

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.⑩ 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.

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 are several explanations for the current study does not report data on angiographic vasospasm or delayed cerebral ischemia, but worse an-

See the corresponding article in this issue, pp 386–390.

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.\textsuperscript{10} Delayed cerebral ischemia secondary to angiographic vasospasm with or without microthromboembolism and cortical spreading ischemia can compound the injury days later.\textsuperscript{15} 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.\textsuperscript{14} 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.\textsuperscript{10}

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.\textsuperscript{9} 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.\textsuperscript{9} The authors of the current report also found, in the same set of patients, that endothelial nitric oxide synthase polymorphisms affect outcome.\textsuperscript{1} Data on the effect of this polymorphism on angiographic vasospasm, delayed cerebral ischemia, and outcome after SAH are conflicting.\textsuperscript{1} 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.\textsuperscript{4,5} Much of the data were collected retrospectively, even though these patients were part of a prospective descriptive study.\textsuperscript{1} 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.\textsuperscript{1} 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.\textsuperscript{11} 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 was 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.

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### Disclosure

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### References


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Response

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We thank Dr. Macdonald for his extensive summary of the role of haptoglobin and his very thoughtful comments regarding our work. We appreciate and agree with his view that this is an important database due to its size. In addition, we agree that the information should lead to more investigation into the role of haptoglobin in the pathology of SAH. The role of multiple gene interactions contributing to damage in SAH is of great interest to us, and our current database permits more extensive analysis of multiple gene interactions in future work. The work reported in this paper explored single gene effects of the HG gene and supports further studies of this gene in multiple gene interaction studies. Dr. Macdonald voiced a few very specific issues with this study that we would like to address.

Our current data set includes individuals enrolled since 1999. At that time, the study design included collection of enrollment Fisher grade and Hunt and Hess grade for all patients. Our work is limited by this fact. Data and samples were collected prospectively. DNA collected from these individuals, in association with the prospectively collected data, has allowed ongoing genetic association studies with a relatively large sample size. We included the full sample of individuals for whom DNA material was available and used the 3-month outcome time point to maximize our sample size. Certainly studies with data extending beyond 3 months will more fully answer the question regarding the association between haptoglobin and recovery from SAH.

The mRS and GOS include death as a single classification. Our data collection allows us to extract this factor from either score, but for individuals who die during the very acute period (that is, within the first few weeks after hemorrhage and in the hospital setting but before the first outcome visit) we must resort to medical record review to identify them.

We also found it interesting that subjects with the a2-a2 genotype were older and had more subarachnoid blood. It may be that this genetic variant may have other effects that lead to a later onset of aneurysm rupture and more blood. While these are prognostic factors after SAH, we found that genotype remained a significant predictor even when controlling for these variables, as well as sex and Hunt and Hess grade, in our multivariate analyses. We believe this to be a strength of our findings as it shows additional contributions of genetic variance to the recovery process.

We purposefully used the 3-month time frame and did not consider angiographic vasospasm to maximize our sample size. As with most longitudinal studies, subjects are lost to follow-up as time goes on, and the 3-month time frame enabled us to utilize the largest sample for our analysis. We had similar issues with angiographic vasospasm in that the available data only included angiographic vasospasm data for 50% of the population. While it would be of great interest to further explore the role of haptoglobin, and haptoglobin genotype, after SAH with more acute measures of poor response, the loss of power guided us to remain focused on 3-month recovery period in this sample. Additional analyses by our group and others would serve the SAH population well, examining haptoglobin genotype and vasospasm, delayed cerebral ischemia, and other acute complications, as well as outcome assessments farther out after SAH.

We agree that the relationship between GOS and mRS scores in our sample is a curious finding. A graph of these data (Fig. 1) provides some visual description of our data. The different thresholds lead to individuals being categorized slightly differently by these two scales. We believe that this small difference may be enough to impact statistical significance in this particular sample even when the sample was dichotomized into “good” (mRS scores of 0–2) and “poor” (mRS scores of 3–6) outcomes.

A graph of the percentage of individuals in each haptoglobin genotype group by mRS score shows that most of those with the a1-a1 genotype had mRS scores of 0–2, and hence outcome fell into the good category (Fig. 2).

We believe our paper contributes to the growing body of literature showing that haptoglobin plays a role in response to SAH and that there are genotypic differences in

8. Kassell NF, Mohr JP, Kempf DE, et al: The importance of the role of haptoglobin and his very thoughtful comments regarding our work. We appreciate and agree with his view that this is an important database due to its size. In addition, we agree that the information should lead to more investigation into the role of haptoglobin in the pathology of SAH. The role of multiple gene interactions contributing to damage in SAH is of great interest to us, and our current database permits more extensive analysis of multiple gene interactions in future work. The work reported in this paper explored single gene effects of the HG gene and supports further studies of this gene in multiple gene interaction studies. Dr. Macdonald voiced a few very specific issues with this study that we would like to address.

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We believe our paper contributes to the growing body of literature showing that haptoglobin plays a role in response to SAH and that there are genotypic differences in
that response contributing to the variable outcome profile after SAH. It is likely, as pointed out by Dr. Macdonald, that the contributions of multiple genes impact multiple biological pathways that interact to produce varying recovery and variable outcomes after SAH. As we learn more about genetic/genomic science, studies such as this will inform a body of work aimed at identifying a genetic/genomic signature that can be modified to improve outcomes for individual patients recovering from SAH.

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