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EDITORIAL COMMENT

Haptoglobin, the Good and the Bad

Is it Evidence Based?*

Robert Roberts, MD

Ottawa, Ontario, Canada

Haptoglobin (Hp) is a molecule that circulates in the blood, to which most of us, particularly cardiologists, pay very little attention. Nevertheless, it has been the focus of considerable controversy in past decades as to whether it contributes to coronary artery disease (CAD) and, in particular, the formation of coronary atherosclerosis (1,2).

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Hp, synthesized in the liver, is a sialoglycoprotein found in the alpha-1 globulin fraction of serum with hemoglobin (Hb)-binding capacity, and its main physiological function is to dispose of free-Hb released from the red blood cells into the plasma. Hp is a positive acute-phase protein induced by cytokines such as interleukin-6. Hp forms a strong, noncovalent 1:1 complex with Hb, which is disposed of in the liver (3). Hb is consistently being released from red blood cells, as they reach the end of their lifespan, which averages 90 days. There is adequate Hp in the blood to bind 3 g of Hb, which under normal circumstances prevents free-Hb from circulating in the blood. Hp evolved as a very important protective mechanism to prevent the deleterious effects associated with circulating free-Hb (2).

Free-Hb stimulates lipid peroxidation (3) and is associated with immune and inflammatory responses, which can lead to significant tissue injury (2). Free-Hb, as occurs in rhabdomyolysis, can be associated with renal damage because it flows through the glomeruli. This is prevented by the complex of Hp–Hb because it is too large to be filtered in the kidney and instead is transported to the liver for appropriate disposal.

The Hp gene is located on chromosome 16, on the long (q) arm, at band 22.2 (16q22.2), and the gene has 2 forms (alleles) referred to as Hp1 and Hp2. These alleles give rise to 3 phenotypes: individuals homozygous for Hp1 (Hp1-1); heterozygous for Hp (Hp2-1); or homozygous for Hp2 (Hp2-2). The protein consists of 2 chains, α and β, with the β chain having a much higher molecular weight than the α chain. The gene (Hp1) that encodes for the β chain is identical in all species, as is the protein. The Hp2 allele, which is specific for humans, encodes for an α chain of high molecular weight and has ~1,700 additional base pairs (2). The Hp protein encoded by the Hp2 allele binds less Hb than that of Hp1 and is less efficient in both preventing oxidation and in promoting the uptake of the Hp–Hb complex by the CD163 receptor on monocytes (4,5). The frequency of these alleles in the white population is estimated at 15% for Hp1-1, 50% for Hp2-1, and 35% for Hp2-2.

The relationship between Hp and coronary heart disease (CHD) is believed to be mediated through atherosclerosis. There are several mechanisms through which Hp may influence atherosclerosis; a decrease in Hp binding to Hb can lead to increased lipid peroxidation with immune and inflammatory responses. The Hp–Hb complex binds to a receptor (CD163) on monocytes and macrophages (2), culprits involved in the pathogenesis of atherosclerosis. Lastly, Hp is also known to form a complex with apolipoprotein L, which alters low-density lipoprotein cholesterol (6).

Studies relating the 2 polymorphisms of Hp to heart disease have been confusing. One of the first studies relating Hp to heart disease was performed in 1984 in Belgium (1). It involved 11,302 subjects and concluded that individuals homozygous for the Hp1 genotype (Hp1-1) had twice the mortality rates from CAD over that of other alleles. In contrast, subsequent findings by Chapelle et al. (7) claimed the Hp2 genotype was associated with increased risk for CAD. In 2004, the Framingham study examined Hp polymorphisms and CHD in a total of 3,283 individuals, including 443 diabetic subjects and 2,840 nondiabetic subjects (8). Overall, they found no significant cardiac risk between the different Hp polymorphisms in the combined population of diabetic and nondiabetic subjects. However, subgroup analysis found a higher frequency of the Hp1-1 genotype among diabetic individuals with CHD. Among the nondiabetic participants, the Hp2 genotype was associated with increased prevalence of CHD, independent of conventional risk factors. A study by Holme et al. (9) found that low plasma levels of Hp were associated with increased risk for myocardial infarction. However, despite a large population of 342,125 subjects, of whom nearly 6,500 were diabetic, there was no relationship between Hp and diabetes.

There is overwhelming data from a variety of studies to indicate that 40% to 60% of susceptibility to CAD is genetic (10). Utilization of the high-throughput, single-nucleotide polymorphism array introduced in 2005 enabled performance of unbiased genome-wide association studies

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From the University of Ottawa Heart Institute, Ottawa Heart Institute Research Corporation, and the Ruddy Canadian Cardiovascular Genetics Centre, Ottawa, Ontario, Canada. Dr. Roberts is a consultant to Cumberland Pharmaceuticals and has received grant support as follows: Canadian Institutes of Health Research #MOP82810; Canada Foundation for Innovation #11966.

(GWAS) in pursuit of genes associated with complex diseases such as CAD. Before this time, there was more than a decade of studies using the candidate gene approach, which claimed that >100 genes were associated with increased risk for CAD. In subsequent GWAS studies, none of these genes was confirmed to have an association with increased risk for CAD (11,12). The candidate gene approach has thus fallen out of favor, and a new group of disease-associated genes have been discovered by GWAS, with confirmation in large independent populations. GWAS has discovered >1,600 genes associated with increased risk for >160 diseases. Among these, 46 genes were found to be associated with increased risk for CAD (13). Despite these discoveries, these genes do not account for most of the expected heritability of CAD or other diseases (14,15).

The study by Cahill et al. (16), in this issue of the *Journal*, has some very intriguing findings that could transform the treatment of diabetes. Their overall study conclusion was that individuals having the Hp2-2 genotype and glycosylated hemoglobin (HbA_{1C}) ≥6.5% have a 10-fold increased risk of CHD compared with individuals with at least 1 Hp1 allele and HbA_{1C} <6.5%. This relative risk ratio makes Hp2-2 a risk factor comparable to cholesterol in individuals with HbA_{1C} ≥6.5%. Patients with the Hp2-2 genotype and HbA_{1C} <6.5% exhibited no increased risk for CHD. Diabetic subjects treated with metformin to HbA_{1C} <6.5% with the Hp2-2 genotype also exhibited much less risk for CHD. A major implication was that good control of diabetes in individuals with the Hp2-2 genotype would decrease their risk for ischemic heart disease.

Where have these data associating Hp2 and diabetes been until now and why now? These findings are startling and require a careful analysis of the methods used. The overall approach adopted by Cahill et al. (16) is the candidate gene approach followed by replication in an independent population with subsequent meta-analysis. The initial population consisted of 407 individuals with myocardial infarction (n = 343) or sudden cardiac death (n = 64) selected from the much larger Nurses' Health Study (NHS) and matched 1:1 with asymptomatic healthy controls, who were also selected from the NHS. In the NHS cases, there were 25 individuals with the Hp2-2 genotype and HbA_{1C} ≥6.5% compared with only 2 individuals in the control group with the Hp2-2 genotype and HbA_{1C} ≥6.5%. This indicates a 10-fold increased relative risk of ischemic heart disease in individuals with the Hp2-2 genotype and HbA_{1C} ≥6.5%. However, the sample size was too small to provide a definitive conclusion. The second population selected for replication had a sample size of 2,070 subjects, all of whom had diabetes but only 29 cardiac events during follow-up. This is unusual, because one normally selects an independent population similar to the original discovery population. In this second population, there were 18 cardiac events in 452 individuals with Hp2-2 and HbA_{1C} ≥6.5% versus only 5 cardiac events in 999 individuals with the Hp1-1 or Hp1-2 genotype and with HbA_{1C} ≥6.5%. This finding indicated a 7-fold increased relative risk of cardiac events in those with the Hp2-2 genotype and HbA_{1C} ≥6.5%. A meta-analysis was performed of these 2 studies, including an additional sample of 412 individuals (206 cases; 206 controls). The meta-analysis showed individuals with Hp2-2 and HbA_{1C} levels ≥6.5% also exhibited a 7-fold increase in cardiac events.

The increased cardiac risk of the Hp2 genotype has not been observed in any of the GWAS for either CAD or diabetes. An international consortium, CARDIoGRAM, performed a meta-analysis (14) of several GWAS to discover genetic risk variants for CAD (discovery population of 87,095 and a replication sample of 56,682). There are several single-nucleotide polymorphisms on the array from the Hp region, yet none was shown to be associated with CAD. Practically all of these GWAS included subjects with diabetes (12). The recent CARDIoGRAMplusC4D, which had a total population of 194,427, also did not observe any association of Hp with CAD or diabetes (13). It is noteworthy that in none of these international studies were the diabetic subjects stratified into those with HbA_{1C} <6.5% and those with HbA_{1C} ≥6.5%. It is reasonable to assume many of the diabetic subjects would have HbA_{1C} ≥6.5%. These results of Cahill et al. (16) are very promising for the treatment of diabetes but also provocative. The nested case-control candidate gene approach with such few cases (Hp2 genotype and HbA_{1C} ≥6.5%) beckons for confirmatory studies before considering any recommendations to alter diabetic management.

Reprint requests and correspondence: Dr. Robert Roberts, University of Ottawa Heart Institute, 40 Ruskin Street, Ottawa, Ontario K1Y 4W7, Canada. E-mail: rroberts@ottawaheart.ca.

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