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Haptoglobin Genotype Is a Consistent Marker of Coronary Heart Disease Risk Among Individuals With Elevated Glycosylated Hemoglobin

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Objectives This study sought to investigate into the biologically plausible interaction between the common haptoglobin (Hp) polymorphism rs#72294371 and glycosylated hemoglobin (HbA_{1c}) on risk of coronary heart disease (CHD).

Background Studies of the association between the Hp polymorphism and CHD report inconsistent results. Individuals with the Hp2-2 genotype produce Hp proteins with an impaired ability to prevent oxidative injury caused by elevated HbA_{1c}.

Methods HbA_{1c} concentration and Hp genotype were determined for 407 CHD cases matched 1:1 to controls (from the NHS [Nurses' Health Study]) and in a replication cohort of 2,070 individuals who served as the nontreatment group in the ICARE (Prevention of Cardiovascular Complications in Diabetic Patients With Vitamin E Treatment) study, with 29 CHD events during follow-up. Multivariate models were adjusted for lifestyle and CHD risk factors as appropriate. A pooled analysis was conducted of NHS, ICARE, and the 1 previously published analysis (a cardiovascular disease case-control sample from the Strong Heart Study).

Results In the NHS, Hp2-2 genotype (39% frequency) was strongly related to CHD risk only among individuals with elevated HbA_{1c} ($\geq$6.5%), an association that was similar in the ICARE trial and the Strong Heart Study. In a pooled analysis, participants with both the Hp2-2 genotype and elevated HbA_{1c} had a relative risk of 7.90 (95% confidence interval: 4.43 to 14.10) for CHD compared with participants with both an Hp1 allele and HbA_{1c} <6.5% (p for interaction = 0.004), whereas the Hp2-2 genotype with HbA_{1c} <6.5% was not associated with risk (relative risk: 1.34 [95% confidence interval: 0.73 to 2.46]).

Conclusions Hp genotype was a significant predictor of CHD among individuals with elevated HbA_{1c}. (J Am Coll Cardiol 2013;61:728–37) © 2013 by the American College of Cardiology Foundation

Haptoglobin (Hp) is an abundant plasma protein that protects against oxidative damage mediated by extracorpuscular hemoglobin (1). A common polymorphism (rs#72294371) exists at the *Hp* locus and consists of 2 alleles, denoted 1 and 2, that are defined by the absence or presence of a 1700 base pair partial intragenic duplication, thus forming 3 possible Hp genotypes (1-1, 2-1, and 2-2) that produce 3 distinctly different proteins that vary in size, shape, and function (2). Interestingly, this common Hp polymorphism is posited to have arisen early in human evolution from a selective advantage of the Hp2 allele

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against infectious disease, but in modern times, it may confer increased risk of several noninfectious, inflammatory, and chronic disease complications (3). Compared with the Hp1-1 genotype, and to a lesser extent the intermediary Hp2-1 genotype, individuals with the Hp2-2 genotype produce a protein that is dysfunctional in protecting against hemoglobin-driven oxidative damage, leading to increased inflammation and oxidative stress, reduced ability of high-density lipoprotein (HDL) to promote reverse cholesterol efflux, and plaque instability in several in vitro and in vivo systems (4–8). This dysfunction in the Hp2-2 protein is accentuated when hemoglobin is glycosylated (6).

The Hp2-2 genotype has been consistently associated with increased risk of cardiovascular complications, such as myocardial infarction (MI), among individuals with type 2 diabetes (2,9). However, the relationship between Hp genotype and coronary heart disease (CHD) in people without diabetes is unclear and controversial (2,10). The impact of maintaining strict glycemic control on the prevention of cardiovascular complications in individuals with type 2 diabetes is also inconsistent (11,12). A possible explanation for this inconsistency is that strict glycemic control may only be beneficial in reducing CHD risk in a subset of individuals most susceptible to vascular damage from hyperglycemia, such as those with the Hp2-2 genotype.

We explored the potential statistical interaction between the Hp genotype and glycemia, as quantified by glycosylated hemoglobin (HbA_{1c}) concentration, on the risk of CHD within 1 large prospective cohort of apparently healthy women. We then replicated this analysis in a second and more focused cohort of individuals with diabetes to determine if individuals with the Hp2-2 genotype and elevated HbA_{1c} are at increased risk of incident CHD. Finally, we conducted a meta-analysis of results from these 2 cohorts with those from a previously published nested case-control study of Hp genotype and cardiovascular disease (CVD).

Methods

Cohort: the NHS. The NHS (Nurses' Health Study) is a prospective cohort of 121,700 female U.S. registered nurses who were aged 30 to 55 years at baseline in 1976. Information on anthropometric and lifestyle factors is obtained through self-administered questionnaires every 2 years and diet every 4 years. From 1989 to 1990, a blood sample was provided by 32,826 women. Women who had an incident MI (n = 343) or fatal CHD (n = 64) between the date of blood draw and June 2004 were identified and matched 1:1 to controls for age, smoking status, fasting status, and month of blood draw, as described elsewhere (13). The majority (96%) of nurses in this case-control sample are white. Anthropometric and lifestyle variables were derived from the questionnaire administered in 1990, with missing information substituted from previous questionnaires. The validity of the questionnaires and the reproducibility of the measurements have been reported previously (14,15), as has the measurement of the standard biochemical risk variables (13). The institutional review board of the Brigham and Women's Hospital and the Human Subjects Committee Review Board of the Harvard School of Public Health approved the study protocol.

Abbreviations and Acronyms

CHD = coronary heart disease
CI = confidence interval
CVD = cardiovascular disease
HbA_{1c} = glycosylated hemoglobin
HDL = high-density lipoprotein
Hp = haptoglobin
LDL = low-density lipoprotein
MI = myocardial infarction
RR = relative risk

Cohort: the ICARE study. ICARE (Prevention of Cardiovascular Complications in Diabetic Patients With Vitamin E Treatment; NCT00220831) is a large clinical trial of vitamin E in participants with type 2 diabetes; it was conducted within 47 primary healthcare clinics of the Clalit Health Services in Israel, as described previously (16). These participants were white men and women aged 22 to 95 years with type 2 diabetes at baseline who were enrolled in the Clalit Health Plan Diabetes Registry and were followed up prospectively for CHD events. Only participants with the Hp2-2 genotype were randomized to receive vitamin E or placebo; participants with the Hp1-1 and Hp2-1 genotypes were not included in the randomization. The ICARE sample of participants included in the current analysis consists of the 2,070 individuals remaining who did not receive vitamin E (the untreated Hp1-1 and Hp2-1 patients and the Hp2-2 patients receiving placebo). The vitamin E–treated Hp2-2 patients were not included in the current analysis to avoid introducing bias because these patients have been shown to have lower risk of CHD due to the administered vitamin E. The study protocol was approved by the independent ethics committee of the Carmel Medical Center in Clalit Health Services and the Israeli Ministry of Health. All participants provided written informed consent.

Hp typing. Hp genotype, recently given the identifier rs#72294371 (17), was determined in both cohorts by using gel electrophoresis of hemoglobin-enriched serum (18). This procedure produces a signature banding pattern for each Hp type and has been shown to corroborate completely with the Hp genotype determined by polymerase chain reaction (19). An unambiguous Hp type was obtained on >99.5% of all samples. A pilot study to test the Hp genotyping method in the NHS found 99% concordance between duplicate samples of 76 individuals. Genotype frequencies in the NHS were in Hardy-Weinberg equilibrium in the whole sample ($p = 0.49$) and also within cases ($p = 0.84$) and controls ($p = 0.52$) separately. Genotype frequencies in ICARE were not in Hardy-Weinberg equilibrium, but this was driven by the study design that excluded one half of the Hp2-2 participants because they had been given vitamin E treatment in the ICARE clinical trial.

CHD case assessment. As previously described in NHS (20) and ICARE (16), CHD was similarly defined in the 2

datasets as nonfatal MI or fatal CHD. In brief, World Health Organization criteria (21) (symptoms plus either diagnostic electrocardiographic changes or altered levels of cardiac enzymes) were used to diagnose CHD. Nonfatal NHS events were confirmed through review of medical records, with deaths confirmed by using medical records, the National Death Index, and from death certificates. All information was reviewed by NHS investigators who determined the primary cause of death. ICARE cases were ascertained by reviewing all hospitalizations of study participants; the hospitalization discharge summary was used for adjudication of events by a panel of physicians. MI was defined by the typical rise and fall of serum markers of myocardial necrosis with at least 1 of the following: 1) typical ischemic symptoms; 2) development of pathologic Q waves on the electrocardiogram; or 3) electrocardiographic changes diagnostic of ischemia. All death cases were ascertained by using the national death registry. For out-of-hospital deaths, adjudication was based on interviews with the participant's physician and family.

Statistical analysis. Participant characteristics were compared between genotypes by using a general linear model for continuous variables and chi-square tests for categorical variables, unless there was a cell with $n < 5$, in which case the Fisher exact test was used. For skewed variables, p values from log-transformed analyses, geometric means, and reverse-transformed 95% confidence intervals were reported. All analyses were conducted by using SAS version 9.1 (SAS Institute, Inc., Cary, North Carolina) at a 2-tailed alpha level of 0.05.

Because of the nested case-control study design, relative risks (RRs) of CHD for the NHS were estimated by using incidence rate ratios from logistic regression with adjustment for the matching factors. In the prospective ICARE cohort, Cox proportional hazards models were used, with days in study as the time-dependent variable to estimate hazard ratios as estimates of RR. For the NHS, unconditional logistic regression was used to allow for maximum statistical power in stratified analyses and because 6 participants ended up unmatched due to missing data for their match. Because of limited power to test for interactions across strata of Hp genotype (due to the Hp1-1 genotype frequency [≤15%]), we combined the Hp1-1 genotype with the Hp 2-1 genotype for these analyses.

In addition to matching factors (age, smoking, fasting status, and month of blood draw), NHS analyses were adjusted for body mass index, alcohol intake, diabetes, history of hypercholesterolemia, and history of hypertension. The following variables were considered as potential covariates for the NHS analysis but ultimately were not included in the model because they did not influence the association: marital status, physical activity, cholesterol-lowering medication use, insulin and oral diabetes medication use, menopausal status, hormone therapy, and parental MI at <60 years of age. ICARE analyses were adjusted for sex, age, smoking status, hypertension, MI before enrollment, statin use, and metformin use. The following variables were considered as potential covariates for the ICARE analysis but ultimately were not included in the model: length of time with diabetes, blood pressure medications, and stroke.

For analyses stratified according to HbA_{1c}, we used 6.5% as the cutoff because this has been defined as the value leading to a diagnosis of diabetes, as well as the level at which complications of diabetes arise, as established by the International Expert Committee composed of members of the American Diabetes Association, the European Association for the Study of Diabetes, and the International Diabetes Federation (22).

Pooled analysis. To pool the risk estimates from multiple study cohorts, we used the weighted average of regression estimates in a random-effects meta-analysis, testing for heterogeneity (23). In addition to NHS and ICARE, we searched Medline through March 2012 for published data of Hp genotype and CVD in cohorts with results stratified according to either HbA_{1c} or diabetes status. We also searched the reference lists from the limited number of studies of Hp genotype in the literature. We found 1 suitable publication, and it was from the Strong Heart Study (SHS) (24). The publication contained Hp genotype frequencies for 206 CVD cases and 206 matched controls, and we used these frequencies to calculate the unadjusted odds ratio and 95% confidence intervals (CIs) for the SHS sample. In the SHS sample, the endpoint was total CVD (CHD and incident stroke events), but >85% of events were CHD related. HbA_{1c} concentrations were not available for all participants, but results were published according to diabetes status, which served as a proxy for HbA_{1c}: it has been reported that in the SHS participants specifically, a diabetes diagnosis has similar risk of CVD as the cutoff of HbA_{1c} 6.5% (25). SHS was a population-based prospective longitudinal study of CVD in American Indians. Detailed descriptions of the SHS cohort, survey methods, and CVD case ascertainment and laboratory techniques have been described previously (25).

Results

The NHS. Baseline characteristics of the NHS participants are described in Table 1 according to Hp rs#72294371 genotype and case status. The distribution of Hp phenotype frequencies was 15% (Hp1-1), 46% (Hp2-1), and 39% (Hp2-2) (data not shown), and the Hp genotype frequency did not differ between cases and controls. Among cases but not controls, Hp1-1 participants were significantly older than those with the Hp2-1 or Hp2-2 genotype. As expected based on previous reports from this cohort (26), cases and controls differed with respect to classic cardiovascular risk factors (data not shown). When adjusted for covariates and case-control status, serum low-density lipoprotein (LDL) cholesterol was the only biomarker to differ across Hp genotypes ($p = 0.05$), with lower concentrations in Hp1-1

Table 1 Baseline Characteristics by Hp Genotype and Case Status Among Women Age 44 to 69 Years at Blood Draw From a Nested Case-Control Study of CHD Events in the NHS, 1990 to 2004

	Cases				Controls			
Characteristic	Hp1-1 (n = 58)	Hp2-1 (n = 188)	Hp2-2 (n = 161)	p Value	Hp1-1 (n = 66)	Hp2-1 (n = 189)	Hp2-2 (n = 156)	p Value
Age* (yrs)	62.1 ± 6.3	59.3 ± 6.4	59.4 ± 6.6	0.01	60.8 ± 5.9	59.5 ± 6.7	59.5 ± 6.5	0.33
Smoking status*								
Never	27 (47)	63 (34)	54 (34)	0.44	26 (39)	66 (35)	55 (35)	0.83
Past	18 (31)	74 (39)	62 (38)		21 (32)	75 (40)	62 (40)	
Current	13 (22)	51 (27)	45 (28)		19 (29)	48 (25)	39 (25)	
BMI (kg/m^2)	26.1 (24.9–27.4)	26.0 (25.3–26.7)	26.1 (25.3–26.9)	0.97	25.5 (24.5–26.5)	24.7 (24.1–25.3)	25.0 (24.4–25.6)	0.39
Activity (MET h/week)	9.2 (6.3–13.5)	7.4 (6.0–9.17)	9.8 (7.8–12.3)	0.20	10.7 (7.9–14.6)	9.6 (8.03–11.6)	11.9 (9.77–14.6)	0.30
Alcohol (g/day)	2.46 (1.58–3.82)	4.88 (3.97–6.00)	4.16 (3.31–5.24)	0.02	4.83 (3.42–6.81)	4.45 (3.59–5.51)	5.10 (4.09–6.35)	0.68
Parental MI <60 yrs of age	9 (16)	41 (22)	32 (20)	0.58	7 (11)	22 (12)	25 (16)	0.39
History of hypercholesterolemia	25 (43)	96 (51)	99 (61)	0.03	22 (33)	79 (42)	63 (40)	0.48
History of hypertension	30 (52)	94 (50)	82 (51)	0.97	18 (27)	47 (25)	46 (29)	0.63
History of diabetes	10 (17)	24 (13)	24 (15)	0.66	4 (6)	13 (7)	7 (5)	0.64
Oral diabetic drug use	2 (3)	13 (7)	5 (3)	0.01	0 (0)	2 (1)	2 (1)	0.18
Cholesterol-lowering drug use	1 (2)	8 (4)	13 (8)	0.12	1 (2)	4 (2)	5 (3)	0.70
Post-menopausal hormone use†	17 (29)	59 (31)	50 (31)	0.43	24 (36)	60 (32)	59 (38)	0.83
CRP (mg/l)	0.30 (0.22–0.41)	0.24 (0.20–0.28)	0.26 (0.21–0.31)	0.36	0.19 (0.15–0.25)	0.19 (0.16–0.22)	0.16 (0.14–0.19)	0.49
Triglycerides (mg/dl)	129.5 (113.7–147.6)	121.4 (112.9–130.5)	123.1 (113.8–133.1)	0.69	107.4 (95.7–120.5)	105.4 (98.5–112.9)	104.3 (96.7–112.4)	0.92
HbA_{1c} (%)	5.96 (5.69–6.24)	5.76 (5.61–5.91)	5.76 (5.62–5.94)	0.43	5.46 (5.33–5.59)	5.53 (5.45–5.61)	5.43 (5.34–5.51)	0.23
HbA_{1c} ≥6.5%	10 (17)	23 (40)	25 (43)	0.001	3 (22)	9 (64)	2 (14)	0.01
Total cholesterol (mg/dl)	228 ± 53	232 ± 37	237 ± 40	0.33	222 ± 35	227 ± 42	229 ± 40	0.54
HDL cholesterol (mg/dl)	53 ± 14	52 ± 15	52 ± 15	0.89	60 ± 18	59 ± 16	60 ± 17	0.90
LDL cholesterol (mg/dl)	139 ± 50	143 ± 33	147 ± 36	0.29	130 ± 32	136 ± 40	137 ± 35	0.42
Apolipoprotein B (mg/dl)	111 ± 36	114 ± 34	116 ± 33	0.68	104 ± 33	103 ± 30	107 ± 30	0.45
Adiponectin (ng/ml)	7,142 (6,325–8,064)	7,125 (6,661–7,623)	7,515 (6,986–8,083)	0.54	8,355 (7,553–9,245)	8,541 (8,045–9,066)	8,649 (8,099–9,240)	0.85

Values are mean ± SD, n (%), or geometric mean (95% confidence interval [CI]). Participant characteristics were compared between genotypes by using a general linear model for continuous variables and chi-square tests for categorical variables, unless there was a cell with n < 5, in which case the Fisher exact test was used. For skewed variables (physical activity, alcohol, high sensitivity C-reactive protein [CRP], body mass index [BMI], glycosylated hemoglobin [HbA_{1c}], triglycerides, and adiponectin), p values from log-transformed analyses and geometric means with reverse-transformed 95% CIs are displayed. *Case-control matching variable (also matched for fasting status and date at blood draw). †In post-menopausal women only.

CHD = coronary heart disease; HDL = high-density lipoprotein; Hp = haptoglobin; LDL = low-density lipoprotein; NHS = Nurses' Health Study.

patients (133 ± 3 mg/dl) compared with Hp2-1 patients (140 ± 2 mg/dl) and Hp2-2 individuals (143 ± 2 mg/dl) (Online Table 1).

Table 2 presents the multivariate RR of CHD for the Hp2-2 genotype compared with the Hp1 allele carriers. Among all Hp2-2 carriers, the RR of CHD was 1.02 (95% CI: 0.76 to 1.36) compared with Hp1 allele carriers (Hp 1-1 and Hp2-1 genotypes). We further stratified the data according to known CHD risk factors. Among participants with HbA_{1c} ≥6.5% (n = 72), the RR for Hp2-2 was 10.12 (95% CI: 1.08 to 94.97), whereas no association for Hp genotype was observed among participants with HbA_{1c} status <6.5% (p for interaction = 0.04). We did not observe a similar interaction for reported diabetes history, but 38% of the women with diabetes had HbA_{1c} <6.5%. Because only 24 participants reported using oral diabetic medication, we could not conduct an analysis further stratified according to medication use.

Figure 1A shows the joint effects of HbA_{1c} level and Hp genotype. Among women with Hp2-2 genotype and elevated HbA_{1c}, the RR of a CHD event was 10.72 (95% CI: 2.46 to 46.77) compared with participants with an Hp1 allele and HbA_{1c} <6.5%. Compared with this same reference group, participants with the Hp2-2 genotype who had HbA_{1c} <6.5% were not at increased risk of CHD (RR: 0.95 [95% CI: 0.70 to 1.28]), and participants with an Hp1 allele but with elevated HbA_{1c} had a borderline increased risk (RR: 2.05 [95% CI: 0.99 to 4.24]).

Replication in the ICARE Study. The distribution of Hp genotype frequencies was 13% (Hp1-1), 55% (Hp2-1), and 32% (Hp2-2) in the ICARE dataset, and the only baseline characteristics that differed among the genotypes were age and smoking (Table 3). Compared with participants with the Hp1-1 or Hp2-1 genotype, the multivariate RR of CHD for the Hp2-2 genotype was 6.76 (95% CI: 2.88 to 15.87) in this diabetic cohort. Although we had less power, we stratified by similar risk factors as we did for the NHS analyses (Table 4). Among participants <69 years of age (mean age) at baseline, the RR for Hp2-2 was 13.72 (95% CI: 3.06 to 61.54) compared with Hp1 allele carriers, whereas the corresponding RR for the older half of participants was 3.73 (95% CI: 1.27 to 11.01). Among diabetic patients not taking metformin, the RR for CHD was 15.84 (95% CI: 3.54 to 70.83) for the Hp2-2 genotype compared with the Hp1 allele carriers, and those taking metformin had an RR of 3.77 (95% CI: 1.26 to 11.30).

To confirm the interaction between Hp genotype and glycemic control, we performed several additional analyses. Among participants with elevated HbA_{1c} (≥6.5%), the RR for CHD was 7.55 (95% CI: 2.79 to 20.47) for Hp2-2 individuals compared with the Hp1 allele carriers, whereas

Table 2 Multivariate RR* of CHD With 95% CIs for Hp Genotypes, Together and Stratified According to CVD Risk Factors, in a Nested Case-Control Study of CHD in Women Aged 44 to 69 Years at Blood Draw From the NHS, 1990 to 2004

	Hp1-1 + Hp2-1		Hp2-2			
Characteristic	Ca/Co	RR	Ca/Co	RR	95% CI	p for Interaction
All participants						
Matching factors only	246/255	Ref.	161/156	1.07	0.81–1.42	—
Multivariate adjusted*	246/255	Ref.	161/156	1.02	0.76–1.36	—
Age at baseline						
<60 yrs	100/112	Ref.	77/67	1.21	0.71–1.78	0.40
≥60 yrs	146/143	Ref.	84/89	0.92	0.63–1.36	
Smoking						
Never	90/92	Ref.	54/55	0.97	0.59–1.60	0.87
Past	92/96	Ref.	62/62	0.97	0.59–1.57	
Current	64/67	Ref.	45/39	1.18	0.67–2.10	
Diabetes†						
No	205/236	Ref.	130/146	0.97	0.71–1.32	0.38
Yes	41/19	Ref.	31/10	2.05	0.73–5.72	
HbA_{1c}						
<6.5%	213/243	Ref.	136/154	0.95	0.70–1.29	0.04
≥6.5%	33/12	Ref.	25/2	10.12	1.08–94.97	
History of hypercholesterolemia						
No	125/154	Ref.	62/93	0.80	0.52–1.21	0.12
Yes	121/101	Ref.	99/63	1.32	0.86–2.03	
LDL concentrations						
Normal (<160 mg/dl)	173/193	Ref.	110/117	0.96	0.68–1.37	0.57
High (≥160 mg/dl)	73/62	Ref.	51/39	1.14	0.64–2.01	

*Adjusted model included age, BMI, smoking status, alcohol intake, diabetes, history of hypercholesterolemia, and history of hypertension, unless stratified by 1 of these variables. †For analysis of risk of CHD by diabetes status, cases were updated as having diabetes if they developed diabetes before their CHD event, and controls were updated as having diabetes if diagnosed with diabetes before their matched case had a CHD event.

CI = confidence interval; CVD = cardiovascular disease; Ref. = reference; RR = relative risk; other abbreviations as in Table 1.

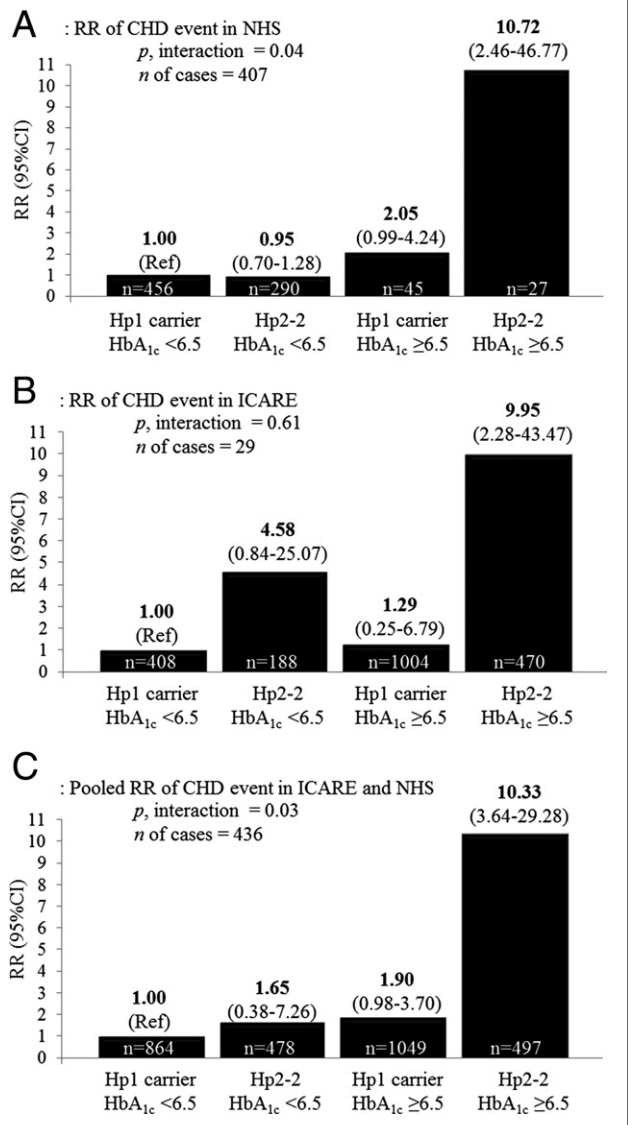

Figure 1 **Multivariate RR of CHD Events According to Hp Genotype and HbA_{1c} Status**

(A) A nested case-control study of coronary heart disease (CHD) in women aged 44 to 69 years from the NHS (Nurses' Health Study) (1990–2004), adjusted for age, body mass index, smoking, alcohol, history of hypercholesterolemia, and history of hypertension. **(B)** Men and women with diabetes aged 22 to 95 years from the ICARE study (2005–2006), adjusted for sex, age, smoking status, hypertension, myocardial infarction before enrollment, statin use, and metformin use. **(C)** ICARE and NHS pooled together. HbA_{1c} = glycosylated hemoglobin; Hp = haptoglobin; RR = relative risk.

no significant effect of Hp genotype was observed among participants with HbA_{1c} <6.5% (p for interaction = 0.61). When grouped by the combination of Hp genotype and HbA_{1c} status, the RR for CHD was 9.95 (95% CI: 2.28 to 43.47) among those with Hp2-2 genotype and HbA_{1c} ≥6.5%, compared with participants with an Hp1 allele and HbA_{1c} <6.5% (Fig. 1B). Compared with the same reference group, participants with an Hp1 allele and elevated HbA_{1c} were not at significantly increased risk of CHD (RR: 1.29 [95% CI: 0.25 to 6.79]). No sex-based differences in results were present.

Pooled analysis. When the NHS and ICARE cohorts were pooled in a meta-analysis, participants with both the Hp2-2 genotype and high HbA_{1c} had a pooled RR of 10.33 (95% CI: 3.64 to 29.28) compared with participants with both an Hp1 allele and HbA_{1c} <6.5% (pooled p for interaction = 0.03) (Fig. 1C). We did not detect heterogeneity between the studies (p = 0.94).

Based on the previously published SHS data (24), we calculated that in the SHS participants, those with both the Hp2-2 genotype and diabetes (as a proxy for elevated high HbA_{1c} because in SHS participants specifically, a diabetes diagnosis has similar risk of CVD as the cutoff of 6.5% for HbA_{1c}) had an RR of 7.01 (95% CI: 3.49 to 14.06) compared with participants with both an Hp1 allele and no diabetes (Fig. 2). When results from the NHS, ICARE, and SHS were combined, this same RR was 7.90 (95% CI: 4.43 to 14.10; heterogeneity p = 0.93, interaction p = 0.004).

Discussion

In 2 independent large prospective cohorts with a broad range of HbA_{1c} concentrations, we found that individuals with the rs#72294371 Hp2-2 genotype and elevated HbA_{1c} (≥6.5%) had a >10-fold increased risk of CHD compared with those with an Hp1 allele and HbA_{1c} <6.5%. Participants with the Hp2-2 genotype and HbA_{1c} <6.5% were not at increased risk of CHD, even in cases of clinically diagnosed diabetes, thus demonstrating that the underlying pathophysiological effects associated with the impaired function of the Hp2-2 protein are greatest among individuals with HbA_{1c} ≥6.5%, regardless of diabetes status. Results from the previously reported SHS data support additional replication. To the best of our knowledge, no other studies have reported results examining Hp genotype, HbA_{1c}, and risk of CHD.

Biological mechanism. Hp functions to block oxidation by iron associated with hemoglobin (8), and striking differences between the Hp genotypes in their ability to perform this function in the setting of hyperglycemia have been demonstrated both in vitro and in vivo (Fig. 3). In vitro, this functional discrepancy is due to a decreased ability of the Hp2-2 protein to stabilize heme in the heme pocket of HbA_{1c} (6,27). In vivo, both in hyperglycemic animal models and humans, those with the Hp2-2 genotype fail to efficiently clear the Hp2-2–hemoglobin complex via the monocyte/macrophage CD163 Hp–hemoglobin scavenger receptor, resulting in increased plasma redox active iron. Diabetic individuals with the Hp2-2 genotype may be at increased risk of CHD from this increase in plasma redox active Hp–hemoglobin complex if it binds to HDL levels and thus results in the oxidative modification and loss of

Table 3 Baseline Characteristics According to Hp Genotype Among Men and Women With Diabetes Aged 22 to 95 Years From the ICARE Study, 2005 to 2006*

Characteristic	Hp1-1 (n = 270)	Hp2-1 (n = 1,142)	Hp2-2 (n = 658)	p Value
Sex	125 (46)	535 (47)	317 (48)	0.82
Age (yrs)	68.9 ± 8.7	68.2 ± 8.8	69.6 ± 8.1	0.007
Smoking (yes)	16 (6)	107 (9)	84 (13)	0.004
Time since diabetes diagnosis (yrs)	8.9 (8.1–9.7)	8.4 (8.0–8.8)	8.6 (8.1–9.1)	0.52
Hypertension	199 (74)	857 (75)	496 (75)	0.86
Statin use	200 (74)	882 (77)	509 (77)	0.51
Metformin use	213 (79)	885 (78)	511 (78)	0.88
HbA_{1c} (%)	7.18 (7.04–7.33)	7.24 (7.18–7.33)	7.26 (7.16–7.35)	0.67
Total cholesterol (mg/dl)	189 ± 33	186 ± 37	187 ± 34	0.50
LDL cholesterol (mg/dl)	103 ± 24	102 ± 29	103 ± 26	0.83
HDL cholesterol (mg/dl)	46 ± 11	46 ± 11	46 ± 11	0.61
MI before study	31 (11)	158 (14)	96 (15)	0.46
MI during follow-up	3 (1)	4 (1)	17 (3)	<0.0001
CHD death during follow-up	0 (0)	0 (0)	5 (1)	0.003
Total CHD event†	3 (1)	4 (1)	22 (3)	<0.0001

Values are n (%), mean ± SD, or geometric mean (95% CI). *Participant characteristics were compared between genotypes by using a general linear model for continuous variables and chi-square tests for categorical variables, unless there was a cell with n < 5, in which case the Fisher exact test was used. For skewed variables (time since diabetes diagnosis and HbA_{1c}), p values from log-transformed analyses and geometric means with reverse-transformed 95% CIs are displayed. †Myocardial infarction (MI) or coronary heart disease (CHD) death during follow-up.
Abbreviations as in Table 1.

function of the HDL in promoting reverse cholesterol transport. This hypothesis is supported by evidence demonstrating that the HDL levels of Hp2-2 diabetic individuals are extensively oxidized, and antioxidant therapy among Hp2-2 diabetic individuals and mice prevents HDL oxidative modification and restores HDL function (27). Hp genotype is linked to CHD by additional mechanisms that may be exacerbated by chronically high blood glucose. For example, Hp motivates microangiogenesis (28,29), and because the Hp1-1 genotype has the highest Hp concen-

Table 4 Multivariate RR* of CHD Event with 95% CIs for Hp Genotypes, Together and Stratified by CVD Risk Factors, in a Prospective Study of Diabetic Men and Women Age 22 to 95 Years From the ICARE Study, 2005 to 2006

	Hp1-1 + Hp2-1		Hp2-2			
Characteristic	Event/No Event	RR	Event/No Event	RR	95% CI	p for Interaction
All participants						
Unadjusted	7/1405	Ref.	22/636	6.83	2.92–15.98	—
Multivariate adjusted	7/1405	Ref.	22/636	6.76	2.88–15.87	—
Age at baseline						
<69 yrs	2/716	Ref.	12/289	13.72	3.06–61.54	0.16
≥69 yrs	5/689	Ref.	10/347	3.73	1.27–11.01	
Previous MI						
No	5/1218	Ref.	15/547	6.39	2.30–17.74	0.89
Yes	2/187	Ref.	7/89	8.23	1.68–40.46	
Smoking						
No	5/1284	Ref.	17/557	7.64	2.81–20.75	0.57
Yes	2/121	Ref.	5/79	4.50	0.80–25.30	
HbA_{1c}						
<6.5%	2/406	Ref.	4/184	4.30	0.77–24.01	0.61
≥6.5%	5/999	Ref.	18/452	7.55	2.79–20.47	
Years with diabetes						
≤8 yrs	5/791	Ref.	11/366	4.59	1.58–13.29	0.39
>8 yrs	2/614	Ref.	17/270	12.74	2.71–58.75	
Metformin use						
No	2/312	Ref.	13/134	15.84	3.54–70.83	0.16
Yes	5/1093	Ref.	9/502	3.77	1.26–11.30	

*A proportional hazards model was used, adjusted for sex, age, smoking status, hypertension, previous MI, statin use, and metformin use, unless stratified by 1 of these variables.
Abbreviations as in Tables 1, 2, and 3.

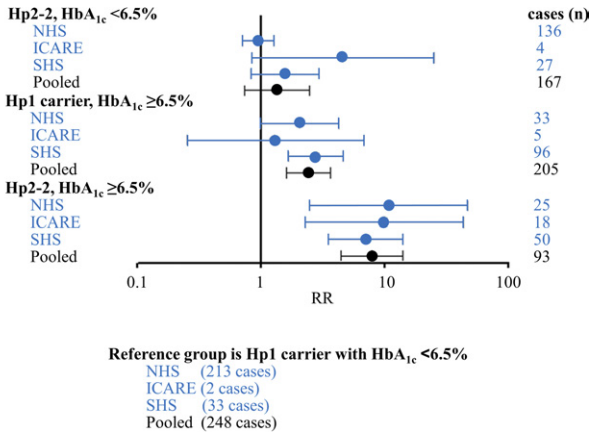

Figure 2 **RR of CHD Event According to Hp Genotype and HbA_{1c} Status in Studies to Date**

In a nested case-control study of CHD in women from the NHS, in men and women with diabetes from the ICARE study, in a nested case-control study of cardiovascular disease (CVD) in men and women from the Strong Heart Study (SHS) in which diabetes status serves as a proxy for HbA_{1c} ≥6.5%, and in all 3 studies pooled together (in the pooled analysis, the p for interaction = 0.004). The NHS analysis used logistic regression adjusted for age, body mass index, smoking, alcohol, history of hypercholesterolemia, and history of hypertension; the ICARE analysis used a proportional hazards model adjusted for sex, age, smoking status, hypertension, myocardial infarction before enrollment, statin use, and metformin use. The SHS analysis was unadjusted by using abstracted data (24). Abbreviations as in Figure 1.

trations (10,29), the Hp1 allele may delay onset of MI due to a better developed collateral circulation (18).

In the NHS, Hp genotype was associated with LDL cholesterol concentrations. Some previous studies (30), although not all (31), have found a similar association between Hp genotype and cholesterol concentrations. We did not observe an association in the ICARE analysis, but this cohort was limited to diabetic individuals only, the majority of who were receiving cholesterol-lowering medication. If an association between Hp genotype and LDL cholesterol exists, it may be due to linkage disequilibrium because the Hp gene is located in close proximity to the lecithin-cholesterol acyltransferase and cholesteryl ester transfer protein genes (32). However, in that case, we would expect to detect a difference in HDL concentrations among the Hp genotypes, which we did not observe.

Implications. Large randomized controlled trials of intensive glycemic control therapy among diabetic individuals have found significant reduction in cardiovascular outcomes in some (11), but not all (12,33), cohorts. This inconsistency may be due in part to different unknown characteristics among patient subgroups that have not yet been explored, as suggested in a meta-analysis of such trials by Ray et al. (34). The Hp genotype frequencies differ among populations (35) and may potentially explain differences in efficacy of glycemic control reported in the literature. Replication in a randomized trial of glycemic control, such as the ACCORD (Action to Control Cardiovascular Risk in Diabetes) study or the DCCT (Diabetes Control and Complications Trial), is required to confirm this finding. If replicated, Hp genotyping could potentially assist in identifying genetically susceptible individuals who would most benefit from clinical management of HbA_{1c} in the prevention of CHD.

Study strengths and limitations. Strengths of the CURRENT analysis include comprehensive data gathered prospectively with a long duration of follow-up, replication in a second cohort, and a validated Hp genotyping method. The availability of results from a third and previously published study extends the validity of the analysis, although further confirmation in larger cohorts is warranted, especially because the ICARE and SHS cohorts had a limited number of incident CHD cases, and in the pooled analysis in the current study, the majority of cases were derived from the NHS. The RR calculated for the SHS was not multivariate adjusted. However, there is little confounding and attenuation of the multivariate RR in the NHS and ICARE analyses; therefore, the crude RR for the SHS was likely minimally biased by confounding factors. The current study may have been underpowered to detect interactions between Hp genotype and CHD risk factors. Furthermore, for biomarkers assessed in stored plasma, such as HbA_{1c}, we only had a single measurement at baseline. Thus, random error caused by normal fluctuations over time would cause underestimation of true RRs. However, HbA_{1c} reflects glycemic control over an average of the 90-day red blood cell lifespan and is less susceptible to daily changes. We found intraclass correlations of 0.73 for repeated HbA_{1c} samples measured 3 years apart, which suggests good within-person reproducibility.

Conclusions

In 2 independent cohorts, we observed that participants with both the Hp2-2 genotype and an HbA_{1c} ≥6.5% had a 10-fold increased risk of CHD compared with those with at least 1 Hp1 allele and HbA_{1c} <6.5%. This finding, if further replicated in studies such as ACCORD or DCCT, may help explain inconsistencies in the literature for the association of glycemic control with CVD outcomes, as well as inconsistencies in previous studies of Hp genotype and CHD. If replicated, Hp genotyping could potentially assist in targeting cost-efficient clinical management of diabetes among the most genetically sensitive individuals.

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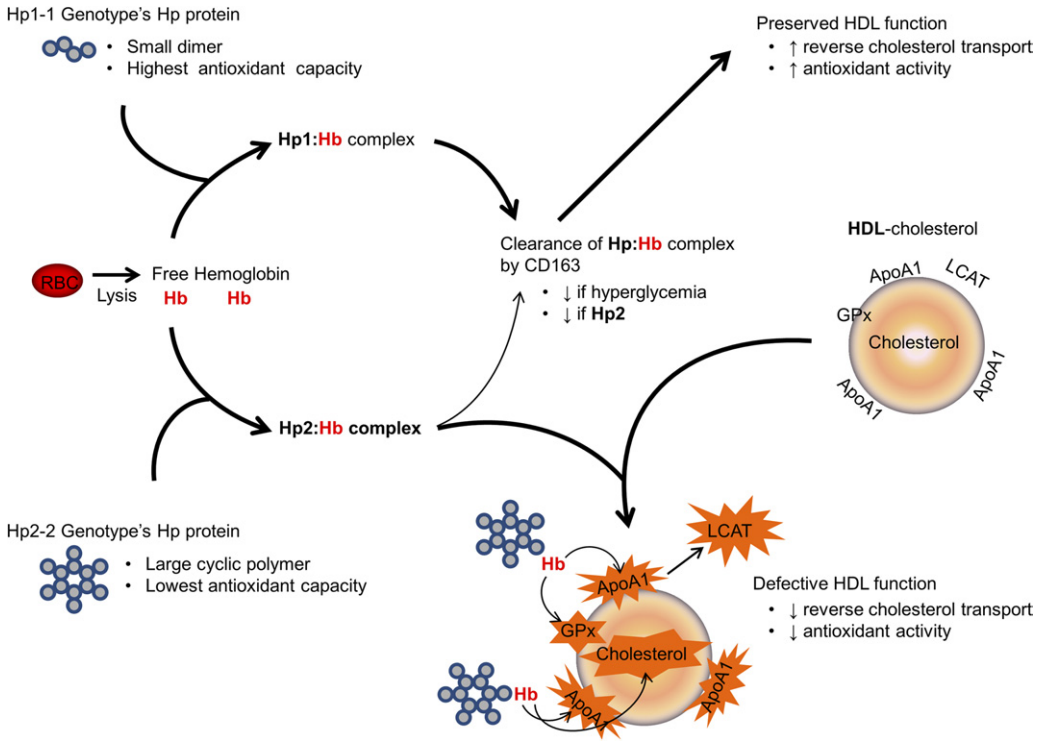

Figure 3 **Proposed Biological Mechanism to Explain Increased Risk of CHD in Hyperglycemic Individuals With the Hp2-2 Genotype**

Hemoglobin (Hb) released intravascularly from erythrocytes (red blood cells [RBC]) is rapidly bound by haptoglobin (Hp) protein to form an Hp–Hb complex that is cleared by scavenger receptor CD163. However, this clearance by CD163 is impaired in Hp2 as well as under hyperglycemic conditions in vivo, resulting in increased amounts of circulating Hp2:Hb complex in Hp2-2 individuals with hyperglycemia. Moreover, we have shown that glycosylation of Hb impairs the ability of the Hp2-2 protein to act as an antioxidant, thus resulting in increased oxidative activity of the glycosylated Hp2:Hb complex. This pro-oxidant Hp2:Hb complex can bind to high-density lipoprotein (HDL) and produce reactive oxygen species that oxidize cholesterol and its related components such as apolipoprotein A (ApoA1), glutathione peroxidase (GPx), and lecithin-cholesterol acyltransferase (LCAT), thereby decreasing the function of HDL as both an antioxidant and in reverse cholesterol transport. The Hp2-1 protein is a linear polymer, intermediate in size and antioxidant capacity (2). CHD = coronary heart disease. Adapted from Asleh et al. (27).

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Key Words: acute myocardial infarction ■ coronary disease ■ epidemiology ■ genetic association ■ genotype ■ glycoproteins.

APPENDIX

For a supplemental table, please see the online version of this article.