Brief Genetics Report

Haptoglobin Polymorphism Predicts 30-Day Mortality and Heart Failure in Patients With Diabetes and Acute Myocardial Infarction

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Patients with diabetes presenting with acute myocardial infarction (AMI) have an increased rate of death and heart failure. Patients with diabetes homozygous for the haptoglobin (Hp) 1 allele (Hp 1-1) develop fewer vascular complications. We tested the hypothesis that Hp type is related to the outcome of patients with diabetes presenting with AMI. We prospectively assessed the relationship between Hp type and 30-day mortality and heart failure in 1,437 patients with AMI (506 with diabetes). Multivariate logistic regression identified a significant interaction between Hp type and diabetes status on these outcome measures. Hp type was not related to outcome among patients without diabetes. In contrast, Hp 1-1 was associated with a strong protective effect with regard to the primary end point of death (OR 0.14, $P = 0.015$) and for death and heart failure (OR 0.35; 95% CI 0.15–0.86, $P = 0.018$) among patients with diabetes. Finally, among patients with diabetes, Hp 1-1 was associated with smaller infarct size. This study demonstrates that in patients with diabetes and AMI, the Hp type is an important determinant of clinical outcome and infarct size. *Diabetes* 54:2802–2806, 2005

P atients with diabetes presenting with acute myocardial infarction (AMI) have a poor in-hospital and long-term prognosis (1). The excess in-hospital mortality and morbidity correlates primarily with an increased incidence of congestive heart failure (1,2), with heart failure developing at about twice the rate in patients with diabetes than in patients without diabetes

(1). Diabetes is also a risk factor for cardiogenic shock in the setting of acute ischemic syndromes (3).

The susceptibility to diabetic complications is partially controlled by complex unknown genetic factors (4,5). One such genetic factor appears to be a functional allelic polymorphism in the haptoglobin (Hp) gene (6–11). In humans, there are two major alleles, denoted 1 and 2, for the Hp gene (12,13). We have recently shown that patients who are homozygous for the Hp 1 allele (Hp 1-1) are at a lower risk of developing both microvascular (6,7,10) and macrovascular complications associated with diabetes (8,9,14). We have proposed that susceptibility to diabetic vascular disease conferred by the Hp type is the result of marked differences in the antioxidant protection against hemoglobin-induced oxidation provided by the Hp 1 and Hp 2 allelic protein products (15–17). Specifically, we have shown in vitro and in vivo in mice genetically modified at the Hp locus that the Hp 1 and Hp 2 protein products differ in a diabetes-dependent fashion in their ability to prevent the release of redox active iron from hemoglobin and in the rate at which the hemoglobin-Hp complex is cleared via the CD163 scavenger receptor on monocyte/macrophages (15–17).

In the present study, we sought to prospectively test the hypothesis that Hp type is related to the outcome of patients presenting with AMI. Because previous studies have shown that the effect of Hp type might be especially important in patients with diabetes (6–10), we assessed whether the effect of Hp type in patients with AMI varied according to diabetes status using traditional interaction testing and stratified analyses.

Between July 2001 and June 2004, a total of 1,437 consecutive patients who presented with AMI were enrolled in the study. The clinical characteristics of the entire study population segregated by Hp type are listed in Table 1. There were no differences in these baseline characteristics between groups with different Hp types with the exception of fewer previous infarctions in the Hp 2-2 group. Of the 506 patients with known diabetes, 72 patients were receiving insulin treatment with or without oral agents (14%), 316 were receiving oral agents (63%), and 118 were on diet therapy (23%).

Over 30 days, 128 patients (8.9%) died and 357 patients (24.8%) developed heart failure. Likelihood ratio tests

TABLE 1
Baseline clinical characteristics according to Hp type

Characteristic	Hp type			P value
	Hp 1-1	Hp 2-1	Hp 2-2	
n	139	716	582	
Age (years)	60 ± 13	61 ± 13	62 ± 12	0.30
Men	111 (80)	554 (77)	457 (79)	0.77
Prior infarction	32 (23)	175 (24)	109 (19)	0.05
Diabetes	53 (38)	244 (34)	209 (36)	0.59
Diet treated	15 (29)	53 (22)	50 (24)	0.57
Oral agents	32 (60)	160 (66)	124 (59)	0.31
Insulin	6 (11)	31 (13)	35 (17)	0.38
Smokers	68 (49)	328 (46)	248 (43)	0.30
Hypertension	68 (49)	382 (53)	307 (53)	0.63
Anterior infarction	56 (40)	300 (42)	275 (47)	0.10
ST-elevation infarction	92 (66)	509 (71)	423 (73)	0.31
Systolic blood pressure (mmHg)	132 ± 27	130 ± 29	130 ± 28	0.75
Heart rate (bpm)	78 ± 20	79 ± 19	78 ± 19	0.64
Thrombolysis or primary angioplasty*	67 (48)	345 (44)	280 (48)	0.99

Data are n (%) or means ± SD. Trends for categorical variables were calculated with using the Cochran-Armitage trend test. *In-patients were given either streptokinase or tissue plasminogen activator with ST-elevation infarction thrombolysis.

showed a significant interaction between Hp 1-1 and diabetes with regard to 30-day mortality in unadjusted ($P = 0.01$) and adjusted ($P = 0.02$) logistic regression models. Stratified Kaplan-Meier analyses indicated that in the group of patients with diabetes, Hp 1-1 was associated with lower mortality rates at 30 days (Fig. 1). The adjusted odds ratio for 30-day mortality for the Hp 1-1 group was 0.14 ($P = 0.015$) in patients with diabetes and 1.4 ($P = 0.48$) in patients without diabetes.

Results of multivariate modeling of the entire study population for the combined end point of death and heart failure found a strong protective effect of Hp 1-1 type with regard to the primary end point of 30-day mortality or heart failure (adjusted OR for Hp 1-1 0.54 [95% CI 0.33–0.89], $P = 0.01$), whereas diabetes was associated with increased mortality and heart failure (adjusted OR 1.67 [95% CI 1.27–2.21], $P = 0.0002$).

The interaction between Hp type and diabetes status was statistically significant in unadjusted ($P = 0.007$) and adjusted ($P = 0.02$) logistic regression models. Results of multivariate logistic regression models by stratification according to diabetes status are shown in Table 2. Hp 1-1 was associated with a small, nonsignificant reduction in 30-day death or heart failure among patients without diabetes. By contrast, Hp 1-1 was associated with a strong protective effect with regard to the primary outcome of death or heart failure among patients with diabetes after adjusting for numerous baseline clinical characteristics (Table 2).

The effect of the Hp polymorphism on infarct size as assessed by echocardiography was determined in a subgroup of patients ($n = 1,120$) with the first myocardial infarction. Wall motion score index was lower among patients with Hp 1-1 compared with patients with Hp 2-1 ($P < 0.0001$) and Hp 2-2 ($P = 0.01$) (Fig. 2A). To test whether diabetes modifies the effect of the Hp polymorphism on infarct size, we examined the effect of Hp type according to diabetes status in a two-way ANCOVA incorporating the main effect of Hp type and diabetes status. Two-way ANCOVA main effects indicated that Hp type ($P < 0.0001$) and diabetes status ($P = 0.01$) were signifi-

cantly associated with higher wall motion score index. There was a significant interaction between Hp type and diabetes status in an unadjusted model containing only the main effects and an interaction term ($P = 0.02$) as well as in the adjusted model ($P = 0.05$).
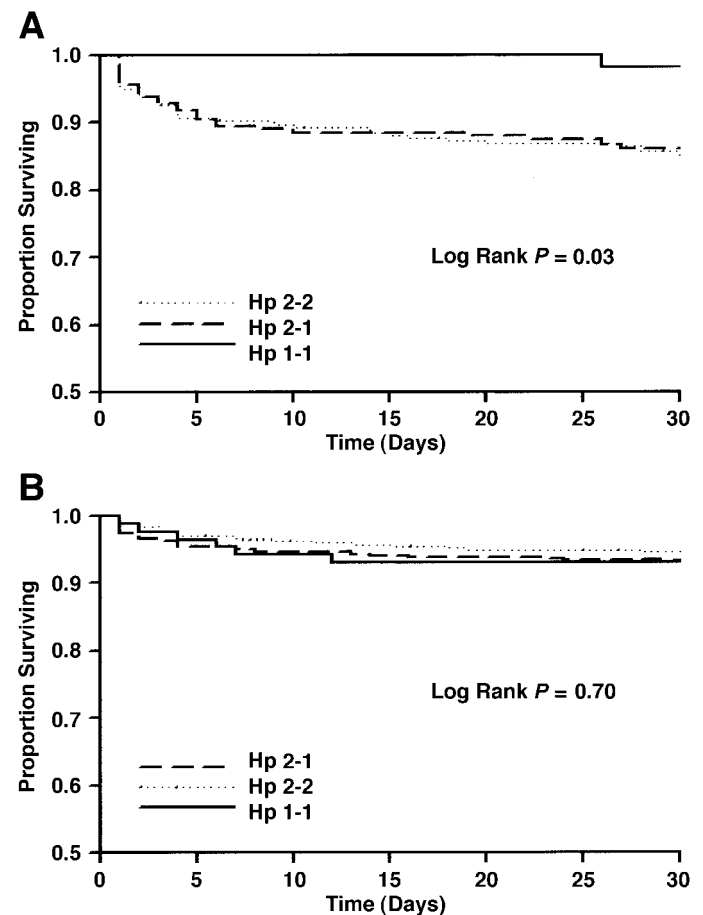

FIG. 1. Kaplan-Meier cumulative survival curves for 30-day mortality of patients with diabetes (A) and without diabetes (B) (comparison by log-rank test).

TABLE 2
Unadjusted and adjusted logistic regression for 30-day mortality and heart failure according to Hp type and diabetes status*

Hp type	n	Events (%)	Unadjusted OR	P value	Adjusted OR	P value
Diabetes	506					
Hp 2-2	209	82 (39)	1.0		1.0	
Hp 2-1	244	106 (43)	1.19 (0.82–1.73)	0.37	1.33 (0.87–2.06)	0.19
Hp 1-1	53	8 (15)	0.28 (0.12–0.61)	0.0005	0.35 (0.15–0.86)	0.018
No diabetes	931					
Hp 2-2	373	84 (23)	1.0		1.0	
Hp 2-1	472	104 (22)	0.97 (0.70–1.35)	0.87	0.99 (0.68–1.43)	0.95
Hp 1-1	86	18 (21)	0.91 (0.51–1.61)	0.75	0.94 (0.49–1.81)	0.85

Data are n (%) or OR (95% CI) unless otherwise indicated. *The final model was adjusted for age, sex, history of hypertension, smoking habit, previous infarction, presence of anterior infarction, ST-elevation infarction, heart rate, and blood pressure on admission and use of reperfusion therapy.

Figure 2*B* shows adjusted means of wall motion score index obtained from the two-way ANCOVA model. In patients without diabetes, there was no significant difference in infarct size between patients with Hp 1-1 and patients with 2-1 ($P = 0.12$) or 2-2 ($P = 0.99$). However, Hp 1-1 was associated with a markedly lower infarct size compared with patients with Hp 2-1 ($P = 0.009$) or 2-2 ($P = 0.02$) among patients with diabetes.

Myocardial infarct size is a key prognostic factor in patients with acute infarction, most prominently in the hospital and throughout the first few months following infarction. The results of the present study suggest that in patients with diabetes, infarct size is dependent in part on Hp type. Patients with diabetes and Hp 2-1 or Hp 2-2 sustain larger infarcts, which may partially explain the higher mortality and heart failure in these patients. By contrast, patients with diabetes and Hp 1-1 have an infarct size comparable with that of nondiabetic patients.

The magnitude of the observed Hp type–dependent difference in wall motion index score might not be large enough to explain the increase in death and heart failure associated with Hp 2-1 and Hp 2-2 in the presence of diabetes. It does indicate, however, that preservation of left ventricular systolic function is an important determinant in the protective effect associated with Hp 1-1 in the setting of AMI.

The protective effect of having Hp 1-1 in patients with diabetes may be related to processes occurring before the onset of AMI. For example, patients with Hp 1-1 may have less extensive coronary disease (8,9,15) or a more developed collateral circulation (11); both factors are major determinants of the amount of myocardial necrosis after AMI.

Alternatively, the salutary effects of Hp 1-1 may also be related to events that take place in the ischemic myocardium after the onset of AMI. The antioxidant activity of the different Hp types differs markedly, with the Hp 1-1 protein conferring greater antioxidant properties compared with the other forms of the protein (15–17). Oxidative stress plays a key role in processes that may affect outcome in the acute phase of AMI including platelet activation (18), reperfusion injury (19), and apoptosis (20), thus giving a relative advantage to patients with Hp 1-1.

FIG. 2. *A*: Mean wall motion score index (95% CI) in patients with first myocardial infarction segregated by Hp type. Patients with Hp 1-1 had a smaller wall motion score index compared with both patients with Hp 2-1 and Hp 2-2. *$P < 0.0001$ compared with Hp 2-1 phenotype; †$P = 0.01$ compared with Hp 2-2 phenotype *B*: Unadjusted and adjusted means of wall motion score index and 95% CIs according to Hp type and diabetes status. Wall motion score index was adjusted for age, sex, previous history of hypertension, smoking status, previous angina, presence of ST-elevation infarction, anterior infarction, and use of reperfusion therapy using ANCOVA under a general linear model. (*P* values are based on the Bonferroni adjustment for multiple comparisons).

RESEARCH DESIGN AND METHODS

This study was performed prospectively from July 2001 to June 2003 at the Rambam Medical Center in Haifa, Israel. We have recently briefly reported mortality data on a subset of the patients included here (21). However, this prior report did not include any analysis of heart failure or left ventricular function. Moreover, due to the smaller sample size of the initial cohort, the prior analysis was insufficiently powered to address potential interaction affects between Hp type and diabetes on cardiovascular end points.

All patients presenting to the intensive coronary care unit with acute myocardial infarction were eligible for entry into the study. The investigational review committee on human research approved the study protocol. Myocardial infarction was diagnosed based on the Joint European Society of
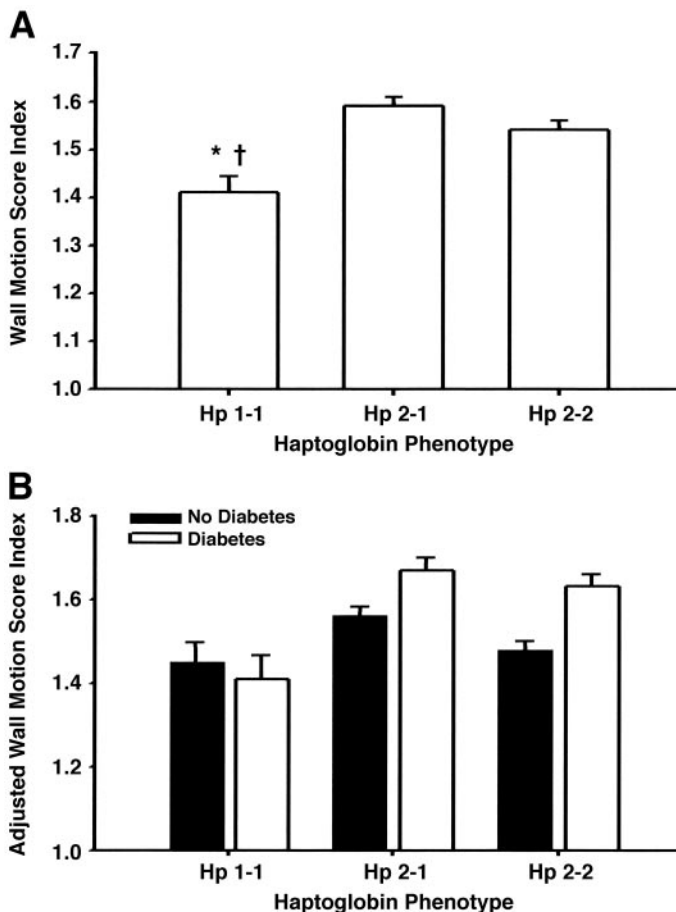
Cardiology/American College of Cardiology criteria (22). Patients admitted >24 h from symptom onset were excluded.

Definition of diabetes. Patients who had a clinical diagnosis of diabetes before enrollment into the study were classified as having diabetes. Patients were classified as having diabetes if the patient had been informed of the diagnosis by a physician before the admission and was taking oral antihyperglycemic agents, insulin, or receiving diet therapy.

Hp typing. Hp phenotyping was performed on 10 μl citrated plasma by polyacrylamide gel electrophoresis (11). We have previously established a 100% correspondence between the Hp phenotype as determined from serum by polyacrylamide gel electrophoresis and the Hp genotype as determined from DNA by PCR (23).

Study end points. The primary end point of this study was death and the composite of death and heart failure occurring within 30 days of admission. Heart failure was diagnosed by the auscultation of rales over more than half of the lung field and pulmonary congestion on chest radiograph. Following hospital discharge, clinical end point information was acquired by reviewing the national death registry and by contacting each patient individually and independently reviewing the hospital course if the patient had been rehospitalized. The secondary end point was infarct size as determined by echocardiographic examination. This analysis was restricted to patients with no previous history of myocardial infarction.

Echocardiographic examination. Assessment of left ventricular function by transthoracic echocardiography was performed on day 2–3 of hospitalization in most patients or earlier if clinically indicated. The distribution of the Hp types and the incidence of major adverse clinical events of patients who did not undergo echocardiography were not different from those who did. Regional myocardial wall motion and left ventricular ejection fraction were determined from the echocardiogram by an observer blinded to the Hp type. For analysis of left ventricular function and wall motion abnormalities, we used the segmentation model according to the American Society of Echocardiography (24), with a higher index being associated with more severe contractile impairment.

Statistical analysis. Baseline characteristics according to Hp type were compared by use of ANOVA for continuous variables and χ^2 test for categorical variables. Stepwise, multivariate logistic regression modeling was the primary statistical analysis used to determine the independent relationship between Hp type and other baseline characteristics to the primary end point. Multivariate linear regression analysis was used to determine the independent effect of Hp type on left ventricular function. Demographic and clinical variables that were considered to be possibly related to the primary end point or to left ventricular functional outcome, or both, were incorporated into the model, and the independent effect of Hp type was determined. Clinical variables for testing were chosen on the basis of their previously determined effect on 30-day mortality (25) and included age; sex; previous history of diabetes, hypertension, and myocardial infarction; smoking status; systolic blood pressure and heart rate upon admission; presence of ST-elevation infarction and anterior infarction; and use of reperfusion therapy.

Our analysis also focused on the possible interaction between Hp type and diabetes. The existence of an interaction between Hp type and diabetes was formally evaluated with the use of logistic regression models incorporating terms for the main effect of Hp type, the main effect of diabetes, and the interaction between Hp type and diabetes.

The influence of diabetes on Hp type–related differences in outcome was also assessed by a stratified analysis. In the stratified analysis, the predictive model developed for the entire study population was tested in subgroups of study patients defined by the presence or absence of diabetes. Differences were considered statistically significant at the two-sided $P < 0.05$ level. Statistical analyses were performed using the SPSS statistical software version 11.5.

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