



## Haptoglobin: A Major Susceptibility Gene for Diabetic Cardiovascular Disease

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### Haptoglobin polymorphism

Haptoglobin is a serum protein for which there are two common alleles in humans, denoted 1 and 2 [1]. The genotype of an individual may therefore be described as being Hp 1-1 (homozygous for the 1 allele), Hp 2-2 (homozygous for the 2 allele), or Hp 2-1 (the heterozygote). The haptoglobin gene locus on chromosome 16q22 has 5 exons encoding the 1 allele or 7 exons encoding the 2 allele [2]. The 2 allele appears to have been generated from the 1 allele by an intragenic duplication event of exons 3 and 4. Hp typing of over 100,000 individuals over the past 35 years has shown considerable geographic and ethnic variation in the frequencies of the two alleles [2]. In Israel, the two alleles are in a balanced polymorphism with approximately 30% of the alleles being type 1 and 70% type 2, thus 9% of the Israeli population is Hp 1-1, 49% is Hp 2-2 and 42% is Hp 2-1 [2]. This allele frequency distribution is quite similar to that found in Europe and the United States. The distribution of these two alleles in individuals with diabetes is not different to that in the general population [3].

### Molecular basis for the interaction between haptoglobin phenotype and diabetes in cardiovascular disease

Haptoglobin is an antioxidant as a direct result of its ability to prevent hemoglobin-driven oxidation [2,4]. The mechanism by which haptoglobin serves as an antioxidant is believed to be via stabilization of the heme moiety within the hemoglobin protein [5]. This ability of haptoglobin to protect against hemoglobin-driven oxidative stress is lost when hemoglobin becomes heavily glycosylated, as occurs in the diabetic state [6]. We have recently demonstrated that diabetic individuals with Hp 1-1 are exposed to less hemoglobin-driven oxidative stress than diabetic Hp 2-2 individuals because the Hp1-1-glycosylated hemoglobin complex is more rapidly scavenged by the macrophage as compared to the Hp 2-2-glycosylated hemoglobin complex [6].

CVD = cardiovascular disease

### Clinical studies in humans associating Hp phenotype and diabetic CVD

We have established in four independent prospective longitudinal studies and one large cross-sectional population study that haptoglobin genotype is an independent risk factor for cardiovascular disease and that this relationship is specific for diabetes.

#### Strong Heart Study

The Strong Heart Study was a population-based longitudinal study of CVD in American Indians [7]. We analyzed the haptoglobin phenotype in a matched case-control study of stored serum samples from this study. In multivariate analyses controlling for conventional CVD risk factors and diabetes mellitus characteristics, haptoglobin phenotype was a highly statistically significant predictor of CVD in diabetes [8] (Table 1). The odds ratio of having CVD in diabetes with the haptoglobin phenotype 2-2 was 5.0 times greater than that in diabetes with the haptoglobin 1-1 phenotype ( $P = 0.002$ ). An intermediate risk of CVD was associated with the haptoglobin 2-1 phenotype. No significant association between Hp type and CVD risk was found in non-diabetic individuals.

#### Munich Stent Study

The goal of this study was to determine if the haptoglobin genotype was predictive of major adverse cardiac events after coronary artery stenting in individuals with diabetes. This study was initiated based on two prior small studies showing a relationship between angioplasty and Hp type with regard to the need for repeat angioplasty in individuals with diabetes [9,10]. A consecutive series

**Table 1.** Relative risk of incident CVD according to DM status and Hp type in the Strong Heart Study adjusted for all CVD risk factors and DM characteristics

Variable	OR	95% CI	P
DM and Hp 2-1 (vs. DM and Hp 1-1)	1.63	(0.74–3.63)	0.228
DM and Hp 2-2 (vs. DM and Hp 1-1)	4.96	(1.85–13.33)	0.002
DM and Hp 2-2 (vs. DM and Hp 2-1)	3.04	(1.30–7.09)	0.010
No DM, Hp 2-1 (vs. no DM, Hp 1-1)	1.46	(0.44–4.87)	0.542
No DM, Hp 2-2 (vs. no DM, Hp 1-1)	2.73	(0.81–9.26)	0.107
No DM, Hp 2-2 (vs. no DM, Hp 2-1)	1.88	(0.73–4.80)	0.190

of 935 treated (oral agents and/or insulin) diabetic patients were followed for 1 year after stenting for major adverse cardiac events defined as target vessel revascularization, myocardial infarction, and death. In multivariate analysis controlling for all known determinants of outcome after stent placement we found that haptoglobin genotype was a highly significant independent predictor of major adverse cardiac events in the 1 year period following stent placement in individuals with diabetes. We found significant differences in the risk of developing myocardial infarction and in the need for target vessel revascularization in the 1 year period following stent placement [Table 2] [11].

### Acute Myocardial Infarction

The goal of this study was to determine if the haptoglobin phenotype was predictive of myocardial infarction size and adverse cardiac events in the peri-infarct period in patients with diabetes. Haptoglobin phenotype was determined in a consecutive series of over 620 individuals presenting with acute myocardial infarction to the coronary intensive care unit (224 with diabetes) of the Rambam Medical Center in Haifa, Israel. Major adverse cardiac events (death, re-infarction, revascularization) occurring within 30 days were prospectively identified. Infarction size and the severity of left ventricular dysfunction were assessed by peak creatine kinase, echocardiography and Killip score. We found a significant interaction effect between Hp type and diabetes in predicting mortality and major adverse cardiac events at 30 days [Table 3] [12]. Specifically, the mortality rate in diabetic Hp 1-1 patients was significantly less than in diabetic patients with the Hp 2 allele, but there was no difference between the Hp types in these outcomes in non-diabetic individuals. For the combined MACE endpoint the relative risk was 5 times greater in diabetic Hp 2-2 as compared to diabetic Hp 1-1 individuals ( $P < 0.0001$ ). There was no difference in mortality or in the combined endpoint according to Hp type in non-diabetic patients. Diabetic patients with Hp 1-1 also had significantly smaller and less severe myocardial infarctions as compared to those with Hp 2-1 or Hp 2-2.

### HOPE (Heart Outcomes Prevention Evaluation)

The HOPE study evaluated the effects of daily administration of 400 IU of vitamin E and/or ramipril for 4.5 years on the development of myocardial infarction, stroke and cardiovascular death in people with CVD or diabetes plus one other risk factor [13]. We Hp phenotyped all participants for whom blood was stored in the HOPE study (Canadian participants). In patients receiving double placebo (i.e., no ramipril or vitamin E), Hp phenotype was a significant predictor of the primary composite outcome (Hp 1-1 20.8%, Hp 2-1 16.4%, Hp 2-2 31.9%,  $P = 0.03$ ). There was no significant association between the primary composite outcome and Hp type in the entire HOPE cohort (non-diabetic patients plus diabetic patients) (Hp 1-1 20.8%, Hp 2-1 15.8%, and Hp 2-2 19.0%,  $P$  for trend = 0.31) [A.P. Levy, unpublished observation]. The effect of antioxidants on this risk is discussed below.

**Table 2.** Incidence of MACE in the 1 year follow-up of 935 consecutive diabetic patients undergoing coronary artery stent placement

	Hp 1-1	Hp 2-1	Hp 2-2	P
n	129	424	382	
Death	7 (5.4%)	28 (6.6%)	15 (3.9%)	0.22
Acute MI	0 (0%)	20 (4.7%)	32 (8.4%)	<0.0001
TVR	21 (16.3%)	82 (19.3%)	91 (23.8%)	0.040
MACE	27 (20.9%)	112 (26.4%)	120 (31.4%)	0.015

**Table 3.** Incidence of MACE at 30 days in 224 consecutive diabetic patients presenting with myocardial infarction

Endpoint	Hp 1-1 (n=28)	Hp 2-1 (n=83)	Hp 2-2 (n=113)	P value
Death (%)	0	19 (23)	28 (25)	0.01
Re-infarction (%)	0	2 (2)	6 (5)	0.31
Revascularization (%)	3 (11)	23 (28)	35 (31)	0.09
Composite (%)	3 (11)	42 (51)	62 (55)	0.0001<

### Framingham Offspring Cohort

We sought to examine the relationship between Hp type, diabetes status and coronary artery disease in a cross-sectional analysis of a large community-based cohort from the Framingham Heart Offspring Study [14]. We determined the Hp phenotype in over 3,200 participants who attended the seventh examination of the study. This study therefore assessed CVD prevalence (since this was a cross-sectional study) rather than incidence of CVD in individuals with and without diabetes segregated by Hp type. We found a significant difference in the nature of association between Hp type and CVD in individuals with diabetes and those without ( $P = 0.01$  for interaction term of Hp type and diabetes) [15].

Taken together, these five studies suggest that Hp genotype may be useful in predicting which patients with diabetes will develop cardiovascular disease. Patients at high risk based on the Hp type may benefit from more aggressive glycemic control, lower targets for coronary disease risk factor modification (i.e., target cholesterol or blood pressure), and earlier revascularization strategies.

### Antioxidants in the prevention of diabetic CVD

An increase in oxidative stress represents an attractive unifying mechanism explaining the coordinate activation of several signal transduction pathways known to mediate diabetic micro- and macrovascular disease [16]. Antioxidant supplementation with vitamin E and other antioxidants has been demonstrated to retard and prevent the development of diabetic vascular disease in several animal models. However, several recent placebo-controlled trials have shown that neither vitamin E alone nor in combination with other antioxidant vitamins reduces the incidence of major adverse cardiovascular events in people with or without a history of diabetes [13,17]. The Heart Outcomes Prevention Evaluation (HOPE) trial was one such study which did not demonstrate any cardiovascular benefit of daily administration of 400 IU vitamin E for 4.5 years in either the overall study population or in study participants with diabetes [13].

MACE = major adverse cardiac events

Several mechanisms have been proposed to explain the failure of vitamin E to show benefit, including the possibility that any benefit may only be demonstrable in specific patient subgroups with particularly high levels of oxidative stress [18]. An example of such a subgroup would include that defined by a haplotype at a polymorphic genetic locus in which there exists functionally different allelic variants encoding proteins involved in protection against oxidative stress.

### Demonstration that Hp genotype may predict benefit from Vitamin E

Participants in the HOPE study were randomized to vitamin E and/or ramipril in a 2x2 factorial design [13]. Whereas ramipril was found to result in an approximately 30% reduction in cardiovascular endpoints of CVD death and myocardial infarction, no benefit from antioxidant supplementation with vitamin E was noted in the entire HOPE population or in the diabetic cohort alone [13]. We were able to determine the relative risk reduction associated with vitamin E therapy according to haptoglobin type in patients with and without diabetes in all of the Canadian participants of the HOPE study (3,176 participants – the only HOPE participants for whom blood was stored) [Levy AP, et al, manuscript submitted]. We found that there was no benefit of vitamin E supplementation detected in either the entire group or in the diabetic subset. However, in diabetic participants with Hp 2-2, vitamin E supplementation was associated with a statistically significant reduction in cardiovascular death (relative risk 0.45, 95% confidence interval 0.23–0.90) and non-fatal myocardial infarction (RR 0.57, 95% CI 0.33–0.97). Vitamin E supplementation appears to have provided significant benefit in the HOPE study in protecting against adverse cardiovascular outcomes in a subgroup of diabetic participants identified by haptoglobin typing. These analyses were not preplanned prior to completion of the HOPE study and therefore require confirmation. However, they were based on, and are consistent with observations made in several large epidemiologic studies as well as current biologic models of the effect of vitamin E. Thus, if these findings are validated prospectively in other trials of vitamin E for the prevention of CVD in diabetic individuals [17], Hp phenotyping may be a useful tool to identify individuals who will benefit from vitamin E.

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RR = relative risk  
CI = confidence interval

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*What is work and what is not work are questions that perplex the wisest of men*

*BhagavadGita (Sanskrit for Song of the Lord), probably composed around 300 BC and one of the philosophic texts of Hinduism*