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Implications of Haptoglobin Genotype and Cardiovascular Risk in Patients with Diabetes

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Abstract

Haptoglobin (HAP) is genetically polymorphic with three primary genotypes, HAP 1-1, 2-1 and 2-2. Each genotype differs phenotypically in HAP structure and ability to perform its main function, scavenging free hemoglobin (Hb) released from old red blood cells. Patients with both diabetes and the genotype 2-2 appear to be at an increased cardiovascular risk than those with the other genotypes or patients without diabetes. This risk appears elevated with worse glycemic control. The exact mechanism for this increased risk is unknown but there are several proposed causes. Vitamin E has shown to reduce cardiovascular events in patients with both diabetes and the 2-2 genotype but the safety of implementing such therapy remains unknown. Recent post-hoc evaluation of a landmark study originally designed to assess the benefits and risks of more aggressive glycemic control suggests there may be a cardiovascular benefit in patients with diabetes and the 2-2 genotype that is not seen in those with the other genotypes. This information, if confirmed with post-hoc evaluation of other similar landmark studies as well as evaluation of genotype differences in recent cardiovascular safety studies with glucagon-like peptide agonists or sodium-glucose cotransporter inhibitors, could provide clinicians with an avenue to better identify patients most at risk for cardiovascular events and who may benefit the most from more aggressive glycemic control or use of other antihyperglycemic agents.

Keywords: type 2 diabetes mellitus, cardiovascular disease, haptoglobin, genotype, phenotype

Abbreviations: HAP: haptoglobin; Hb: hemoglobin; MI: myocardial infarction; HDL: high-density lipoprotein; LCAT: lecithin-cholesterol acyltransferase; HOPE: heart outcomes prevention evaluation; WHS: women's health

study; ICARE: Israel cardiovascular events reduction with vitamin E; ACCORD: action to control cardiovascular risk in diabetes; VADT: veterans affairs diabetes trial

Introduction

Consensus recommendations for reducing cardiovascular risk in patients with diabetes have long called for control of more traditional cardiovascular risk factors such as blood pressure and cholesterol, smoking cessation, and antiplatelet therapy in appropriate patients [1]. The use of antihyperglycemic agents shown to reduce cardiovascular morbidity and/or mortality are now also recommended in those with known cardiovascular disease or at high risk for such. Despite efforts to reduce cardiovascular risk and outcomes, residual risk remains and there persists a need to assess other potential strategies that may limit these outcomes. One that merits further investigation includes testing for genetic susceptibility of cardiovascular risk [2].

Haptoglobin (HAP), a protein produced primarily by the liver, facilitates the clearance of hemoglobin (Hb) and the heme iron it carries [3]. HAP is thought to provide protection through sequestration of free Hb and minimizing heme iron release from Hb. This then prevents oxidative tissue and endothelial damage, decreases iron deposition in renal proximal tubule cells, and reduces Hb scavenging of nitric oxide [2]. The HAP-Hb complex is removed from systemic circulation by macrophages *via* their CD163 receptor. HAP is genetically polymorphic with two primary alleles, HAP 1 and HAP 2. Differences in functionality exist between these two alleles which are then subclassified into three major genotypes depending on if a patient is homozygous for the HAP 1 allele (HAP 1-1) or homozygous for the HAP 2 allele (HAP 2-2) or heterozygous (HAP 2-1) [4]. The different genotypes differ phenotypically. The HAP 1-1 genotype forms a protein dimer, HAP 2-1 a linear polymer, and HAP 2-2 a cyclic polymer with the HAP 1-1 protein being considered to have greater anti-inflammatory and antioxidant properties [5]. Between each genotype, differences in-vitro\in-vivo effects of antioxidant, scavenging, and immunomodulatory properties have been seen [2, 4]. No differences have been found between the HAP 1 and HAP 2 proteins in binding affinity to Hb; however, the HAP 1-1 protein can bind more Hb than the HAP 2-1 or HAP 2-2 types [2]. The smaller HAP 1-1 protein is also thought to be more effective at getting into areas where the HAP 2-2 protein may be restricted due to its larger size [2]. The HAP 1-1 protein is more effective in preventing the Hb oxidation and heme release from the HAP-Hb complex, is cleared twice as fast from circulation, and is more efficient in promoting the uptake of the HAP-Hb complex by the CD163 receptor [2, 6].

The prevalence of the three HAP genotypes varies throughout the world, with the highest prevalence of HAP 1 allele found in Africa and South America and the lowest in Southeast Asia [2, 4, 5]. Reviews have shown in most northwestern European countries the allocation of HAP genotypes to be ~16% for HAP 1-1, 48% for HAP 2-1, and 36% for HAP 2-2 [5]. Between patients with and without diabetes, no differences in frequency of the two HAP genotypes has been found [4].

Haptoglobin Genotype and Cardiovascular Event Risk

The association of HAP genotype and risk for cardiovascular events in patients with diabetes has been evaluated in several studies. A post-hoc assessment of HAP genotype was evaluated in the Strong Heart Study, a longitudinal study of cardiovascular disease in Native Americans [7]. Controlling for traditional cardiovascular risk factors, the investigators found a five-fold higher risk for a cardiovascular event in patients with diabetes (type not specified) and the HAP 2-2 genotype compared to patients with diabetes and HAP 1-1 genotype. HAP 2-2 genotype in patients without diabetes conferred no increased cardiovascular risk. In subjects with diabetes, the HAP 2-1 genotype did not show an increased risk for events compared to the HAP 1-1 genotype. In patients with diabetes and acute myocardial infarction (MI), another study found the HAP 1-1 genotype was associated with a lower risk for 30-day mortality and heart failure and appeared protective compared to patients with the Hap 2-2 genotype [8]. As in the previous study, the risk was not increased in subjects without diabetes regardless of genotype. In a multivariate analysis controlling for known cardiovascular risk factors, HAP 2-2 genotype in patients with diabetes (type not specified) was predictive

of MI one year after percutaneous coronary angioplasty [9]. The risk for increased cardiovascular events with the HAP 2-2 genotype has been found in patients with type 1 diabetes mellitus showing a two-fold higher risk for an event compared to subjects with the HAP 1-1 genotype [10]. This increased cardiovascular risk in subjects with the HAP 2-2 genotype appears to be affected by glycemic control. Two separate studies found the risk for a cardiovascular event was higher in individuals with the HAP 2-2 genotype and a hemoglobin A1c greater or equal to 6.5% suggesting the risk is higher with worse glycemic control [11, 12].

Proposed Mechanism of Increased Atherosclerosis with HAP Polymorphism

The demonstrated increased risk of atherosclerosis and cardiovascular disease in patients with diabetes and the HAP 2-2 genotype most likely arises secondary to endothelial dysfunction from a culmination of increased oxidative stress, reduced effectiveness of reverse cholesterol transport, and increased inflammation [2].

Diabetes increases the turnover rate and reduces the lifespan of red blood cells, which increases the release of free Hb, the iron content of which is a potent oxidant [13, 14] The increase in redox active iron in the diabetic state is further increased in patients with the HAP 2-2 genotype [15]. This is due to multiple issues; the overall reduced affinity for all genotypes for glycated Hb, reduced effective clearance of the HAP 2-Hb complex compared to the HAP 1-Hb complex *via* the CD163 scavenger receptor, and decreased ability of HAP 2-Hb to prevent oxidation of lipoproteins [6, 16].

HAP is a high-density lipoprotein (HDL) associated protein and has a binding site on apolipoprotein (apoA1); in individuals with the HAP 2-2 genotype the amount of Hb associated with HDL is increased along with redox active iron and lipid peroxides [17]. It has been proposed that the binding of the HAP 2-Hb complex could interfere with lecithin-cholesterol acyltransferase (LCAT) activity and this decrease in enzymatic activity has been demonstrated in people with diabetes and the HAP 2-2 genotype [18, 19]. This decrease in LCAT activity would impair the maturation of HDL and the ability to promote cholesterol efflux [18, 19]. In addition, the HAP 2-Hb-HDL complex may be proatherogenic and prothrombotic by limiting nitric oxide (NO) availability through sequestration that may then result in further endothelial dysfunction [20, 21].

The HAP 2-2 genotype may also impact inflammation. A proinflammatory state is potentiated with the HAP 2-2 genotype in patients with diabetes through increased activation of CD163 leading to a cascade of calcium mobilization, inositol triphosphate production, and inflammatory cytokine release [6]. Conversely, the HAP 1-1 genotypic complex results in increased interleukin 10 release, which is considered anti-inflammatory [22].

Vitamin E and Cardiovascular Outcomes with the HAP 2-2 Genotype

The use of vitamin E as an antioxidant to reduce cardiovascular risk fell out of favor 15–20 years ago after large, well designed clinical trials in a variety of patient populations failed to show a reduction in cardiovascular events and a subsequent meta-analysis found an increased mortality risk with its use [23, 24]. It has been suggested, however, in patients with diabetes who have the HAP 2-2 genotype, vitamin E supplementation may have an effect at reducing the dysfunctional effects on HDL associated with this genotype and as a result this may be a population where vitamin E could have a beneficial role [25]. In two previous clinical trials that failed to show a cardiovascular benefit of vitamin E in the general population evaluated, retrospective subgroup assessment focusing solely on those with both diabetes and the HAP 2-2 genotype showed conflicting results. HAP genotype was evaluated in 3,167 of the over 9400 subjects in the Heart Outcomes Prevention Evaluation (HOPE) trial assessing 400 IU daily of vitamin E over a 4.5-year period [26, 27]. In the subgroup with both the HAP 2-2 genotype and diabetes, vitamin E use was associated with a significant 30% relative risk reduction in the primary outcome (non-fatal MI, non-fatal stroke, or death from cardiovascular causes), a 55% decrease in cardiovascular death, and a 43% reduction in non-fatal MI. The subgroup analysis failed to show a risk reduction in stroke. A similar post-hoc analysis of the 277 diabetic HAP 2-2 genotype subjects in the Women's Health Study (WHS) failed to show a significant reduction in cardiovascular events with the use of vitamin

E (600 IU daily) over a 10-year timespan [28, 29]. However, this subgroup analysis was likely vastly underpowered to detect a significant cardiovascular benefit as there were very few patients with diabetes included in the study. The only prospective study designed to specifically evaluate vitamin E supplementation in patients with both diabetes and the HAP 2-2 genotype is the Israel Cardiovascular Events Reduction with Vitamin E (ICARE) trial [30]. This study evaluated 400 IU daily of vitamin E *vs.* placebo in 1434 patients, at least 55 years old, with the HAP 2-2 genotype and assessed the same primary outcome as the HOPE study. The study was stopped early after an 18-month interim analysis showed the primary outcome occurred in significantly fewer patients receiving vitamin E (2.2%) compared to placebo (4.7%), $p = 0.01$. The study also found a statistically significant reduction in MI (1.4%, $p = 0.04$) but did not show a significant reduction in stroke, cardiovascular death, or revascularization procedures. The study was too short and small to adequately assess safety in this population. A subsequent meta-analysis, assessing data pooled from HOPE, WHS, and ICARE, showed a 34% relative reduction in the composite outcome of cardiovascular death, non-fatal MI, or non-fatal stroke in those with diabetes and the HAP 2-2 genotype [31]. No effect of vitamin E was noted in patients with diabetes but with other HAP genotypes.

While the clinical trial data above is interesting, the limitations in size, whether prospective or retrospective in design, and length of study remain apparent. A long-term (3 or more years), larger, prospective clinical trial to evaluate vitamin E's role in cardiovascular risk reduction in subjects with diabetes and the HAP 2-2 genotype is needed to truly evaluate both efficacy and safety. It has been suggested that at least 6000 subjects would be necessary to power such a study. Given the high price burden of this type of investigation and the unlikeliness any pharmaceutical company would profit from such a low monetary value intervention, performing such a trial may be cost-prohibitive [25]. Until or if such a study is performed and confirms a true cardiovascular benefit with minimal risk in this population, the routine use of vitamin E cannot be supported.

Intensive Glycemic Control, Cardiovascular Outcomes, and the HAP Genotype

To date, studies specifically designed to prospectively assess the effects of more intensive glycemic control in type 2 diabetes on cardiovascular outcomes have shown conflicting results. The Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial showed no overall benefit in reducing the cardiovascular composite primary outcome (non-fatal stroke, non-fatal MI, or death from cardiovascular disease) with intensive glycemic control {target A1c < 6%} compared to less aggressive control (target A1c 7–7.9%). The study did show a 24% relative reduction in the risk for non-fatal MI [32]. However, a 22% relative increase in death from any cause was found in the more intensive control group compared to less aggressive control. Intensive glycemic control *vs.* more standard glycemic control in either the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (known as ADVANCE) trial or the Veterans Affairs Diabetes Trial (VADT) failed to show an effect, positive or negative, on cardiovascular outcomes or mortality [33, 34].

Recently a group of investigators reanalyzed the ACCORD trial data to determine if the effect of intensive *vs.* standard glucose lowering therapy and the occurrence of cardiovascular events could be explained by different HAP genotypes [35]. The original ACCORD trial included a total of 10,251 patients. The National Institutes of Health's Open Biologic Specimen and Data Repository Information Coordinating Center provided the ACCORD trial's data and frozen serum blood samples for HAP genotyping. The current investigation restricted their analysis to the largest ethnic group (non-Hispanic white subjects) within the trial due to possible confounding and heterogeneity in linkage disequilibrium patterns because of ancestral and/or racial differences. This led to a population of 5,806 patients for analysis. HAP genotyping was determined using an assay method differentiating the HAP types 1-1, 2-1, and 2-2. HAP 1-1 and 2-1 were combined to create a group of HAP 1 allele carriers. 37% of subjects had the HAP 2-2 genotype, 15% the HAP 1-1, and 48% had the HAP 2-1 genotype. The patients were grouped according to their glycemic treatment assignment and their HAP genotyping. Baseline patient characteristics showed 36% of subjects were female, with a mean age of 63 years and a diabetes duration of approximately 9 years. Overall, the incidence rate (per 10,000 patient years) of outcomes were lowest for non-fatal cardiovascular events (coronary heart disease, cardiovascular disease, or non-fatal MI) in the HAP 2-2 genotype/intensive therapy group. The HAP 1 genotype in both the intensive and standard therapy

groups had the next lowest incidences whereas the HAP 2-2 genotype/standard therapy group had the highest incidence rates. A 29% relative lower risk of coronary heart disease, a 29% lower risk for cardiovascular disease and a 36% lower risk of non-fatal MI was associated with allocation to intensive glycemic therapy and also having the HAP 2-2 genotype. There was a neutral effect on these non-fatal outcomes in subjects in the HAP 1 allele group. The investigators found no significant effect of glycemic control on fatal cardiovascular disease or total mortality in those with the HAP 2-2 genotype. Reciprocally, the HAP 1 allele intensive therapy group had the highest fatal cardiovascular disease and mortality rates. Intensive therapy was associated with a 50% higher risk of cardiovascular mortality and 40% higher risk of total mortality compared to standard therapy in the HAP 1 allele carriers. The post-hoc analysis of the ACCORD study based on HAP genotype and glycemic control suggest intensive glycemic control may prevent cardiovascular events in HAP 2-2 genotypes but has no effect on mortality in this group, while HAP 1 allele carriers showed no non-fatal cardiovascular benefit but an increased mortality risk.

Conclusion

The HAP 2-2 genotype appears to lead to a more phenotypically dysfunctional HAP complex than the other two genotypes, HAP 1-1, and HAP 2-1. There is a positive correlation between those patients with both diabetes and the HAP 2-2 genotype and cardiovascular events. A risk that appears to be worse with hyperglycemia while the polymorphism does not appear to affect the risk in those without diabetes. The exact physiological reason for the increased atherosclerotic risk with the HAP 2-2 genotype is not fully understood but likely multifactorial. While vitamin E shows some degree of cardiovascular risk reduction in those with both diabetes and HAP 2-2, the existing data are either of too short in duration or size to provide sufficient information to recommend its use given the possible increased mortality found in prior studies. Recent post-hoc assessment of the ACCORD study suggests aggressive glycemic control may benefit patients with type 2 diabetes and HAP 2-2 in terms of non-fatal cardiovascular events but may be harmful to those with the HAP 2-1 or 1-1 genotypes. The post-hoc ACCORD study is limited in subject ethnic diversity and geographically limited to just North America. An evaluation of this concept in the VADT and ADVANCE trials, also designed to evaluate if improved glycemic control reduces cardiovascular events but showed neutral results, is warranted. Such an assessment of the ADVANCE study would be useful as the study was more diverse in subject ethnic diversity and geography [33]. If such studies confirmed what was found in the post-hoc evaluation of the ACCORD study, it may serve clinicians with a way to identify patients, after HAP genotype evaluation, where aggressive glycemic control could be recommended as another way to reduce cardiovascular risk. A prospective clinical study specifically designed to assess cardiovascular outcomes in patients with diabetes and the HAP 2-2 genotype is what is needed to best affect clinical practice and professional consensus recommendations. Until such time, genotyping for HAP polymorphism remains an interesting field of study but may not be useful in current clinical care of patients with diabetes. A similar post-hoc assessment of HAP genotype in the cardiovascular safety studies of sodium glucose co-transporter 2 inhibitors or glucagon-like peptide agonists with demonstrated cardiovascular benefit would also be an interesting research avenue to pursue as perhaps it would identify those patients who would benefit the most from such intervention. It could also explain some of the differing outcomes observed in the trials between agents [36].

Conflicts of Interest

None of the authors have a personal, professional, or financial relationship that could potentially be construed as a conflict of interest.

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