Haptoglobin Genotype Is Predictive of Major Adverse Cardiac Events in the 1-Year Period After Percutaneous Transluminal Coronary Angioplasty in Individuals With Diabetes

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OBJECTIVE — The goal of this study was to determine whether the haptoglobin (Hp) genotype was predictive of restenosis and major adverse cardiac events (MACEs) after percutaneous transluminal coronary angioplasty (PTCA) in individuals with diabetes.

RESEARCH DESIGN AND METHODS — A consecutive series of 935 diabetic patients treated with oral agents and/or insulin were followed for 1 year after PTCA. The primary study end point was angiographic restenosis, MACEs and secondary study end points were defined as target vessel revascularization, myocardial infarction, and death. Two alleles exist at the Hp gene locus, denoted 1 and 2. The Hp genotype (Hp 1–1, Hp 2–1, or Hp 2–2) was determined by PCR.

RESULTS — In multivariate analysis controlling for all known determinants of outcome after PTCA, we found that the Hp genotype was a highly significant independent predictor of MACEs in the 1-year period after PTCA in individuals with diabetes. This was predominantly due to differences in the risk of myocardial infarction during that period: Hp 1–1, 0 of 129 (0%); Hp 2–1, 20 of 424 (4.7%); and Hp 2–2, 32 of 382 (8.4%); $P < 0.0001$.

CONCLUSIONS — The Hp genotype seems to be highly predictive of adverse cardiac events, particularly myocardial infarction, in the 1-year period after PTCA. Determination of the Hp genotype may be useful in the evaluation of new therapies to reduce cardiovascular risk after PTCA.

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Patients with diabetes have a three- to fivefold increase in risk of atherosclerotic cardiovascular disease (CVD) compared with nondiabetic individuals (1,2). Diabetes is also recognized as a risk factor for poor outcome after percutaneous transluminal coronary angioplasty (PTCA) or coronary artery bypass grafting (CABG) (3–10). The Bypass Angioplasty Revascularization Investigation (BARI) directly compared PTCA and CABG in patients with multivessel disease and demonstrated a clear advantage of CABG over PTCA in patients with treated diabetes (9,10). Accordingly, CABG is currently recommended over multivessel PTCA in all diabetic patients with multivessel coronary artery disease.

The haptoglobin (Hp) gene has two major classes of alleles, denoted 1 and 2 (11). The protein products of these two alleles differ dramatically in terms of their structure and biological function. For example, the protein product of the Hp 1 allele is a superior antioxidant to that produced by the Hp 2 allele (12). We have shown that the Hp 2 allele is positively correlated with the development of several diabetic vascular complications of a micro- and macrovascular nature (13–15).

In a matched case-control sample from the Strong Heart Study (16,17), a longitudinal population study of North American Indians, we have recently demonstrated that Hp genotype is an independent predictor of incident CVD in the setting of diabetes. Specifically, study participants with diabetes who were homozygous for the Hp 2 allele (Hp 2-2) were shown to have a fivefold greater risk of CVD than participants with diabetes homozygous for the Hp 1 allele (Hp 1-1). An intermediate risk was seen in diabetic individuals who were heterozygous (Hp 2-1) at the Hp locus (17).

We proposed that the Hp genotype could identify a cohort of diabetic patients at lower cardiovascular risk after PTCA. We tested this hypothesis in a consecutive series of 935 treated diabetic patients followed for 1 year after PTCA for major adverse cardiac events (MACEs), defined as target vessel revascularization (TVR), myocardial infarction (MI), and death.

RESEARCH DESIGN AND METHODS

Study population

This study was approved by the Deutsches Herzzentrum Munchen Hospital in

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Abbreviations: CABG, coronary artery bypass grafting; CVD, cardiovascular disease; Hp, haptoglobin; MACE, major adverse cardiac event; MI, myocardial infarction; PTCA, percutaneous transluminal coronary angioplasty; TVR, target vessel revascularization.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Baseline characteristics of patients*

	Hp 1-1	Hp 2-1	Hp 2-2	*P*
n	129	424	382	
Insulin only	37	186	138	
Age	67.3 ± 10.1	67.6 ± 9.4	67.3 ± 9.4	0.85
Men	93 (72%)	287 (68%)	272 (71%)	0.46
Hypertension	106 (82%)	353 (83%)	322 (84%)	0.84
Hypercholesterolemia	92 (71%)	312 (74%)	270 (71%)	0.64
Cholesterol (mg/dl)	203.1 ± 52.3	202.4 ± 52.6	204.3 ± 48.6	0.86
Active smoker	64 (50%)	146 (34%)	142 (37%)	0.008
Unstable angina	38 (30%)	111 (26%)	117 (31%)	0.36
Acute MI	31 (24%)	49 (12%)	56 (15%)	0.002
Angiographic characteristics				
Left ventricular ejection fraction	54.9 ± 16.0	55.3 ± 14.6	54.3 ± 15.0	0.62
Lesion length	12.2 ± 6.1	13.6 ± 7.8	14.0 ± 8.7	0.10
Reference diameter	2.94 ± 0.51	2.94 ± 0.53	2.94 ± 0.52	0.99
Minimal lesion diameter	0.73 ± 0.52	0.76 ± 0.57	0.75 ± 0.57	0.81
Vess affected				0.41
1	20 (16%)	68 (16%)	60 (16%)	
2	33 (26%)	148 (35%)	121 (32%)	
3	76 (59%)	208 (49%)	201 (52%)	
Procedural characteristics				
Stented length	20.5 ± 13.3	22.0 ± 12.6	22.6 ± 13.9	0.27
Pressure	13.5 ± 2.7	13.2 ± 3.0	13.2 ± 3.0	0.53
Minimal lesion diameter after stenting	2.81 ± 0.53	2.87 ± 0.51	2.83 ± 0.53	0.46

Data are means ± SD or *n* (%) unless otherwise indicated. Lesion length, minimal lesion diameter, and reference diameter are given in mm; maximal balloon pressure is given in atm.

Munich, Germany. Patients consented to genetic studies. During the period from April 1996 to August 2000, DNA was stored from several thousand patients (diabetic and nondiabetic) who had undergone coronary artery stent placement for the treatment of stable or acute coronary syndromes. The primary purpose of DNA procurement during this period was to identify genetic markers predictive of restenosis and adverse coronary events after stent placement, and several genetic markers have been investigated using this dataset (18–20). We report on a consecutive series of 935 diabetic patients treated with oral agents and/or insulin from this cohort. The diabetic classification, similar to what was used in the Bypass Angioplasty Revascularization Investigation (BARI) (10), was based on patient history and strengthened by the objective evidence of use of insulin or oral hypoglycemic medication.

Study protocol

The primary end point of this study was angiographic restenosis, and the secondary end point was adverse clinical events 1 year after stent placement. Six months after the initial stent placement, repeat angiography was performed in 695 patients (74.3%). Restenosis was defined as a loss of >50% of the luminal area. Clinical end points of death, TVR, and acute MI were obtained during follow-up 1 year after stent placement. Acute MI was defined to include cases with abnormal Q waves or an acute coronary event with an increase in creatine kinase to more than threefold the normal value. A combined end point was defined as a MACE, including acute MI, death, and TVR.

Hp genotyping

Hp genotyping was performed by PCR in all patients with diabetes (21). We have confirmed the accuracy of our PCR method by performing standard Hp phenotyping on 300 of the same patients and have found 100% concordance between the two methodologies.

Genomic DNA was extracted from peripheral blood leukocytes using the QIAamp DNA Blood Kit (Qiagen, Hilden, Germany). Oligonucleotide primers A (5′ GAGGGGAGCTTGCCTTTCCATTG3′) and B (5′GAGATTTTGAGCCCTGGCT GGT 3′) were used for the amplification of a 1,757-bp Hp 1 allele–specific sequence. Primers C (5′CCTGCCTCGTAT TAACTGCACCAT3′) and D (5′CCGAG TGCT CCACATAGCCATGT3′) were used to amplify a 349-bp Hp 1 allele–specific sequence.

Statistical analysis

The χ^2 test (or Fisher's exact test) was used to determine whether a difference in baseline clinical and angiographic characteristics existed between groups. The χ^2 test for trends was used to determine whether a graded association existed between the number of Hp 2 alleles and the risk of each of the major adverse clinical events. Multivariate analysis, according to the Cox proportional hazard model, was performed to determine the relative risk of MACE according to Hp genotype. All baseline demographic, clinical, and angiographic parameters (Table 1) were included in our model as potentially confounding factors. For all statistical analyses, $P < 0.05$ was considered significant.

RESULTS — The demographic and angiographic characteristics of the study cohort, segregated by Hp genotype at the time of presentation for PTCA, are shown in Table 1. The frequency of the three Hp genotypes did not deviate from Hardy-Weinberg expectation: Hp 1-1 (14%), Hp 2-1 (45%), and Hp 2-2 (41%).

As shown in Table 2, we found that the risk of several adverse cardiac end points after PTCA in diabetic patients was dependent on the number of Hp 2 alleles. First, we observed a graded risk of the need for TVR positively correlated with the number of Hp 2 alleles ($P = 0.040$). This genotype-specific effect on TVR was due to differences between the Hp genotypes in the need for repeat PTCA ($P = 0.029$), because there was no significant difference between the Hp genotypes in the need for CABG within the 1-year period after PTCA. These differences between Hp genotypes in the need for repeat PTCA could not be explained by differences in the angiographic restenosis rate between the Hp genotypes, as shown by quantitative coronary angiography performed 6 months after PTCA. Second, we observed a statistically significant increased risk of MI positively correlated

Table 2—*Adverse clinical events occurring in the 1-year period after PTCA*

	Hp 1-1	Hp 2-1	Hp 2-2	*P*
Restenosis	34 (38%)	115 (35%)	107 (38%)	0.72
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
Repeat PTCA	18 (14%)	73 (17.2%)	83 (21.7%)	0.029
MACE	27 (20.9%)	112 (26.4%)	120 (31.4%)	0.015

Data are *n* (%). Clinical events are for all 935 diabetic patients in the 1-year follow-up after stent placement, whereas restenosis was determined for the 695 of these patients who underwent repeat angiography.

with the number of Hp 2 alleles in the 1-year period after PTCA. The incidence of MI was 0% in Hp 1-1, 4.7% in Hp 2-1, and 8.4% in Hp 2-2 ($P < 0.0001$). Finally, for the combined end point of MACE, we found a statistically significant graded risk dependent on the number of Hp 2 alleles. The incidence of MACE in the 1-year period after PTCA was 21% in Hp 1-1 patients, 26% in Hp 2-1 patients, and 31% in Hp 2-2 patients ($P = 0.015$).

To determine whether Hp genotype was independently related to MACE, a Cox proportional hazards model was generated using all of the baseline demographic, clinical, and angiographic parameters of the study population described in Table 1 as potentially confounding factors. After adjustments for these potential confounders, Hp genotype remained an independent predictor of MACE at 1 year ($P = 0.032$). The adjusted relative risk of MACE comparing Hp 1-1 to Hp 2-2 was 1.73 (95% CI 1.13–2.65).

In a consecutive series of 5,585 nondiabetic patients undergoing PTCA in the same facility over the same time interval as the diabetic patients described herein, incidence of major adverse clinical events was as follows: MI (4.9%), TVR (17%), and MACE (23%). Therefore, compared with this series of nondiabetic patients, diabetic patients with the Hp 2 allele had a significantly greater incidence of adverse clinical events ($P < 0.06$ for MI, $P < 0.001$ for TVR, and $P < 0.001$ for MACE). However, diabetic patients who were homozygous for the Hp 1 allele did not have a higher incidence of adverse clinical events as compared with the nondiabetic population.

CONCLUSIONS — We have demonstrated in a prospective longitudinal study of >900 diabetic patients undergoing stent placement that the Hp genotype is predictive of the need for TVR and/or repeat PTCA as well as the risk of MI and the combined end point of MACE. For all of these adverse cardiac end points, we observed a graded effect positively correlated with the number of Hp 2 alleles such that patients homozygous for the Hp 1 allele were at lowest risk, patients homozygous for the Hp 2 allele were at highest risk, and heterozygous patients were at intermediate risk. These results thus confirm and extend the generalizability of our previous findings associating the Hp phenotype and incident CVD in North American Indians in the Strong Heart Study (17).

We have not validated our hypothesis based on preliminary findings from a much smaller cohort of 36 diabetic patients (4 patients with Hp 1-1), suggesting that the incidence of angiographic restenosis in diabetic patients with Hp 1-1 is lower (22). However, although the incidence of angiographic restenosis was not different between the three Hp types, in the present study we did observe a significant difference in the rate of repeat angioplasty ($P = 0.029$) according to Hp type. This is consistent with our initial reports on Hp and the prevalence of restenosis (13,23), which were not angiographic follow-up studies but rather were based on the analysis of patients who presented to the catheterization laboratory for clinically indicated reasons (i.e., acute coronary syndrome) after having had a prior angioplasty at any time in the past (range 4 months to several years).

If Hp 1-1 is protective against acute MI, then why is the percentage of Hp 1-1 patients at baseline (Table 1) significantly higher than for Hp 2-1 and Hp 2-2? This apparent paradox is the result of differences between disease prevalence and incidence. Risk of MI can only be determined prospectively and after incident events. The increased prevalence of acute MI in the Hp 1-1 cohort at the time of patient recruitment may be the result of increased early mortality from acute MI in diabetic patients with the Hp 2 allele, which thereby prevented a disproportionate number of diabetic patients with the Hp 2 allele from being enrolled in the current study. In a separate study (24), we have found in a consecutive series of more than 220 diabetic individuals presenting with acute MI that the acute mortality rate in diabetic patients with the Hp 2 allele was significantly higher than that of diabetic patients with Hp 1-1. One possible explanation contributing to the decreased rate of MI in diabetic patients with the Hp 1 allele may be differences in coronary artery collateral density between the different Hp types, as previously reported (25), because coronary artery collaterals are a major determinant of the ability to adapt to acute myocardial ischemia (26).

Although we have not examined nondiabetic individuals in the current study, we have previously examined the relationship between CVD and Hp type in nondiabetic individuals, and taken together, these data suggest that there is a difference in the nature of the interaction between diabetic and nondiabetic individuals and the Hp type in the development of CVD. First, in the Strong Heart Study, we did not find a relationship between the incidence of CVD and Hp type in nondiabetic patients (17). Second, in a prospective angiographic study of restenosis in 178 nondiabetic patients, we found no relationship between Hp type and restenosis (22). Third, we have examined the relationship between coronary artery collaterals and Hp type in 138 nondiabetic individuals and have found no relationship (25). Fourth, in the acute MI study of >620 consecutive individuals with acute MI (diabetic and nondiabetic), Hp type was predictive of MACE at 30 days only in diabetic individuals. Fifth, in the Framingham Offspring Cohort (Levy AP, Carey D, Lotan R, Larsen MG, Benjamin EJ, manuscript submitted), a cross-sectional analysis examining the association of prevalent CVD and Hp type in individuals with and without diabetes, we have demonstrated a statistically significant interaction between Hp and diabetes in determining the prevalence of CVD. Finally, we have recently proposed a plausible biological mechanism explaining the interaction of diabetes and Hp type on incident CVD provided by the convergence of two independent processes: *1*) the impairment of the ability of Hp to neutralize hemoglobin in the dia-

betic state, and 2) more rapid scavenging of Hp 1-1-Hb complexes as compared with Hp 2-2-Hb complexes by the Hp hemoglobin scavenger receptor CD163 present on the monocyte/macrophage (27). Taken together, these two processes result in there being a greater potential for oxidative tissue damage in the blood vessel wall in the setting of Hp 2-2 versus Hp 1-1 specifically in the diabetic state (27).

Finally, these data suggest that Hp genotyping may be useful theranostically in the targeting of pharmacological therapies to patients at high risk for adverse outcomes. Hp is an antioxidant, and the different Hp proteins differ markedly in terms of the antioxidant protection they provide; Hp 2-2 individuals were provided the least amount of antioxidant protection (12,27). Accordingly, it would be of considerable interest to assess the efficacy of antioxidant supplementation given prospectively after PTCA to this high-risk cohort of diabetic individuals with Hp 2-2.

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