 (48%)	41 (49%)	73 (44%)
Hp1-1	25 (23%)	7 (28%)	18 (21%)	35 (21%)
Hp0-0	1 (1%)		1 (1%)	
Frequency of *Hp2* allele	0.53	0.48	0.55	0.57

Percentages are given in parentheses.
Genotypes: *Hp1-1*: both alleles encoding for α^1. *Hp1-2*: one allele encoding for α^1 and the other for α^2. *Hp2-2*: both alleles encoding for α^2. *Hp0-0*: no amplification of one of the alleles.

HELLP syndrome, none of the specific Hp alleles could be amplified and this patient therefore most probably bears the rare Hp null genotype (*Hp0-0*).[1] The prevalence of the *Hp2-2* genotype in women with a history of severe pre-eclampsia was no different from that of control women (28% versus 35%) with an odds ratio (95% CI) of 0.7 (0.4 to 1.2). When subsets of women with a history of pre-eclampsia or a history of HELLP syndrome with pre-eclampsia were analysed separately, similar prevalences of the *Hp2-2* genotype (24% and 29%, respectively) were found as compared to controls, resulting in odds ratios (95% CI) of 0.6 (0.2 to 1.6) and 0.7 (0.4 to 1.3), respectively.

The Hp2 allele frequency was 0.57 in the control women. Women with a history of severe pre-eclampsia had a similar allele frequency (0.53), whereas subsets of women with pre-eclampsia and those with pre-eclampsia and the HELLP syndrome both showed a similar Hp2 allele frequency (0.48 and 0.55, respectively).

DISCUSSION

The pathogenesis of pre-eclampsia and cardiovascular related diseases, for example, essential hypertension and coronary artery disease, share common characteristics including thrombophilia, endothelial damage, and oxidative stress.[5 8–11] Furthermore, follow up studies show that women who had pre-eclampsia during their pregnancy are at higher risk of developing vascular related diseases.[12 13] Higher incidence of the Hp2-2 phenotype was associated with an increased risk for pregnancy induced hypertension[6] and severity of cardiovascular diseases like established essential hypertension[14] or coronary artery disease.[15] Therefore, we hypothesised that a higher incidence of the *Hp2-2* genotype could be present in women with severe pre-eclampsia as compared to control women. However, we could not find a higher incidence of the *Hp2*-2 genotype in women with severe pre-eclampsia as compared to control women, whereas a similar prevalence was found in a subset of women with pre-eclampsia and the HELLP syndrome. Control women showed a similar *Hp2* allele frequency to that reported in other control populations by other investigators.[1 14]

Reasons for the discrepancy between the results of Chandra *et al*[6] and our results may be that different racial groups were examined and that different methods were used for analysis, being phenotyping by Chandra *et al*[6] versus genotyping in our study. In addition, pre-eclampsia and HELLP syndrome are multifactorial diseases in which placental development and maternal defence against oxidative stress among many other factors may play an important role.[3 9 16] Hp1-1 and Hp2-2 phenotypes have different efficiencies for the various functional properties of Hp[1]; therefore, each phenotype may be protective at another stage of the disease. Hp has angiogenetic properties, with Hp2-2 being the most potent polymorphic variant; consequently Hp2-2 may be most beneficial for placental development. However, during the clinical stage of pre-eclampsia the antioxidant properties of Hp, that is, binding of free haemoglobin and direct scavenging of free radicals, may be more important. Since Hp1-1 has the highest affinity to bind haemoglobin, it may provide the best protection against oxidative stress.[1]

In conclusion, we did not find evidence that the *Hp2-2* genotype is associated with an increased risk for the development of either pre-eclampsia or the HELLP syndrome.

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