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Haptoglobin 2-2 genotype and the risk of Coronary Artery Disease in the Diabetes Control and Complications Trial/ Epidemiology of Diabetes Interventions and Complications Study (DCCT/EDIC)

Trevor J. Orchard, MD[1], **Jye-Yu C. Backlund, MPH**[2], **Tina Costacou, PhD**[1], **Patricia Cleary, MS**[2], **Maria Lopes-Virella, MD, PhD**[3], **Andrew P. Levy, MD, PhD**[4], **John M. Lachin, Sc. D**[2], and **and the DCCT/EDIC Research Group***

[1]Graduate School of Public Health, University of Pittsburgh, Haifa, Israel

[2]The Biostatistics Center, The George Washington University, Haifa, Israel

[3]Medical University of South Carolina, Haifa, Israel

[4]Israel Institute of Technology, Haifa, Israel

Abstract

Aims/hypothesis—Haptoglobin (Hp) 2-2 genotype has been shown to increase coronary artery disease (CAD) risk in numerous type 2 diabetes studies but in only one type 1 diabetes cohort. We assessed the association of Hp 2-2 with incident CAD over 26 years of follow-up in 1,303 Caucasian participants of the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) study.

Methods—DCCT randomized volunteers with type 1 diabetes to intensive versus conventional therapy within two cohorts: 'primary prevention' with 1–5 years diabetes duration and 'secondary intervention' with 1–15 years diabetes duration and early retinopathy, with or without albuminuria, but no advanced complications. CAD was defined as myocardial infarction (MI) or death judged to be from CAD, silent MI, angina, coronary revascularization, or congestive heart failure due to CAD.

Correspondence Author: John M. Lachin, Sc.D., Research Professor of Biostatistics and of Epidemiology, and of Statistics, The George Washington University Biostatistics Center, 6110 Executive Blvd., Rockville MD 20852, jml@bsc.gwu.edu, Voice: 301-816-8081.

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Author Contributions: Dr Lachin had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

T.J.O. researched the data, assisted with analysis and wrote the manuscript; J-Y.C.B. analyzed the data and wrote the manuscript; T.C. assisted with analysis and wrote the manuscript; P.C. reviewed/edited the manuscript; M.L.V. reviewed/edited the manuscript; A.P.L. researched the data, assisted with analysis and wrote the manuscript; J.M.L. researched the data, assisted with analysis and reviewed/ edited the manuscript.

Results—In the entire DCCT cohort, Hp 2-2 was not significantly associated with incident CAD or MI. However, in pre-specified exploratory subgroup analyses, an increased MI risk was suggested in the secondary cohort for those with Hp 2-2.

Conclusions/Interpretation—The analysis does not statistically confirm an overall association between Hp 2-2 and incident CAD, however, some suggestions of associations were observed in secondary analyses.

Introduction

Individuals with type 1 diabetes are at increased risk for cardiovascular complications even though, with the exception of hyperglycemia, traditional risk factor profiles are similar to, or even better than, those in the general population (1, 2). In 2005, the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) study demonstrated that intensive therapy yields substantial cardiovascular benefit in this patient population (3). Unfortunately, despite the reduction in CVD risk resulting from intensive diabetes management, aimed at achieving glycemic control as close to the non-diabetic range as possible, cardiovascular event rates in type 1 diabetes continue to be reported as being higher than that observed in the general population (4), suggesting that other factors, including genetic, may also play a role. One such genetic factor may comprise a copy number repeat polymorphism in the haptoglobin (Hp) gene (rs72294371).

Hp is a plasma glycoprotein considered to have anti-inflammatory and anti-oxidative properties (5). As inflammation and oxidation are also implicated in the development of atherosclerosis, it is possible that Hp may play an important role in determining susceptibility to early atherosclerotic disease. In humans, Hp is polymorphic with two major alleles which differ by the presence or absence of 1.7 kb partial intragenic duplication, and three genotypes denoted Hp 1-1, 2-1 and 2-2 (6). Structural and functional differences exist between the two Hp protein allele products, with the Hp 2-2 protein having less effective anti-inflammatory/anti-oxidative properties than Hp 1-1 (7–12).

These functional differences are thought to underlie the strong, consistent, findings of increased cardiovascular disease risk, particularly myocardial infarction, among Hp 2-2 genotype carriers as compared to non-Hp 2-2 genotype carriers in cohort studies of type 2 diabetes (13–16). In type 1 diabetes, the Pittsburgh Epidemiology of Diabetes Complications (EDC) study has been the only thus far to evaluate the role of the Hp genotype in the development of CAD, providing similar evidence for an increased risk with the Hp 2-2 genotype (17). In EDC, the Hp 2-2 genotype was also a strong predictor of declining renal function and progression to end-stage renal disease (18), findings which were recently confirmed in the DCCT/EDIC study (19), as well as of mortality attributed to cardiovascular or renal causes (20).

In the present investigation we sought to: 1) confirm the association between Hp genotype and CAD incidence previously demonstrated in type 1 diabetes mellitus (DM) in the EDC study cohort in the DCCT/EDIC cohort; and 2) determine whether the associations between Hp genotype and CAD and myocardial infarction are modified by either the original DCCT

treatment group (intensive or conventional) or cohort (primary prevention or secondary intervention) in the DCCT/EDIC cohort.

Research Design and Methods

Detailed descriptions of the methods of the DCCT and EDIC follow-up study have been published previously (19, 21–23). The DCCT, a randomized controlled clinical trial conducted between 1983 and 1993, compared the effects of an intensive diabetes treatment regimen (n=730) with those of conventional therapy (n=711) on the development and progression of microvascular complications. The primary prevention cohort included 726 subjects with diabetes for 1–5 years, no retinopathy, and <40 mg albuminuria per 24 h, and the secondary intervention cohort included 715 participants who had diabetes for 1–15 years with mild to moderate non-proliferative retinopathy and urinary albumin excretion rate <200 mg/d. At baseline, eligibility criteria excluded patients with a history of cardiovascular disease, hypertension or hypercholesterolemia (22).

Of the 1441 patients with type 1 diabetes, who were 13 to less than 40 years old at the time of randomization, 1422 completed the DCCT; the mean follow-up was 6.5 years. During the DCCT, intensive (INT) and conventional (CONV) therapies achieved mean HbA1c levels of 7% and 9% respectively (21). During a bridge period between the DCCT and EDIC, the original conventional group patients were instructed in intensive therapy and ongoing diabetes care was transferred back to the subject's local providers (23). In 1994, 1394 subjects of the surviving cohort agreed to participate in the long term EDIC observational follow-up study. All participants were encouraged to follow an intensive therapy regimen, and HbA1c has been similar in both groups within 5 years after the initiation of EDIC. This report includes data obtained through April 31, 2010 from 1303 Caucasian participants. Individuals who were determined to be admixed between Caucasian and other ethnic groups by population genetic approaches, using the software Eigenstrat (24), seeding with genotype data from the 3 major populations genotyped in HapMap phase II (25) (n=136), were excluded because of their small number and racial variation in Hp genotypes.

Study Procedures

Glycated hemoglobin was measured quarterly during DCCT and annually during EDIC (23, 26). Renal function (serum creatinine) was measured annually throughout DCCT and EDIC (27). Serum creatinine levels, age, sex and race were used to estimate glomerular filtration rate by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (28). Urinary albumin excretion rate was measured annually during DCCT and every other year during EDIC from a clinic-based, four hour timed, urine collection. Microalbuminuria and macroalbuminuria were defined as ≥30 mg in a 24-hour period at two consecutive study visits and ≥300 mg in a 24- hour period at a single visit, respectively (27).

Coronary artery disease (CAD) events were determined on the basis of clinical history and ECG recordings obtained annually or from death records. All hospitalized events and death records were reviewed by the Mortality and Morbidity Committee (MMC) to determine the presence of a CAD event defined as any of the following: fatal myocardial infarction (MI) or death judged to be from CAD by the MMC, non-fatal MI based on ECG finding and cardiac

enzyme level from the hospital records, silent MI based on significant annual serial changes in the ECG read by a central reading center, angina confirmed by ischemic changes with exercise tolerance testing or by clinically significant obstruction on coronary angiography, coronary revascularization, or congestive heart failure due to CAD.

Method for Hp typing

Serum samples from the 1303 subjects in this study were typed for Hp genotype by polyacrylamide gel electrophoresis (PAGE) and ELISA with no knowledge of patient identity. Two samples could not be Hp typed using either method. There was a difference in the Hp type obtained by PAGE and ELISA for five samples and for the analyses performed herein, the results obtained by PAGE were used. In 12 samples where an Hp type could not be obtained by PAGE, the Hp type used in this analysis was obtained by ELISA. Both the PAGE (29) and ELISA methods have been described in detail previously (19).

Statistical analysis

Due to the relatively small size of the cohort and resulting inadequate power to assess effects of the three individual genotypes, we pre-specified that the combined Hp 1-1 and Hp 2-1 group (or non-Hp 2-2) would be compared to the high risk Hp 2-2 genotype for all analyses. Clinical characteristics between the Hp 2-2 and non-Hp 2-2 groups were compared using the Wilcoxon rank sums test for continuous variables and the Chi-Square test for categorical variables. The cumulative incidence of CAD events between the Hp 2-2 and non-Hp 2-2 groups was estimated by the Kaplan-Meier method (30) and differences between groups were tested using the log-rank test (30). Cox proportional hazards models (30–31) were used to assess the effect of Hp type on the risk of CAD and MI/silent MI events without (unadjusted) and with (adjusted) other covariates to estimate the hazard ratio (HR) between Hp types, and group differences were assessed using the Wald test. The models also were adjusted for population stratification using three principal components of ancestry previously derived for DCCT/EDIC (32–33). The models were evaluated by diabetes treatment (INT vs. CON) and study cohort (primary prevention and secondary intervention) separately, were tested for interaction between Hp and treatment group or study cohort, and were tested for three-way interactions among Hp, treatment group, and study cohort.

All analyses were performed using the SAS software (version 9.3; SAS Institute, Cary, NC) and R package (34). A two-sided $p \leq 0.05$ was considered nominally statistically significant.

Results

Among the 1303 Caucasian subjects, 169 (13%) were Hp genotype 1-1, 618 (47%) were Hp 2-1, and 516 (40%) were Hp 2-2, conforming to Hardy-Weinberg equilibrium (p=0.92). Baseline associations comparing Hp 2-2 vs. non-Hp 2-2 study participants are presented in Table 1. There were no significant differences in baseline characteristics between Hp 2-2 and non-Hp 2-2.

Table 2 shows the overall number and proportion of participants with the Hp 2-2 versus non Hp 2-2 genotype who experienced any CAD event (n=149) or MI/silent MI (n=89) during a mean of 26 years of follow-up in the DCCT/EDIC. Figure 1 shows a small but consistent

difference between the time to the first CAD event among Hp 2-2 carriers and non-Hp 2-2 genotype carriers that was, however, non-significant (log-rank test p=0.205). Figure 2 explores this difference in time to the first CAD event further within treatment groups and study cohorts. Little difference was seen within the conventional treatment group for CAD in either the primary (p=0.49) or secondary cohort (p=0.53). However, in the intensive group, a borderline significant difference in CAD risk was seen between Hp genotypes in the secondary cohort (p=0.049 by the log-rank test), but not in the primary cohort (p=0.99). Thus, two-way interaction terms between the Hp genotype and either treatment group or study cohort on CAD were non-significant (p=0.17 for both interactions, Table 2), while a three-way interaction among Hp genotype, treatment group, and study cohort was nominally significant (p=0.049, Table 2), suggesting that the association between Hp and CAD incidence differed within subgroups of intensive vs. conventional therapy and primary vs. secondary cohort.

A borderline non-significant difference between Hp genotypes was observed for MI/silent MI alone (p=0.054) overall (Table 2), while the unadjusted HR was significantly increased for Hp 2-2 compared to non-Hp 2-2 in the secondary cohort (HR=2.02, CI: 1.28, 3.38, p=0.008). A two-way interaction between the Hp genotype and treatment group was not significant for the outcome of MI/silent MI (p=0.52), while the two-way interaction between Hp and study cohort as well as the three-way interaction among Hp genotype, treatment group, and study cohort were borderline non-significant (p=0.08 and p=0.07, respectively), suggesting that the effect of Hp on the incidence of MI/Silent MI may differ in those in the primary vs. secondary cohort as well as within treatment/cohort subgroups.

Adjusted Cox Proportional Hazards models are also shown in Table 2. There was little effect when adjusting for key covariates, which include albumin excretion rate and estimated glomerular filtration rate, and/or treatment group or cohort in the overall CAD analyses. Similarly, the associations observed within the cohort and treatment subgroups remained essentially unaltered after covariate adjustment. For the outcome of MI/silent MI, in the entire study population, adjustment for covariates reduced the HR for Hp 2-2 vs. non-Hp 2-2 from 1.51 (0.99, 2.28) to 1.42 (0.93, 2.16) while in the secondary cohort, the HR for MI/silent MI, remained significantly elevated at 1.91 (1.13, 3.22 p=0.016).

Discussion

The Pittsburgh EDC study demonstrated a significantly increased risk of CAD events in those carrying the Hp 2-2 vs. the Hp 1-1 genotype, with the fully adjusted HR of 2.21 (95% CI.1.05, 4.65, p=0.04) (17). However, in this analysis of the Caucasian DCCT/EDIC cohort, we failed to show a significantly increased risk of CAD events in those carrying the Hp 2-2 genotype. The proportion of cases by specific Hp genotype in DCCT/EDIC was too small to permit separate analyses by the three Hp genotypes as was done in EDC, where the proportion developing events was nearly three times as high (30% vs. 11% for CAD). However, the risk of CAD appeared somewhat increased in those Hp 2-2 DCCT/EDIC participants randomized to intensive therapy (HR 1.48; 0.91, 2.42) and in those recruited into the secondary prevention cohort (HR 1.47; 0.99, 2.18), where the majority of events occurred. The Hp 2-2 effect also appeared stronger for MI (HR 1.51; 0.99, 2.28) than for

total CAD, particularly for those in the secondary intervention cohort where a highly significant association was evident (HR 2.02; 1.20, 3.38 p=0.008).

Clearly the overall results lack statistical significance and are not confirmatory of the Hp 2-2 association for all CAD. However, it should be noted that in the EDC cohort, the Hp 2-2 genotype was associated with a significantly increased 36% in the incidence of CAD in type 1 individuals, somewhat lower than the 47% borderline significant increase seen in the current report for the DCCT/EDIC secondary cohort. Furthermore, given the overall lower event rate in DCCT/EDIC (11% vs. 30%), the current analysis had only 45% power to detect a statistically significant increase in CAD in participants with the Hp 2-2 genotype similar to the magnitude seen in EDC.

A critical difference between the EDC and DCCT/EDIC cohorts is the diabetes duration at baseline, which was 19 years in EDC compared to only 6 years in DCCT/EDIC. Participants in DCCT/EDIC are only now reaching the duration of diabetes that resulted in the association between Hp genotype and CAD incidence previously demonstrated in the EDC type 1 diabetes mellitus study. A stronger association was observed in the secondary DCCT/EDIC cohort, albeit only in the intensive treatment group, in which participants had a longer duration of diabetes and more advanced disease, and were thus more akin to the EDC population. This perhaps lends credence as to why the analysis of the entire DCCT/EDIC population failed to confirm the Hp 2-2 association with CAD. In addition, it should be noted that, in contrast to EDC, the DCCT/EDIC study excluded those at high risk of CAD at baseline.

The current findings concerning treatment group differences are of interest and require further exploration. The risk of CAD associated with Hp 2-2 seen in the intensively treated group (HR 1.48 (0.91–2.42), p=0.11) appears greater than that in the conventionally treated group (HR 1.05; 0.68, 1.61, p=0.83). Though this difference is clearly not significant, there was a nominally significant decrease in the time to a first CAD event in Hp 2-2 vs. non-Hp 2-2 (p=0.049 by log-rank analysis) in the intensively treated secondary cohort. A number of potential explanations of these findings arise.

First, it maybe that the weaker (relative to glycemic) Hp 2-2 effect can be observed only in those on intensive therapy, in whom the glycemic related risk is reduced. This would primarily be seen, as observed, in the secondary cohort where the majority of events occurred. Another potential explanation is that intensive treatment had no effect on the event rate in persons at genetically high risk by virtue of having Hp 2-2 (12.7% with intensive vs. 12.9% with conventional treatment) but did have a borderline significant effect in non-Hp 2-2 individuals (8.5% with intensive vs. with 12.6% conventional treatment, p=0.06).

It may also be possible that intensive treatment initiated very early on in the natural history of type 1 diabetes (as in the primary prevention cohort) provides greater benefits to those genetically susceptible to complication development (i.e. Hp 2-2). In contrast, starting intensive therapy post manifestation of early complications (as in the secondary intervention cohort) may not benefit these individuals as much, thus allowing the increased risk associated with Hp 2-2 to become apparent. Nevertheless, as the current study was not

sufficiently powered to detect an interaction between Hp genotype and treatment group (study power of less than 20%), these results need confirmation and no conclusions concerning differential effects of diabetes therapy by Hp genotype can be drawn at this time.

Finally, the finding that the relationship between the Hp genotype and CAD is stronger when assessing myocardial infarction as the outcome rather than using a broader definition of CAD is supported by mechanistic data, including evidence that Hp genotype stimulates opposing responses to intraplaque hemorrhage (35), a recognized event immediately prior to plaque rupture and myocardial infarction (36). These differences suggest that the likelihood of plaque rupture and myocardial infarction is higher in Hp 2-2, a hypothesis which is supported by studies in Hp 2-2 transgenic mice.

A limitation of these analyses is the small number of incident events, especially within the primary cohort and the intensively treated group, severely limiting our power to detect statistically significant associations. Nevertheless, the effect size observed for an Hp – CAD association in the secondary cohort, which more closely resembles the EDC population, was of the same direction and of greater magnitude than that previously reported within the EDC study. The small event number further affected our ability to conduct multivariable analyses when stratifying by both study cohort and treatment group (Table 2D). However, as no differences in baseline risk factors were observed by Hp genotype (Table 1), it is not clear that such adjustment would greatly alter study findings.

In conclusion, this DCCT/EDIC analysis does not statistically confirm an overall association between Hp 2-2 and incident CAD. However, subgroup analyses in the DCCT/EDIC study suggest that increased CAD risk in the Hp 2-2 vs. non-Hp 2-2 groups may be stronger in those recruited into the secondary cohort, those randomized to intensive therapy and for the harder outcome of myocardial infarction. These findings should be explored in other cohorts.

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Conflict of Interest Disclosures: TJO consultant (Advisory Board) for Eli Lilly Inc. and Profil Institute for Scientific Research. APL's institution, Israel Institute of Technology, Haifa, Israel, owns a patent which claims that haptoglobin genotype can predict risk of diabetes complications.

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Highlights

- We investigated the association between the Hp 2-2 genotype and CAD incidence in the DCCT/EDIC cohort.
- Hp 2-2 was not significantly associated with incident CAD.
- An increased MI risk was suggested in the secondary cohort for those with Hp 2-2.

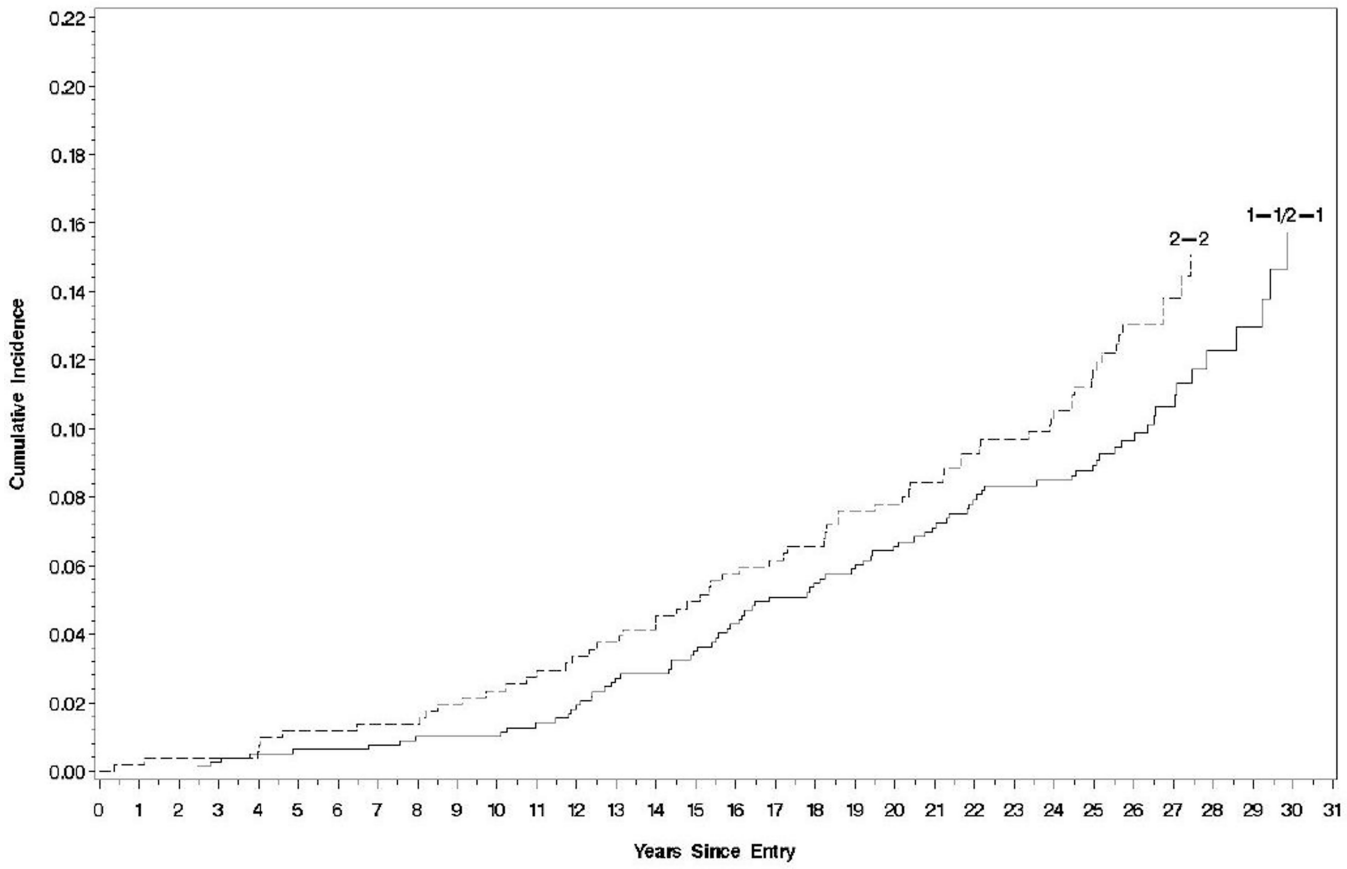

Figure 1.

Time to the first CAD event by haptoglobin genotype Hp 2-2 vs. non-HP 2-2 (Log-rank test p-value, p=0.205).

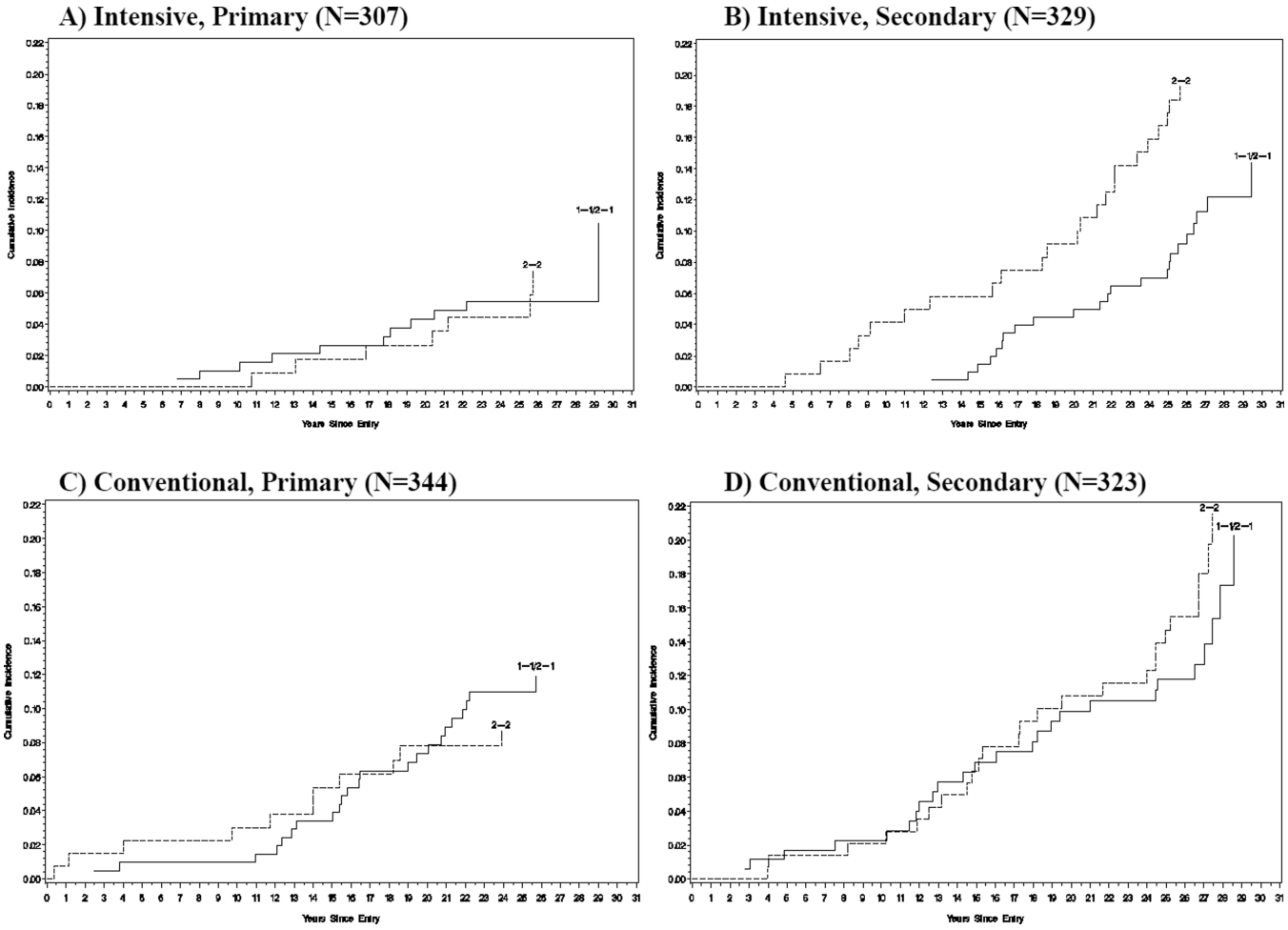

Figure 2.

Time to the first CAD event by haptoglobin genotype Hp 2-2 (dashed lines) vs. non-Hp 2-2 (solid lines), stratified by intensive vs. conventional treatment group and primary vs. secondary cohort. Number of observations and log-rank test p-value are: A) Intensive and Primary, N=307, p=0.992. B) Intensive and Secondary, N=329, p=0.049. C) Conventional and Primary, N=344, p=0.486. D) Conventional and Secondary, N=323, p=0.528.

Table 1

Baseline Characteristics by Hp genotype

	HP 1-1 or HP 2-1 (N=787)	HP 2-2 (N=516)	p-value*
Intensive treatment group	399 (51)	237 (46)	0.092
Age (years)	27 ± 7	27 ± 7	0.667
BMI (kg/m^2)	23.4 ± 2.8	23.4 ± 2.8	0.730
Duration of type 1 diabetes in months	66 ± 49	70 ± 51	0.259
Female	379 (48)	230 (45)	0.205
Baseline HbA1c (%)	8.8 ± 1.5	8.9 ± 1.6	0.104
Baseline HbA1c (mmol/mol)	72.7 ± 16.9	74.3 ± 17.6	0.104
Primary cohort	402 (51)	249 (48)	0.319
Clinical neuropathy = yes	48 (6)	32 (6)	0.958
Smoker = yes	145 (18)	101 (20)	0.604
LDL (mg/dL)	108 ± 29	111 ± 30	0.082
HDL (mg/dL)	50 ± 12	50 ± 12	0.652
Triglycerides (mg/dL)	82 ± 49	82 ± 48	0.682
Cholesterol (mg/dL)	175 ± 33	178 ± 35	0.132
Blood pressure (mm Hg)			
Systolic	114 ± 12	115 ± 11	0.171
Diastolic	73 ± 9	72 ± 9	0.990
Estimated Glomerular Filtration Rate (mL/min/1.73m^2)	125.5 ± 14.0	126.0 ± 13.3	0.441
Albumin excretion rate (mg/24 h)	15.8 ± 17.3	15.9 ± 20.8	0.862

Data are N (%) and mean ± SD.

*Wilcoxon for continuous variables and chi-square test for categorical variables.

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Table 2

Cox Proportional Hazards models of CAD and of MI/silent MI for Haptoglobin 2-2 genotype versus Haptoglobin 1-1/2-1 genotype, both unadjusted and adjusted for other factors.

A. Overall

	CAD								
		Unadjusted		Adjusted for covariates * and treatment group		Adjusted for covariates * and study cohort		Adjusted for covariates * and treatment group and lipid-lowering medication †	
	N(%)	Hazard Ratio (95% C.I.)	p-value	Hazard Ratio (95% C.I.)	p-value	Hazard Ratio (95% C.I.)	p-value	Hazard Ratio (95% C.I.)	p-value
Total	149(11.4)								
HP 1-1/HP 2-1	83(10.6)	1 Reference		1 Reference		1 Reference		1 Reference	
HP 2-2	66(12.8)	1.23(0.89, 1.70)	0.205	1.17(0.85, 1.63)	0.338	1.20(0.86, 1.66)	0.282	1.18(0.85, 1.63)	0.335
HP Interaction by group or cohort				0.169		0.169			
Group by Cohort by HP 3 way interaction						0.049			
	MI/Silent MI								
		Unadjusted		Adjusted for covariates * and treatment group		Adjusted for covariates * and study cohort		Adjusted for covariates * and treatment group and lipid-lowering medication †	
	N(%)	Hazard Ratio (95% C.I.)	p-value	Hazard Ratio (95% C.I.)	p-value	Hazard Ratio (95% C.I.)	p-value	Hazard Ratio (95% C.I.)	p-value
Total	89(6.8)								
HP 1-1/HP 2-1	45(5.7)	1 Reference		1 Reference		1 Reference		1 Reference	
HP 2-2	44(8.5)	1.51(0.99, 2.28)	0.054	1.42(0.93, 2.16)	0.106	1.43(0.94, 2.18)	0.093	1.43(0.94, 2.17)	0.099
HP Interaction by group or cohort				0.519		0.077			
Group by Cohort by HP 3 way interaction						0.073			

B. by Treatment Group

	CAD									
	Intensive Treatment Group					Conventional Treatment Group				
		Unadjusted		Adjusted for covariates *			Unadjusted		Adjusted for covariates *	
	N(%)	Hazard Ratio (95% C.I.)	p-value	Hazard Ratio (95% C.I.)	p-value	N(%)	Hazard Ratio (95% C.I.)	p-value	Hazard Ratio (95% C.I.)	p-value
N	64(10.1)					85(12.7)				
HP 1-1 /HP 2-1	34(8.5)	1 Reference		1 Reference		49(12.6)	1 Reference		1 Reference	
HP 2-2	30(12.7)	1.48(0.91, 2.42)	0.115	1.43(0.88, 2.36)	0.152	36(12.9)	1.05(0.68, 1.61)	0.831	0.99(0.63, 1.54)	0.947

	MI/Silent MI									
	Intensive Treatment Group					Conventional Treatment Group				
		Unadjusted		Adjusted for covariates *			Unadjusted		Adjusted for covariates *	
	N(%)	Hazard Ratio (95% C.I.)	p-value	Hazard Ratio (95% C.I.)	p-value	N(%)	Hazard Ratio (95% C.I.)	p-value	Hazard Ratio (95% C.I.)	p-value
N	38(6.0)					51(7.6)				
HP 1-1 /HP 2-1	19(4.8)	1 Reference		1 Reference		26(6.7)	1 Reference		1 Reference	
HP 2-2	19(8.0)	1.67(0.88, 3.15)	0.116	1.62(0.85, 3.10)	0.143	25(9.0)	1.37(0.79, 2.38)	0.258	1.25(0.71, 2.20)	0.433

C. by Study Cohort

	CAD									
	Primary Prevention Cohort					Secondary Prevention Cohort				
		Unadjusted		Adjusted for covariates *			Unadjusted		Adjusted for covariates *	
	N(%)	Hazard Ratio (95% C.I.)	p-value	Hazard Ratio (95% C.I.)	p-value	N(%)	Hazard Ratio (95% C.I.)	p-value	Hazard Ratio (95% C.I.)	p-value
N	52(8.0)					97(14.9)				
HP 1-1 /HP 2-1	34(8.5)	1 Reference		1 Reference		49(12.7)	1 Reference		1 Reference	

C. by Study Cohort

HP 2-2	18(7.2)	0.86(0.48, 1.52)	0.594	0.83(0.46, 1.49)	0.526	48(18.0)	1.47(0.99, 2.18)	0.059	1.45(0.97, 2.18)	0.070

	MI/Silent MI									
	Primary Prevention Cohort					Secondary Prevention Cohort				
		Unadjusted		Adjusted for covariates *			Unadjusted		Adjusted for covariates *	
	N(%)	Hazard Ratio (95% C.I.)	p-value	Hazard Ratio (95% C.I.)	p-value	N(%)	Hazard Ratio (95% C.I.)	p-value	Hazard Ratio (95% C.I.)	p-value
N	30(4.6)					59(9.0)				
HP 1-1 /HP 2-1	20(5.0)	1 Reference		1 Reference		25(6.5)	1 Reference		1 Reference	
HP 2-2	10(4.0)	0.80(0.38, 1.72)	0.572	0.78(0.36, 1.70)	0.529	34(12.7)	2.02(1.20, 3.38)	0.008	1.91(1.13, 3.22)	0.016

D. by Treatment Group and Study Cohort (Unadjusted)

	CAD											
	Intensive Treatment Group						Conventional Treatment Group					
	Primary Prevention Cohort, Unadjusted			Secondary Prevention Cohort, Unadjusted			Primary Prevention Cohort, Unadjusted			Secondary Prevention Cohort, Unadjusted		
	N(%)	Hazard Ratio (95% C.I.)	p-value	N(%)	Hazard Ratio (95% C.I.)	p-value	N(%)	Hazard Ratio (95% C.I.)	p-value	N(%)	Hazard Ratio (95% C.I.)	p-value
N	18(5.9)			46(14.0)			34(9.9)			51(15.8)		
HP 1-1 /HP 2-1	11(5.7)	1 Reference		23(11.1)	1 Reference		23(11.0)	1 Reference		26(14.6)	1 Reference	
HP 2-2	7(6.1)	1.01(0.39, 2.59)	0.992	23(18.9)	1.78(1.00, 3.17)	0.052	11(8.2)	0.78(0.38, 1.59)	0.487	25(17.2)	1.19(0.69, 2.07)	0.528

	MI/Silent MI											
	Intensive Treatment Group						Conventional Treatment Group					
	Primary Prevention Cohort, Unadjusted			Secondary Prevention Cohort, Unadjusted			Primary Prevention Cohort, Unadjusted			Secondary Prevention Cohort, Unadjusted		
	N(%)	Hazard Ratio (95% C.I.)	p-value	N(%)	Hazard Ratio (95% C.I.)	p-value	N(%)	Hazard Ratio (95% C.I.)	p-value	N(%)	Hazard Ratio (95% C.I.)	p-value

D. by Treatment Group and Study Cohort (Unadjusted)

N	11(3.6)			27(8.2)			19(5.5)			32(9.9)		
HP 1-1 /HP 2-1	8(4.2)	1 Reference		11(5.3)	1 Reference		12(5.7)	1 Reference		14(7.9)	1 Reference	
HP 2-2	3(2.6)	0.59(0.16, 2.22)	0.435	16(13.1)	2.56(1.19, 5.53)	0.017	7(5.2)	0.95(0.37, 2.41)	0.912	18(12.4)	1.60(0.79, 3.21)	0.189

*Unadjusted PH models assessed the difference in HP 2-2 versus 1-1 and 2-1 combined without adjustment for other factors. Baseline covariates employed in adjusted models were gender, DCCT baseline age, duration, BMI, HbA1c, LDL, systolic blood pressure, smoking, triglycerides, albumin excretion rate, glomerular filtration rate, and three principal components of ancestry.

†Lipid lowering medication is a time-dependent covariate.