Haptoglobin Genotype and Renal Function Decline in Type 1 Diabetes

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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 m² from baseline estimated [by the Cockcroft-Gault equation] glomerular filtration rate [eGFR] in those with baseline eGFR >60 ml/min per 1.73 m²).

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

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 α2-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 (Hp1 and Hp2) 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 anti-hypertensive medication. Stable HbA1c was measured by ion exchange chromatography (Isolab, Akron, OH) and subsequently by automated high-performance liquid chromatography (Diazymat; 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.

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creatinine excretion and albumin-to-creatinine ratio was used (microalbuminuria) in 10% of the samples. Urine collections were deemed inadequate based on defined as starting dialysis or undergoing renal transplantation. Early renal function decline was defined as the incidence of ≥30 ml/min per 1.73 m² 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 (Genta 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 logtransformed. Univariate associations were determined using the Student t test and χ² 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 HbA1c, blood pressure, non-HDL cholesterol, serum creatinine, eGFR, and AER. The incidence 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 m² 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 m² and ESRD incidence in Table 2. Generally, incident case subjects with both microalbuminuria and macroalbuminuria were more likely to have higher levels of HbA1c, 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,

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**TABLE 1**

Participant characteristics at study entry by subsequent microalbuminuria and macroalbuminuria status

|                      | Microalbuminuria | Macroalbuminuria |
|----------------------|------------------|------------------|
| **n**                | 163              | 111              |
| **Age (years)**      | 25.0 ± 7.7       | 25.8 ± 8.3       |
| **Age at onset (years)** | 8.5 ± 4.2       | 8.3 ± 4.1        |
| **Diabetes duration (years)** | 16.3 ± 6.9     | 17.6 ± 7.6       |
| **Follow-up time (years)** | 15.5 ± 5.0      | 7.3 ± 4.8        |
| **Female subjects**  | 50.9 (83)        | 55.9 (62)        |
| **BMI (kg/m²)**      | 23.1 ± 3.2       | 23.2 ± 3.3       |
| **Waist-to-hip ratio** | 0.81 ± 0.06     | 0.82 ± 0.06      |
| **Ever smokers**     | 28.8 (47)        | 31.5 (35)        |
| **HbA1 (%)**         | 9.7 (1.4)        | 10.8 (1.8)       |
| **Insulin dose per weight** | 0.81 (0.65–0.95) | 0.80 (0.66–0.95) |
| **Systolic blood pressure (mmHg)** | 106.9 ± 10.7   | 109.1 ± 10.3  |
| **Diastolic blood pressure (mmHg)** | 67.9 ± 8.4     | 69.8 ± 7.8      |
| **Hypertension**     | 3.7 (6)          | 3.6 (6)          |
| **HDL cholesterol (mg/dl)** | 55.2 ± 12.1     | 54.0 ± 9.8      |
| **Non-HDL cholesterol (mg/dl)** | 116.0 ± 25.5    | 128.1 ± 94.3   |
| **ACE/ARB use**      | 1.3 (2)          | 0.9 (1)          |
| **Serum creatinine (mg/dl)** | 0.80 (0.70–1.0) | 0.80 (0.60–0.90) |
| **eGFR by Cockcroft-Gault (ml/min per 1.73 m²)** | 121.1 ± 37.8 | 124.6 ± 38.7  |
| **AER (µg/min)**     | 7.2 (5.1–10.1)   | 9.0 (6.2–11.6)   |
| **White blood cell count x 10³/mm³** | 5.6 (4.8–6.6) | 6.1 (5.2–7.2)  |
| **Fibrinogen (mg/dl)** | 250.0 (200.0–300.0) | 265.0 (220.0–300.0) |
| **Hp genotype**      |                  |                  |
| 1/1                  | 77.4 (24)        | 22.6 (7)         |
| 2/1                  | 55.5 (71)        | 44.5 (57)        |
| 2/2                  | 59.1 (68)        | 40.9 (47)        |
| **HbA1 (%)**         |                  |                  |
| **Insulin dose per weight** |              |                  |
| **Systolic blood pressure (mmHg)** |              |                  |
| **Diastolic blood pressure (mmHg)** |              |                  |
| **Hypertension**     |              |                  |
| **HDL cholesterol (mg/dl)** |              |                  |
| **Non-HDL cholesterol (mg/dl)** |              |                  |
| **ACE/ARB use**      |              |                  |
| **Serum creatinine (mg/dl)** |              |                  |
| **eGFR by Cockcroft-Gault (ml/min per 1.73 m²)** |              |                  |
| **AER (µg/min)**     |              |                  |
| **White blood cell count x 10³/mm³** |              |                  |
| **Fibrinogen (mg/dl)** |              |                  |

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 (392 noncases and 60 incident cases). *The Wilcoxon two-sample test was used for nonnormally distributed variables; data are median (interquartile range). †Fisher exact test. AB, 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, HbA1c, 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
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

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**TABLE 3**

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

| Outcome                        | Model 1 | Model 2 | Model 3 |
|--------------------------------|---------|---------|---------|
| Microalbuminuria (n = 270, 111 incident events) | | | |
| Hp genotype                    |         |         |         |
| 1/1 Referent                   |         |         |         |
| 2/1                            | 2.09 (0.95–4.59) | 2.19 (0.996–4.80) | 2.08 (0.95–4.56) |
| 2/2                            | 1.84 (0.83–4.07) | 1.95 (0.88–4.32) | 1.67 (0.75–3.71) |
| 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 HbA1, 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                   |         |         |         |
| 2/1                            | 0.77 (0.35–1.70) | 0.78 (0.35–1.72) | 0.77 (0.35–1.72) |
| 2/2                            | 0.84 (0.38–1.86) | 0.78 (0.35–1.72) | 0.78 (0.35–1.73) |
| 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 HbA1, 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 $\geq$30 ml/min per 1.73 m$^2$ from baseline eGFR (n = 441, 187 incident events) | | | |
| Hp genotype                    |         |         |         |
| 1/1 Referent                   |         |         |         |
| 2/1                            | 1.59 (0.97–2.60) | 1.30 (0.78–2.18) | 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) |
| 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 HbA1, 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                   |         |         |         |
| 2/1                            | 0.72 (0.39–1.69) | 1.14 (0.48–2.74) | 1.24 (0.51–2.98) |
| 2/2                            | 1.15 (0.50–2.61) | 2.17 (0.93–5.04) | 2.74 (1.17–6.45) |
| A1C 672.450                    | 522.184 | 508.657 | 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 HbA1, hypertension, and HDL and non-HDL cholesterol | | | |
| Model 3 allowed for variables in model 2 in addition to white blood cell count and fibrinogen | | | |
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TABLE 4
Incidence of renal outcomes by Hp genotype

| Outcome incidence | n   | 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  |
| Macrolaminuria     |     |     |     |     |       |
| 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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REFERENCES
1. Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. Diabetes 1999;48:1–9
2. Lonn E, Yusuf S, Hoogwerf B, Pogue J, Yi Q, Zinman B, Bosch J, Dagenais G, Mann JF, Gerstein HC, HOPE Study, MICRO-HOPE Study. Effects of vitamin E on cardiovascular and microvascular outcomes in high-risk patients with diabetes: results of the HOPE study and MICRO-HOPE substudy. Diabetes Care 2002;25:1919–1927
3. Levy AP. Application of pharmacogenomics in the prevention of diabetic cardiovascular disease: mechanistic basis and clinical evidence for utilization of the haptoglobin genotype in determining benefit from antioxidant therapy. Pharmacol Ther 2006;112:501–512
4. Langlois MR, Delanghe JR. Biological and clinical significance of haptoglobin polymorphism in humans. Clin Chem 1996;42:1589–1600
5. Kristiansen M, Graversen JH, Jacobsen C, Sonne O, Hoffman HJ, Law SK, Moestrup SK. Identification of the haemoglobin scavenger receptor. Nature 2004;406:198–201
6. Ashle R, Levy AP. In vivo and in vitro studies establishing haptoglobin as a major susceptibility gene for diabetic vascular disease. Vasc Health Risk Manag 2005;1:119–28
7. Ashle R, Marsh S, Shilkrot M, Binah O, Guetta J, Lejbkowicz F, Enav B, Shehadeh N, Kanter Y, Lache O, Cohen O, Levy NS, Levy AP. Genetically determined heterogeneity in Hb scavenging and susceptibility to diabetic cardiovascular disease. Circ Res 2006;98:1193–1200
8. 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
9. Levy AP, Purushothaman KR, Levy NS, Purushothaman M, Strauss M, Ashle R, Marsh S, Cohen O, Moestrup SK, Moller HJ, Zias EA, Benhayon D, Fuster V, Moreno PR. Downregulation of the Hb scavenger receptor in individuals with diabetes and the Hp 2–2 genotype: implications for the response to intraplaque hemorrhage and plaque vulnerability. Circ Res 2007;101:106–110
10. Ashle R, Miller-Lotan R, Aviram M, Hayek T, Yulish M, Levy JE, Miller B, Blum S, Milman U, Shapira C, Levy AP. Haptoglobin genotype is a regulator of reverse cholesterol transport in diabetes in vitro and in vivo. Circ Res 2006;99:1419–1425
11. Levy AP, Hochberg I, Jablonski K, Resnick HE, Lee ET, Best L, Howard BV, Strong Heart Study. Haptoglobin phenotype is an independent risk factor for cardiovascular disease in individuals with diabetes: the Strong Heart Study. J Am Coll Cardiol 2002;40:1984–1990
12. Suleiman M, Aronson D, Ashle R, Kapeliovich MR, Roguin A, Meisel SR, Shochat M, Aronson D, Alshiek J, Bennett L, Kostenko M, Landau M, Keidar S, Levy Y, Khemlin A, Radan A, Levy AP. Vitamin E supplementation reduces cardiovascular events in a subgroup of middle-aged individuals with both type 2 diabetes
mellitus and the haptoglobin 2–2 genotype: a prospective double-blinded clinical trial. Arterioscler Thromb Vasc Biol 2008;28:341–347
15. Costacou T, Ferrell RE, Orchard TJ. Haptoglobin genotype: a determinant of cardiovascular complication risk in type 1 diabetes. Diabetes 2008;57:1702–1706
16. Orchard TJ, Dorman JS, Maser RE, Becker DJ, Drash AL, Ellis D, LaPorte RE, Kuller LH. Prevalence of complications of IDDM by sex and duration: Pittsburgh Epidemiology of Diabetes Complications Study II. Diabetes 1990;39:1116–1124
17. Wagener DK, Sacks JM, LaPorte RE, Macgregor JM. The Pittsburgh Study of insulin-dependent diabetes mellitus: risk for diabetes among relatives of IDDM. Diabetes 1982;31:136–144
18. Borhani NO, Kass EH, Langford HG, Payne GH, Remington RD, Stamler J, HDFP Cooperative Group. The hypertension detection and follow-up program. Prev Med 1976;5:207–215
19. Warnick GR, Albers JJ. Heparin–Mn2+/H11001 quantitation of high-density-lipoprotein cholesterol: an ultrafiltration procedure for lipemic samples. Clin Chem 1978;24:900–904
20. National Institutes of Health and Department of Health. Lipid Research Clinics Program. Washington, DC, US Government Printing Office, 1975
21. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974;20:470–475
22. Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem 1973;19:476–482
23. Ellis D, Buffone GJ. A new approach to the evaluation of proteinuric states. Clin Chem 1977;23:666–670
24. Ellis D, Coonrod BA, Dorman JS, Kelsey SF, Becker DJ, Ayner ED, Orchard TJ. Choice of urine sample predictive of microalbuminuria in patients with insulin-dependent diabetes mellitus. Am J Kidney Dis 1989;13:321–328
25. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16:31–41
26. Koch W, Latz W, Elchinger M, Roguin A, Levy AP, Schömig A, Kastrati A. Genotyping of the common haptoglobin Hp 1/2 polymorphism based on PCR. Clin Chem 2002;48:1377–1382
27. Victor J, Cheong W, Chen JJS, Levy N, Miller-Lotan R, Levy AP, Blum S, Orchard TJ, Evans RW, Costacou T, Hauth BA. Clinical results of a rapid screening assay for haptoglobin 2–2: a cardiovascular disease risk marker in diabetes (Abstract). Diabetes 2009;58(Suppl. 1):A176
28. Levy AP, Roguin A, Hochberg I, Herer P, Marsh S, Nakhoul FM, Skorecki K. Haptoglobin phenotype and vascular complications in patients with diabetes. N Engl J Med 2000;343:969–970
29. Conway BR, Savage DA, Brady HR, Maxwell AP. Association between haptoglobin gene variants and diabetic nephropathy: haptoglobin polymorphism in nephropathy susceptibility. Nephron Exp Nephrol 2007;105:e75–e79
30. Koda Y, Soejima M, Yamagishi S, Amano S, Okamoto T, Inagaki Y, Yamada K, Kimura H. Haptoglobin genotype and diabetic microangiopathies in Japanese diabetic patients. Diabetologia 2002;45:1039–1040
31. Ellis D, Forrest KY, Erbey J, Orchard TJ. Urinary measurement of transforming growth factor-β and type IV collagen as new markers of renal injury: application in diabetic nephropathy. Clin Chem 1998;44:953–956
32. Kramer HJ, Nguyen QD, Curhan G, Hsu CY. Renal insufficiency in the absence of albuminuria and retinopathy among adults with type 2 diabetes mellitus. JAMA 2003;289:3273–3277
33. Retnakaran R, Cull CA, Thorne KJ, Adler AI, Holman RR, UKPDS Study Group. Risk factors for renal dysfunction in type 2 diabetes: U.K. Prospective Diabetes Study 74. Diabetes 2006;55:1832–1839
34. 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
35. Nakhoul FM, Miller-Lotan R, Awad H, Asleh R, Jad K, Nakhoul N, Asaf R, Abu-Saleh N, Levy AP. Pharmacogenomic effect of vitamin E on kidney structure and function in transgenic mice with the haptoglobin 2–2 genotype and diabetes mellitus. Am J Physiol Renal Physiol 2009;296:F830–F838