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Glycaemic control modifies the haptoglobin 2 allele-conferred susceptibility to coronary artery disease in Type 1 diabetes

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Abstract

Aims—We aimed to assess whether the association of the haptoglobin 2 allele with coronary artery disease is modified by glycaemic control in a prospective cohort study of individuals with childhood-onset Type 1 diabetes.

Methods—Coronary artery disease events (death from coronary artery disease, confirmed myocardial infarction, stenosis ≥50%, revascularization) were assessed between 1986 and 2013 among 480 individuals with Type 1 diabetes (baseline age 28 years; diabetes duration 19 years). Better glycaemic control was defined as an updated mean HbA_{1c} during follow-up of <8% (64 mmol/mol).

Results—In crude models, the incidence of coronary artery disease increased with the number of haptoglobin 2 alleles (hazard ratio 1.34, 95% CI 1.05–1.71). This association was more pronounced in those with better than in those with worse glycaemic control (*P* interaction = 0.05) and remained essentially unaltered after multivariable adjustments (hazard ratio 2.65, 95% CI 1.30–5.41 in those with better glycaemic control and hazard ratio 1.20, 95% CI 0.93–1.56 in those with worse glycaemic control).

Conclusions—These results suggest that, although better control may reduce the incidence of coronary artery disease in Type 1 diabetes, a residual risk related to the Hp 2 allele remains.

Introduction

It has been shown in several prospective studies that the haptoglobin (Hp) 2 allele increases the risk of coronary artery disease (CAD) among individuals with a diabetes diagnosis or glucose levels indicative of the presence of diabetes, while similarly strong associations have not been observed in the general population [1–7]. A major function of Hp is to bind free haemoglobin, inhibiting heme iron release, and thus, reducing oxidative tissue damage [8]. Of the three major Hp genotypes (Hp 1-1, Hp 2-1 and Hp 2-2), the Hp 1-1 protein possesses superior antioxidant and anti-inflammatory properties compared with the other two genotypes [8–9]. Although these functional differences across Hp genotypes appear immaterial in the absence of diabetes, the elevated levels of haemoglobin, especially HbA_{1c}, in diabetes and their effect on Hp's antioxidant function are thought to contribute to this

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Competing interests

None declared.

apparent interaction by diabetes status and to the observed increased cardiovascular risk with the Hp 2 allele in diabetes [9]. In the present study, we investigated whether the risk associated with Hp 2 is further related to the level of glycaemic control in a cohort with Type 1 diabetes.

Research Design and Methods

The present investigation was based on data collected over the 25-year follow-up of the Epidemiology of Diabetes Complications (EDC) study of childhood-onset Type 1 diabetes ($n=658$). The EDC is a historical, prospective cohort of incident cases of childhood-onset Type 1 diabetes [mean (range) age at onset 8 (0.25– 6.26) years], diagnosed or seen within 1 year of diagnosis (1950–1980) at the Children's Hospital of Pittsburgh. Surveys (biennial) or clinical examinations (biennial for 10 years, then at 18 and 25 years) followed a first clinical assessment, conducted between 1986 and 1988, when the average participant age and diabetes duration were 28 and 19 years, respectively. The University of Pittsburgh institutional review board approved the study protocol and all study participants provided written informed consent.

Demographic, healthcare, diabetes self-care and medical history information was ascertained biennially. Blood pressure was measured using a random zero sphygmomanometer after a 5-min rest [10], and hypertension was defined as blood pressure $>140/90$ mm Hg or use of antihypertensive medications. Stable HbA_{1c} levels were measured using ion exchange chromatography (Isolab; Akron, OH, USA) and subsequently by automated high-performance liquid chromatography (Diamat; BioRad, Hercules, CA, USA). The two assays were highly correlated ($r=0.95$). Beyond the 10-year follow-up, HbA_{1c} was measured using the DCA 2000 Analyser (Bayer, Tarrytown, NY, USA). The DCA and Diamat assays were also highly correlated ($r=0.95$). Original HbA_{1c} (1986–1998) and HbA_{1c} values from 1998–2004 were converted to Diabetes Control and Complications Trial-aligned standard HbA_{1c} values before analyses [4]. The updated mean HbA_{1c} values were calculated as the mean of all HbA_{1c} values available until censorship for non-cases, or for the examination cycle prior to an incident CAD event for cases. 'Better' glycaemic control was defined as an updated mean HbA_{1c} below the 25th percentile of $<8\%$ (<64 mmol/mol). Cholesterol, triglycerides [11–12] and HDL cholesterol [13] were determined enzymatically. Non-HDL cholesterol was calculated as total cholesterol minus HDL cholesterol. Urinary albumin was measured by immunonephelometry [14] and creatinine by an Ectachem 400 Analyzer (Eastman Kodak Co., Rochester, NY, USA). GFR values were estimated [15].

High molecular weight genomic DNA was isolated using the PureGene kit (Gentra Systems, Minneapolis, MN, USA), and Hp was genotyped using an amplification method [16]. Genotypes were assigned visually by comparison with controls of known genotype. CAD was defined as myocardial infarction confirmed by Q-waves on electrocardiogram (Minnesota codes 1.1 or 1.2) or hospital records, angiographic stenosis $\geq 50\%$, revascularization or death from CAD.

Statistical analysis

The chi-squared test or Fisher's exact test, as appropriate, was used to determine univariate associations. Cox proportional hazards models were constructed to assess any modification of the effect of Hp on CAD incidence by level of glycaemic control, by including an interaction term (Hp $\times$ mean HbA_{1c} category), along with the lower order terms, and following adjustment for diabetes duration. Multivariable Cox models were also subsequently constructed, to assess the independence of the Hp–CAD association. Survival time was defined as the time in years from study entry to either an incident event or censorship (including death or end of follow-up).

Results

Of 658 study participants, 486 had DNA available for the assessment of Hp genotype. Six of those individuals had pre-existing CAD at study entry and were thus excluded from further analyses. Compared with individuals with DNA available, those without DNA were more likely to have had longer diabetes duration, higher levels of HbA_{1c}, blood pressure, non-HDL cholesterol, albuminuria and worse kidney function at study entry.

The characteristics of the remaining 480 individuals by Hp genotype are shown in Table S1. During 25 years of follow-up (mean follow-up, 19 years), 155 (32.3%) developed an incident event. In the univariate analysis, incidence increased incrementally with the number of Hp 2 alleles ($P = 0.04$, $P_{\text{trend}} = 0.10$) while in time-to-event analyses adjusting for diabetes duration, a 34% increased CAD risk was observed with each additional Hp 2 allele (Table 1). This trend appeared stronger with the number of Hp 2 alleles with better [hazard ratio (HR) 2.50, 95% CI 1.23–5.08] compared with worse (HR 1.19, 95% CI 0.92–1.54) glycaemic control over time, after adjustment for diabetes duration ($P_{\text{interaction}} = 0.05$). Examination of the effect of glycaemia on CAD incidence within each Hp genotype showed that, although better control was generally associated with lower event rates, statistical significance was reached only among the less prevalent Hp 1-1 carriers.

These associations were maintained in multivariable Cox models allowing for duration, gender, BMI, HbA_{1c}, hypertension, lipids, albumin excretion rate and estimated GFR. Thus, in the overall cohort, CAD risk increased by 35% with each additional Hp 2 allele (95% CI 1.05–1.73; $P = 0.02$). After stratification by glycaemic control status over time, the risk increased significantly with the number of Hp 2 alleles among those in better control (HR 2.65, 95% CI 1.30–5.41; $P = 0.007$), but not in those with worse control overtime (HR 1.20, 95% CI 0.93–1.56; $P = 0.17$).

Discussion

Among individuals with childhood-onset Type 1 diabetes followed prospectively for 25 years, we observed that the association between the Hp 2 allele and the incidence of hard CAD endpoints is modified by an individual's long-term glycaemic control status. Contrary to our expectations, however, the risk of CAD associated with the Hp 2 allele appeared greater among individuals with better, not worse, glycaemic control over time. In addition, although incidence increased with worse glycaemic control across all three Hp genotypes,

statistical significance was observed only among Hp 1-1 carriers. The small sample size, however, precludes definitive conclusions.

The findings of the present study appear contrary to a previous report where, in two cohorts, an increased hazard associated with the Hp 2-2 genotype was restricted to individuals with Type 2 diabetes or HbA_{1c} levels >48 mmol/mol (6.5%) and thus indicative of the presence of diabetes [6]; however, it should be noted that our population comprised individuals with a long duration of Type 1 diabetes, among whom only four had mean HbA_{1c} concentrations < 48 mmol/mol (6.5%). The present results cannot be directly compared, therefore, with results derived from the previous investigation. Nonetheless, it is difficult to reconcile the present observations that HbA_{1c} levels > 48 mmol/mol (6.5%) are required to reveal the Hp 2–CAD association, yet the strength of the same association is inversely related to levels above the 48 mmol/mol (6.5%) threshold. Combined, these findings suggest that a range of glycaemic levels exists, within which the risk associated with this genetic marker becomes manifest, yet below which (i.e. absence of diabetes) this genetic effect is completely offset and above which this effect is somewhat attenuated (i.e. the group with worse glycaemic control in the present analyses).

Undoubtedly, the small sample size of the present study population, especially when stratified by long-term glycaemic control status and Hp genotype limits our ability to draw definitive conclusions; however, the present and previously reported findings on the risk conferred by the Hp 2 allele in diabetes cannot be ignored, and these results are concerning both because of the consistency and strength of this association [1–7] as well as because Hp 2 allele carriers represent the vast majority (>80%) of individuals with diabetes. Importantly, clinical trial evidence for cardiovascular disease prevention in Type 1 diabetes has, until now, focused only on glycaemic control [17]. The present data underscore the importance of understanding other factors in cardiovascular risk assessment, beyond hyperglycaemia. The importance of the Hp 2 allele with regard to CAD risk can be expected to increase with time as more patients achieve better control. This then raises the question as to what alternative strategies could reduce or eliminate the Hp 2 allele-associated genetic susceptibility. In Type 2 diabetes, clinical trial evidence points to a reduction in cardiovascular events with vitamin E supplementation among Hp 2-2 carriers [5,18–19]. Similar trial evidence does not exist in Type 1 diabetes, although in a small intervention trial, vitamin E appeared to improve HDL-mediated reverse cholesterol transport, a proposed mechanism by which Hp relates to CAD, among Hp 2-2 carriers [20]. In conclusion, while the present findings require validation in further cohorts, the effects of the genetic polymorphism in the Hp gene raises new avenues regarding cardiovascular disease prevention in Type 1 diabetes, particularly for those with residual risk, despite better glycaemic control.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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What's new?

- The haptoglobin (Hp) 2 allele has been shown to increase the risk of coronary artery disease (CAD) among individuals with diabetes, while, in the absence of diabetes, an Hp 2 allele–CAD association was not apparent.
- We assessed whether glycaemic control modifies the Hp genotype–CAD association in people with Type 1 diabetes.
- We noted significant modification of the Hp effect by long-term glycaemic levels, such that the adverse Hp 2 allele effect was more pronounced in people with better glycaemic control.
- Our results suggest that, although better glycaemic control may reduce CAD incidence in Type 1 diabetes, a residual risk associated with the Hp 2 allele remains.

Table 1

Incidence of hard coronary artery disease endpoints by haptoglobin genotype and level of glycaemic control over time

	Hp 1-1	Hp 2-1	Hp 2-2	P trend	HR (95% CI) [*]	HR (95% CI) [†]
Total cohort	23.7 (14/59)	32.2 (69/214)	35.7 (74/207)	0.10	1.34 (1.05-1.71)	1.35 (1.05-1.73)
By HbA _{1c} over time						
Total cohort						
<8% [‡] (n=117)	0.0 (0/11)	23.2 (13/56)	28.0 (14/50)	0.09	2.50 (1.23-5.08)	2.65 (1.30-5.41)
8% (n=363)	29.2 (14/48)	35.4 (56/158)	38.2 (60/157)	0.27	1.19 (0.92-1.54)	1.20 (0.93-1.56)
P [§]	0.05	0.09	0.19			

Hp, haptoglobin; HR, hazard ratio.

^{*} HR (95% CI) for coronary artery disease incidence with the number of Hp 2 alleles, adjusting for diabetes duration.

[†] HR (95% CI) for coronary artery disease incidence with the number of Hp 2 alleles, allowing for diabetes duration, gender, BMI, HbA_{1c}, hypertension, lipids, albumin excretion rate and estimated GFR.

[‡] An HbA_{1c} value of 8% corresponds to 64 mmol/mol.

[§] P-value for an association between HbA_{1c} level overtime and coronary artery disease incidence within Hp genotype.