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The Haptoglobin genotype predicts cardio-renal mortality in type 1 diabetes

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ABSTRACT

Aims: The Haptoglobin (*HP*) 2-2 genotype increases cardiovascular diabetes complication incidence. In type 1 diabetes, *HP* 2-2 also predicts declining kidney function and end-stage renal disease. We investigated whether *HP* 2-2 predisposes to cardio-renal mortality, while considering other causes of death as competing risks.
Methods: Individuals with type 1 diabetes and *HP* data available (n = 486; mean baseline age, 27 and duration, 19 years) were selected for study. Vital status was assessed as of 8/31/2014. The underlying cause of death was determined and classified based on standardized procedures.
Results: During 25 years of follow-up, 79 (16.3%) cardio-renal deaths and 43 (8.8%) deaths related to other causes occurred. Although total mortality did not differ by *HP* (25.4% with *HP* 1 versus 24.6% with *HP* 2-2, p = 0.84), a greater proportion of *HP* 2-2 carriers exhibited a cardio-renal death (19.0 versus 14.2, p = 0.05). In time-to-event analyses, *HP* 2-2 was associated with a statistically significant increase of the sub-distribution hazard ratio for cardio-renal mortality (HR = 1.64, p = 0.03), although this effect was somewhat attenuated after multivariable adjustment (HR = 1.58, p = 0.05).
Conclusions: Our results suggest that in addition to predicting the incidence of cardio-renal complications, *HP* 2-2 also increases susceptibility for cardio-renal mortality in type 1 diabetes. These findings require validation in other cohorts.

1. Introduction

Haptoglobin is an acute phase plasma glycoprotein whose name (from the Greek verb "άπτειν", haptein, to bind) conveys its major function, binding free hemoglobin, thereby inhibiting heme iron release and thus reducing free hemoglobin's oxidative potential (Langlois & Delanghe, 1996). The haptoglobin–hemoglobin complex formed through this binding is subsequently primarily cleared from circulation by attaching to the CD163 receptor on monocytes/macrophages while a secondary pathway for excretion is through the kidneys (Langlois & Delanghe, 1996). Two major haptoglobin alleles exist in humans: the *HP* 1 and the *HP* 2, the latter formed from the duplication of exons 3 and 4 of the *HP* 1 allele (Langlois & Delanghe, 1996). As *HP* is a copy number variant (CNV), currently available GWAS versions cannot tag *HP* CNV (Adams et al., 2013; Cahill, Jensen, et al., 2013; Rodriguez et al., 2012). *HP* is a known functional polymorphism, however, with well described structural and functional differences across the genotypes. Thus, the *HP* 1-1 protein (dimer) has been ascribed superior antioxidant and anti-inflammatory properties compared to the other two genotypes (*HP* 2-1 and *HP* 2-2), whereas the *HP* 2 allele is a more efficient angiogenic factor (Asleh et al., 2008; Langlois & Delanghe, 1996; Philippidis et al., 2004).

Several prospective studies of type 2 diabetes have provided evidence that the *HP* 2-2 genotype directly predicts the development of cardiovascular events (Adams et al., 2013; Cahill, Levy, et al., 2013; Levy et al., 2002; Roguin et al., 2003; Suleiman et al., 2005), likely owing to its inferior antioxidant capacity and inefficient clearance of hemoglobin (Asleh et al., 2008; Langlois & Delanghe, 1996). To date, the role of this polymorphism in the pathogenesis of cardiovascular disease in type 1 diabetes remains largely unexplored. Our finding in the Epidemiology of Diabetes Complications (EDC) study of increased susceptibility to coronary artery disease (CAD) conferred by the *HP* 2 allele (Costacou, Ferrell, & Orchard, 2008) has yet to be validated in other prospective cohorts, although a similarly increased risk was observed for the incidence of coronary artery calcification in the Coronary Artery Calcification in Type 1 Diabetes (CACTI) study (Simpson et al., 2011). Despite the abundance of data rendering *HP* 2 as a risk factor for CAD, other EDC findings suggest a protective role for this allele against the extent of white matter hyperintensities (Costacou, Rosano, et al., 2015) and stroke incidence (Costacou, Secrest, Ferrell, & Orchard, 2014). This contrasting finding may be mediated by the *HP* 2 allele's enhanced angiogenic activity (Langlois & Delanghe, 1996).

Duality of interest: The authors have no conflicts of interest.

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In contrast to heart disease for which abundant evidence exists in type 2 diabetes, prospective data for an association between the *HP* genotype and kidney disease appear thus far limited to studies in type 1 diabetes, where our observation of a twofold increased risk of declining kidney function and end-stage renal disease with *HP* 2-2 (Costacou, Ferrell, Ellis, & Orchard, 2009) was recently confirmed in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) study (Orchard et al., 2013). However, whether *HP* has the ability to predict cardio-renal-specific mortality in diabetes has yet to be evaluated. We therefore assessed the presence of such an association in a childhood-onset type 1 diabetes cohort recruited in the late 1980s and followed prospectively for 25 years.

2. Material and Methods

The Pittsburgh EDC, on which the present investigation was based, is a representative, historical, cohort of incident cases of childhood-onset type 1 diabetes, diagnosed or seen within one year of diagnosis (1950–80) at Children's Hospital of Pittsburgh (Orchard et al., 1990). A first clinical EDC assessment took place in 1986–88 (mean age, 28 and diabetes duration, 19 years). Surveys (biennial) and clinical examinations (biennial for 10 years and again at 18 and 25 years) followed for over 25 years. The University of Pittsburgh IRB approved the study protocol.

At each assessment, participants provided demographic, health care, self-care, and medical history information. During the clinical examinations, blood pressure was measured with a random zero sphygmomanometer, after a five minute rest. Hypertension was defined as >140/90 mmHg or antihypertensive medication use. Stable glycosylated hemoglobin (HbA1) was measured by ion exchange chromatography (Isolab, Akron, OH) and subsequently by automated high-performance liquid chromatography (Diamat, BioRad, Hercules, CA). The two assays were highly correlated ($r = 0.95$). HDL cholesterol (HDLc) was determined by a precipitation technique (Warnick & Albers, 1978); cholesterol and triglycerides enzymatically (Allain, Poon, Chan, Richmond, & Fu, 1974; Bucolo & David, 1973); and non-HDLc was calculated as total minus HDLc. Urinary albumin was measured by immunonephelometry (Ellis & Buffone, 1977) and creatinine was assayed by an Ectachem 400 Analyzer (Eastman Kodak Co., Rochester, NY). Glomerular filtration rate was estimated (eGFR) (Levey et al., 2009). High molecular weight genomic DNA was isolated using the PureGene kit (Gentra Systems, Minneapolis, MN), and *HP* was genotyped by an amplification method. Genotypes were assigned visually by comparison to controls of known genotype (Koch et al., 2002). We have previously shown good agreement between our genotyping and a sandwich enzyme immunoassay (EIA) for the haptoglobin gene product (kappa = 0.92, $p < 0.0001$) (Victor et al., 2009).

Vital status was assessed as of August 31, 2014. In addition to death certificates, medical records, autopsy/coroner's reports, and/or interviews with next-of-kin regarding the circumstances surrounding the death were also obtained when possible. The underlying cause of death was determined and classified (i.e. cardiovascular, renal, acute complication, accident/suicide, infection, cancer, other diabetes related, or other non-diabetes related) by a Mortality Classification Committee of at least two physician epidemiologists using all available data, based on standardized procedures as previously published (Diabetes Epidemiology Research International Mortality Study Group, 1991). For the present analysis, cardio-renal deaths comprised all cases for which the underlying cause was due to either cardiovascular or renal complications. The distribution of the underlying and secondary causes of death among deceased participants is displayed in Table 1.

Table 1
Distribution of the underlying and/or secondary causes of death among deceased (n = 122).

	Total cohort (n = 122)	*HP* 1-1/2-1 (n = 70)	*HP* 2-2 (n = 52)
Underlying cause (%, n)			
Cardio-renal complications	64.7 (79)	55.7 (39)	76.9 (40)
-Cardiovascular (n)	63	30	33
-Renal (n)	16	9	7
Acute complications	9.8 (12)	14.3 (10)	3.8 (2)
-Diabetic ketoacidosis (n)	5	5	
-Diabetic coma (n)	2	2	
-Hypoglycemia (n)	5	3	2
Accident/suicide	4.9 (6)	5.7 (4)	3.8 (2)
Infection	10.7 (13)	10.0 (7)	11.5 (6)
Cancer	1.6 (2)	2.9 (2)	0.0 (0)
Other/Non-diabetes related/Unknown	4.9 (6)	7.1 (5)	1.9 (1)
-Hemorrhagic gastritis/ulcer (n)	1	0	1
-Respiratory failure (n)	1	1	0
-Multiple sclerosis (n)	1	1	0
-Unknown (n)	3	3	0
Other diabetes related	3.3 (4)	4.3 (3)	1.9 (1)
Underlying or secondary cause (%, n)			
Cardio-renal complications	82.8 (101)	72.9 (51)	96.1 (50)
-Cardiovascular	75.4 (92)	70.0 (49)	82.7 (43)
-Renal	45.1 (55)	35.7 (25)	57.7 (30)
Other (i.e. no mention of cardio-renal complications)	17.2 (21)	27.1 (19)	3.8 (2)

3. Statistical analysis

Analyses were conducted using the SAS statistical software version 9.4. Differences in participant baseline characteristics by subsequent mortality status were assessed with simple descriptive statistics. Univariate associations were determined using parametric (linear regression) or non-parametric (the Kruskal–Wallis test) approaches for normally and non-normally distributed continuous variables, respectively, and the χ^2 test for categorical variables. To evaluate the association between the *HP* genotype and cardio-renal mortality risk, deaths from causes other than cardiovascular or renal were treated as competing events. Although prior evidence suggested an inverse *HP* 2-2–stroke association (Costacou et al., 2014), six of the seven stroke deaths occurred among *HP* 2-2 carriers in this population and, therefore, stroke was not isolated from other cardiovascular causes of death. To analyze these competing risks data, procedure PHREG was used to model the cumulative incidence function, by defining the sub-distribution hazard according to a method developed by Fine and Gray (1999). To better account for the strong effect of diabetes duration on the risk of death, duration of diabetes was used as the time axis. Variables that were univariately associated with the outcome were included as covariates and a final model was produced after backward elimination. A graphic presentation of cardio-renal-specific cumulative mortality hazards was also obtained from the final multivariable competing risk model.

4. Results

Of 658 study participants, 486 had DNA available for *HP* genotyping. Compared to those without DNA, individuals with DNA available had shorter diabetes duration, lower HbA_1, blood pressure, non-HDL, and AER levels, and higher eGFR levels. Approximately 12.1% and 43.4% were homozygous for the *HP* 1 and *HP* 2 allele, respectively. Given the small proportion of *HP* 1-1 carriers and previous observations that the greatest complication risk is associated with *HP* 2 homozygosity, all analyses were conducted comparing *HP* 2-2 carriers to individuals with at least one *HP* 1 allele.

During 25 years of follow-up, 122 (25.1%) deaths occurred: 79 attributed to cardio-renal complications and 43 due to other causes. Differences in baseline participant characteristics by survival status are shown in Table 2. As expected, deceased individuals were more