Original Article

Haptoglobin Polymorphism in Individuals with Type 2 Diabetic Microangiopathy

Ahmad Ahmadzadeh Amiri[1,2], Mohammad Bagher Hashemi-Soteh[2], Mohammad Reza Haghshenas[2,3], Fatemeh Daneshvar[4], Afsaneh Rastegar[5], Toraj Farazmand[2]

[1]Department of Ophthalmology, Mazandaran University of Medical Sciences, [2]Molecular and Cell Biology Research Center and Medical Faculty, Mazandaran University of Medical Sciences, Mazandaran, [3]Department of Microbiology, Molecular Cell-Biology Research Center, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, [4]General Medicine, Research Committee, Faculty of Health, Tehran University of Medical Sciences, Tehran, [5]General Medicines, Student Research Committee, Faculty of Medicine, Mazandaran University of Medical Sciences, Mazandaran, Iran

Abstract

Background: Haptoglobin is an acute phase protein with antioxidant and immunomodulatory properties. Gene polymorphism may be a risk factor for diabetic vascular disease in Iranian population. **Aims:** The study investigates the existence or not of an association between haptoglobin genotypes and prevalence of diabetic microangiopathy in individuals with type 2 diabetic microangiopathy. **Materials and Methods:** We included 206 type 2 diabetic patients (>5 years duration) categorized into two groups according to the presence or absence of diabetic microvascular complications. The cases of interest were diabetic neuropathy, retinopathy, and nephropathy identified during clinical and or laboratory examination. In addition, 114 age- and sex-matched individuals were selected to serve as a control group. Haptoglobin genotyping was done using an amplification gel electrophoresis. **Results:** The frequency of haptoglobin phenotype 2-1 in diabetic patients was 70/206 (33.9%) as compared with 54/114 (47.3%) in nondiabetics ($P = 0.01$). However, the frequency of haptoglobin phenotype 2-2 was greater in diabetics (126/114, 61.1%) than in those without diabetes (56/114, 49.1%; $P = 0.02$). Patients with diabetic microangiopathy; however, did not differ significantly between haptoglobin phenotype groups. **Conclusions:** Haptoglobin phenotype 2-2 is considered to be a major susceptibility gene for type 2 diabetic patients. Moreover, haptoglobin phenotype 2-1may be good prognostic factors for the development of diabetes mellitus.

Keywords: Diabetes mellitus, Diabetic microvascular complications, Genetic polymorphisms, Haptoglobin genotypes

Introduction

Haptoglobin (Hp) is a plasma a2-glycoprotein synthesized primarily by hepatocytes.[1] Synthesis is stimulated by infection or inflammation (the acute phase response). After release into the circulation it has a half-life of 2-4 days.[2] Hp binds oxygenated, free hemoglobin with a very high affinity of approximately 1×10^{-15} mol/l.[3]

Access this article online	
Quick Response Code:	**Website:** www.najms.org
	DOI: 10.4103/1947-2714.118929

Free hemoglobin can be harmful to the body, promoting the accumulation of hydroxyl radicals via the Fenton reaction ($H_2O_2 + Fe^{2+}$ fi $Fe^{3+} + OH$) + OH), resulting in oxidative tissue damage.[4] Once irreversibly bound to Hp, free hemoglobin loses its oxidizing ability.[4,5] The Hp-hemoglobin (Hp-Hb) complex is removed from the circulation by binding to a receptor (CD163) found on the cell surface of monocytes and macrophages.[6] Hp was first described as polymorphic by Smithies who used starch gel electrophoresis to separate the various types and the existence of two autosomal genes with incomplete penetrance was proposed by Smithies and Walker. In humans, two alleles (marked as 1 and 2) exist for the Hp gene. These give rise to three major phenotypes in man still commonly referred to as Hp1-1, Hp2-1, and Hp2-2.[7] It is an acute phase protein synthesized mainly in the liver and found at levels of 30-300 mg/dl

in normal human serum, Hp serum levels are increased up to three- to eight-fold during the acute phase reaction and in response to injury.[8] The hepatic synthesis of Hp is induced by interleukin-6 (IL-6), IL-1, and tumor necrosis factor-α (TNF-α). Hp is also expressed in specific nonhepatic cells, including adipocytes and lung cells, and its levels are increased after inflammation similar to that observed in hepatocytes.[9] Moreover, Hp is highly expressed in arteries after sustained flow changes induced by shear stress and nitric oxide, which influence IL-6 expression. Arterial Hp is believed to play a role in cell migration and arterial restructuring.[10]

Although diabetic disease duration is the main risk factor for the development of diabetic microvascular complications (MVCs); including nephropathy, neuropathy, and retinopathy; glycemic control; blood pressure; and other co-risk factors are modifier to disease duration. Hyperglycemia appears to be a necessary but not sufficient condition for the development of diabetic vascular disease.[11-15] There exists a growing body of evidence that some genetic susceptibility had modifying effects on diabetic vascular disease develop.[16-18] Considerable evidences have shown the importance of the generation of reactive oxygen species in the development of diabetic vascular complication.[19]

There exists considerable *in vitro* and *in vivo* data supporting differences in the antioxidant, scavenging, and immunomodulatory properties between the different Hp types. Several researches demonstrated in prospective and cross-sectional population studies that a polymorphism in the Hp gene is an independent risk factor for diabetic vascular disease.[20,21] The prevalence of diabetes mellitus (DM) in the Iranian population is estimated to be relatively high; around 8% in the population aged between 30 and 69 years.[22,23] To our knowledge, there are no studies investigating the influence of Hp polymorphism on this subpopulation to date. In order to verify, if there is an association between Hp genotypes and the presence of diabetic related MVCs in general and each specific diabetic MVC in particular, we studied type 2 diabetic patients, with and without diabetic microangiopathy.

Materials and Methods

Ethical certificate was obtained from the ethics committee of the molecular biology research center of Mazandaran University of Medical Sciences. This case-control study was conducted in Sari city where subjects in this study were Iranian people living in Mazandaran province, and all provided informed consent to participate.

The cases were 206 diabetic related MVCs/MVC under focus and the controls were type 2 DM without the respective MVCs/MVC under focus. The 206 subjects with type 2 DM had already been diagnosed and were outpatients of the Diabetes Research Center of Imam Hospital, Sari City, between December 2010 and August 2012 were recruited. Patients were also compared with a control group of 114 healthy individuals who had been studied from the same geographical region as a secondary target for comparison of the entire type 2 DM group with nondiabetics group. The MCVs of interest were diabetic neuropathy, retinopathy, and nephropathy identified during clinical and or laboratory examination. We estimated that a sample size of 218 cases and 116 controls would give 90% power to detect an OR of 2.0 at 5% level of significance given a prevalence of Hp in the community of 7-70% and in individuals with diabetic MVCs of 6-10%.[24]

The inclusion criteria were defined as all diabetic patients with at least 5 years disease duration. Patients with pregnancy, heritable diseases (such as Biedle), using chronic drugs (such as corticosteroids), uveitis and retinal dystrophy, nondiabetic related renal diseases, nondiabetic neuropathy, and those aged 75 years or older were excluded from study.

Patients underwent a comprehensive clinical and ophthalmic evaluation. The following demographic, clinical, and laboratory information was documented for each subject: Mean age, sex, diabetes, age of onset, and duration, presence of hypertension (blood pressure was corrected according to sex and age, presence of chronic complications (neuropathy and proteinuria)), funduscopy, average glycosylated HbA1c and insulin usage. Blood pressure was measured at every clinic visit, with hypertension being defined as systolic pressure 140 mmHg or greater or diastolic pressure 90 mmHg or greater. The MVCs of interest were diabetic neuropathy, retinopathy, and nephropathy identified during clinical and or laboratory examination.

Fundus evaluation was performed by trained ophthalmologist and, in case of abnormalities, documented by fundus fluersceine angiography. Retinopathy was defined by the presence of characteristic changes, including hemorrhages, exudates, laser marks, and fibrous proliferation, detected by ophthalmoscopy through dilated pupils. In the presence of diabetic retinopathy, it was classified as nonproliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR).[25]

All patients provided two separate 24 h urine collection samples. Urinary albumin concentration was measured by immunoturbidimetry with intra- and interassay coefficient of variance of 3.3 and 6.7%, respectively. The lowest limit of detection was 2.5 mg/l.

Nephropathy was defined as an albumin excretion rate 30-300 mg/l/24 h as microalbuminuria and >300 mg/l/24 h as gross albuminuria in a timed urine collection after excluding urinary tract infection.[26]

Clinical evaluation for peripheral neuropathy includes examination for paresthesia, sensation loss, weakness, and decreased or absent deep tendon reflexes. The clinical staging of peripheral neuropathy was defined according to involvement of the 14 nerves (both median, ulnar, peroneal, posterior tibial motor nerves and both median, ulnar, sural sensory nerves) as follows: Stage 0 (no neuropathy); stage 1 (mild), one or two nerves affected; stage 2 (moderate), three or four nerves affected; stage 3 (severe), five or more nerves affected. Neurophysiologic studies with nerve conduction velocity (NCV) were performed with the Nicolet Viking IIe (Nicolet Biomedical, Madison, WI) machine. Motor conduction studies were measured with surface electrodes on the muscles and supramaximal stimulation of the corresponding nerves. Median and ulnar nerves were stimulated at the wrist and elbow, with motor action potential being recorded over the abductor pollicis brevis and abductor digiti minimi, respectively. Peroneal and tibial nerves were stimulated at the ankle and knee, with motor action potential being recorded over the extensor digitorum brevis and adductor hallucis, respectively. Sensory NCV were performed on the median, ulnar, and sural nerves; with orthodromic stimulation at finger II, finger V, and lateral malleolus; respectively. The sensory action potentials of the median and ulnar nerves were recorded at the wrist and at the lower leg for the sural nerve.[14]

The tests described in autonomic dysfunction are based on the responses of the heart rate and blood pressure to a variety of stimuli. The first three reflect cardiac parasympathetic integrity, while the other two start to give abnormal results with more severe sympathetic nerve damage. While each test may be used individually, we think that parasympathetic and sympathetic nerve tests should be performed when possible, hence giving complete information about the state of the autonomic nervous system. A test reflecting cardiac parasympathetic damage was performed with the patient lying quietly on a couch, while the heart rate is recorded continuously on an electrocardiograph. The patient is then asked to stand up unaided, and the point at starting to stand is marked on the electrocardiogram. The shortest R-R interval at or around the 15th beat and the longest R-R interval at around the 30th beat after starting to stand, is measured with a ruler. The characteristic heart rate response is expressed by the 30:15 ratios. Immediate heart rate response to standing (30:15 ratios) > 1 was normal and <1.00 was abnormal. The tests reflecting sympathetic damage was performed by measuring the patient's blood pressure with a sphygmomanometer while he is lying down quietly and again when he stands up. The postural fall in blood pressure is taken as the difference between the systolic blood pressure in the lying and standing position. Blood pressure response to standing (fall in systolic blood pressure) <10 mmHg was normal and >30 mmHg was abnormal.[15]

The blood samples (5 ml) were drawn from the antecubital vein from all subjects into ethylenediaminetetraacetic acid (EDTA) and processed for Hp genotyping. A fasting blood sample was collected aseptically from all subjects and was also collected on EDTA and analyzed within a few hours to estimate the percentage of glycosylated hemoglobin (HbA1c) using a fast ion exchange resin separation method,[27] serum samples were separated and immediately frozen until analyzed at central lab of medical school, Sari.

The genomic DNA was extracted from the remaining white blood cells using a Puregene DNA purification kit (Gentra Systems, Inc., Minneapolis, MN). The DNA samples were stored and shipped at 4°C. All kits and reagents were used according to their manufacturer's instructions. HP genotyping were determined using conventional polymerase chain reaction (PCR). Amplification of the 1757-bp HP1 allele-specific fragment was performed in a volume of 20 μl, containing 4 μl of 5× Prime STAR Buffer (Mg^{2+} plus) (Takara, Shiga, Japan), 250 nmol/l each of primers A and B, and about 0.1-10 ng genomic DNA. The temperature profile was 35 cycles of denaturing at 98°C for 10 s and annealing and extension at 72°C for 2 min. The HP2 allele-specific fragment was amplified in a volume of 20 and 10 μl of Gotaq® Green Master mix (Promega, Madison, WI), 250 nmol/l each of primers C and D,[28] and about 0.1-10 ng genomic DNA. The temperature profile was 95°C for 1 min, followed by 35 cycles of denaturing at 96°C for 10 s, annealing and extension at 65°C for 30 s, and then final extension cycle at 72°C for 1 min. Primers for the conventional PCR were synthesized by Hokkaido System (Sapporo, Hokkaido, Japan). HP del zygosity was determined by duplex PCR using Hp-del-U, Hp-del-L, Hp-Ex1-U, and Hp-Ex1-L primers as described by Koda *et al*.[29] Resultant PCR products were size-fractionated by agarose gel electrophoresis.

Statistical analysis

Data are expressed as the mean ± standard deviation (SD), or proportions for categorical variables. HP genotypes variation was calculated using the chi-square test. Categorical valuables were compared using Fisher's exact test. Nonparametric data were analyzed by the odds ratio (Or) test. Associations between the HP genotypes and DM, blood pressure, serum lipid values,

and diabetic microangiopathic change were evaluated. A *P* < 0.05 was considered to be statistically significant.

Results

The main clinical and biochemical data of the type 2 diabetic patients are shown in Table 1. This finding emphasis that type 2 DM with MVCs where slightly older, have longer DM duration, and less controlled as can be evident by HbA1C though not statistically significant.

Data analyses after adjusting for age and gender showed that the statistical significance of diabetes duration in non-MVCs patients with DM to MVCs patients with DM was 0.001, and that the elevated HbA1c in non-MVCs patients with DM to MVCs patients with DM was 0.003 [Table 1]. These confirmed already known associations between MVCs and diabetes duration and elevated HbA1c levels in DM. There were no significant differences in HbA1c levels, hypertension, hypercholesterolemia, or duration of diabetes between patients with different Hp genotypes.

Among the diabetic participants; retinopathy, nephropathy, and neuropathy were found in 109 (52.9%), 57 (28.8%), and 103 (58.5%) individuals, respectively. The patients with diabetic neuropathy were either peripheral or autonomic in 103 (58.5%) and 61 (34.6%) individuals, respectively. In diabetic patients, 65.4% had at least one form of MVCs.

Table 2 shows the results of the Hp genotype distribution for diabetes patients and control subjects. The allele frequencies were also categorized according to the presence/absence of MVCs. The distribution of the three Hp genotypes was 4.4, 38.7, and 56.9% for Hp 1/1, 2/1, and 2/2, respectively. This result has shown that the Hp2-2 allele frequency is highly significant for type 2 diabetes versus control cases (61.1 vs. 49.1%), whereas Hp1-2 allele appears inversely (33.9 vs. 47.3%); *P* = 0.057.

Table 1: Clinical and biochemical data of type 2 diabetic patients with and without MVCs

Characteristics	Control (*n*=114)	DM with MVCs (*n*=134)	DM without MVCs (*n*=71)	*P*-value
Age (years)	59.16±13.21	56.97±10.46	55.69±8.49	0.376
Sex (M/F)	45/69	42/92	21/50	0.46
Diabetes duration (<10 years/>10 years)	—	59/75	49/21	0.001
HbA1c (<7.5%/>7.5%)	—	41/93	35/35	0.003
Hypertension (no/yes)	51/58	98/36	51/20	0.870
Hypercholesterolemia (no/yes)	23/40	56/26	25/16	0.432

DM: Diabetes mellitus; MVCs: Microvascular complications; M: Male; F: Female. Data are expressed as mean±standard deviation (SD) or % (*n*). Data are in percentage (*n*) unless otherwise indicated

Table 2: Haptoglobin genotype in a group of Iranian diabetic patients with or without MVCs and in healthy controls

Groups / Variables	Hp1-1 (*n*)	Hp2-1 (*n*)	Hp2-2 (*n*)	*P*-value
DM without DR	4/97 (4.1%)	37/97 (38.1%)	56/97 (57.7%)	0.469
vs				
DM with DR	6/109 (5.5%)	33/109 (30.3%)	70/109 (64.2)	
DM without DN	7/141 (4.9%)	49/141 (34.7%)	85/141 (60.2%)	1.000
vs				
DM with DN	3/57 (5.2%)	20/57 (35%)	34/57 (59.6%)	
DM without DNp	4/73 (5.4%)	21/73 (28.7%)	48/73 (65.7%)	0.701
vs				
DM with DNp	5/103 (4.8%)	36/103 (34.9%)	62/103 (60.1%)	
DM without DNa	6/115 (5.2%)	33/115 (28.6%)	76/115 (66%)	0.371
vs				
DM with DNa	3/61 (4.9%)	24/61 (39.3%)	34/61 (55.7%)	
DM without MVCs	3/71 (4.2%)	23/71 (32.3%)	45/71 (63.3%)	0.937
vs				
DM with MVCs	7/134 (5.2%)	46/134 (34.3%)	81/134 (60.4%)	
Controls	4/114 (3.5%)	54/114 (47.3%)	56/114 (49.1%)	0.057
vs.				
DM	10/206 (4.8%)	70/206 (33.9%)	126/206 (61.1%)	

DM: Diabetes mellitus; MVCs: Microvascular complications; DR: Diabetic retinopathy; DN: Diabetic nephropathy; DNp: Peripheral diabetic neuropathy; DNa: Autonomic diabetic neuropathy. Data are in percentage (*n*) unless otherwise indicated

Factors for MVCs	Non-DM-DM			Non-DR-DR			Non-DN-DN			Non-DNp-DNp			Non-DNa-DNa			Non-MVCs -MVCs		
	P-values	OR	95% CI	*P*-values	OR	95% CI	*P*-values	OR	95% CI	*P*-values	OR	95% CI	*P*-values	OR	95% CI	*P*-values	OR	95% CI
Hp1-1	0.400	0.713	0.218-2.326	0.449	0.738	0.202-2.698	0.931	0.940	0.234-3.771	0.853	1.139	0.294-4.385	0.932	1.064	0.257-4.412	0.752	0.800	0.201-3.195
Hp2-1	0.013	1.749	1.096-2.790	0.149	1.420	0.796-2.533	0.964	0.985	0.517-1.874	0.388	0.752	0.393-1.438	0.151	0.620	0.323-1.193	0.877	0.917	0.497-1.690
Hp2-2	0.025	0.613	0.386-0.973	0.209	0.761	0.436-1.535	0.934	1.028	0.548-1.923	0.453	1.270	0.680-2.370	0.177	1.548	0.819-2.923	0.399	1.132	0.625-2.051

Table 3: Predictive values (odds ratio (OR)) of risk factors for MVCs using multiple logistic regression analysis

DM: Diabetes mellitus; MVCs: Microvascular complications; DR: Diabetic retinopathy; DN: Diabetic nephropathy; DNp: Peripheral diabetic neuropathy; DNa: Autonomic diabetic neuropathy; Hp: Haptoglobin; CI: Confidence interval

Predictive values of risk factors for MVCs using multiple logistic regression analysis are shown in Table 3. These data demonstrate that the relative risk of Hp 2-2 in diabetes was significantly greater than in nondiabetes, (confidence interval (CI) 0.386-0.973, OR = 0.613; *P* = 0.025) and the relative risk of Hp 2-1 in diabetes was significantly lesser than in nondiabetes (CI 1.096-2.790, OR = 1.749; *P* = 0.013).

Discussion

In this case-control of subjects with type 2 diabetes, we demonstrate no association between the Hp genotype and MVCs in general or any specific MVCs. Previous studies assessing the association between the Hp phenotype and the presence or incidence of renal disease have produced conflicting findings. In a small, cross-sectional study of 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).[30,31] Similar results were reported from an Irish type 1 diabetes case-control study.[32] Conversely, separate study of American and Japanese individuals with type 2 diabetes did not observe an increased risk associated with the common Hp phenotype (*P* = 0.77 and 0.43, respectively).[33,34] Similarly, we were also not able to detect association for either microalbuminuria or macroalbuminuria incidence in our individuals with a long duration of type 2 diabetes, perhaps suggesting that at a more advanced stage of diabetes, early Hp-susceptible cases of nephropathy may have been excluded.

It has been shown that the Hp1-1 phenotype is associated with a reduced risk for the development of DR in a group of Nakhoul *et al.*, DM1 patients.[35] The authors of this study suggested that the functional differences in antioxidant capacity between the phenotypes could be responsible for this association. On the other hand, Koda *et al.*, investigating Japanese patients with DM2 did not find a protective effect of the HP1 allele. On the contrary, the Hp2-2 phenotype frequency was higher in patients without DR.[34] Similar results were reported

from an Brazilian diabetes case-control study.[36] We could not find any significant differences in genotype frequencies for the DM patients with or without DR and all other types of MVCs analyzed here.

The only statistically significant results we obtained were in relation to the presence of diabetes that such differences may be important. This study has shown that the Hp2-2 allele is a high risk factor for type 2 diabetes in Iran whereas Hp1-2 allele appears protective. There are many reports on HP phenotypes, diabetes, and its complications from many parts of the world.[37-44] This was similar with Quaye *et al.*, study that concluded that HP2-2 allele was a risk factor for type 2 diabetes in Ghana, whereas the HP1-1 allele appeared protective.[37] However, this finding contrasts with previous observation in American-Indian studies where HP allele was a risk factor. These differences highlight role of genetic and ethnic variation determining susceptibility to type 2 diabetes.[40,41]

Glycosylated Hb is a more potent source of redox active iron and Hp2-2 has less ability to inhibit the release of such iron from glycosylated Hb.[43] The lack of simple associations between Hp type and disease may reflect the complex interactions between Hp and the immune system as well as antioxidant functions. There was no significant difference in between the Hp genotypes for any of the MVCs risk factors or DM characteristics as determined both by univariate analysis and by multinomial logistic regression analysis. The only statistically significant results we obtained were in relation to the already known associations between MVCs and diabetes duration and elevated HbA1c levels in DM, respectively. In conclusion, the lack of association between MVCs and polymorphism of the Hp gene indicates that Hp genotypes may not be genetic markers of predisposition to diabetic microangiopathy in this Iranian population. However, in respect to diabetes incidence, it would therefore be emphasizing that the difference in Hp distribution between type 2 diabetics and nondiabetics is very interesting.

Acknowledgement

References

1. Putnam FW. Haptoglobin. In: The Plasma Proteins: Structure, Function and Genetic Control. New York: Academic Press; 1975. p. 2-50.

2. 2. Garby L, Noyes W D. Studies on hemoglobin metabolism.1. The kinetic properties of the plasma hemoglobin pool in normal man. Journal of Clinical Investigation1959; 38:1479-83.

3. Okazaki T, Yanagisawa Y, Nagai T. Analysis of the affinity of each haptoglobin polymer for hemoglobin by two-dimensional affinity electrophoresis. Clin Chim Acta 1997; 258:137-44.

4. Sadrzadeh SM, Graf E, Panter SS, Hallaway PE, Eaton JW. Hemoglobin: A biologic Fenton reagent. J Biol Chem 1984; 259:14354-6.

5. Gutteridge JM. The antioxidant activity of haptoglobin towards hemoglobin-stimulated lipid-peroxidation. Biochim Biophys Acta 1987;917:219-23.

6. Kristiansen M, Graversen JH, Jacobsen C, Sonne O, Hoffman HJ, Law SK, *et al.* Identification of the haemoglobin scavenger receptor. Nature 2001;409:198-201.

7. Smithies O, Walker NF. Notation for serum protein groups and the genes controlling their inheritance. Nature 1956; 178:694-5.

8. Dobryszycka W. Biological functions of haptoglobin—new pieces to an old puzzle. Eur J Clin Chem Clin Biochem 1997; 35:647-54.

9. Kalmovarin N, Friedrichs WE, O'Brien HV, Linehan LA, Bowman BH, Yang F. Extrahepatic expression of plasma protein genes during inflammation. Inflammation 1991; 15:369-79.

10. Smeets MB, Pasterkamp G, Lim SK, Velema E, van Middelaar B, de Kleijn DP. Nitric oxide synthesis is involved in arterial haptoglobin expression after sustained flow changes. FEBS Lett 2002;529:221-4.

11. American Diabetes Association, Diagnosis and classification of diabetes mellitus. Diab Eye 2004;27:5-10.

12. Fioretto P, Dodson PM, Ziegler D, Rosenson RS. Residual microvascular risk in diabetes: Unmet needs and future directions. Nat Rev Endocrinol 2010;6:19-25.

13. Fong DS, Aiello L, Garden TW, King GL, Blankenship G, Gavallenano JD, *et al.* Diabetic retinopathy. Diabetes Care 2003;26:S99-102.

14. Bao XH, Wong V, Wang Q, Low LC. Prevalence of peripheral neuropathy with insulin-dependent diabetic mellitus. Pediatr Neurol 1999;20:204-9.

15. Ewing DJ, Clarke BF. Diagnosis and management of diabetic autonomic neuropathy. Br Med J (Clin Res Ed) 1982;285:916-8.

16. Chowdhury TA, Dyer PH, Kumar S, Barnett AH, Bain SC. Genetic determinants of diabetic nephropathy. Clin Sci (Lond) 1999;96:221-30.

17. Parving HH, Tarnow L, Rossing P. Genetics of diabetic nephropathy. J Am Soc Nephrol 1996;7:2509-17.

18. Marre M. Genetics and the prediction of complications in type 1 diabetes. Diabetes Care 1999;22:B53-8.

19. Guigliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complication. Diabetes Care 1996; 19:257-67.

20. Costacou T, Ferrel RE, Orchard TJ. Haptoglobin genotype: A determinant of cardiovascular complication risk in type 1 diabetes. Diabetes 2008;57:1702-6.

21. Levy AP, Purushothaman KR, Levy NS, Purushothaman M, Strauss M, Asleh R, *et al.* Downregulation of the hemoglobin scavenger receptor in individuals with diabetes and the Hp2-2 genotype: Implications for the response to intraplaque hemorrhage and plaque vulnerability. Circ Res 2007;101:106-10.

22. Golozar A, Khademi H, Kamangar F, Poutschi H, Islami F, Abnet CC, *et al.* Diabetes mellitus and its correlates in an Iranian adult population. PLoS One 2011;6:e26725.

23. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 2010;87:4-14.

24. Carter K, Worwood M. Haptoglobin: A review of the major allele frequencies worldwide and their association with diseases. Int J Lab Hematol 2007;29:92-110.

25. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications, Trial Research Group. N Engl J Med 1993; 329:977-86.

26. Cheung CK, Swaminathan R. Rapid, economical immunoturbidimetric method for microalbuminuria. Clin Chem 1987;33:204.

27. Maquart FX, Gillery P, Bernard JF, Mante JP, Borel JP. A method for specially measuring of hemoglobin A1C with a disposable commercial ion-exchange column. Clin Chim Acta 1980;108:329-32.

28. Koch W, Latz W, Eichinger M, Roguin A, Levy AP, Schömig A, *et al.* Genotyping of the common haptoglobin Hp ½ polymorphism based on PCR. Clin Chem 2002;48:1377-82.

29. Koda Y, Watanabe Y, Soejima M, Shimada E, Nishimura M, Morishita K, *et al.* Simple PCR detection of haptoglobin gene deletion in anhaptoglobinemic patients with antihaptoglobin antibody that causes anaphylactic transfusion reactions. Blood 2000;95:1138-43.

30. 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 2005;1:19-28.

31. Levy AP, Roguin A, Hochberg I, Herer P, Marsh S, Nakhoul FM, *et al.* Haptoglobin phenotype and vascular complications in patients with diabetes. N Engl J Med 2000; 343:969-70.

32. 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:e75-9.

33. Costacou T, Ferrell RE, Ellis D, Orchard TJ. Haptoglobin genotype and renal function decline in Type 1 diabetes. Diabetes 2009;58:2904-9.

34. Koda Y, Soejima M, Yamagishi S, Amano S, Okamoto T, Inagaki Y, *et al*. Haptoglobin genotype and diabetic microangiopathies in Japanese diabetic patients. Diabetologia 2002;45:1039-40.

35. Nakhoul FM, Marsh S, Hochberg I, Leibu R, Miller BP, Levy AP. Haptoglobin genotype as a risk factor for diabetic retinopathy. JAMA 2002;284:1244-5.

36. Wobeto VP, Rosim ET, Melo MB, Calliani EP, Sonati Mde F. Haptoglobin polymorphism and diabetic retinopathy in Brazilian patient. Diabetes Res Clin Pract 2007;77:385-8.

37. Quaye IK, Ababio G, Amoah AG. Haptoglobin 2-2 phenotype is a risk factor for type 2 diabetes in Ghana. J Atheroscler Thromb 2006;13:90-4.

38. Awadallah S, Hamad M. The prevalence of type II diabetes mellitus is haptoglobin phenotype-independent. Cytobios 2000;101:145-50.

39. Iyengar S, Hamman RF, Marshall JA, Baxter J, Majumder PP, Ferrell RE. Genetic studies of type-2 (non-insulin-dependent) diabetes mellitus: Lack of association with 7 genetic markers. Diabetologia 1989;32:690-3.

40. Stern MP, Ferrell RE, Rosenthal M, Haffner SM, Hazuda HP. Association between NIDDM, RH blood group, and haptoglobin phenotype. Results from the San Antonio Heart Study. Diabetes 1986;35:387-91.

41. Chakraborty R, Ferrell RE, Stern MP, Haffner SM, Hazuda HP, Rosenthal M. Relationship of prevalence of non-insulin-dependent diabetes mellitus to Amerindian admixture in the Mexican Americans of San Antonio, Texas. Genet Epidemiol 1986;3:435-54.

42. Khan GA, Khan N, Ishaq M, Sastry EP, Rani TS. Increased frequency of haptoglobin phenotype Hp 1-1 in diabetic patients with complications. Int J Diab Dev 2002;22:101.

43. 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;96:435-41.

44. Adinortey MB, Gyan BA, Adjimani JP, Nyarko PE, Sarpong C, Tsikata FY, *et al*. Haptoglobin polymorphism and association with complications in ghanaian type 2 diabetic patients. Indian J Clin Biochem 2011;26:366-72.

Staying in touch with the journal

1) **Table of Contents (TOC) email alert**
   Receive an email alert containing the TOC when a new complete issue of the journal is made available online. To register for TOC alerts go to **www.najms.org/signup.asp.**

2) **RSS feeds**
   Really Simple Syndication (RSS) helps you to get alerts on new publication right on your desktop without going to the journal's website. You need a software (e.g. RSSReader, Feed Demon, FeedReader, My Yahoo!, NewsGator and NewzCrawler) to get advantage of this tool. RSS feeds can also be read through FireFox or Microsoft Outlook 2007. Once any of these small (and mostly free) software is installed, add **www.najms.org/rssfeed.asp** as one of the feeds.