NEWS & VIEWS

Does the Concentration of Oxidative and Inflammatory Biomarkers Differ by Haptoglobin Genotype in Type 1 Diabetes?

Tina Costacou, Rhobert W. Evans, and Trevor J. Orchard

Abstract

The haptoglobin (*Hp*) 2 allele directly predicts coronary artery disease in type 1 diabetes, potentially due to its decreased antioxidative/anti-inflammatory properties. We measured the concentrations of oxidative/inflammatory biomarkers (urinary 15-isoprostane F_{2t} [IsoP], α - and γ -tocopherol, tumor necrosis factor α [TNF- α], high-sensitivity C-reactive protein [hsCRP], white blood cell [WBC] count, fibrinogen, and adiponectin) thrice during 20 years of follow-up among 454 individuals with childhood-onset type 1 diabetes (mean baseline age, 28 years and diabetes duration, 19 years). Differences in biomarkers by *Hp* were assessed both at baseline (*i.e.*, the first time point of measurements) and over time (with mixed models). No differences by *Hp* were observed at baseline with the exception of a significant trend toward higher IsoP concentrations with the number of *Hp* 2 alleles ($p=0.01$). In multivariable mixed models, the concentrations of IsoP ($\beta=0.05$, $p=0.01$) and WBC count ($\beta=0.20$, $p=0.06$) overtime increased incrementally with the number of *Hp* 2 alleles. No other biomarker assessed related to *Hp*. Reported elevated IsoP and WBC count concentrations over time among *Hp* 2 allele carriers lead to the hypothesis that the antioxidative and anti-inflammatory capacity of the *Hp* 2 is inferior to that of the *Hp* 1 allele in type 1 diabetes. *Antioxid. Redox Signal.* 23, 1439–1444.

Introduction

OVER THE PAST DECADE, the haptoglobin (*Hp*) genotype has been gaining acceptance as a determinant of diabetic cardiovascular risk. In type 1 diabetes, *Hp* has been suggested to be a strong predictor of coronary artery disease (CAD), declining kidney function, and end-stage renal disease (ESRD) (5, 6). It has been proposed that the biologic rationale underlying the observed *Hp* genotype–cardiorenal diabetes complications association relates to functional differences across the three *Hp* genotypes (7).

Hp is an acute-phase glycoprotein, whose plasma concentrations increase twofold to fivefold in response to inflammatory stimuli (7). The main role of *Hp* is to bind free hemoglobin following erythrocyte destruction, inhibiting heme iron release and reducing the heme-mediated generation of reactive oxygen species and subsequent oxidative tissue damage (7). The *Hp*-hemoglobin complex formed through this binding is cleared from circulation by either the monocyte/macrophage scavenger receptor CD163 or hepatocytes (7).

Three common *Hp* genotypes in humans (*Hp* 1-1, 2-1, and 2-2) are derived from two classes of alleles: *Hp* 1 and *Hp* 2 (7). Evidence has been accumulating that the antioxidant and anti-inflammatory properties of *Hp* are genotype dependent. Thus, the *Hp* 1 protein allele product is a more efficient antioxidant and anti-inflammatory factor, whereas *Hp* 2 is associated with enhanced angiogenic activity (1, 7). As both oxidative stress and inflammation have been implicated in the pathophysiology of atherosclerosis, impaired antioxidative activity and anti-inflammatory macrophage signaling associated with the *Hp* 2-2 genotype may constitute one of the underlying mechanisms by which this genotype is associated with increased cardiovascular risk.

With the exception of potentially higher low-density lipoprotein concentrations among *Hp* 2-2 carriers, however, few other differences have been observed across *Hp* genotypes (5, 6), although a detailed assessment of variations in oxidative/inflammatory biomarkers by *Hp* in type 1 diabetes is currently lacking. We therefore conducted repeated measurements of eight oxidative/inflammatory biomarkers (the

Innovation

The haptoglobin (*Hp*) 2 allele is thought to increase cardiovascular disease risk due to a deficiency in its antioxidant and anti-inflammatory properties compared with the *Hp* 1 allele. However, no data currently exist on the concentrations of multiple oxidative/inflammatory markers over time by *Hp* genotype in humans. We provide data on the concentration of eight oxidative/inflammatory biomarkers (urinary isoprostanes, α - and γ -tocopherol, tumor necrosis factor α [TNF- α], high-sensitivity C-reactive protein [hsCRP], white blood cell count, fibrinogen, and adiponectin) over time by *Hp* genotype among individuals with type 1 diabetes. Our findings suggest that the antioxidant and anti-inflammatory capacity of the *Hp* 2 is inferior to that of the *Hp* 1 allele.

plasma antioxidants, α - and γ -tocopherol; the lipid peroxidation biomarker, urinary 15-isoprostane F_{2t} [IsoP]; and the biomarkers of inflammation, tumor necrosis factor α [TNF- α], high-sensitivity C-reactive protein [hsCRP], white blood cell [WBC] count, fibrinogen, and adiponectin) over a period of 20 years by *Hp* genotype in adults with childhood-onset type 1 diabetes.

Results and Discussion

Distribution of, and participant characteristics by, the Hp genotype

Of 658 Pittsburgh Epidemiology of Diabetes Complications (EDC) study participants, the *Hp* genotype and samples for the assessment of biomarkers at three time points were available in 454, although samples were not always adequate for analyses of all biomarkers at all three time points. Of these 454, 11.7% were homozygous for the *Hp* 1 allele and 41.6% for the *Hp* 2 allele. In agreement with previous reports (5, 6), no differences in participant characteristics were observed by *Hp* genotype at the first time point of biomarker assessment, with the exception of a trend toward greater incident CAD events with the number of *Hp* 2 alleles (Table 1). Few differences were also noted in oxidative/inflammatory biomarkers by *Hp* and were restricted to a trend toward greater urinary IsoP concentrations with the number of *Hp* 2 alleles.

Oxidative/inflammatory biomarkers over time by Hp genotype

To evaluate whether differences in measured oxidative/inflammatory biomarkers existed by *Hp* genotype over time, we conducted repeated measures analyses using mixed models. We observed that *Hp* 2 allele carriers exhibited higher creatinine-adjusted urinary IsoP concentrations compared with their *Hp* 1-1 counterparts ($\beta=0.05$, $p=0.01$). Diabetes duration, female gender, a history of smoking, HbA1c, systolic blood pressure, and estimated glomerular filtration rate (eGFR) were also positively related to urinary IsoP.

IsoPs are currently considered the gold standard biomarker of oxidative stress and lipid peroxidation *in vivo* and have been previously associated with both subclinical atherosclerosis and manifest CAD (2). In type 1 diabetes, we have demonstrated the presence of an inverse association between the ratio of α -

tocopherol to urinary IsoP as a marker of response to oxidative stress and the development of CAD (4). Thus, although no differences were observed in α -tocopherol concentrations by *Hp* in the present investigation, the noted divergence in urinary IsoP trajectories between the *Hp* 1-1 and *Hp* 2-1/2-2 groups certainly compliments previous type 2 diabetes findings of increases in lipid peroxides associated with high-density lipoprotein (HDL) among *Hp* 2-2 carriers (1).

WBC count also increased with the number of *Hp* 2 alleles ($\beta=0.20$, $p=0.06$). Table 2 shows the results of the full and reduced (after backward elimination) models. A graphic display of trajectories of urinary IsoP and WBC count over the follow-up period by the presence or absence of the *Hp* 2 allele is shown in Figures 1 and 2, respectively. *Hp* 1-1 carriers appear to exhibit lower urinary IsoP and WBC concentrations throughout follow-up. Conversely, the trajectories of other measured biomarkers of oxidation/inflammation did not appear to consistently differ over the follow-up period by *Hp* (data not shown).

Cross-sectional reports of oxidative/inflammatory biomarkers by Hp genotype

No other studies, to date, have produced data on repeated measurements of oxidation/inflammation biomarkers over time by *Hp* genotype. However, similar to our results, no significant differences were observed in fibrinogen and CRP concentrations by *Hp* genotype in an earlier study of male Caucasians (3). Conversely, in a small ($n=56$) cross-sectional study of chronic kidney disease patients, hsCRP was elevated among *Hp* 2-2 compared with *Hp* 1-1 carriers, although no differences existed with the heterozygous group (9). In another cross-sectional study of obese adults, greater concentrations of TNF- α and interleukin 6 (IL-6) (but not hsCRP) were observed in *Hp* 2-2 versus *Hp* 1-1 carriers, with no differences noted with the heterozygous group (8). In this latter study, the gender distribution, age, and measures of body fat differed significantly by *Hp*. Thus, although the authors adjusted for these factors in multivariable analyses, questions are raised as to whether residual confounding may have been, at least partially, responsible for the observed associations with TNF- α and IL-6 as these biomarkers have been previously implicated in obesity and insulin resistance.

Limitations and conclusion

Despite the improved precision derived from our repeated risk factor measurements, biomarker assessment at three time points over a 20-year follow-up may not adequately represent an individual's oxidative/inflammatory status. Another limitation of these data lies in the restriction of measured markers to eight from a vast array of currently known oxidative/inflammatory biomarkers. Thus, whether other biomarkers better reflect differences in the antioxidant/anti-inflammatory capacity of the three *Hp* alleles cannot be derived from these data. Nevertheless, our findings suggest that the *Hp* 2 allele is associated with increased inflammation and oxidative stress in type 1 diabetes. Should these findings be replicated in further prospective studies, they could, at least partially, explain the strong and independent association of the *Hp* genotype with the incidence of cardiorenal complications.

TABLE 1. PARTICIPANT CHARACTERISTICS BY *Hp* GENOTYPE AT THE BASELINE ASSESSMENT (I.E., THE FIRST TIME POINT OF MEASUREMENTS)

	<i>Hp</i> 1-1 (n = 53)	<i>Hp</i> 2-1 (n = 212)	<i>Hp</i> 2-2 (n = 189)	Overall p-value	p-Trend
Age (years)	27.0 (8.2)	28.9 (8.4)	27.4 (7.6)	0.11	0.60
Duration (years)	17.6 (12.5, 25.8)	19.7 (13.7, 26.1)	17.6 (13.6, 24.4)	0.30	0.62
Females, % (n)	50.9 (27)	51.4 (109)	50.3 (95)	0.97	0.87
Body-mass index (kg/m ²)	23.6 (3.5)	24.0 (3.2)	23.7 (3.1)	0.46	0.80
Ever smoking, % (n)	30.2 (16)	35.5 (75)	34.0 (64)	0.76	0.80
HbA _{1c}					
%	8.7 (7.8, 9.8)	8.4 (7.7, 9.5)	8.6 (7.8, 9.8)	0.66	0.75
mmol/mol	72 (62, 84)	68 (61, 80)	70 (62, 84)		
Insulin dose per body weight	0.75 (0.61, 0.95)	0.74 (0.58, 0.91)	0.78 (0.63, 0.95)	0.29	0.30
Systolic blood pressure (mmHg)	112 (103, 119)	111 (104, 120)	110 (104, 120)	0.81	0.51
Diastolic blood pressure (mmHg)	72 (65, 84)	72 (65, 78)	72 (67, 79)	0.79	0.40
Blood pressure medication use, % (n)	9.4 (5)	10.6 (22)	8.1 (15)	0.71	0.56
Hypertension, % (n)	20.7 (11)	16.5 (35)	12.2 (23)	0.23	0.09
Pulse (beats/min)	77 (72, 84)	76 (69, 82)	76 (68, 82)	0.42	0.28
HDL cholesterol (mg/dl)	53.5 (46.9, 64.1)	53.6 (45.7, 61.0)	51.6 (44.9, 60.8)	0.49	0.33
LDL cholesterol (mg/dl)	102.9 (89.5, 124.2)	109.1 (92.0, 130.4)	112.8 (92.0, 134.2)	0.41	0.29
Non-HDL cholesterol (mg/dl)	118.7 (101.5, 151.7)	124.0 (105.9, 153.5)	131.2 (105.5, 157.0)	0.21	0.11
Total cholesterol (mg/dl)	179 (153, 202)	181 (160, 208)	185 (160, 213)	0.44	0.21
Triglycerides (mg/dl)	80 (56, 107)	77 (57, 109)	80 (62, 117)	0.72	0.94
Albumin excretion rate (μg/min)	12.7 (7.5, 378.2)	12.1 (6.1, 114.8)	12.4 (7.1, 135.6)	0.84	0.95
eGFR (ml/min/1.73 m ²)	110.7 (87.0, 124.4)	110.0 (85.1, 125.0)	114.2 (91.8, 128.9)	0.37	0.31
Complication prevalence					
CAD, % (n)	0.0 (0)	0.9 (2)	3.2 (6)	0.20	0.05
Microalbuminuria, % (n)	48.0 (24)	52.9 (90)	53.9 (83)	0.76	0.52
Macroalbuminuria, % (n)	29.2 (14)	22.2 (43)	23.0 (40)	0.58	0.56
ESRD, % (n)	4.1 (2)	1.0 (2)	1.7 (3)	0.28	0.52
Biomarkers					
Urinary 15-isoprostane F _{2t} (ng/mg creatinine)	7.4 (6.0, 10.1)	8.5 (5.8, 12.0)	8.8 (6.5, 11.7)	0.12	0.01
α-Tocopherol (mg/L)	8.2 (6.9, 9.3)	8.2 (6.5, 10.2)	8.4 (6.8, 10.6)	0.18	0.09
γ-Tocopherol (mg/L)	2.2 (1.7, 3.0)	2.0 (1.5, 2.7)	2.1 (1.6, 2.8)	0.19	0.92
TNF-α (pg/ml)	1.4 (1.2, 1.7)	1.4 (1.1, 1.9)	1.4 (1.1, 1.9)	0.85	0.49
hsCRP (mg/L)	1.2 (0.53, 2.2)	1.3 (0.52, 3.2)	1.3 (0.45, 2.7)	0.58	0.42
White blood cell count × 10 ³ /mm ²	6.0 (5.1, 6.9)	6.2 (5.3, 7.5)	6.4 (5.2, 7.3)	0.36	0.16
Fibrinogen (mg/dl)	280 (225, 330)	270 (230, 330)	270 (225, 330)	0.91	0.50
Adiponectin (μg/ml)	8.8 (5.0, 11.7)	7.8 (5.4, 10.7)	7.3 (5.1, 9.8)	0.56	0.46

Data are mean (SD), median (interquartile range), or percent (n).

CAD, coronary artery disease; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; HDL, high-density lipoprotein; *Hp*, haptoglobin; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; TNF-α, tumor necrosis factor α.

Notes

Design and methods

This investigation was based on participants of the EDC study, a representative, retrospectively defined incident cohort of childhood-onset type 1 diabetes cases, diagnosed at the Children's Hospital of Pittsburgh (1950–1980). A baseline examination was conducted in 1986–1988 (*n* = 658; mean age, 28 years and diabetes duration, 19 years). Subsequently, participants have been reexamined or surveyed biennially for up to 20 years. The University of Pittsburgh IRB approved the study protocol.

At each assessment, participants provided demographic, healthcare, self-care, and medical history information, as previously described in detail (4). Blood pressure was measured with a random zero sphygmomanometer, and hypertension was defined as blood pressure >140/90 mmHg or antihypertensive medication use. Stable glycosylated hemo-

globin (HbA_{1c}) was measured by ion exchange chromatography (Isolab, Akron, OH) for the first 18 months and for the subsequent 10 years by automated high-performance liquid chromatography (Diamat; BioRad, Hercules, CA); the two assays were highly correlated (*r* = 0.95). For follow-up beyond the 10 years, HbA_{1c} was measured with the DCA 2000 analyzer (Bayer, Tarrytown, NY). The DCA and Diamat assays were highly correlated (*r* = 0.95). Original HbA_{1c} (1986–1998) and HbA_{1c} values (1998–2004) were converted to DCCT-aligned standard HbA_{1c} values using regression formulae derived from duplicate assays.

HDL cholesterol was determined by a precipitation technique; cholesterol and triglycerides, enzymatically; and non-HDL cholesterol was calculated as the total minus HDL cholesterol. Urinary albumin was measured by immunonephelometry, and creatinine was assayed by an Ektachem 400 analyzer (Eastman Kodak Co., Rochester, NY). GFR was estimated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI)

TABLE 2. INDEPENDENT CORRELATES OF LOGARITHMICALLY TRANSFORMED URINARY ISO_P OVER TIME: FINAL FULL AND REDUCED MULTIVARIABLE MIXED MODELS

Dependent variable	Independent variables	Full model		Reduced model	
		β Estimate (st. error)	p-Value	β Estimate (st. error)	p-Value
Urinary 15-isoprostane F _{2t} (n = 454)	Number of <i>Hp</i> 2 alleles	0.05 (0.02)	0.03	0.05 (0.02)	0.01
	Diabetes duration (years)	0.006 (0.002)	0.002	0.007 (0.002)	0.0004
	Women vs. men	0.14 (0.03)	<0.0001	0.15 (0.03)	<0.0001
	Ever vs. never smokers	0.10 (0.03)	0.0008	0.09 (0.03)	0.001
	HbA1c (%)	0.06 (0.008)	<0.0001	0.05 (0.008)	<0.0001
	Systolic blood pressure (mmHg)	0.003 (0.001)	0.006	0.003 (0.001)	0.005
	Diastolic blood pressure (mmHg)	-0.003 (0.001)	0.03	-0.004 (0.001)	0.01
	Pulse (beats/min)	0.00009 (0.001)	0.93	Not made available	
	HDL cholesterol (mg/dl)	0.001 (0.001)	0.15	Not made available	
	eGFR (ml/min/1.73 m ²)	0.003 (0.0005)	<0.0001	0.003 (0.0005)	<0.0001
	Type of urine sample				
	24 h	Referent		Not made available	
White blood cell count (n = 453)	Overnight	-0.08 (0.03)	0.12		
	Timed postclinic	0.04 (0.04)	0.88		
	Number of <i>Hp</i> 2 alleles	0.19 (0.11)	0.07	0.20 (0.11)	0.06
	Diabetes duration (years)	-0.004 (0.01)	0.68	Not made available	
	Women vs. men	0.60 (0.16)	0.0001	0.61 (0.15)	<0.0001
	Body-mass index	0.04 (0.02)	0.02	0.05 (0.02)	0.02
	Ever vs. never smokers	0.90 (0.14)	<0.0001	0.90 (0.14)	<0.0001
	HbA1c (%)	0.10 (0.04)	0.008	0.11 (0.04)	0.007
	Insulin dose per body weight	1.3 (0.28)	<0.0001	1.36 (0.27)	<0.0001
	Systolic blood pressure (mmHg)	0.01 (0.005)	0.03	Not made available	
	Diastolic blood pressure (mmHg)	-0.01 (0.007)	0.12	Not made available	
	Pulse (beats/min)	0.02 (0.005)	<0.0001	0.02 (0.005)	<0.0001
	HDL cholesterol (mg/dl)	-0.01 (0.005)	0.004	-0.01 (0.005)	0.007
	Non-HDL cholesterol (mg/dl)	0.005 (0.002)	0.002	0.005 (0.002)	0.001
	eGFR (ml/min/1.73 m ²)	-0.005 (0.002)	0.04	-0.005 (0.002)	0.02

All models included a term for follow-up time (*i.e.*, time of measurement).
IsoP, urinary 15-isoprostane F_{2t}.

creatinine equation. Microalbuminuria was defined as the albumin excretion rate (AER) of 20–200 μ g/min (30–300 mg/24 h) and macroalbuminuria as AER >200 μ g/min (>300 mg/24 h) in at least two of three validated timed urine collections. ESRD was defined as dialysis or kidney transplantation. CAD

was determined by myocardial infarction confirmed by Q-waves on the electrocardiogram (Minnesota codes 1.1 or 1.2) or hospital records, angiographic stenosis $\geq$ 50%, or revascularization. *Hp* was genotyped by an amplification method; when DNA was unavailable, the *Hp* phenotype was assessed.

FIG. 1. Urinary isoprostane (ng/mg creatinine) trajectories by *Hp* genotype (adjusted for diabetes duration, gender, smoking status, HbA1c, systolic and diastolic blood pressure, and eGFR). Gray circles, *Hp* 1-1; gray triangles, *Hp* 2-1/2-2. eGFR, estimated glomerular filtration rate; *Hp*, haptoglobin.

FIG. 2. White blood cell count ($\times 10^3/\text{mm}^2$) trajectories by *Hp* genotype (adjusted for diabetes duration, gender, smoking status, HbA1c, insulin dose/weight, pulse, HDL and non-HDL cholesterol, and eGFR). Gray circles, *Hp* 1-1; gray triangles, *Hp* 2-1/2-2. HDL, high-density lipoprotein.

The concentrations of eight biomarkers (α - and γ -tocopherol, urinary IsoP, TNF- α , hsCRP, WBC count, fibrinogen, and adiponectin) were measured at three time points during the 20-year follow-up from samples collected at each clinical visit and stored at -70°C until the present analyses. Given the availability, samples were chosen to reflect an early, midpoint, and late assessment during a participant's follow-up. The concentrations of α - and γ -tocopherol in EDTA-anticoagulated plasma samples were measured under subdued lighting (interassay CV between runs of 6.0% and 2.8%, respectively); TNF- α (interassay CV of 15%), using ELISA (R&D Systems, Minneapolis, MN); and hsCRP using reagents from Carolina Liquid Chemicals (Brea, CA) and an AU400 from Olympus America, Inc., (Melville, NY) (interassay CV of 3.0%). Fibrinogen was determined with a biuret colorimetric procedure and a clotting method, and WBC count was obtained using a counter S-plus IV. Adiponectin was assessed with a sandwich ELISA test, using reagents obtained from ALPCO Diagnostics (Salem, NH; # 47-ADPH-9755) (interassay CV of 10.9%). Urine samples were obtained in 24 h ($\sim 58\%$) or overnight ($\sim 42\%$) collections. The same type of timed sample was used at all time points for a given individual. Levels of urinary 15-isoprostane F_{2t} were measured using a competitive ELISA procedure developed by Oxford Biomedical Research (Oxford, MI), which has a correlation (r^2) of >0.8 with GC-MS procedures. The 15-isoprostane F_{2t} assay appeared stable regardless of the length of sample storage.

Statistical analysis

Urinary IsoPs (ng/ml) were normalized for urinary creatinine concentration and expressed as nanograms per milligram of creatinine. Univariate associations between measurements conducted at the first time point (baseline) and the *Hp* genotype were determined using the appropriate methods for continuous and categorical variables. Spearman rank correlations were used to investigate the association

between each biomarker and participant characteristics. The procedure MIXED in SAS was used to assess the association between the *Hp* genotype and repeated measurements of each biomarker (as the dependent variables) after adjustment for risk factors significantly univariately associated with either the *Hp* genotype or the measured biomarker. Mixed models were also used to graph trajectories of the main independent variables over time by *Hp*. Trajectories were plotted as a function of the follow-up time, and cubic splines with continuous second derivatives were used to smooth lines. Non-normally distributed outcome variables were logarithmically transformed to more closely satisfy the assumption of normality.

Acknowledgments

The authors thank Ms. B. Hauth, Department of Epidemiology, University of Pittsburgh, and Ms. R. de la Vega, Department of Epidemiology, University of Pittsburgh, for expert laboratory analyses. The authors are forever indebted to the participants of the Pittsburgh Epidemiology of Diabetes Complications (EDC) study who have tirelessly volunteered their time for more than 20 years. This research has been supported by NIH grants, DK34818 and DK082900.

Authors' Contributions

T.C. conceived the hypothesis, researched data, and wrote the manuscript. R.W.E. researched data and edited the manuscript. T.J.O. researched data and edited the manuscript. T.C. is the guarantor of this work.

References

1. Asleh R, Blum S, Kalet-Litman S, Alshiek J, Miller-Lotan R, Asaf R, Rock W, Aviram M, Milman U, Shapira C, Abassi Z, and Levy AP. Correction of HDL dysfunction in individuals with diabetes and the haptoglobin 2-2 genotype. *Diabetes* 57: 2794–2800, 2008.

2. Basarici I, Altekin RE, Demir I, and Yilmaz H. Associations of isoprostanes-related oxidative stress with surrogate sub-clinical indices and angiographic measures of atherosclerosis. *Coron Artery Dis* 18: 615–620, 2007.
3. Braeckman L, De Bacquer D, Delanghe J, Claeys L, and De Backer G. Associations between haptoglobin polymorphism, lipids, lipoproteins and inflammatory variables. *Atherosclerosis* 143: 383–388, 1999.
4. Costacou T, Evans RW, Schaffer GL, and Orchard TJ. Oxidative stress and response in relation to coronary artery disease in type 1 diabetes. *Diabetes Care* 36: 3503–3509, 2013.
5. Costacou T, Ferrell R, and Orchard TJ. Haptoglobin genotype: a determinant of cardiovascular complication risk in type 1 diabetes. *Diabetes* 57: 1702–1706, 2008.
6. Costacou T, Ferrell RE, Ellis D, and Orchard TJ. Haptoglobin genotype and renal function decline in type 1 diabetes. *Diabetes* 58: 2904–2909, 2009.
7. Langlois MR and Delanghe JR. Biological and clinical significance of haptoglobin polymorphism in humans. *Clin Chem* 42: 1589–1600, 1996.
8. Lazalde B, Huerta-Guerrero HM, Simental-Mendía LE, Rodríguez-Morán M, and Guerrero-Romero F. Haptoglobin 2-2 genotype is associated with TNF- α and IL-6 levels in subjects with obesity. *Dis Markers* 2014: 912756, 2014; DOI: 10.1155/2014/912756.
9. Strandhave C, Svensson M, Krarup H, and Christensen JH. Haptoglobin genotype and risk markers of cardiovascular disease in patients with chronic kidney disease. *Int J Nephrol* 2013: 650847, 2013; DOI: 10.1155/2013/650847.

Address correspondence to:
Dr. Tina Costacou
Department of Epidemiology
University of Pittsburgh
3512 Fifth Avenue
Pittsburgh, PA 15213

E-mail: costacout@edc.pitt.edu

Date of first submission to ARS Central, May 17, 2015; date of acceptance, May 24, 2015.

Abbreviations Used

AER = albumin excretion rate
 CAD = coronary artery disease
 CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration
 EDC = Epidemiology of Diabetes Complications
 eGFR = estimated glomerular filtration rate
 ESRD = end-stage renal disease
 GC-MS = gas chromatography–mass spectrometry
 HDL = high-density lipoprotein
 Hp = haptoglobin
 hsCRP = high-sensitivity C-reactive protein
 IL-6 = interleukin 6
 IsoP = urinary 15-isoprostane F_{2t}
 LDL = low-density lipoprotein
 TNF- α = tumor necrosis factor α
 WBC = white blood cell