Haptoglobin 2-2 Genotype Is Associated with Increased Risk of Type 2 Diabetes Mellitus in Northern Chinese

Xiaohong Shi,¹ Liang Sun,¹ Li Wang,² Feng Jin,³ Junfeng Sun,¹
Xiaoquan Zhu,¹ Lei Tang,¹ Yanchun Qu,⁴ and Ze Yang¹

The aim of this study was to investigate the association between haptoglobin (*Hp*) gene polymorphism and occurrence of type 2 diabetes mellitus (T2DM) in a northern Chinese population. We studied the association of the *Hp* gene polymorphism with T2DM in 584 unrelated T2DM patients and 690 control subjects with normal glucose tolerance among northern Chinese. The patients were diagnosed in accordance with the guidelines of the American Diabetes Association. The clinical characteristics of the study population were recorded, and the *Hp* genotype was determined. The frequencies of the genotypes in the group of T2DM patients and the controls were as follows: *Hp*2-2, 51.7% and 44.1%; *Hp*2-1, 39.7% and 45.1%; and *Hp*1-1, 8.6% and 10.9%, respectively. There was significant difference for the genotypic and allelic distribution between the two groups ($p=0.021$ and $p=0.007$, respectively). Even after readjusting for the confounding effects of age, gender, and body mass index, a significant effect of genotypes on T2DM was still found in the recessive model for the *Hp*2 allele tested ($p=0.002$). Those who had the *Hp*2-2 genotype had a significantly higher risk for T2DM than those with other genotypes (odds ratio=1.441, 95% confidence interval=1.143–1.817). The results showed that the *Hp*2-2 genotype is associated with increased risk of T2DM in the northern Chinese Han population.

Introduction

HAPTOGLOBIN (*Hp*), AN ACUTE-PHASE plasma protein, is synthesized mainly in the liver (Mustafa *et al.*, 2004; Levy *et al.*, 2010). *Hp* is characterized by its strong binding affinity for free hemoglobin (Hb) released after hemolysis (Bowman and Kurosky, 1982). In humans, the *Hp* gene is located on chromosome 16q22.3 (Bowman and Kurosky, 1982; Mustafa *et al.*, 2004). The two common alleles for *Hp*1 and *Hp*2 result in three major genotypes: *Hp*1-1, *Hp*2-1, and *Hp*2-2 (Bowman and Kurosky, 1982). At the protein level, there are three major phenotypes: *Hp*1-1, *Hp*2-1, and *Hp*2-2. The *Hp* phenotype is inherited in a Mendelian pattern (Langlois and Delanghe, 1996; Mustafa *et al.*, 2004).

The best known function of *Hp* is to bind and aid in clearance of free Hb, stemming from common, low-grade intravascular hemolysis (Bowman and Kurosky, 1982; Mustafa *et al.*, 2004; Levy *et al.*, 2010). This can prevent kidney damage by free Hb in the circulation passed through the glomerular filter. Furthermore, free Hb can induce peroxidation of serum low-density lipoprotein (LDL) in the circulation forming the oxidized LDL, which has strong toxic side effects and plays a crucial role in the development of endothelial

dysfunction. The dysfunction of endothelial cells is one of the characteristic of early stage type 2 diabetes mellitus (T2DM) (Sjoholm and Nystrom, 2005; Quaye *et al.*, 2006). Thus, *Hp* functions as an antioxidant and an important vascular endothelial protector by binding Hb and preventing LDL oxidation (Mustafa *et al.*, 2004; Asleh *et al.*, 2005, 2006; Levy *et al.*, 2007, 2010).

Considerable data *in vitro* and *in vivo* suggest that different *Hp* types exhibit some differences in function. In healthy men, the *Hp*2-2 type is related to the highest serum iron and ferritin levels compared to other *Hp* types (Fagoonee *et al.*, 2005). Although each type of *Hp* protein can bind to free Hb, the *Hp*1-1 has the highest affinity for free Hb, and is superior to the *Hp*2-2 protein in neutralizing its oxidative effects (Okazaki and Nagai, 1997; Melamed-Frank *et al.*, 2001; Asleh *et al.*, 2005; Levy *et al.*, 2007; Milman *et al.*, 2008). The functional differences among the *Hp* types have important biological and clinical consequences.

Hp genotypes and phenotypes have been reported to be associated with various diseases, including diabetic vascular complications (Levy *et al.*, 2000, 2002; Costacou *et al.*, 2008; Farbstein and Levy, 2010), diabetic nephropathy (Nakhoul *et al.*, 2001; Bessa *et al.*, 2007; Conway *et al.*, 2007), gestational

¹Key Laboratory of Geriatrics, Institute of Geriatrics, Beijing Hospital, Ministry of Health, Beijing, China.

²Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China.

³Key Laboratory of Mental Health, Institute of Psychology, Chinese Academy of Sciences, Beijing, China.

⁴Tianjin Institute of Urology, The Second Hospital of Tianjin Medical University, Tianjin, China.

diabetes mellitus (Mustafa *et al.*, 2004), diabetes (Quaye *et al.*, 2006), malaria (Cox *et al.*, 2007, 2008), anaemia (Atkinson *et al.*, 2006), and HIV infection (Friis *et al.*, 2003).

Diabetes mellitus (DM) is a complicated multifactor syndrome characterized by elevated levels of serum glucose. More than 90% of patients with the disease are categorized as having T2DM. T2DM is caused mainly by impairment in both insulin action and insulin secretion. Multiple lines of evidence confirm that inherited factors play an important role in the pathogenesis of T2DM (Das and Elbein, 2006). Due to the phenotypic and genotypic heterogeneity of the disease, the association of *Hp* with T2DM has not been replicated in different ethnic populations.

In a study conducted in Ghana (Quaye *et al.*, 2006), the *Hp2* allele was reported as a risk factor for T2DM. Subjects with *Hp2-2* genotype had significantly higher risk for T2DM (odds ratio [OR]=6.1, $p=0.001$), while the *Hp1* allele appeared protective (OR=0.56, $p=0.06$). In Amerindians and Mexican Americans, the *Hp1* allele was observed to be a risk factor for T2DM in a dose-dependent manner (Chakraborty *et al.*, 1986; Stern *et al.*, 1986). In Mongolian, Brazilian, and Jordanian study (Iyengar *et al.*, 1989; Awadallah and Hamad, 2000; Nakamura *et al.*, 2009), no significant association between *Hp* phenotype and T2DM was found.

To date, no risk association between the *Hp* gene polymorphism and T2DM among the northern Chinese population has been reported.

Therefore, the aim of this study was to investigate the prevalence of the *Hp* gene polymorphism and to determine whether the polymorphism was associated with T2DM in northern Chinese.

Materials and Methods

Study subjects

A total of 1274 participants, 584 patients with T2DM (273 men and 311 women, age 58.82 ± 12.28 years) and 690 normal glucose-tolerant control subjects (352 men and 338 women, age 55.18 ± 10.99 years) without kinship were randomly recruited from the epidemiological database of T2DM in urban communities in Beijing, Dalian, and Harbin area of northern China. All subjects in this study were Chinese Han in origin. The protocol was approved by the ethics committee of Beijing Hospital, Ministry of Health, P.R. China, and all participants gave their written informed consent.

T2DM was diagnosed in accordance with the guidelines of the American Diabetes Association (2005). Subjects were defined as having T2DM if they had a history of T2DM, or fasting plasma glucose (FPG) ≥ 7.0 mM, or 2-h plasma glucose ≥ 11.1 mM during an oral glucose tolerance test (OGTT). The inclusion criteria of normal glucose tolerance (NGT) were as follows: over 40 years old, no history of diagnosis of diabetes, no diabetes in first-degree relatives, FPG < 5.6 mM, and 2-h plasma glucose < 7.8 mM during an OGTT.

Clinical measurements

All study participants were examined in the morning after overnight fasting. Each of the participants was administered a health questionnaire. Personal and family history of diabetes was obtained. Height, weight, waist circumference, and hip circumference were measured by standard procedures. Body

mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Blood pressure was measured after 5 min rest in the sitting position, using an automated sphygmomanometer. Blood samples were taken from all participants under the condition of overnight fasting. An OGTT was performed in all subjects except for the individuals with history of T2DM. The procedure of OGTT was using a 75-g anhydrous glucose load, dissolved in 250 mL water drunk within 5 min. Blood samples were taken just before oral glucose and 2 h after oral glucose.

Plasma glucose concentrations were measured by the glucose oxidase method. Total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, and LDL cholesterol were tested using a Hitachi 912 Autoanalyser (Roche Diagnostics GmbH, Mannheim, Germany).

Genotyping

Genomic DNA was extracted and purified from peripheral blood leukocytes according to standard methods. *Hp* was genotyped by allele-specific polymerase chain reaction (PCR), according to the method of Werner Koch *et al.* (2002). To optimize the experimental results, we modified the original method in PCR systems and PCR programs. Primer pair A (5'-GAGGGGAGCTTGCCCTTCCATTG-3' and 5'-GAGATTTTGTAGCCCTGG CTGGT-3') were used to amplify a 1757-bp *Hp1* allele-specific sequence and a 3481-bp *Hp2* allele-specific sequence. Because it was not possible to determine conclusively whether the 3481-bp band was also present when the 1757-bp band was detected, so a second primer pair B (5'-CCTGCCTCGTATTAACTGCACCAT-3' and 5'-CCGAGTGCTCCACATAGCCATGT-3') was also used for the complete genotyping. In a PCR with primer pair B, a 349-bp product for the *Hp2* allele-specific sequence could be generated. PCR with primer pairs A and B were carried out in separate reactions. PCR was performed using 20-ng genomic DNA in a total volume of 20 μL , including 1XPCR buffer, 2 mM MgCl_2 , 0.15 μM of each primer, 0.2 mM dNTP, 2.5U Taq polymerase (for primer pairs A), or 1.0 U Taq polymerase (for primer pairs B). PCR conditions for primer pairs A were as follows: initial denaturation at 95°C for 2 min, followed by 35 cycles of two-step thermocycling procedure for denaturation at 95°C for 1 min, extension at 69°C for 3.5 min, and final extension at 72°C for 7 min. For genotype assignments, the PCR products for primer pair A were separated in 1.0% agarose gels. PCR conditions for primer pair B were as follows: initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 20 s, annealing at 69°C for 20 s, and extension at 72°C for 20 s, followed by a final extension at 72°C for 7 min. For genotype assignments, the PCR products for primer pair B were separated and detected on 8% ethidium bromide-stained polyacrylamide gel electrophoresis. We randomly selected 10% of the samples to verify the accuracy of genotyping; all the genotypic results were consistent.

Statistical analysis

Data were introduced into the database of Microsoft Excel 2000. All analyses were performed using SPSS for Windows (Version 11.5). Hardy-Weinberg equilibrium was tested using the Chi-square goodness-fit test. Genotypic distributions in different groups were compared using the Chi-square test. Student's *t*-test and analysis of variance (ANOVA) were used

TABLE 1. COMPARISON OF CLINICAL CHARACTERISTIC BETWEEN PATIENTS WITH TYPE 2 DIABETES MELLITUS AND NONDIABETIC CONTROLS

Parameter	T2DM (n=584)	Nondiabetic controls (n=690)	p-Value
Age (years)	58.82±12.28	55.18±10.99	<0.001
Gender (M/F)	273/311	352/338	0.129
BMI (kg/m ²)	25.35±3.59	24.23±3.04	<0.001
Waist-hip ratio	0.90±0.07	0.82±0.06	<0.001
Waist circumference (cm)	90.21±10.18	81.07±8.90	<0.001
SBP (mmHg)	134.34±23.24	111.15±17.43	<0.001
DBP (mmHg)	81.69±12.67	79.94±13.01	0.017
Fasting plasma glucose (mM)	8.81±3.45	4.63±0.69	<0.001
Postprandial 2-h glucose (mM)	13.78±5.05	4.66±1.17	<0.001
Triglyceride (mM)	2.37±2.18	2.28±1.75	0.524
Total-cholesterol (mM)	4.80±1.57	4.15±1.81	<0.001
HDL cholesterol (mM)	1.36±0.33	1.42±0.31	0.095
LDL cholesterol (mM)	2.88±0.86	2.70±0.67	0.043

Except for the data of gender, data are expressed as means±SD. For continuous variables, unpaired *t*-test and analysis of variance were used to compare the difference between groups. For the data of gender, Chi-square test was used to compare the difference between groups.

T2DM, type 2 diabetes mellitus; LDL, low-density lipoprotein; HDL, high-density lipoprotein; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

to compare continuous variables expressed as means and standard deviations. ORs and 95% confidence intervals (CIs) were calculated. Binary logistic regression analysis was performed to compare frequencies after adjustment for age, gender, and BMI between patients and normal controls. All tests were two-tailed and *p*<0.05 was considered statistically significant.

Results

The clinical characteristics of the study population are presented in Table 1. Besides the glucose level, the patients with T2DM had higher BMI (*p*<0.001), WHR (*p*<0.001), waist circumference (*p*<0.001), systolic blood pressure (SBP) (*p*<0.001), diastolic blood pressure (DBP) (*p*=0.017), T-cholesterol (*p*<0.001), and LDL (*p*=0.043) compared with nondiabetic controls.

The genotype and allele frequencies of *Hp* for T2DM patients and the nondiabetic controls are presented in Table 2. The genotypic distribution was in Hardy-Weinberg equilibrium in both groups (T2DM, *p*=0.567; NGT, *p*=0.732). The frequencies of the genotypes in the group of T2DM patients and the nondiabetic controls were as follows: *Hp*2-2, 51.7% and 44.1%; *Hp*2-1, 39.7% and 45.1%; and *Hp*1-1, 8.6% and 10.9%, respectively. Chi-square analysis indicated that there was significant difference for the genotypic and allelic distribution between the two groups (*p*=0.021 and *p*=0.007, re-

spectively). Further analysis for the genetic models (recessive model and dominant model) showed that a significant effect of genotypes on T2DM was found in the recessive model for the *Hp*2 allele tested (*p*=0.006). The frequency of the *Hp*2-2 genotype in the patients was significantly higher than that in controls. The odds ratio (OR=1.360, 95% CI=1.090–1.696) indicated that the *Hp*2-2 genotype is a risk factor for T2DM in northern Han Chinese.

We also used multivariate logistic regression analysis to exclude the confounding effects of age, gender, and BMI differences on the genotypic expression between the patients and controls (Table 3). Similarly, after readjusting for the confounding effects, significant effect of genotypes on T2DM was found in the recessive model (*p*=0.002). Those who had *Hp*2-2 genotype still had a significantly higher risk for T2DM than those with other genotypes (OR=1.441, 95% CI=1.143–1.817). These results suggest that the *Hp*2 allele increases the risk of T2DM under a recessive model of inheritance in northern Han Chinese.

Phenotype variables of individuals with different genotypes were compared by one-way ANOVA in patients and controls for the two genetic models (recessive model and dominant model), respectively. Among nondiabetic controls (Table 4), the group of *Hp*2-2 genotype carriers had significantly higher FPG (4.80±0.72), SBP (112.92±19.44), total cholesterol (4.32±1.83), LDL cholesterol (2.92±0.76), and lower DBP (77.84±12.72) levels compared with the group of

TABLE 2. DISTRIBUTION OF THE HAPTOGLOBIN GENOTYPE AND ALLELE FREQUENCIES IN PATIENTS WITH TYPE 2 DIABETES MELLITUS AND NONDIABETIC CONTROLS

	<i>HWE</i> (p)	n	<i>Genotype</i> , n (%)			<i>Allele</i> , n (%)		<i>Recessive model</i> (<i>Hp</i> 2-2 vs. <i>Hp</i> 2-1/ <i>Hp</i> 1-1)		<i>Dominant model</i> (<i>Hp</i> 2-1/ <i>Hp</i> 2-2 vs. <i>Hp</i> 1-1)	
			<i>Hp</i> 2-2	<i>Hp</i> 2-1	<i>Hp</i> 1-1	<i>Hp</i> 2	<i>Hp</i> 1	OR (95% CI)	p-value	OR (95% CI)	p-value
T2DM	0.567	584	302 (51.7)	232 (39.7)	50 (8.6)	836 (71.6)	332 (28.4)	1.360 (1.090–1.696)	0.006	1.302 (0.894–1.897)	0.169
NGT	0.732	690	304 (44.1)	311 (45.1)	75 (10.9)	919 (66.6)	461 (33.4)				

p-value and OR (95% CI) are calculated using Fisher's exact test with a recessive model for *Hp*2 allele and a dominant model for *Hp*2 allele. *Hp*, haptoglobin; NGT, normal glucose tolerance; OR, odds ratio; CI, confidence interval.

TABLE 3. LOGISTIC REGRESSION ANALYSIS RESULTS OF THE HAPTOGLOBIN POLYMORPHISM IN ALL SUBJECTS

Variable	p	OR	95% CI
Age (years)	<0.001	1.030	1.020–1.040
Gender (M/F)	0.035	0.780	0.620–0.983
BMI (kg/m ²)	<0.001	1.112	1.073–1.152
Waist circumference (cm)	<0.001	1.113	1.096–1.131
<i>Hp</i> ^a	0.002	1.441	1.143–1.817
Age (years)	<0.001	1.028	1.018–1.038
Gender (M/F)	0.039	0.785	0.623–0.988
BMI (kg/m ²)	<0.001	1.112	1.074–1.152
Waist circumference (cm)	<0.001	1.103	1.088–1.119
<i>Hp</i> ^b	0.266	1.251	0.843–1.857

Logistic regression analysis: risk (OR) of T2DM.

Dependent variable: T2DM (1) versus NGT (0).

Independent variables: gender [(male(1), female (0))].

^aA recessive model for *Hp*2 allele: *Hp*2-2(1) versus *Hp*2-1/*Hp*1-1(0).

^bA dominant model for *Hp*2 allele: *Hp*2-2/*Hp*2-1(1) versus *Hp*1-1(0).

*Hp*2-1/*Hp*1-1 genotype carriers ($p < 0.05$). For the analysis of dominant model for *Hp*2, a significant difference was found only in the LDL level between groups. All other clinical characteristics were similar among the different groups and no significant associations were observed, while in T2DM patients all clinical characteristics were similar among the different *Hp* genotype groups and no significant associations were observed (Table 5).

Discussion

To our knowledge, the present study is the first association study of the *Hp* gene polymorphism and T2DM in northern Chinese. This study showed that the *Hp*2-2 genotype is a risk factor for T2DM in northern Han Chinese.

First, we analyzed the general distribution of the genotype and allele of the *Hp* gene between T2DM patients and controls. We found significant difference for the genotypic and allelic distribution between the case and control groups. Further, we found significant effect on T2DM in the recessive model for the *Hp*2 allele ($p = 0.006$). Then, when we used multivariate logistic regression analysis to exclude the confounding effects of age, gender, and BMI differences on the genotypic expression between the case and control groups,

the distribution of the *Hp*2-2 (OR = 1.441, $p = 0.002$) genotype still showed significant difference.

Our results are similar to the study conducted in Ghana (Quaye *et al.*, 2006), whereas they conflict with the results obtained in the two previous studies in Amerindians and Mexican Americans (Chakraborty *et al.*, 1986; Stern *et al.*, 1986), which found that the *Hp*1 allele was a risk factor for T2DM. Also, our results contradict those of three other previous studies in Brazil, Jordanian, and Mongolian (Iyengar *et al.*, 1989; Awadallah and Hamad, 2000; Nakamura *et al.*, 2009), which failed to find any association between *Hp* genotype and T2DM. In addition, there are marked ethnic differences in the distribution of the *Hp* genotype in different populations. For the *Hp*1 allele, the highest incidence reported was 92% in Mexican Lacandon, and the lowest was 7% in Indian Irulas (Langlois and Delanghe, 1996). This might be attributable to the genetic heterogeneity character of T2DM among different ethnic groups. Another possible explanation might lie in a variety of environmental factors, especially diet structure, also involved in the etiology of T2DM. When interacting with different environmental factors, the same genetic factor might have a different contribution to the etiology of a complex disease. Compared to the aforementioned studies, the sample size of our study was relatively larger, so our results were quite reliable.

We compared the phenotype variables of individuals with different genotypes in patients and controls, respectively. We found that among nondiabetic controls, the group of *Hp*2-2 genotype carriers had higher FPG, SBP, total cholesterol, LDL cholesterol, and lower DBP levels compared with those of other *Hp* genotypes (*Hp*2-1/*Hp*1-1). FPG is the direct diagnostic parameter for T2DM. Although the FPG level in *Hp*2-2 genotype carriers was within normal range, it was significantly higher than that of other *Hp* genotypes carriers. It suggested that *Hp*2-2 genotype is a potential risk for the rise of FPG. In addition, we found that the *Hp*2-2 genotype was associated with increased levels of total cholesterol and LDL cholesterol ($p < 0.05$), which are also risk factors for T2DM. Our study provided some evidence that the *Hp*2-2 genotype was associated with increased risk of developing T2DM.

In T2DM patients, no significant difference was observed in clinical and biochemical characteristics among *Hp* genotypes. Given the fact that most of the T2DM patients had taken medication, the relationship between genotype and

TABLE 4. COMPARISON OF MEAN VALUES FOR CONTINUOUS VARIABLES ACCORDING TO HAPTOGLOBIN GENOTYPES FOR NONDIABETIC CONTROLS

Parameter	<i>Hp</i> 2-2 (<i>n</i> = 304)	<i>Hp</i> 2-1/ <i>Hp</i> 1-1 (<i>n</i> = 386)	<i>P</i> _{Recessive}	<i>Hp</i> 2-1/ <i>Hp</i> 2-2 (<i>n</i> = 615)	<i>Hp</i> 1-1 (<i>n</i> = 75)	<i>P</i> _{Dominant}
BMI (kg/m ²)	24.16 ± 3.16	24.28 ± 2.94	0.595	24.20 ± 3.00	24.47 ± 3.34	0.468
Waist-hip ratio	0.83 ± 0.06	0.82 ± 0.06	0.482	0.82 ± 0.06	0.83 ± 0.06	0.186
Waist circumference (cm)	81.95 ± 8.84	80.89 ± 9.04	0.553	80.89 ± 8.85	82.59 ± 9.17	0.148
Fasting plasma glucose (mM)	4.80 ± 0.72	4.49 ± 0.63	<0.001	4.63 ± 0.70	4.60 ± 0.63	0.704
Postprandial 2-h glucose (mM)	4.64 ± 1.20	4.67 ± 1.16	0.789	4.65 ± 1.15	4.76 ± 1.32	0.545
SBP (mmHg)	112.92 ± 19.44	109.76 ± 15.57	0.021	110.98 ± 17.84	112.51 ± 13.66	0.475
DBP (mmHg)	77.84 ± 12.72	81.58 ± 13.02	<0.001	79.94 ± 12.95	81.53 ± 13.46	0.265
Triglyceride (mM)	2.26 ± 1.66	2.31 ± 1.87	0.738	2.28 ± 1.76	2.25 ± 1.72	0.907
T-cholesterol (mM)	4.32 ± 1.83	3.93 ± 1.77	0.041	4.17 ± 1.82	3.96 ± 1.78	0.476
HDL cholesterol (mM)	1.44 ± 0.32	1.40 ± 0.31	0.502	1.42 ± 0.32	1.42 ± 0.27	0.988
LDL cholesterol (mM)	2.92 ± 0.76	2.52 ± 0.52	0.001	2.75 ± 0.67	2.31 ± 0.51	0.009

TABLE 5. COMPARISON OF MEAN VALUES FOR CONTINUOUS VARIABLES ACCORDING TO HAPTOGLOBIN GENOTYPES FOR TYPE 2 DIABETES MELLITUS PATIENTS

Parameter	Hp2-2 (n=302)	Hp2-1/Hp1-1 (n=282)	$P_{\text{Recessive}}$	Hp2-1/Hp2-2 (n=534)	Hp1-1 (n=50)	P_{Dominant}
BMI (kg/m ²)	25.54±3.53	25.14±3.64	0.173	25.36±3.61	25.17±3.38	0.720
Waist-hip ratio	0.90±0.07	0.90±0.06	0.671	0.90±0.07	0.90±0.06	0.749
Waist circumference (cm)	90.58±10.62	89.81±9.70	0.369	90.29±10.26	89.29±9.39	0.508
Fasting plasma glucose (mM)	8.64±3.55	8.98±3.33	0.235	8.81±3.46	8.73±3.39	0.877
Postprandial 2-h glucose (mM)	13.40±4.92	14.11±5.16	0.263	13.60±4.97	15.53±5.66	0.074
SBP (mmHg)	136.21±23.21	132.34±23.15	0.055	134.64±23.67	131.02±22.91	0.302
DBP (mmHg)	82.21±13.33	81.13±11.93	0.311	81.81±12.80	80.40±11.28	0.461
Triglyceride (mM)	2.44±2.56	2.30±1.65	0.561	2.41±2.23	1.99±1.38	0.324
T-cholesterol (mM)	4.87±1.40	4.73±1.73	0.398	4.84±1.56	4.38±1.59	0.133
HDL cholesterol (mM)	1.36±0.30	1.35±0.37	0.920	1.38±0.34	1.23±0.21	0.057
LDL cholesterol (mM)	2.90±0.87	2.86±0.85	0.813	2.90±0.87	2.77±0.81	0.499

phenotype in these patients may not be the true performance. We believe that the results of the control group more likely reflect the actual information.

Oxidative damage appears to play an important role in the development of T2DM (Rosen *et al.*, 2001; Quaye *et al.*, 2006). We also propose that the role of Hp in T2DM risk might lies in its ability to act as an antioxidant (Mustafa *et al.*, 2004; Quaye *et al.*, 2006). Free Hb-induced oxidation is more effectively inhibited by Hp1-1 compared with Hp2-2, which has been reported to be a less effective antioxidant *in vitro* and *in vivo* (Okazaki and Nagai, 1997; Melamed-Frank *et al.*, 2001; Mustafa *et al.*, 2004; Asleh *et al.*, 2005; Levy *et al.*, 2007; Milman *et al.*, 2008; Nakhoul *et al.*, 2009). With the lower anti-oxidative potential, those with Hp2-2 genotype may therefore have the higher risk for T2DM.

We conclude that the Hp2-2 genotype is associated with increased risk of T2DM in Northern Chinese. This was independent of age, gender, and BMI.

Acknowledgments

This research was supported by the grants from the Major State Basic Research Development Program of China (973 Program) (No. 2006CB503901) and the National Natural Science Foundation of China (No. 81061120527; No. 30801208).

Disclosure Statement

No competing financial interests exist.

References

- Asleh R, Guetta J, Kalet-Litman S, Miller-Lotan R, Levy AP (2005) Haptoglobin genotype- and diabetes-dependent differences in iron-mediated oxidative stress *in vitro* and *in vivo*. *Circ Res* 96:435–441.
- Asleh R, Miller-Lotan R, Aviram M, Hayek T, Yulish M, Levy JE, Miller B, Blum S, Milman U, Shapira C, Levy AP (2006) Haptoglobin genotype is a regulator of reverse cholesterol transport in diabetes *in vitro* and *in vivo*. *Circ Res* 99:1419–1425.
- Atkinson SH, Rockett K, Sirugo G, Bejon PA, Fulford A, O'Connell MA, Bailey R, Kwiatkowski DP, Prentice AM (2006) Seasonal childhood anaemia in West Africa is associated with the haptoglobin 2-2 genotype. *PLoS Med* 3:e172.
- Awadallah S, Hamad M (2000) The prevalence of type II diabetes mellitus is haptoglobin phenotype-independent. *Cytobios* 101:145–150.
- Bessa SS, Hamdy SM, Ali EM (2007) Haptoglobin gene polymorphism in type 2 diabetic patients with and without nephropathy: an Egyptian study. *Eur J Intern Med* 18:489–495.
- Bowman BH, Kurosky A (1982) Haptoglobin: the evolutionary product of duplication, unequal crossing over, and point mutation. *Adv Hum Genet* 12:189–261.
- Chakraborty R, Ferrell RE, Stern MP, Haffner SM, Hazuda HP, Rosenthal M (1986) Relationship of prevalence of non-insulin-dependent diabetes mellitus to Amerindian admixture in the Mexican Americans of San Antonio, Texas. *Genet Epidemiol* 3:435–454.
- Conway BR, Savage DA, Brady HR, Maxwell AP (2007) Association between haptoglobin gene variants and diabetic nephropathy: haptoglobin polymorphism in nephropathy susceptibility. *Nephron Exp Nephrol* 105:e75–e79.
- Costacou T, Ferrell RE, Orchard TJ (2008) Haptoglobin genotype: a determinant of cardiovascular complication risk in type 1 diabetes. *Diabetes* 57:1702–1706.
- Cox SE, Doherty C, Atkinson SH, Nweneka CV, Fulford AJ, Ghattas H, Rockett KA, Kwiatkowski DP, Prentice AM (2007) Haplotype association between haptoglobin (Hp2) and Hp promoter SNP (A-61C) may explain previous controversy of haptoglobin and malaria protection. *PLoS ONE* 2:e362.
- Cox SE, Doherty CP, Atkinson SH, Nweneka CV, Fulford AJ, Sirugo G, Rockett KA, Kwiatkowski DP, Prentice AM (2008) Haptoglobin genotype, anaemia and malaria in Gambian children. *Trop Med Int Health* 13:76–82.
- Das SK, Elbein SC (2006) The genetic basis of type 2 diabetes. *Cellscience* 2:100–131.
- Fagoonee S, Gburek J, Hirsch E, Marro S, Moestrup SK, Laurberg JM, Christensen EI, Silengo L, Altruda F, Tolosano E (2005) Plasma protein haptoglobin modulates renal iron loading. *Am J Pathol* 166:973–983.
- Farbstein D, Levy AP (2010) The genetics of vascular complications in diabetes mellitus. *Cardiol Clin* 28:477–496.
- Friis H, Gomo E, Nyazema N, Ndhlovu P, Krarup H, Madsen PH, Michaelsen KF (2003) Iron, haptoglobin phenotype, and HIV-1 viral load: a cross-sectional study among pregnant Zimbabwean women. *J Acquir Immune Defic Syndr* 33:74–81.
- Iyengar S, Hamman RF, Marshall JA, Baxter J, Majumder PP, Ferrell RE (1989) Genetic studies of type 2 (non-insulin-dependent) diabetes mellitus: lack of association with seven genetic markers. *Diabetologia* 32:690–693.

- Koch W, Latz W, Eichinger M, Roguin A, Levy AP, Schomig A, Kastrati A (2002) Genotyping of the common haptoglobin Hp 1/2 polymorphism based on PCR. *Clin Chem* 48:1377–1382.
- Langlois MR, Delanghe JR (1996) Biological and clinical significance of haptoglobin polymorphism in humans. *Clin Chem* 42:1589–1600.
- Levy AP, Asleh R, Blum S, Levy NS, Miller-Lotan R, Kalet-Litman S, Anbinder Y, Lache O, Nakhoul FM, Asaf R, Farbstein D, Pollak M, Soloveichik YZ, Strauss M, Alshiek J, Livshits A, Schwartz A, Awad H, Jad K, Goldenstein H (2010) Haptoglobin: basic and clinical aspects. *Antioxid Redox Signal* 12:293–304.
- Levy AP, Hochberg I, Jablonski K, Resnick HE, Lee ET, Best L, Howard BV (2002) Haptoglobin phenotype is an independent risk factor for cardiovascular disease in individuals with diabetes: The Strong Heart Study. *J Am Coll Cardiol* 40:1984–1990.
- Levy AP, Levy JE, Kalet-Litman S, Miller-Lotan R, Levy NS, Asaf R, Guetta J, Yang C, Purushothaman KR, Fuster V, Moreno PR (2007) Haptoglobin genotype is a determinant of iron, lipid peroxidation, and macrophage accumulation in the atherosclerotic plaque. *Arterioscler Thromb Vasc Biol* 27:134–140.
- Levy AP, Roguin A, Hochberg I, Herer P, Marsh S, Nakhoul FM, Skorecki K (2000) Haptoglobin phenotype and vascular complications in patients with diabetes. *N Engl J Med* 343:969–970.
- Melamed-Frank M, Lache O, Enav BI, Szafrank T, Levy NS, Ricklis RM, Levy AP (2001) Structure-function analysis of the antioxidant properties of haptoglobin. *Blood* 98:3693–3698.
- Milman U, Blum S, Shapira C, Aronson D, Miller-Lotan R, Anbinder Y, Alshiek J, Bennett L, Kostenko M, Landau M, Keidar S, Levy Y, Khemlin A, Radan A, Levy AP (2008) 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* 28:341–347.
- Mustafa S, Vukovich T, Prikoszovich T, Winzer C, Schneider B, Esterbauer H, Wagner O, Kautzky-Willer A (2004) Haptoglobin phenotype and gestational diabetes. *Diabetes Care* 27: 2103–2107.
- Nakamura H, Soejima M, Munkhtulga L, Iwamoto S, Koda Y (2009) Haptoglobin polymorphism in Mongolian population: comparison of the two genotyping methods. *Clin Chim Acta* 408:110–113.
- Nakhoul FM, Miller-Lotan R, Awad H, Asleh R, Jad K, Nakhoul N, Asaf R, Abu-Saleh N, Levy AP (2009) 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* 296:F830–F838.
- Nakhoul FM, Zoabi R, Kanter Y, Zoabi M, Skorecki K, Hochberg I, Leibur R, Miller B, Levy AP (2001) Haptoglobin phenotype and diabetic nephropathy. *Diabetologia* 44:602–604.
- Okazaki T, Nagai T (1997) Difference in hemoglobin-binding ability of polymers among haptoglobin phenotypes. *Clin Chem* 43:2012–2013.
- Quaye IK, Ababio G, Amoah AG (2006) Haptoglobin 2-2 phenotype is a risk factor for type 2 diabetes in Ghana. *J Atheroscler Thromb* 13:90–94.
- Rosen P, Nawroth PP, King G, Moller W, Tritschler HJ, Packer L (2001) The role of oxidative stress in the onset and progression of diabetes and its complications: a summary of a Congress Series sponsored by UNESCO-MCBN, the American Diabetes Association and the German Diabetes Society. *Diabetes Metab Res Rev* 17:189–212.
- Sjoholm A, Nystrom T (2005) Endothelial inflammation in insulin resistance. *Lancet* 365:610–612.
- Stern MP, Ferrell RE, Rosenthal M, Haffner SM, Hazuda HP (1986) Association between NIDDM, RH blood group, and haptoglobin phenotype. Results from the San Antonio Heart Study. *Diabetes* 35:387–391.

Address correspondence to:
Ze Yang, M.D., Ph.D.
Key Laboratory of Geriatrics
Institute of Geriatrics
Beijing Hospital
Ministry of Health
Beijing 100730
China

E-mail: yangze016@163.com