Haptoglobin Genotype
A Determinant of Cardiovascular Complication Risk in Type 1 Diabetes

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

OBJECTIVE—Haptoglobin is a plasma protein that binds free hemoglobin, thereby inhibiting hemoglobin-induced oxidative damage. We investigated the association between the haptoglobin genotype and the incidence of coronary artery disease (CAD) in a cohort of individuals with childhood-onset type 1 diabetes.

RESEARCH DESIGN AND METHODS—Participants from the Epidemiology of Diabetes Complications Study who were free of CAD at study entry and had DNA available were selected (n = 453, mean age 27.1 years, and diabetes duration 18.8 years). CAD was defined as angina, ischemic electrocardiogram, myocardial infarction confirmed by Q-waves on electrocardiogram or hospital records, angiographic stenosis >50%, or revascularization.

RESULTS—The proportions of the cohort with the haptoglobin 1/1, 2/1, and 2/2 genotypes were 11.5, 41.3, and 47.2%, respectively. During 18 years of follow-up, there were 135 (29.8%) incident CAD events. Univariately, the proportion of CAD events increased from 15.4 to 28.3 and 34.6% for haptoglobin 1/1, 2/1, and 2/2, respectively (P = 0.02, P-trend = 0.007). Cumulative incidence (including 33 baseline prevalent cases) also increased from 24.1 to 32.3 and 39.1%, respectively (P = 0.07, P-trend = 0.02). In Cox proportional hazards models adjusting for traditional CAD risk factors, the haptoglobin 2/2 genotype was associated with increased CAD incidence compared with the haptoglobin 1/1 genotype (hazard ratio [HR] 2.21, 95% CI 1.05–4.65, P = 0.04). Although the risk associated with the haptoglobin 2/1 genotype did not reach significance (1.78, 0.84–3.79, P = 0.13), there remained a significant trend across the three groups (P = 0.03).

CONCLUSIONS—These data support the hypothesis that the haptoglobin genotype influences cardiovascular risk in type 1 diabetes. ***Diabetes*** **57:1702–1706, 2008**

Although individuals with type 1 diabetes exhibit increased morbidity and mortality from cardiovascular disease compared with the general population, the underlying pathogenesis of atherosclerosis in type 1 diabetes is still poorly understood (1). Recently, a number of studies have shown increased cardiovascular disease risk among diabetic individuals carrying the haptoglobin 2/2 genotype (2–5).

Haptoglobin, from the Greek word *haptein* (to bind), is a plasma protein that owes its name to its hemoglobin-binding properties. These properties have led to its description as an antioxidant protein because binding to free hemoglobin inhibits hemoglobin-induced oxidative tissue damage (6). Haptoglobin is mainly synthesized by hepatocytes, its levels increasing during inflammation or infection (7). Thus, the involvement of haptoglobin in states of oxidation and inflammation has generated interest in its potential association with vascular disease, especially under conditions of increased glycemic levels. In humans, two alleles for haptoglobin have been described that give rise to different haptoglobin proteins and three major genotypes, haptoglobin 1/1, 2/1, and 2/2 (8). Up to now, reported studies in diabetes have been conducted in individuals with presumed type 2 diabetes (2–5). We thus assessed the association between the haptoglobin genotype and coronary artery disease (CAD) incidence in a cohort of individuals with childhood-onset type 1 diabetes.

RESEARCH DESIGN AND METHODS

The study population was a historical, prospective cohort based on incident cases of childhood-onset (<17 years of age) type 1 diabetes diagnosed or seen within 1 year of diagnosis at Children's Hospital of Pittsburgh between 1950 and 1980 (9–10), shown to be representative of the type 1 diabetes population of Allegheny County, Pennsylvania (11). This cohort was first examined for the Pittsburgh Epidemiology of Diabetes Complications study between 1986 and 1988, when the mean age was 28 years and the mean diabetes duration was 19 years. Participants were subsequently reexamined biennially for 10 years, with a more limited follow-up for a further 8 years. The University of Pittsburgh institutional review board approved the study protocol.

Before each of the scheduled clinic visits, participants were sent questionnaires concerning demographic, health care, self-care, and medical history information. Blood pressure was measured with a random-zero sphygmomanometer, according to the Hypertension Detection and Follow-up Program protocol, after a 5-min rest (12). Hypertension was defined as ≥140/90 mmHg or use of antihypertensive medication. Stable glycosylated hemoglobin (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 (heparin and manganese chloride) with a modification (13) of the Lipid Research Clinics method (14). Cholesterol and triglycerides were measured enzymatically (15,16). Non-HDL cholesterol was calculated as total minus HDL cholesterol. Smoking status (ever/never) was obtained via self-report. The glucose disposal rate was estimated by a regression equation (with terms for waist-to-hip ratio, HbA_1, and hypertension) derived from hyperinsulinemic-euglycemic clamp studies on 24 individuals chosen to represent the full spectrum of insulin resistance, as represented by insulin resistance risk factors (17). White blood cell count was obtained using a Coulter Counter model S-Plus IV and fibrinogen using a biuret colorimetric procedure and a clotting method. Serum and urinary albumin were measured by immunonephelometry (18), and creatinine was assayed by an Ectachem 400 Analyzer (Eastman Kodak, Rochester, NY).

CAD was determined by study physician–diagnosed angina, myocardial

From the [1]Department of Epidemiology, University of Pittsburgh, Pittsburgh, Pennsylvania; and the [2]Department of Human Genetics, University of Pittsburgh, Pittsburgh, Pennsylvania.

Corresponding author: Tina Costacou, PhD, Diabetes and Lipid Research Bldg., 3512 Fifth Ave., Pittsburgh, PA 15213. E-mail: costacout@edc.pitt.edu.

Published ahead of print at http://diabetes.diabetesjournals.org on 10 March 2008. DOI: 10.2337/db08-0095.

Received for publication 22 January 2008 and accepted in revised form 3 March 2008.

CAD, coronary artery disease.

TABLE 1
Baseline risk characteristics by haptoglobin genotype in a cohort of individuals with type 1 diabetes

Risk characteristics	*n* according to haptoglobin genotype			Haptoglobin genotype		
	1/1	2/1	2/2	1/1	2/1	2/2
Age (years)	52	187	214	26.1 ± 7.4	28.2 ± 8.0	26.3 ± 7.4†
Age at onset (years)	52	187	214	7.8 ± 4.0	8.8 ± 4.1	8.0 ± 4.0
Diabetes duration (years)	52	187	214	18.2 ± 7.7	19.4 ± 7.4	18.3 ± 7.6
Percent female	52	187	214	55.8 (29)	47.6 (89)	49.5 (106)
BMI (kg/m^2)	52	187	213	23.6 ± 3.5	23.8 ± 3.3	23.5 ± 3.3
Waist-to-hip ratio	52	186	212	0.80 ± 0.07	0.82 ± 0.07	0.83 ± 0.07
Percent ever smoked	52	187	214	32.7 (17)	37.4 (70)	35.5 (76)
HbA_1 (%)	52	187	212	10.4 ± 2.0	10.1 ± 1.6	10.3 ± 1.8
eGDR (mg × kg^{-1} × min^{-1})	52	186	210	8.2 ± 1.9	8.0 ± 1.8	7.9 ± 1.8
Insulin dose/weight	52	182	205	0.86 ± 0.30	0.76 ± 0.23*	0.81 ± 0.23
Systolic blood pressure (mmHg)	52	187	214	111.9 ± 13.8	112.0 ± 14.4	111.9 ± 13.2
Diastolic blood pressure (mmHg)	52	187	214	71.4 ± 10.9	71.5 ± 10.6	71.8 ± 10.4
Percent hypertensive	52	187	214	11.5 (6)	14.4 (27)	13.1 (28)
Pulse	52	187	214	78.3 ± 8.6	77.1 ± 11.5	77.8 ± 9.1
HDL cholesterol (mg/dl)	51	187	213	55.8 ± 12.2	54.2 ± 11.4	53.8 ± 12.5
Non-HDL cholesterol (mg/dl)	51	187	213	129.3 ± 42.9	128.1 ± 35.3	139.1 ± 43.9†
ACE inhibitor use (%)	52	185	206	1.9 (1)	2.7 (5)	2.9 (6)
Serum creatinine (mg/dl)‡	52	187	213	0.91 ± 0.38	0.89 ± 0.30	0.97 ± 0.77
Glomerular filtration rate by Cockcroft-Gault (ml/min per 1.73 m^2)	52	187	213	113.1 ± 35.3	116.6 ± 37.3	117.2 ± 45.0
AER (μg/min)‡	52	187	214	258.4 ± 600.4	261.7 ± 933.3	310.2 ± 863.9
Hemoglobin (g/dl)	50	186	214	15.0 ± 1.5	15.2 ± 1.6	15.1 ± 1.6
White blood cell × 10^3/mm^2	50	186	214	6.0 ± 2.0	6.4 ± 1.7	6.7 ± 1.9
Fibrinogen (mg/dl)	52	186	214	302.8 ± 96.6	278.1 ± 77.0	280.0 ± 82.7
Beck Depression Inventory‡	41	151	177	6.7 ± 6.5	7.2 ± 6.3	7.0 ± 6.1
Calories expended in physical activity	48	175	202	2,449.2 ± 2,563.1	2,019.0 ± 2,008.2	2,220.4 ± 1,896.3

Data are the means ± SD or % (*n*). *Different from the haptoglobin 1/1 genotype at $P < 0.0167$; †different from the haptoglobin 2/1 genotype at $P < 0.0167$; ‡logarithmically transformed before statistical testing. AER, albumin excretion rate; eGDR, estimated glucose disposal rate.

infarction confirmed by Q-waves on electrocardiogram (Minnesota codes 1.1 or 1.2) or hospital records, angiographic stenosis >50%, coronary artery bypass surgery, angioplasty, ischemic electrocardiogram changes (Minnesota codes 1.3, 4.1–4.3, 5.1–5.3, 7.1), or CAD death.

High–molecular weight genomic DNA was isolated using a PureGene kit (Gentra Systems, Minneapolis, MN), and haptoglobin was genotyped by the amplification method of Koch et al. (19). Genotypes were assigned visually by comparison to controls of known genotype.

Statistical analysis. Variables not following a normal distribution were logarithmically transformed (i.e., albumin excretion rate, serum creatinine, and Beck Depression Inventory). Student's *t* test and χ^2 test (or Fisher's exact test as appropriate) were used to determine univariate associations. The χ^2 test for trend was used to assess whether the proportion of CAD events increased with haptoglobin genotype. Cox proportional hazards models were constructed to assess the multivariable association between haptoglobin genotype and CAD incidence adjusting for traditional CAD risk factors 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. All statistical analyses were conducted using SAS version 9.1 (SAS Institute, Cary, NC).

RESULTS

Of the 604 participants free of CAD at study entry, DNA for haptoglobin genotyping was available for 453 (75%). Compared with those without DNA available, individuals with DNA data had a shorter diabetes duration and higher HbA_1, blood pressure, non-HDL cholesterol, and serum creatinine levels as well as a higher albumin excretion rate. The distribution of the three haptoglobin genotypes was 11.5, 41.3, and 47.2% for haptoglobin 1/1, 2/1, and 2/2, respectively. Generally, no differences were observed in participant characteristics by haptoglobin genotype at study entry, with the exception of younger age and higher non-HDL cholesterol levels in those with the haptoglobin 2/2 compared with the haptoglobin 2/1 genotype and lower insulin dose per body weight in those with the haptoglobin 2/1 compared with the haptoglobin 1/1 genotype (Table 1). During 18 years of follow-up, there were 135 (29.8%) incident CAD events. As presented in Table 2, the proportion of incident CAD events increased from 15.4% for haptoglobin 1/1, to 28.3 and 34.6% for haptoglobin 2/1 and 2/2 genotypes, respectively (χ^2 $P = 0.02$, P-trend = 0.007). To exclude the possibility that survival bias may have led to a spurious association, we stratified the cohort by year of diabetes diagnosis (before or after 1965, as the proportion of deceased was 40% versus only 13%, respectively). Despite the considerably smaller number of events, similar trends were observed, although results did not always reach statistical significance. To further exclude the possibility of bias resulting from the elimination of prevalent cases at study entry, analyses were also conducted for cumulative CAD incidence (including 33 prevalent cases at baseline), and similar findings were obtained; cumulative incidence increased from 24.1% for haptoglobin 1/1, to 32.3 and 39.1% for haptoglobin 2/1 and 2/2, respectively (χ^2 $P = 0.07$, P-trend = 0.02). Finally, similar trends were also observed for hard CAD incidence (myocardial infarction, revascularization, stenosis >50%, and CAD death, and excluding ischemic electrocardiogram changes and angina, $n = 480$).

In multivariable Cox proportional hazards models adjusting for traditional CAD risk factors (Table 3), the haptoglobin 2/2 genotype was associated with increased

TABLE 2
Distribution of incident cases of CAD over 18 years of follow-up among individuals with type 1 diabetes

Complication	Haptoglobin genotype 1/1	2/1	2/2	χ^2 *P* value	*P*-trend
CAD	52	187	214		
Yes (n = 135)	15.4 (8)	28.3 (53)	34.6 (74)	0.02	0.007
Hard CAD	58	196	226		
Yes (n = 102)	13.8 (8)	19.9 (39)	24.3 (55)	0.18	0.07
Participants diagnosed with type 1 diabetes prior to 1965					
CAD	17	61	66		
Yes (n = 80)	29.4 (5)	52.5 (32)	65.2 (43)	0.02*	0.008
Hard CAD	20	63	73		
Yes (n = 61)	25.0 (5)	36.5 (23)	45.2 (33)	0.23*	0.09
Participants diagnosed with type 1 diabetes after 1965					
CAD	35	126	148		
Yes (n = 55)	8.6 (3)	16.7 (21)	21.0 (31)	0.22*	0.08
Hard CAD	38	133	153		
Yes (n = 41)	7.9 (3)	12.0 (16)	14.4 (22)	0.59*	0.28

Data presented are n or % (n). Hard CAD has been defined as myocardial infarction, revascularization, stenosis >50%, or death from CAD, thus excluding angina and ischemia from the total CAD definition. *Fisher's exact test *P* value.

CAD incidence compared with the haptoglobin 1/1 genotype (hazard ratio [HR] 2.21, 95% CI 1.05–4.65, $P = 0.04$). Although the intermediate risk associated with the haptoglobin 2/1 genotype did not reach statistical significance compared with the haptoglobin 1/1 genotype (1.78, 0.84–3.79, *P*-value = 0.13), there was a significant trend across the three groups (*P*-trend = 0.03). Combining haptoglobin 2/1 and 2/2 was also associated with an increased cardiovascular disease risk compared with the haptoglobin 1/1 genotype (2.00, 0.97–4.13, *P*-value = 0.06). In analyses replacing HbA_1, waist-to-hip ratio, and hypertension with estimated glucose disposal rate (insulin sensitivity), both haptoglobin 2/1 and 2/2 were associated with increased CAD risk compared with those with the 1/1 genotype (1.93, 0.91–4.09, $P = 0.08$ for haptoglobin 2/1; 2.31, 1.11–4.82, $P = 0.03$ for haptoglobin 2/2, *P*-trend = 0.03; and 2.13, 1.04–4.39, $P = 0.04$ for the combination of haptoglobin 2/1 and 2/2).

DISCUSSION

In this cohort of individuals with a long duration of type 1 diabetes, we demonstrated an increased risk of cardiovascular disease among individuals carrying the haptoglobin 2/2 genotype compared with those carrying the haptoglobin 1/1 genotype. This association persisted after accounting for traditional cardiovascular risk factors and appeared independent of central obesity, lipid concentrations, and inflammation (white blood cell count), factors which appeared to reduce its effect, as well as of glycemic control. Although the univariately observed risk elevations of individuals with the haptoglobin 2/1 compared with those with the haptoglobin 1/1 genotype did not reach statistical significance after multivariable adjustment, there was a consistent trend. Furthermore, combining the haptoglobin 2/1 and 2/2 genotypes conferred a twofold greater increased cardiovascular risk compared with indi-

TABLE 3
Cox proportional hazards models for the prediction of CAD among individuals with type 1 diabetes

Risk characteristics	Model 1	Model 2	Model 3	Model 4
Haptoglobin genotype (%)				
1/1	Referent	Referent	Referent	Referent
2/1	1.83 (0.87–3.86)	1.77 (0.84–3.74)	1.75 (0.83–3.69)	1.78 (0.84–3.79)*
2/2	2.62 (1.26–5.44)	2.43 (1.17–5.07)	2.38 (1.14–4.96)	2.21 (1.05–4.65)†
Diabetes duration (years)	1.13 (1.10–1.16)	1.12 (1.10–1.15)	1.12 (1.10–1.15)	1.11 (1.08–1.14)
Female sex (%)	Not made available	Not selected	Not selected	Not selected
Waist-to-hip ratio	Not made available	19.90 (2.03–195.41)	19.31 (1.95–191.06)	Not selected
Ever smoked (%)	Not made available	1.51 (1.07–2.14)	1.48 (1.05–2.09)	1.42 (0.99–2.04)
HbA_1 (%)	Not made available	Not made available	1.09 (0.99–1.20)	Not selected
Systolic blood pressure (mmHg)	Not made available	Not made available	Not made available	Not selected
Hypertension (%)	Not made available	Not made available	Not made available	2.22 (1.49–3.30)
HDL cholesterol (mg/dl)	Not made available	Not made available	Not made available	0.98 (0.97–1.00)
Non-HDL cholesterol (mg/dl)	Not made available	Not made available	Not made available	1.007 (1.003–1.011)
Log AER (μg/min)	Not made available	Not made available	Not made available	Not selected
White blood cell count × 10^3/mm^2	Not made available	Not made available	Not made available	1.10 (1.00–1.21)
AIC	1,413.367	1,404.308	1,403.364	1,370.957

Data are hazard ratio (95% CI). Inclusion in the models of insulin dose per body weight, pulse, fibrinogen, calories expended in physical activity, glomerular filtration rate by Cockcroft-Gault, or ACE inhibitor use (variables significantly associated with CAD incidence univariately) did not alter findings. For data marked as not made available, the variable was not made available for selection in the model and thus was not included in the model. n = 443, 132 incident events. *$P = 0.13$; †$P = 0.04$. AER, albumin excretion rate; AIC, Akaike's Information Criterion.

viduals with the haptoglobin 1/1 genotype, suggesting a graded response according to the number of haptoglobin 2 alleles present.

Haptoglobin is an acute-phase protein, its secretion increasing in response to injury or inflammatory insults (7). The main function of haptoglobin, however, is that of an antioxidant, binding to free hemoglobin and thereby inhibiting hemoglobin-induced oxidative damage to tissues (6). Because both inflammation and oxidative injury are thought to contribute to the development of vascular complications (1), the haptoglobin genotype clearly has the potential to be a determinant of diabetic vascular disease risk.

Substantial evidence supports a pathogenetic role for the haptoglobin phenotype. The haptoglobin 2 allele protein product appears to be an inferior antioxidant compared with the haptoglobin 1 allele protein product. Moreover, haptoglobin 1 is more efficient both in preventing heme release from the haptoglobin-hemoglobin complexes and in promoting uptake by the CD163 macrophage receptor (20–22). In addition, haptoglobin 2/2 has been shown to have impaired reverse cholesterol transport in diabetic mice (23).

In humans, four longitudinal studies have established that this polymorphism in the haptoglobin gene is an independent risk factor for cardiovascular disease among individuals with presumed type 2 diabetes, whereas similar relationships have not been identified in persons without diabetes. In a small study of 45 individuals with type 2 diabetes (2), restenosis after a mean of 23 months past angioplasty was present in 82% of individuals with the haptoglobin 2/2 or 2/1 phenotype but in none of those individuals with the haptoglobin 1/1 phenotype ($P < 0.0001$). In a matched case-control analysis of the Strong Heart Study, the haptoglobin 2/2 compared with the haptoglobin 1/1 or 2/1 phenotype was an independent predictor of cardiovascular disease among American Indians with a type 2 diabetes diagnosis, whereas no association was observed in the nondiabetic population (3). Furthermore, in the Munich Stent Study, the haptoglobin 2/2 compared with the haptoglobin 1/1 genotype conferred an almost twofold risk of the incidence of a major adverse cardiac event within 1 year after percutaneous transluminal coronary angioplasty among individuals with type 2 diabetes (5). Haptoglobin phenotype was also shown to predict 30-day mortality and heart failure among individuals with diabetes and acute myocardial infarction, with the haptoglobin 1/1 phenotype granting protection (4). In this report, the haptoglobin 1/1 phenotype was further associated with a smaller infarct size. Once again, these relationships were only observed in the diabetic population.

Although the distribution of haptoglobin alleles does not differ between individuals with and without diabetes (8), it is of interest that the haptoglobin genotype appears to affect outcome only in those with diabetes. We have recently reported that hemoglobin levels appear elevated in type 1 diabetes (24), which may help explain this finding. Further aspects of the diabetic state, including glucosylation of the hemoglobin molecule and a reduction in the proportion of monocytes expressing surface CD163, may also contribute to this apparent effect modification (8,20). Furthermore, the oxidation of LDL by glycosylated hemoglobin is reported not to be completely blocked by binding to haptoglobin (20). Thus, because glycosylated hemoglobin levels are elevated in diabetes and the likelihood of LDL oxidation by this pathway is thereby increased, the impaired removal of glycosylated hemoglobin-haptoglobin complexes from the circulation in those with haptoglobin 2/2 may be especially critical (20). The observation that antioxidant (vitamin E) therapy was only effective in individuals with diabetes and haptoglobin 2/2 is also consistent with the current findings and suggests that such therapy may only be effective in those with inadequate antioxidant defenses (25).

In conclusion, our findings among individuals with type 1 diabetes concur with those in cohorts of type 2 diabetes and suggest that this polymorphism of the haptoglobin gene is an independent determinant of CAD risk. The significance and implication of these results in the ability of antioxidant therapy to lower cardiovascular risk in the susceptible haptoglobin 2/1 and 2/2 groups should be addressed.

ACKNOWLEDGMENTS

This research was supported by National Institutes of Health Grant DK34818.

We thank all study participants for their invaluable contributions as well as the Epidemiology of Diabetes Complications study staff.

REFERENCES

1. Orchard TJ, Costacou T, Kretowski A, Nesto RW: Type 1 diabetes and coronary artery disease. *Diabetes Care* 29:2528–2538, 2006
2. Levy AP: Haptoglobin genotype and vascular complications in patients with diabetes. *N Engl J Med* 343:969–970, 2000
3. Levy AP, Hochberg I, Jablonski K, Resnick HE, Lee ET, Best L, Howard BV; Strong Heart Study: Haptoglobin genotype is an independent risk factor for cardiovascular disease in individuals with diabetes: the Strong Heart Study. *J Am Coll Cardiol* 40:1984–1990, 2002
4. Suleiman M, Aronson D, Asleh R, Kapeliovich MR, Roguin A, Meisel SR, Shochat M, Sulieman A, Reisner SA, Markiewicz W, Hammerman H, Lotan R, Levy NS, Levy AP: Haptoglobin polymorphism predicts 30-day mortality and heart failure in patients with diabetes and acute myocardial infarction. *Diabetes* 54:2802–2806, 2005
5. Roguin A, Koch W, Kastrati A, Aronson D, Schomig A, Levy AP: Haptoglobin genotype is predictive of major adverse cardiac events in the 1-year period after percutaneous transluminal coronary angioplasty in individuals with diabetes. *Diabetes Care* 26:2628–2631, 2003
6. Bowman BH, Kurosky A: Haptoglobin: the evolutionary product of duplication, unequal crossing over, and point mutation. *Adv Hum Genet* 12:189–261, 1982
7. Gabay C, Kushner I: Mechanisms of disease: acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 340:448–454, 1999
8. Asleh R, Levy AP: In vivo and in vitro studies establishing haptoglobin as a major susceptibility gene for diabetic vascular disease. *Vasc Health Risk Manag* 1:19–28, 2005
9. Orchard TJ, Dorman JS, Maser RE, Becker DJ, Ellis D, LaPorte RE, Kuller LH, Wolfson SK Jr, Drash AL: Factors associated with the avoidance of severe complications after 25 years of insulin dependent diabetes mellitus: Pittsburgh Epidemiology of Diabetes Complications Study I. *Diabetes Care* 13:741–747, 1990
10. 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* 39:1116–1124, 1990
11. Wagener DK, Sacks JM, LaPorte RE, MacGregor JM: The Pittsburgh Study of insulin-dependent diabetes mellitus: risk for diabetes among relatives of IDDM. *Diabetes* 31:136–144, 1982
12. Borhani NO, Kass EH, Langford HG, Payne GH, Remington RD, Stamler J: The hypertension detection and follow-up program. *Prev Med* 5:207–215, 1976
13. Warnick GR, Albers JJ: Heparin-Mn2+ quantitaion of high density lipoprotein cholesterol: an ultrafiltration procedure for lipemic samples. *Clin Chem* 24:900–904, 1978
14. National Institutes of Health and Department of Health: *Lipid Research Clinics Program*. Washington, DC, U.S. Govt. Printing Office, 1975
15. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC: Enzymatic determination of total serum cholesterol. *Clin Chem* 20:470–475, 1974

16. Bucolo G, David H: Quantitative determination of serum triglycerides by the use of enzymes. *Clin Chem* 19:476–482, 1973
17. Williams KV, Erbey JR, Becker D, Arslanian S, Orchard TJ: Can clinical factors estimate insulin resistance in type 1 diabetes? *Diabetes* 49:626–632, 2000
18. Ellis D, Buffone GJ: A new approach to the evaluation of proteinuric states. *Clin Chem* 23:666–670, 1977
19. Koch W, Latz W, Eichinger M, Roguin A, Levy AP, Schömig A, Kastrati A: Genotyping of the common haptoglobin Hp 1/2 polymorphism based on PCR. *Clin Chem* 48:1377–1382, 2002
20. Asleh R, Marsh S, Shilkrut 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 hemoglobin scavenging and susceptibility to diabetic cardiovascular disease. *Circ Res* 92:1193–200, 2003
21. 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* 96:435–441, 2005
22. Levy AP, Purushothaman KR, Levy NS, Purushothaman M, Strauss M, Asleh R, Marsh S, Cohen O, Moestrup SK, Moller HJ, Zias EA, Benhayon D, Fuster V, Moreno PR: Downregulation of the hemoglobin scavenger receptor in individuals with diabetes and the Hp 2–2 genotype: implications for the response to intraplaque hemorrhage and plaque vulnerability. *Circ Res* 101:106–110, 2007
23. Asleh 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* 99:1419–1425, 2006
24. Conway B, Fried L, Orchard TJ: Hemoglobin and overt nephropathy complications in type 1 diabetes. *Annals Epidemiol* 18:147–155, 2008
25. Levy AP, Gerstein HC, Miller-Lotan R, Ratner R, McQueen M, Lonn E, Pogue J: The effect of vitamin E supplementation on cardiovascular risk in diabetic individuals with different haptoglobin phenotypes (Letter). *Diabetes Care* 27:2767, 2004