Editorial

Haptoglobin genotype

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Kantor and colleagues study haptoglobin isoforms in 193 patients with subarachnoid hemorrhage (SAH).\(^8\) Patients with the α2-α2 isoform had worse outcome on the modified Rankin Scale (mRS) at 3 months. These findings add to the existing literature showing that this haptoglobin isoform is associated with poor outcome after SAH.\(^2\) Data are not uniform, however, with some studies showing no association of haptoglobin phenotype with outcome.\(^13\) A recent study by Kantor and colleagues\(^1\) found that α2 haptoglobin isoform was associated with poor outcome in a cohort of patients with SAH. These findings are consistent with the known ability of haptoglobin to bind free extracellular hemoglobin and reduce its ability to generate oxygen free radicals and with the hypothesis that angiographic vasospasm is in part due to hemoglobin-mediated oxidative stress.

The biochemistry of haptoglobin is complex. It is a glycoprotein that is a tetramer consisting of 2 α and 2 β chains, synthesized mostly in the liver.\(^6,7\) Since it is an acute-phase protein, haptoglobin increases in plasma during infections, burns, trauma, and probably other major stressors. One function of haptoglobin is to bind free extracellular hemoglobin. This complex binds CD163 on tissue macrophages and circulating monocytes and is thereby taken up into these cells. The gene is on chromosome 16q22 and there are 2 haptoglobin α chain alleles that can combine to form 3 types of haptoglobin: α1-α1, α1-α2, and α2-α2. Humans are the only species identified so far with these 2 alleles; other animals have only the α1 form. The α2 allele arose during evolution by partial duplication (of exons 3 and 4) of α1. Because of the duplication, the molecular weights of the chains vary (9 kDa for α1, 16 kDa for α2, and 45 kDa for the common β chain). The duplication contains a multimerization sequence that leads to further complexity in haptoglobin structure. People homozygous for the α1 allele can form only α1 dimers, whereas heterozygotes form linear polymers that have a variable number of internal α2 chains, with an α1 chain at either end. If someone is α2 homozygous, he/she forms cyclic polymers of varying numbers of α2 chains.

It is sometimes written that the α1 protein binds to hemoglobin more avidly than the α2 protein, but according to Goldenstein et al., the binding affinity is the same for the α alleles.\(^7\) The difference is that the α1 protein more potently inhibits the heme-catalyzed oxidative reactions of hemoglobin. Carriers of the α2 allele have been suggested to be at increased risk of some conditions such as diabetic vascular disease. If that is true, then why would the α2 allele persist? The reason that the α2 allele has persisted may be because it protects against some infectious diseases such as streptococcal infections. In southeast Asia, where such infections are prevalent, 90% of people are α2-α2 whereas in most western countries this rate is only 36%.

There is normally very little haptoglobin in CSF, which has 50,000 times less hemoglobin-binding capacity than the systemic blood circulation.\(^6\) After SAH, there is an increase in systemic haptoglobin concentration, but CSF concentrations actually decrease slightly and the hemoglobin binding capacity of the CSF is still far lower than the amount of free hemoglobin.

Kantor and colleagues speculate that the reason patients with α2 haptoglobin do worse is related to reduced CSF clearance of hemoglobin, increased reactive oxygen species, and more inflammation. This is based on experimental evidence from mice expressing human α2-α2 haptoglobin.\(^3\) The experimental studies showed specifically that mice with human α2-α2 haptoglobin had more large-artery vasospasm and CSF white blood cells after SAH. Galea et al., found evidence for impaired hemoglobin efflux from CSF in patients with α2-α2 haptoglobin.\(^6\) The role of reactive oxygen species is less certain. The current study does not report data on angiographic vasospasm or delayed cerebral ischemia, but worse an-
giographic vasospasm was reported in individuals with the α2-α2 phenotype in a study of 95 patients with SAH. In the latter study, however, haptoglobin genotype was not associated with delayed cerebral ischemia or outcome.

The pathogenesis of brain injury after SAH is multifactorial. Direct destruction of the brain from intracerebral bleeding, herniations, systemic hypoxic ischemic insults, and such aside, the initial phase or early brain injury from SAH is hypothesized to be due to a combination of transient global cerebral ischemia and the effects of subarachnoid blood. Delayed cerebral ischemia secondary to angiographic vasospasm with or without microthromboembolism and cortical spreading ischemia can compound the injury days later. But other factors must contribute to the response to SAH. Evidence for this includes the following. One analysis of prognostic factors for outcome found that poor outcome at 3 months was associated with factors such as age, initial neurological grade, aneurysm site and size, amount of blood shown on the admission CT scan, preexisting hypertension, myocardial infarction, liver disease, fever, and development of delayed cerebral ischemia. These factors explained only about 36% of the variation in outcome. Lo and colleagues analyzed the same data set using Bayesian neural network modeling with fuzzy logic inferences and found evidence of additional variables contributing to outcome.

What accounts for the rest of the variation in outcome? Genetic variations affect human response to disease and, as suggested by the current findings, likely influence outcome after SAH. There is other evidence as well. Some genetic factors that affect the brain response to injury include apolipoprotein E genotype. Meta-analysis of multiple studies in SAH found that patients with the epsilon 4 allele had worse outcome after SAH than those with other genotypes. The authors of the current report also found, in the same set of patients, that endothelial nitric oxide synthase polymorphisms affect outcome. Data on the effect of this polymorphism on angiographic vasospasm, delayed cerebral ischemia, and outcome after SAH are conflicting. It would be important for the authors to analyze their data, including both endothelial nitric oxide synthase polymorphism and haptoglobin genotype, in a multivariate analysis. It is likely that more genetic variations that affect angiographic vasospasm and outcome after SAH will be discovered.

There are a few limitations to this study, one being that some more standardized scales like the World Federation of Neurosurgical Societies clinical grading scale and the modified Fisher Scale are not used. Much of the data were collected retrospectively, even though these patients were part of a prospective descriptive study. Even though mRS scores were collected prospectively, for some reason mortality had to be abstracted from medical records. The variables entered into the multivariate analysis are not fully described. There were some imbalances between the haptoglobin groups that could account for some of the effect, such as the fact that the α2-α2 patients were older and had more SAH documented on CT scans. Both of these are adverse prognostic factors for outcome. The detrimental effect of the α2 isoform persisted, however, after adjustment for these factors in multivariate analysis. Thus, it remains likely that the α2 genotype is associated with worse outcome, although the findings are not perfect given this and the relatively small sample size.

The authors did not include angiographic vasospasm, although they should have the data. I also wondered why they reported outcome at 3 months in this study and 6 months in the first one. Another curious finding was that there was no relationship between the Glasgow Outcome Scale (GOS) score and haptoglobin genotype. Usually in studies of SAH, there is a good correlation between the GOS score and mRS scores. It would be interesting to see graphs of GOS scores versus mRS scores as well as mRS scores versus haptoglobin genotype. It was not clear at what cut point the mRS score was dichotomized. The suggestion that the mRS score correlated with genotype but that the GOS score did not because the former has more levels did not make sense to me since the mRS score was analyzed as a dichotomous variable.

Overall I compliment the authors on conducting the largest analysis of its kind so far and I concur with their conclusion that more studies assessing the effect of genetics on outcome after SAH hopefully will increase our understanding of the disease.

(http://thejns.org/doi/abs/10.3171/2013.7.JNS131178)

Disclosure

Dr. Macdonald receives grant support from the Physicians Services Incorporated Foundation, Brain Aneurysm Foundation, Canadian Institutes for Health Research, and the Heart and Stroke Foundation of Canada; he is Chief Scientific Officer of Edge Therapeutics, Inc.

References