Haptoglobin Genotype and the Rate of Renal Function Decline in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study

Trevor J. Orchard,[1] Wanjie Sun,[2] Patricia A. Cleary,[2] Saul M. Genuth,[3] John M. Lachin,[2] Paula McGee,[2] Andrew D. Paterson,[4] Philip Raskin,[5] Yefim Anbinder,[6] Andrew P. Levy,[6] and the DCCT/EDIC Research Group*

Many patients with type 1 diabetes develop renal disease despite moderately good metabolic control, suggesting other risk factors may play a role. Recent evidence suggests that the haptoglobin (HP) 2-2 genotype, which codes for a protein with reduced antioxidant activity, may predict renal function decline in type 1 diabetes. We examined this hypothesis in 1,303 Caucasian participants in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) study. HP genotype was determined by polyacrylamide gel electrophoresis. Glomerular filtration rate was estimated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation and albumin excretion based on timed urine samples. Participants were followed up for a mean of 22 years. HP genotype was significantly associated with the development of sustained estimated glomerular filtration rate (GFR) <60 mL/min/1.73 m^2 and with end-stage renal disease (ESRD), with HP 2-2 having greater risk than HP 2-1 and 1-1. No association was seen with albuminuria. Although there was no treatment group interaction, the associations were only significant in the conventional treatment group, where events rates were much higher. We conclude that the HP genotype is significantly associated with the development of reduced GFR and ESRD in the DCCT/EDIC study. *Diabetes* 62:3218–3223, 2013

T he Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) study has demonstrated a substantial benefit for intensive therapy aimed at normalization of glycemic levels (1). However, the presence of interindividual variability in the development of diabetic nephropathy (DN), despite similar levels of glycemic control and clustering of DN within families, suggests there are additional risk factors, including genetic predisposition (2). Because reactive oxygen species (ROS) are also implicated (3), polymorphic genetic loci encoding variants in proteins, which may protect against ROS, may be of importance. Haptoglobin (HP) (http://www.ncbi.nlm.nih.gov/gene/3240, 16q22.2), an abundant serum protein whose primary function is to protect against the pro-oxidative activity of extracorpuscular hemoglobin (Hb), is one potential determinant of susceptibility to DN (4). Two alleles (rs72294371), denoted 1 and 2, give rise to three genotypes: 1-1, 2-1, and 2-2 (5). The structure and function of the HP proteins differ markedly, with the HP 2-2 protein having impaired antioxidant activity and lower efficiency in clearing hemoglobin-derived iron (6).

Studies in type 1 and type 2 diabetes cohorts have demonstrated that individuals with diabetes and the HP 2-2 genotype have a two- to threefold increased incidence of cardiovascular disease (CVD) compared with HP 1-1 (6,7). Intriguingly, a recent report from the Epidemiology of Diabetes Complications (EDC) study similarly found that the HP 2-2 genotype predicted decline in renal function and increased risk of end-stage renal disease (ESRD) (8).

In the current study we examine whether the HP genotype is associated with a decline in renal function as the primary outcome, and the development of retinopathy or neuropathy as the secondary outcomes, in the DCCT/EDIC cohort after an average of 22 years of follow-up and, if so, whether the association varied according to the original DCCT treatment group.

RESEARCH DESIGN AND METHODS

Detailed descriptions of the methods of the DCCT and EDIC follow-up study have been published previously (1,9,10). The DCCT, a randomized controlled clinical trial conducted between 1983 and 1993, compared the effects of an intensive diabetes treatment regimen with those of conventional therapy. During the DCCT, intensive (INT) and conventional (CONV) therapies achieved mean HbA$_{1c}$ levels of 7 and 9%, respectively (1).

Of the 1,441 patients with type 1 diabetes who were 13–39 years old at the time of randomization, 1,422 completed the DCCT; the mean follow-up was 6.5 years. At baseline, eligibility criteria excluded patients with a history of CVD, hypertension, or hypercholesterolemia (9). Of the surviving cohort, 1,394 agreed to join the long-term EDIC observational follow-up study in 1994, during which subjects received care from their local providers (10). Approximately 80% in both groups followed an intensive therapy regimen during EDIC, and HbA$_{1c}$ was similar in both groups. This report includes data obtained through 31 April 2010 on the 1,303 Caucasian participants.
Study procedures. HbA$_{1c}$ was measured quarterly during DCCT and in alternate years during EDIC (10,11). Renal function was measured annually during the DCCT and in alternate years in EDIC (12). The urinary albumin excretion rate was determined from a 4-h clinic-based urine collection, with microalbuminuria and macroalbuminuria defined as ≥30 mg in a 24-h period

TABLE 1
Baseline associations with all HP types

	HP 1-1 (n = 169)	HP 2-1 (n = 618)	HP 2-2 (n = 516)	P value*
Intensive treatment group	46 (45)	323 (52)	237 (46)	0.06
Age (years)	26 ± 7	27 ± 7	27 ± 7	0.43
BMI (kg/m^2)	23.0 ± 2.6	23.6 ± 2.8	23.5 ± 2.8	0.08
Duration of type 1 diabetes in months	67 ± 51	66 ± 48	70 ± 51	0.53
Female	83 (49)	296 (48)	230 (45)	0.44
Baseline HbA$_{1c}$ (%)	8.8 ± 1.6	8.8 ± 1.5	8.9 ± 1.6	0.24
Primary cohort	83 (49)	319 (52)	249 (48)	0.52
Clinical neuropathy = yes	33 (20)	112 (18)	101 (20)	0.80
Smoker = yes	7 (4)	41 (7)	32 (6)	0.54
LDL (mg/dL)	104.1 ± 27.0	109.3 ± 29.5	111.5 ± 21.9	**0.016**
HDL (mg/dL)	50.8 ± 12.5	50.4 ± 12.4	50.2 ± 12.1	0.82
Triglycerides (mg/dL)	78.6 ± 43.7	82.6 ± 50.5	82.2 ± 48.1	0.30
Cholesterol (mg/dL)	381.3 ± 213.5	389.7 ± 212.2	417.3 ± 302.1	0.20
Blood pressure (mmHg)				
Systolic	114.8 ± 11.8	113.7 ± 11.6	114.8 ± 11.5	0.26
Diastolic	72.7 ± 8.7	72.5 ± 8.9	72.5 ± 8.8	0.99
GFR (mL/min)	127.7 ± 20.2	128.0 ± 19.9	127.6 ± 19.1	0.98
Albumin excretion rate (mg/24 h)	14.9 ± 14.2	16.0 ± 18.1	15.9 ± 20.8	0.77

Data are n (%) and mean ± SD. The bold P value is statistically significant. *Kruskal-Wallis for continuous variables and Fisher exact for categorical variables.

at two consecutive study visits and ≥300 mg in a 24-h period at a single visit, respectively (12). Retinopathy, clinical neuropathy, and abnormal autonomic response were defined as detailed elsewhere (10,12,13).

An impaired glomerular filtration rate (GFR) was defined as an estimated GFR (eGFR) of <60 mL/min/1.73 m^2 at two consecutive study visits, at least 1 year apart (1). Data on serum creatinine, age, sex, and race were then used to calculate the eGFR using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (14). ESRD was defined as the need for kidney

transplantation or the initiation of maintenance dialysis and was assessed yearly and adjudicated by the DCCT/EDIC Mortality and Morbidity Committee. During the DCCT, use of ACE inhibitors was discouraged, unless clearly required for clinical reasons, and were rarely used. During EDIC, the use of inhibitors of the renin-angiotensin-aldosterone system (RAAS) was assessed yearly by self-report.

Method for HP typing. Serum samples for the 1,303 subjects in this study were typed for HP genotype by PAGE (15) and ELISA (16), with no knowledge

TABLE 2
Incidence of microvascular complications in the Caucasian DCCT/EDIC participants (N = 1,303) by HP type 1-1, 2-1, and 2-2

Complication	HP type 1-1	HP type 2-1	HP type 2-2	P value*
n (%)	169 (100)	618 (100)	516 (100)	
Renal complications				
Sustained albumin excretion rate >30 mg/24 h				0.16
Yes: n = 359	21.9	28.2	28.7	
Albumin excretion rate >300 mg/24 h				0.13
Yes: n = 141	7.7	10.7	12.0	
Sustained eGFR <60 mL/min/1.73 m^2				0.037
Yes: n = 64	3.6	3.9	6.6	
ESRD				0.036
Yes: n = 21	0	1.5	2.3	
Retinopathy complications				
Sustained 3-step progression since DCCT baseline				0.23
Yes: n = 584	42.6	43.7	46.9	
Mild NPDR or worse				0.49
Yes: n = 1,080	81.7	82.5	83.7	
Moderate NPDR or worse				0.16
Yes: n = 634	44.4	48.2	50.6	
PDR				0.15
Yes: n = 231	17.2	15.9	20.2	
Neuropathy				
Abnormal autonomic response				0.36
Yes: n = 505	38.7	44.4	44.4	
Confirmed clinical neuropathy				0.83
Yes: n = 363	29.0	26.9	28.7	

NPDR, nonproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy. *P value is from the Cochran-Armitage trend test.

of patient identity. Two samples could not be HP typed by either method. In five samples, there was a difference in the HP type obtained by PAGE and ELISA, and the analysis reported here is for the results obtained by PAGE. In 12 samples where an HP type could not be obtained by PAGE, the HP type used in this analysis was that obtained by ELISA.

Statistical analysis. Clinical characteristics between HP types were compared using the Kruskal-Wallis test for continuous variables and the Fisher exact test for categorical variables. A Cochran-Armitage trend test or a variation (which is consistent with the literature and minimizes multiple comparisons) was used for the risk of microvascular complications and rate of change in eGFR across HP types. The cumulative incidence of impaired GFR ($<$60 ml/min/1.73 m^2 at two consecutive study visits) and ESRD within HP types was evaluated by the Kaplan-Meier method. Cox proportional hazards models were used to assess the effect of HP type on the risk of impaired GFR separately for the two DCCT treatment groups after adjustment for DCCT baseline eGFR, an interaction term of the HP type and DCCT treatment group, and three principle components of ancestry to account for population structure (details are given in the Supplementary Data). No multivariate model was used for ESRD due to a small number of events (n = 22). No adjustments were made for multiple comparisons.

RESULTS

Among the 1,303 Caucasian subjects, 198 (13.8%) had HP genotype 1-1, 681 (47.3%) had HP 2-1, and 560 (38.9%) had HP 2-2, conforming to Hardy-Weinberg equilibrium (P = 0.92). Baseline associations are presented in Table 1. Only one significant baseline difference was seen across the three HP groups, with higher LDL cholesterol (P = 0.016) with more copies of the 2 allele. A borderline association (P = 0.08) was seen for increasing BMI.

Table 2 reports the number of participants who experienced each microvascular complication in the combined DCCT and EDIC study by HP types 1-1, 2-1, and 2-2. Among all microvascular complications, there was an increasing trend in the proportions developing impaired eGFR (3.6 vs. 3.9 vs. 6.6%, trend P = 0.037) and ESRD (0 vs. 1.5 vs. 2.3%, trend P = 0.036) among those with 0, 1, or 2 copies of the HP 2 allele, respectively. There were no significant differences in retinopathy or neuropathy complications or albumin excretion among the three HP types.

Figure 1 shows the Kaplan-Meier cumulative incidence of impaired eGFR among the three HP types, which incorporates the event or censoring time for those with or without events. Those with HP 2-2 had the highest cumulative incidence (5.3% by 20 years after the start of the DCCT), followed by those with HP 2-1 (3.1%), and those with HP 1-1 (2.5%). Log-rank trend test showed a significant increasing trend of cumulative incidence of impaired GFR associated with the number of HP 2 alleles (trend P = 0.036). Likewise, ESRD showed a similar increasing trend
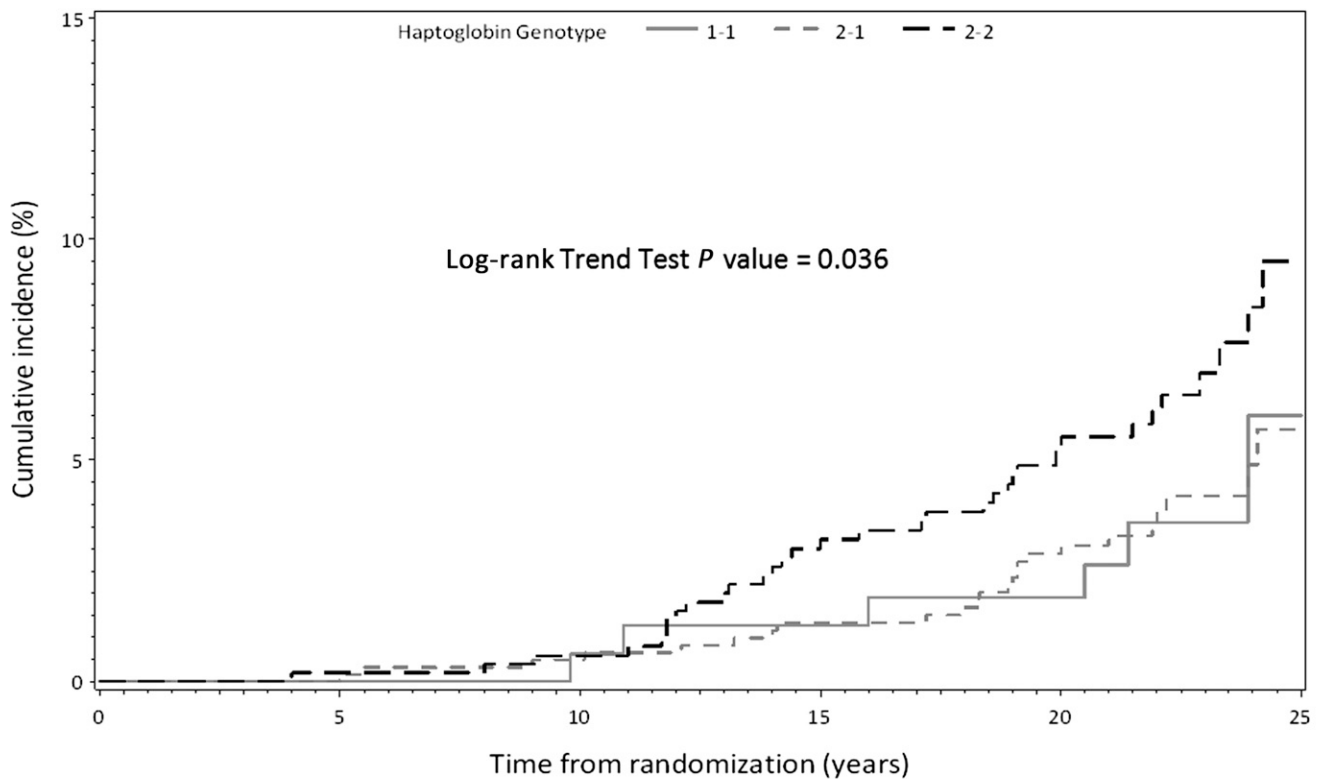
FIG. 1. Cumulative incidence of sustained GFR $<$60 mL/min/1.73 m^2 in the 1,303 Caucasian DCCT/EDIC participants by HP type 1-1, 2-1, and 2-2. P value is from a log-rank trend test.

of cumulative incidence with the number of HP 2 alleles (Fig. 2). The cumulative incidence after 20 years of follow-up was 0% for HP 1-1, 1.6% for HP 2-1, and 2% for HP 2-2 (trend $P = 0.029$).

In multivariate analyses (Supplementary Table 1), Cox proportional hazard models assessed the effect of HP type on the risk of impaired GFR separately for the two former treatment groups after adjustment for DCCT baseline eGFR, an interaction term of HP type and DCCT treatment group, and the three principle components of ancestry. In the former CONV group, a significant increasing risk of eGFR in HP 1-1, 2-1, and 2-2 ($P = 0.005$) was observed, and 63.7% of the variance was explained by albumin excretion rate, which nullified the HP effect ($P = 0.31$). The INT group showed a similar but nonsignificant trend due to a low number of events. No significant treatment group/HP interaction was seen ($P = 0.80$).

We also examined the change over time in eGFR as a continuous measure (Supplementary Table 2). HP differences reached significance in a trend analysis ($P = 0.028$). Likewise, each additional allele of HP 2 was associated with a 0.12 mL/min/1.73 m^2/year greater decline in eGFR in CONV ($P = 0.028$) but not in INT ($P = 0.74$). However, the difference between the two former treatment groups did not reach statistical significance (interaction term $P = 0.38$).

DISCUSSION

We have demonstrated that in the DCCT/EDIC study, the HP 2-2 genotype was associated with a more rapid decline in renal function and an increased incidence of impaired eGFR and ESRD. These observations are of considerable potential clinical relevance because the prevalence of the HP 2-2 genotype is ~40% in Caucasian DCCT/EDIC subjects, similar to that seen in other populations (8). Interventions designed to decrease the increased risk in HP 2-2 genotype may thus have a profound effect on the cumulative public health burden of renal disease in individuals with type 1 diabetes, especially those in poor glycemic control. Other important observations are that this association of HP 2-2 appears to be largely independent of time-dependent albuminuria and that the heterozygous state (HP 2-1) may have an intermediary effect. Finally, even in high-risk individuals based on their HP genotype, intensive therapy was highly effective in reducing the risk of renal disease; that is, glycemic intervention "trumps" genetic risk.
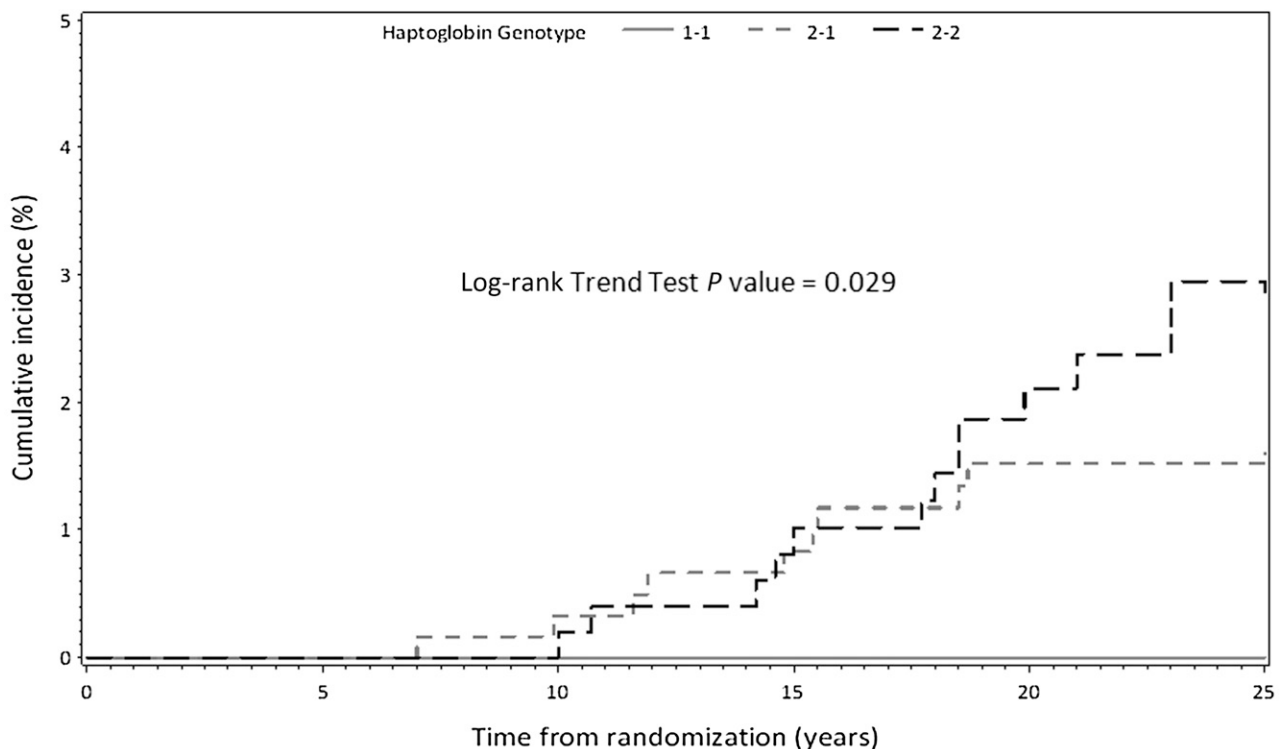
N at risk by HP type:					
1-1: 169	168	159	154	135	18
1-2: 618	616	603	582	512	71
2-2: 516	510	496	481	411	58

FIG. 2. Cumulative incidence of ESRD in the 1,303 Caucasian DCCT/EDIC participants by HP type 1-1, 2-1, and 2-2. *P* value is from a log-rank trend test.

These data are consistent with preclinical, transgenic, and human longitudinal studies. In vitro studies have demonstrated key differences between the HP 1-1 and HP 2-2 proteins in their abilities to block the pro-oxidative activity of extracorpuscular free Hb and to clear this free Hb via the HP-Hb receptor CD163 (6,17). These differences are exaggerated in the setting of conditions mimicking the diabetic state (17). These data are also consistent with those published from the EDC cohort, where in 18 years of follow-up, a strong relationship was described between the HP 2-2 genotype and the incidence of early decline in renal function and ESRD, which remained, or became even stronger, after confounder adjustment (8). In EDC, as here in DCCT/EDIC, it also seems the HP 2-1 group is at intermediate risk. Interestingly, also similar to what has been described in EDC (8), the HP genotype was not significantly associated with microalbuminuria in DCCT/EDIC. There is a growing body of evidence suggesting that factors contributing to the development of microalbuminuria and a functional decline in eGFR may be more distinct than previously thought (18,19). Recent studies, including the DCCT/EDIC study, have demonstrated that reductions in eGFR can occur without preceding microalbuminuria, although ESRD is always preceded by albuminuria of some degree (18,19). The importance of a greater understanding of eGFR and albuminuria is further emphasized by the observations from two major studies that demonstrated that virtually all the excess mortality in type 1 diabetes could be related to the development of microalbuminuria (20,21).

In addition to its potential role in renal disease, the HP genotype has also been demonstrated to be associated with CVD in type 1 diabetes (22,23). Examination of the DCCT/EDIC cohort to investigate the effect of glycemic control on the manifestation, or strength, of the HP effect on CVD risk will need to await the development of more events to facilitate such analyses.

The association of the HP genotype and LDL concentrations or non-HDL cholesterol observed here has been seen in some cohorts (8), but not all (24). Multivariate analysis controlling for LDL did not change the effect sizes of HP on risk of renal dysfunction seen here. This association with HP genotype and LDL appears to be due to a genetic variation in the HPR gene that is tightly linked to the HP locus (25).

The major limitation of this report is the potential selection bias of the DCCT/EDIC cohort because subjects with hypertension and an albumin excretion rate >200 mg/24 h are excluded.

In conclusion, we have confirmed an independent association between HP genotype 2-2 and renal function decline in type 1 diabetes. HP genotyping may be useful in establishing which individuals with diabetes are at the highest risk for developing renal dysfunction.

REFERENCES

1. de Boer IH, Sun W, Cleary PA, et al.; DCCT/EDIC Research Group. Intensive diabetes therapy and glomerular filtration rate in type 1 diabetes. N Engl J Med 2011;365:2366–2376
2. Pezzolesi MG, Skupien J, Mychaleckyj JC, Warram JH, Krolewski AS. Insights to the genetics of diabetic nephropathy through a genome-wide association study of the GoKinD collection. Semin Nephrol 2010;30:126–140
3. Makuc J, Petrovic D. A review of oxidative stress related genes and new antioxidant therapy in diabetic nephropathy. Cardiovasc Hematol Agents Med Chem 2011;9:253–261
4. Langlois MR, Delanghe JR. Biological and clinical significance of haptoglobin polymorphism in humans. Clin Chem 1996;42:1589–1600
5. Bowman BH, Kurosky A. Haptoglobin: the evolutionary product of duplication, unequal crossing over, and point mutation. Adv Hum Genet 1982;12:189–261, 453–454
6. Asleh R, Marsh S, Shilkrut M, et al. Genetically determined heterogeneity in hemoglobin scavenging and susceptibility to diabetic cardiovascular disease. Circ Res 2003;92:1193–1200
7. Levy AP, Asleh R, Blum S, et al. Haptoglobin: basic and clinical aspects. Antioxid Redox Signal 2010;12:293–304
8. Costacou T, Ferrell RE, Ellis D, Orchard TJ. Haptoglobin genotype and renal function decline in type 1 diabetes. Diabetes 2009;58:2904–2909
9. The Diabetes Control and Complications Trial (DCCT). Design and methodologic considerations for the feasibility phase. The DCCT Research Group. Diabetes 1986;35:530–545
10. EDIC Research Group. Epidemiology of Diabetes Interventions and Complications (EDIC). Design and implementation of a long-term follow-up of the Diabetes Control and Complications Trial Cohort. Diabetes Care 1999;22:99–111
11. Goldstein DE, Soeldner S, Cleary PA, Nathan DM; The DCCT Research Group. Feasibility of centralized measurements of glycated hemoglobin in the Diabetes Control and Complications Trial: a multicenter study. Clin Chem 1987;33:2267–2271
12. The Diabetes Control and Complications (DCCT) Research Group. Effect of intensive therapy on the development and progression of diabetic

nephropathy in the Diabetes Control and Complications Trial. Kidney Int 1995;47:1703–1720

13. Albers JW, Herman WH, Pop-Busui R, et al.; Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. Effect of prior intensive insulin treatment during the Diabetes Control and Complications Trial (DCCT) on peripheral neuropathy in type 1 diabetes during the Epidemiology of Diabetes Interventions and Complications (EDIC) Study. Diabetes Care 2010;33:1090–1096

14. Levey AS, Stevens LA, Schmid CH, et al.; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150:604–612

15. Hochberg I, Roguin A, Nikolsky E, Chanderashekhar PV, Cohen S, Levy AP. Haptoglobin phenotype and coronary artery collaterals in diabetic patients. Atherosclerosis 2002;161:441–446

16. Levy NS, Vardi M, Blum S, et al. An enzyme linked immunosorbent assay (ELISA) for the determination of the human haptoglobin phenotype. Clin Chem Lab Med 2013:1–8

17. Asleh R, Guetta J, Kalet-Litman S, Miller-Lotan R, Levy AP. Haptoglobin genotype- and diabetes-dependent differences in iron-mediated oxidative stress in vitro and in vivo. Circ Res 2005;96:435–441

18. Costacou T, Ellis D, Fried L, Orchard TJ. Sequence of progression of albuminuria and decreased GFR in persons with type 1 diabetes: a cohort study. Am J Kidney Dis 2007;50:721–732

19. DCCT/EDIC Research Group, de Boer IH, Sun W, Cleary PA, et al. Intensive diabetes therapy and glomerular filtration rate in type 1 diabetes. N Engl J Med 2011;365:2366–2376

20. Groop PH, Thomas MC, Moran JL, et al.; FinnDiane Study Group. The presence and severity of chronic kidney disease predicts all-cause mortality in type 1 diabetes. Diabetes 2009;58:1651–1658

21. Orchard TJ, Secrest AM, Miller RG, Costacou T. In the absence of renal disease, 20 year mortality risk in type 1 diabetes is comparable to that of the general population: a report from the Pittsburgh Epidemiology of Diabetes Complications Study. Diabetologia 2010;53:2312–2319

22. Costacou T, Ferrell RE, Orchard TJ. Haptoglobin genotype: a determinant of cardiovascular complication risk in type 1 diabetes. Diabetes 2008;57:1702–1706

23. Simpson M, Snell-Bergeon JK, Kinney GL, et al. Haptoglobin genotype predicts development of coronary artery calcification in a prospective cohort of patients with type 1 diabetes. Cardiovasc Diabetol 2011;10:99

24. Delanghe J, Langlois M, Duprez D, De Buyzere M, Clement D. Haptoglobin polymorphism and peripheral arterial occlusive disease. Atherosclerosis 1999;145:287–292

25. Guthrie PA, Rodriguez S, Gaunt TR, Lawlor DA, Smith GD, Day IN. Complexity of a complex trait locus: HP, HPR, haemoglobin and cholesterol. Gene 2012;499:8–13