Haptoglobin Genotype and Renal Function Decline in Type 1 Diabetes

Tina Costacou,[1] Robert E. Ferrell,[2] Demetrius Ellis,[3] and Trevor J. Orchard[1]

OBJECTIVE—Haptoglobin (Hp) binds free Hb, inhibiting Hb-induced oxidative damage. As oxidative stress has been associated with microvascular complications, we evaluated the relationship between Hp genotype and microalbuminuria, macroalbuminuria, end-stage renal disease (ESRD), and early renal function decline in type 1 diabetes.

RESEARCH DESIGN AND METHODS—Participants from the Epidemiology of Diabetes Complications Study with DNA available were studied for the incidence of microalbuminuria (albumin excretion rate [AER] 20–200 μg/min), macroalbuminuria (AER >200 μg/min), ESRD (renal dialysis or transplantation), and renal function decline (a decline ≥30 ml/min per 1.73 m^2 from baseline estimated [by the Cockcroft-Gault equation] glomerular filtration rate [eGFR] in those with baseline eGFR >60 ml/min per 1.73 m^2).

RESULTS—The proportions with the Hp 2/2, 2/1, and 1/1 genotype were 43.4, 44.4, and 12.1%, respectively. During 18 years of follow-up, the incidence of eGFR decline, microalbuminuria, macroalbuminuria, and ESRD was 42.0, 40.5, 16.7, and 12.2%, respectively. No significant univariate differences were observed by Hp genotype. However, in multivariable Cox models, an ~twofold increased risk was observed for the Hp 2/2 compared with the Hp 1/1 genotype for eGFR decline (hazard ratio 1.79 [95% CI 1.06–3.00]) and ESRD (2.74 [1.17–6.45]); no significant associations were observed for microalbuminuria or macroalbuminuria.

CONCLUSIONS—These data suggest that although Hp genotype is not associated with albuminuria per se, it may be an independent determinant of early renal function decline and progression to ESRD. Understanding these apparent contradictory findings may provide further insight into the pathogenesis of renal disease in type 1 diabetes. ***Diabetes*** **58:2904–2909, 2009**

Reactive oxygen species have been implicated in both the etiology and progression of diabetes complications, including nephropathy (1). However, clinical trials assessing the impact of antioxidant supplementation on micro- and macrovascular disease development have generally yielded null results (2).

It has recently been proposed that the effectiveness of an antioxidant regimen may be limited to susceptible subgroups, such as individuals with the haptoglobin (Hp) 2/2 genotype (3). Hp is an acute-phase plasma α_2-glycoprotein that, by binding to free Hb, inhibits Hb-induced oxidative tissue damage (4). Once bound to Hp, the Hp-Hb complex is cleared from circulation either at the liver hepatocyte or through the scavenger receptor CD163 present on monocytes and macrophages (5). In humans, two common allele classes (Hp^1 and Hp^2) at the Hp locus on chromosome 16q22 form three major genotypes: Hp 1/1, Hp 2/1, and Hp 2/2 (4). Substantial evidence supports a pathogenetic role of this polymorphism (6), with the Hp 1 protein allele being more efficient in preventing heme release from Hp-Hb complexes and promoting uptake by the CD163 macrophage receptor (7–9) as well as the antioxidant capacity of Hp 2 allele protein product being restricted by its greater molecular mass (5) and also associated with impaired reverse cholesterol transport (7,10). Moreover, although Hp allele distribution does not differ by diabetes status (6), the Hp 2 allele protein product increases susceptibility to vascular complications only in diabetes (11,12). Finally, daily vitamin E supplementation in type 2 diabetes with the Hp 2/2 genotype significantly reduced cardiovascular event risk (13,14).

We have previously shown that the Hp 2/2 genotype is a determinant of the risk of cardiovascular disease also in type 1 diabetes (15). In this article, we evaluated the relationship between Hp genotype and both renal damage (microalbuminuria and macroalbuminuria) and renal function (end-stage renal disease [ESRD] and early renal function decline) in type 1 diabetes (n = 486).

RESEARCH DESIGN AND METHODS

The Epidemiology of Diabetes Complications Study was based on a historical cohort of incident cases of childhood-onset (<17 years) type 1 diabetes, diagnosed or seen within 1 year of diagnosis (1950–1980) at Children's Hospital of Pittsburgh (16). The cohort has been shown to be representative of the Allegheny County, Pennsylvania, type 1 diabetes population (17). Subsequent to a first clinical assessment (1986–1988, when average participant age and diabetes duration were 28 and 19 years, respectively), biennial examinations were conducted for 10 years, with a further examination at 18 years. The University of Pittsburgh Institutional Review Board approved the study protocol.

Prior to each clinic visit, participants were sent questionnaires concerning demographic, health care, self-care, and medical history information. Blood pressure was measured with a random zero sphygmomanometer after a 5-min rest (18). Hypertension was defined as ≥140/90 mmHg or use of antihypertensive medication. Stable HbA_1 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 was determined by a precipitation technique with a modification (19) of the Lipid Research Clinics method (20). Cholesterol and triglycerides were enzymatically measured (21,22). Non-HDL cholesterol was calculated as total minus HDL cholesterol. White blood cell count was obtained using a counter S-plus IV and fibrinogen using a biuret colorimetric procedure and a clotting method.

From the [1]Department of Epidemiology, University of Pittsburgh, Pittsburgh, Pennsylvania; the [2]Department of Human Genetics, University of Pittsburgh, Pittsburgh, Pennsylvania; and the [3]Children's Hospital of Pittsburgh, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.
Corresponding author: Tina Costacou, costacout@edc.pitt.edu.
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TABLE 1
Participant characteristics at study entry by subsequent microalbuminuria and macroalbuminuria status

	Microalbuminuria			Macroalbuminuria		
	No	Yes	*P*	No	Yes	*P*
n	163	111		309	62	
Age (years)	25.0 ± 7.7	25.8 ± 8.3	0.37	26.2 ± 8.1	27.1 ± 7.4	0.43
Age at onset (years)	8.5 ± 4.2	8.3 ± 4.1	0.64	8.2 ± 4.2	9.3 ± 3.3	0.02
Diabetes duration (years)	16.3 ± 6.9	17.6 ± 7.6	0.21	18.0 ± 7.6	17.8 ± 7.5	0.83
Follow-up time (years)	15.5 ± 5.0	7.3 ± 4.8	<0.0001	15.2 ± 5.0	8.0 ± 5.3	<0.0001
Female subjects	50.9 (83)	55.9 (62)	0.42	52.4 (162)	40.3 (25)	0.08
BMI (kg/m^2)	23.1 ± 3.2	23.2 ± 3.3	0.70	23.5 ± 3.4	23.6 ± 3.1	0.74
Waist-to-hip ratio	0.81 ± 0.06	0.82 ± 0.06	0.27	0.81 ± 0.07	0.84 ± 0.07	0.002
Ever smokers	28.8 (47)	31.5 (35)	0.63	32.0 (99)	38.7 (24)	0.31
HbA_1 (%)	9.7 (1.4)	10.8 (1.8)	<0.0001	10.0 (1.6)	11.1 (2.0)	0.0002
Insulin dose per weight*	0.81 (0.65–0.95)	0.80 (0.66–0.95)	0.77	0.80 (0.63–0.94)	0.80 (0.60–0.98)	0.97
Systolic blood pressure (mmHg)	106.9 ± 10.7	109.1 ± 10.3	0.10	108.8 ± 11.4	110.7 ± 12.9	0.24
Diastolic blood pressure (mmHg)	67.9 ± 8.4	69.8 ± 7.8	0.05	69.5 ± 9.1	70.8 ± 9.1	0.31
Hypertension	3.7 (6)	3.6 (4)	1.00†	5.5 (17)	9.7 (6)	0.21
HDL cholesterol (mg/dl)	55.2 ± 12.1	54.0 ± 9.8	0.37	54.5 ± 11.4	56.1 ± 11.9	0.38
Non-HDL cholesterol (mg/dl)	116.0 ± 25.5	128.1 ± 34.3	0.002	122.9 ± 30.2	141.1 ± 43.2	0.002
ACE/ARB use	1.3 (2)	0.9 (1)	1.00†	1.0 (3)	1.7 (1)	0.52†
Serum creatinine (mg/dl)*	0.80 (0.70–1.0)	0.80 (0.60–0.90)	0.16	0.80 (0.70–1.0)	0.90 (0.70–1.0)	0.39
eGFR by Cockcroft-Gault (ml/min per 1.73 m^2)	121.1 ± 37.8	124.6 ± 38.7	0.46	120.9 ± 37.1	125.0 ± 43.5	0.44
AER (μg/min)*	7.2 (5.1–10.1)	9.0 (6.2–11.6)	0.004	19.2 (5.9–16.1)	23.5 (9.5–71.3)	<0.0001
White blood cell count × 10^3/mm^2*	5.6 (4.8–6.6)	6.1 (5.2–7.2)	0.02	5.8 (5.1–7.0)	6.7 (5.2–8.0)	0.005
Fibrinogen (mg/dl)*	250.0 (200.0–300.0)	265.0 (220.0–300.0)	0.12	250.0 (210.0–305.0)	270.0 (240.0–310.0)	0.06
Hp genotype						
1/1	77.4 (24)	22.6 (7)		81.4 (35)	18.6 (8)	
2/1	55.5 (71)	44.5 (57)		84.8 (145)	15.2 (26)	
2/2	59.1 (68)	40.9 (47)	0.08	82.2 (129)	17.8 (28)	0.77

Data are percent (*n*) or means ± SD unless otherwise indicated. The sample size for ACE/angiotensin receptor blocker medications was 270 for the outcome of microalbuminuria (159 noncases and 111 incident cases) and 362 for the outcome of macroalbuminuria (302 noncases and 60 incident cases). *The Wilcoxon two-sample test was used for nonnormally distributed variables; data are median (interquartile range). †Fisher exact test. ARB, angiotensin receptor blocker.

Urinary albumin was measured by immunonephelometry (23), and creatinine was assayed by an Ectachem 400 Analyzer (Eastman Kodak, Rochester, NY). Microalbuminuria was defined as albumin excretion rate (AER) of 20–200 μg/min (30–300 mg per 24 h) and macroalbuminuria as AER >200 μg/min (>300 mg per 24 h) in at least 2 of 3 validated timed urine collections. In 10% of the samples, urine collections were deemed inadequate based on creatinine excretion and albumin-to-creatinine ratio was used (microalbuminuria, 0.03–0.3 mg/mg; macroalbuminuria, >0.3 mg/mg) (24). ESRD onset was defined as starting dialysis or undergoing renal transplantation. Early renal function decline was defined as the incidence of a decline of ≥30 ml/min per 1.73 m^2 from baseline estimated glomerular filtration rate (eGFR) based on the Cockcroft-Gault equation (25) among participants with normal or mildly reduced renal function at study entry (stages I and II).

High molecular weight genomic DNA was isolated using the PureGene kit (Gentra Systems, Minneapolis, MN), and Hp was genotyped by an amplification method (26). Genotypes were assigned visually by comparison with controls of known genotype and in a random sample showed excellent agreement (97%) with an Eliza method (27).

Statistical analysis. Nonnormally distributed variables were logarithmically transformed. Univariate associations were determined using the Student *t* test and χ^2 or Fisher exact test, as appropriate. Cox proportional hazards models with backward elimination were constructed to assess the multivariable association between Hp genotype and the incidence of each outcome of interest adjusting for traditional risk factors (including eGFR and AER levels at study entry) and univariately significant variables. Survival time was defined as the time in years from study entry to either an incident event or censorship during the 18-year follow-up. Statistical analyses were conducted using SAS (version 9.1; SAS Institute, Cary, NC).

RESULTS

Of 658 study participants, DNA for Hp genotyping was available for 486 (73.9%). Compared with those without DNA available, individuals with DNA data had a shorter diabetes duration and higher HbA_1, blood pressure, non-HDL cholesterol, serum creatinine, eGFR, and AER. The distribution of the Hp genotype was 12.1% Hp 1/1, 44.4% Hp 2/1, and 43.4% Hp 2/2. Generally, no differences were observed in participant characteristics by Hp genotype at study entry with the exception of younger age and higher non-HDL cholesterol in those with the Hp 2/2 compared with the 2/1 genotype and lower insulin dose per weight in those with the Hp 2/1 compared with the 1/1 genotype.

During 18 years of follow-up, 40.5% (n = 111) developed incident microalbuminuria, 16.7% (n = 62) macroalbuminuria, and 12.2% (n = 58) ESRD. Moreover, 188 (42.0%) exhibited an early decline in renal function (≥30 ml/min per 1.73 m^2 from baseline eGFR). Descriptive participant characteristics by incidence of microalbuminuria and macroalbuminuria are shown in Table 1 and by renal function decline ≥30 ml/min per 1.73 m^2 and ESRD incidence in Table 2. Generally, incident case subjects with both microalbuminuria and macroalbuminuria were more likely to have higher levels of HbA_1, non-HDL cholesterol, AER, and inflammatory markers compared with those who remained disease free. Incident case subjects with macroalbuminuria were also older at the time of diabetes onset compared with noncase subjects and had a greater waist-to-hip ratio.

Compared with noncase subjects, incident case subjects with early renal function decline and ESRD were older,

TABLE 2
Participant characteristics at study entry by a subsequent decline ≥30 ml/min per 1.73 m^2 from baseline eGFR and ESRD

	A decline ≥30 ml/min per 1.73 m^2 from baseline eGFR			ESRD		
	No	Yes	*P*	No	Yes	*P*
n	260	188		416	58	
Age (years)	26.2 ± 8.0	27.8 ± 7.3	0.04	26.6 ± 7.8	31.2 ± 6.3	<0.0001
Age at onset (years)	7.9 ± 4.2	9.0 ± 3.8	0.005	8.4 ± 4.1	7.8 ± 3.9	0.29
Diabetes duration (years)	18.3 ± 7.3	18.8 ± 7.6	0.53	18.2 ± 7.4	23.4 ± 7.1	<0.0001
Follow-up time (years)	14.0 ± 5.3	6.5 ± 4.8	<0.0001	15.2 ± 3.9	9.1 ± 4.6	<0.0001
Female subjects	45.8 (119)	54.3 (102)	0.08	49.5 (206)	48.3 (28)	0.86
BMI (kg/m^2)	23.3 ± 3.5	24.3 ± 3.0	0.0008	23.6 ± 3.3	23.9 ± 3.0	0.48
Waist-to-hip ratio	0.82 ± 0.07	0.83 ± 0.07	0.47	0.82 ± 0.07	0.84 ± 0.08	0.09
Ever smokers	33.5 (87)	38.8 (73)	0.24	34.6 (144)	41.4 (24)	0.31
HbA_1 (%)	8.5 (1.4)	9.0 (1.4)	0.0004	10.2 (1.7)	10.6 (1.9)	0.11
Insulin dose per weight*	0.80 (0.62–0.94)	0.77 (0.64–0.94)	0.75	0.80 (0.64–0.94)	0.67 (0.57–0.80)	0.002
Systolic blood pressure (mmHg)	110.6 ± 12.6	113.4 ± 14.9	0.04	110.7 ± 12.8	123.5 ± 16.5	<0.0001
Diastolic blood pressure (mmHg)	71.1 ± 10.5	72.7 ± 10.8	0.10	71.1 ± 10.2	77.9 ± 12.4	0.0001
Hypertension	9.6 (25)	13.8 (26)	0.17	9.1 (38)	50.0 (29)	<0.0001
HDL cholesterol (mg/dl)	55.2 ± 12.4	52.9 ± 11.4	0.05	54.6 ± 12.1	50.2 ± 10.4	0.009
Non-HDL cholesterol (mg/dl)	127.5 ± 34.2	138.9 ± 44.2	0.003	128.8 ± 34.9	166.5 ± 57.6	<0.0001
ACE/ARB use	1.6 (4)	2.2 (4)	0.47†	1.5 (6)	13.8 (8)	<0.0001
Serum creatinine (mg/dl)*	0.90 (0.80–1.10)	0.70 (0.60–0.90)	<0.0001	0.80 (0.70–1.0)	1.3 (0.90–1.80)	<0.0001
eGFR by Cockcroft-Gault (ml/min per 1.73 m^2)	105.3 ± 23.4	140.6 ± 42.9	<0.0001	121.3 ± 38.0	77.7 ± 40.8	<0.0001
AER (μg/min)*	10.6 (6.2–31.0)	19.6 (8.8–363.1)	<0.0001	11.3 (6.8–46.7)	1,030.2 (281.5–1,877.8)	<0.0001
White blood cell count × $10^3/mm^2$*	6.1 (5.2–7.1)	6.2 (5.4–7.6)	0.10	6.1 (5.2–7.2)	7.1 (5.9–8.9)	<0.0001
Fibrinogen (mg/dl)*	250.0 (210.0–310.0)	270.0 (240.0–350.0)	0.0007	270.0 (220.0–310.0)	310.0 (270.0–390.0)	<0.0001
Hp genotype						
1/1	64.8 (35)	35.2 (19)		87.9 (51)	12.1 (7)	
2/1	62.1 (128)	37.9 (78)		90.2 (193)	9.8 (21)	
2/2	51.6 (97)	48.4 (91)	0.06	85.2 (172)	14.9 (30)	0.29

Data are percent (*n*) or means ± SD unless otherwise indicated. The sample size for ACE/angiotensin receptor blocker medications was 437 for a decline ≥30 ml/min per 1.73 m^2 from baseline eGFR (251 noncases and 186 incident cases) and 463 for the outcome of ESRD (405 noncases and 58 incident cases). *The Wilcoxon two-sample test was used for nonnormally distributed variables; data are median (interquartile range). †Fisher exact test. ARB, angiotensin receptor blocker.

with higher systolic blood pressure, non-HDL cholesterol, AER, and inflammatory marker levels and lower HDL cholesterol. Incident case subjects with early renal function decline had an older age of diabetes onset and higher BMI, HbA_1, and eGFR and lower serum creatinine. Conversely, greater diabetes duration and higher levels of diastolic blood pressure and serum creatinine but lower insulin dose per kilogram body weight and eGFR were observed in incident case subjects with ESRD compared with noncase subjects. No univariate association, however, was observed between Hp and the incidence of other renal outcomes at the 0.05 significance level. Hp genotype was also not associated with all-cause mortality ($P = 0.80$) based on 82 (16.9%) deceased individuals.

Multivariable Cox proportional hazards models (Table 3) showed no association between the Hp genotype and microalbuminuria or macroalbuminuria incidence. Conversely, adjusting for univariately significant risk factors, an increased risk of an early renal function decline was observed for individuals carrying the Hp 2/2 compared with the Hp 1/1 genotype (hazard ratio 1.79 [95% CI = 1.06–3.00]). Similarly, the Hp 2/2 conferred over a twofold increased risk of ESRD compared with the Hp 1/1 genotype (2.45 [1.05–5.73]). The risk associated with the Hp 2/1 reached statistical significance for neither early renal function decline nor ESRD incidence.

To examine the possibility of survival bias, we stratified the cohort by diabetes diagnosis year (prior to or after 1965, wherein mortality was 40 vs. 13%, respectively). With the exception of macroalbuminuria, a trend toward higher incidence rates among the Hp 2/2 compared with the Hp 1/1 genotype was generally observed in those diagnosed after 1965 (less subject to survival bias); however, none of the stratified results were statistically significant (Table 4). Similarly, when conducting cumulative incidence analyses (including prevalent cases in outcomes), results demonstrated nonsignificantly higher rates in those carrying the Hp 2/2 compared with the Hp 1/1 genotype with the exception of macroalbuminuria (Table 4).

DISCUSSION

In this cohort of subjects with type 1 diabetes, we failed to show an association between the Hp genotype and either microalbuminuria or macroalbuminuria incidence. However, although not univariately significant, approximately a twofold increased risk emerged for outcomes assessing

TABLE 3
Hazard ratios (95% CIs) from Cox proportional hazard models for the incidence of microalbuminuria, macroalbuminuria, eGFR decline (decline ≥30 ml/min per 1.73 m^2 from baseline eGFR), and ESRD

Outcome	Crude	Model 1	Model 2	Model 3
Microalbuminuria (n = 270, 111 incident events)				
Hp genotype				
1/1	Referent	Referent	Referent	Referent
2/1	2.09 (0.95–4.59)	2.19 (0.996–4.80)	2.08 (0.95–4.56)	1.77 (0.80–3.92)
2/2	1.84 (0.83–4.07)	1.95 (0.88–4.32)	1.67 (0.75–3.71)	1.34 (0.59–3.05)
A1C	1,147.803	1,140.392	1,106.418	1,100.600
Model 1 allowed for diabetes duration, sex, log AER, and eGFR				
Model 2 allowed for variables in model 1 in addition to HbA_1, systolic blood pressure, and HDL and non-HDL cholesterol				
Model 3 allowed for variables in model 2 in addition to white blood cell count				
Macroalbuminuria (n = 364, 61 incident events)				
Hp genotype				
1/1	Referent	Referent	Referent	Referent
2/1	0.77 (0.35–1.70)	0.78 (0.35–1.72)	0.77 (0.35–1.72)	0.72 (0.33–1.60)
2/2	0.84 (0.38–1.86)	0.78 (0.35–1.72)	0.78 (0.35–1.73)	0.73 (0.33–1.62)
A1C	686.874	656.057	640.020	636.806
Model 1 allowed for diabetes duration, sex, smoking status, waist-to-hip ratio, log AER, and eGFR				
Model 2 allowed for variables in model 1 in addition to HbA_1, systolic blood pressure, and HDL and non-HDL cholesterol				
Model 3 allowed for variables in model 2 in addition to white blood cell count				
A decline ≥30 ml/min per 1.73 m^2 from baseline eGFR (n = 441, 187 incident events)				
Hp genotype				
1/1	Referent	Referent	Referent	Referent
2/2	1.59 (0.97–2.60)	1.30 (0.78–2.18)	1.38 (0.82–2.31)	1.38 (0.82–2.31)
2/2	1.59 (0.97–2.60)	1.64 (0.99–2.73)	1.79 (1.06–3.00)	1.79 (1.06–3.00)
A1C	2,102.198	1,897.376	1,896.567	1,896.567
Model 1 allowed for diabetes duration, sex, BMI, log AER, and eGFR				
Model 2 allowed for variables in model 1 in addition to HbA_1, systolic blood pressure, and HDL and non-HDL cholesterol				
Model 3 allowed for variables in model 2 in addition to fibrinogen				
ESRD (n = 467, 57 incident events)				
Hp genotype				
1/1	Referent	Referent	Referent	Referent
2/1	0.72 (0.30–1.69)	1.14 (0.48–2.74)	1.24 (0.51–2.98)	1.32 (0.55–3.16)
2/2	1.15 (0.50–2.61)	2.17 (0.93–5.04)	2.74 (1.17–6.45)	2.45 (1.05–5.73)
A1C	672.450	522.184	509.657	508.736
Model 1 allowed for diabetes duration, sex, smoking status, log AER, and eGFR				
Model 2 allowed for variables in model 1 in addition to HbA_1, hypertension, and HDL and non-HDL cholesterol				
Model 3 allowed for variables in model 2 in addition to white blood cell count and fibrinogen				

renal function decline and ESRD incidence after multivariable adjustments.

Previous studies assessing the association between the Hp phenotype and the presence or incidence of renal disease have produced discrepant findings. In a small, cross-sectional study of normotensive subjects with type 1 or 2 diabetes, none of those with the Hp 1/1 phenotype exhibited signs of nephropathy (0/18) compared with 27% (10/37) of those with Hp 2/1 and 34% (19/55) of those with Hp 2/2 ($P < 0.02$) (6,28). Similar results were reported from an Irish type 1 diabetes case-control study (29). Conversely, a Japanese study of individuals with a long duration (>10 years) of type 2 diabetes did not observe an increased risk associated with the common Hp phenotype ($P = 0.43$) (30). Similarly, we were also not able to detect an association for either microalbuminuria or macroalbuminuria incidence in our cohort of individuals with a long duration of type 1 diabetes, perhaps suggesting that at a more advanced stage of diabetes, early Hp-susceptible cases of microalbuminuria and macroalbuminuria may have been excluded. Indeed, the cumulative incidence of microalbuminuria (including both prevalent cases at study entry and incident cases) appeared higher in our study among participants carrying the Hp 2 allele, although results did not reach statistical significance. However, analogous findings were not observed for the cumulative incidence of macroalbuminuria, suggesting that the Hp 2/2 genotype is not a strong determining factor for progression to macroalbuminuria.

Despite the null associations for the incidence of proteinuria, a strong relationship was noted between the Hp 2/2 genotype and the incidence of both an early decline in renal function and ESRD. Unfortunately, we are not aware of any published reports on the association between the Hp genotype and renal function decline among individuals with diabetes and thus cannot, at present, confirm these findings. However, the possibility of a factor affecting the incidence of renal dysfunction but not that of renal disease per se raises the hypothesis that these are two different disease entities, and thus factors contributing to their development may be distinct. Indeed, almost a decade ago, we suggested that in certain cases tubulopathy may precede glomerulopathy in type 1 diabetes and that even microalbuminuria may be secondary to impaired tubular reabsorption (31). More recently, research studies have shown that reductions in eGFR do occur without preceding microalbuminuria in those with diabetes (32–34). Importantly, a pathophysiological mechanism has been proposed that could account for the increased rate of renal function decline among individuals with diabetes and the

TABLE 4
Incidence of renal outcomes by Hp genotype

Outcome incidence	*n*	Hp genotype 1/1	2/1	2/2	*P*
Microalbuminuria					
Diabetes diagnosis ≤1965	73	11.1 (1)	46.3 (19)	47.8 (11)	0.14*
Diabetes diagnosis >1965	201	27.3 (6)	43.7 (38)	39.1 (36)	0.37
Total cohort	274	22.6 (7)	44.5 (57)	40.9 (47)	0.08
Cumulative incidence	483	59.3 (35)	67.1 (145)	67.3 (140)	0.49
Macroalbuminuria					
Diabetes diagnosis ≤1965	112	0.0 (0)	17.5 (11)	24.3 (9)	0.16*
Diabetes diagnosis >1965	259	25.8 (8)	13.9 (15)	15.8 (19)	0.28
Total cohort	371	18.6 (8)	15.2 (26)	17.8 (28)	0.77
Cumulative incidence	483	40.7 (24)	32.9 (71)	38.0 (79)	0.40
Early renal function decline					
Diabetes diagnosis ≤1965	145	47.1 (8)	34.3 (25)	50.9 (28)	0.15
Diabetes diagnosis >1965	303	29.7 (11)	39.9 (53)	47.4 (63)	0.13
Total cohort	448	35.2 (19)	37.9 (78)	48.4 (91)	0.06
ESRD					
Diabetes diagnosis ≤1965	162	22.2 (4)	17.7 (14)	27.7 (18)	0.33*
Diabetes diagnosis >1965	312	7.5 (3)	5.2 (7)	8.8 (12)	0.50*
Total cohort	474	12.1 (7)	9.8 (21)	14.9 (30)	0.29
Cumulative incidence	483	13.6 (8)	10.7 (23)	17.3 (36)	0.14

Data are percent (*n*) unless otherwise indicated. *Fisher exact test.

Hp 2/2 genotype (7), based on the recognition that renal proximal tubule cells serve as a (secondary to CD163) default mechanism for clearance of the Hp-Hb complex. Because CD163-mediated clearance of the Hp-Hb complex is impaired in subjects with diabetes and the Hp 2/2 genotype, renal proximal tubule cells are used to a greater extent, resulting in a dramatic increase in iron deposition, oxidative stress, and hypertrophy. In fact, Hp 2/2 diabetic mice have been shown to display significantly increased glomerular and proximal tubular hypertrophy and greater deposition of collagen type IV, smooth muscle actin, and increased renal iron (35). Intriguingly, vitamin E administration was shown to slow diabetic renal disease progression among the Hp 2/2 but not the Hp 1/1 mice.

In conclusion, we observed an association between the Hp genotype and renal function decline in individuals with long-standing type 1 diabetes. A caveat of this study is the lack of independent replication of findings in another cohort. Nevertheless, these results raise the possibility that pharmacological administration of vitamin E, shown to reduce cardiovascular disease outcomes in those with type 2 diabetes with the Hp 2/2 genotype (13–14), may also lead to reduced renal disease risk.

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