

Original Article

Haptoglobin 2-2 Phenotype is a Risk Factor for Type 2 Diabetes in Ghana

Isaac K. Quaye¹, Grace Ababio¹, and Albert G. Amoah^{1,2}

¹Department of Medical Biochemistry, University of Ghana Medical School, Ghana.

²Department of Medicine and Therapeutics, University of Ghana Medical School, Ghana.

We have investigated the role of haptoglobin gene polymorphisms in 129 type 2 diabetic patients and 87 non-diabetic subjects, classified by the ADA criteria, in Ghana. The diabetic subjects were recruited consecutively from the National Diabetic Management and Research Center of the University of Ghana Medical School, Korle-Bu, Accra, Ghana and were categorized by their haptoglobin phenotypes. The haptoglobin 2 allele was determined to be a risk factor for type 2 diabetes in Ghana (OR=6.1, 95% CI=1.8-21.2; P=.0001) while the Hp1 allele appeared protective (OR=0.56, 95% CI=0.31-1.0; P=.06). The deleterious role of the Hp2 allele was further evidenced by the reduced risk associated with Hp2-1M mutant heterozygotes, who produce less Hp2 protein than the normal Hp2-1 heterozygote. (OR=0.52, 95% CI=0.27-1.0; P=0.06). The subjects with the homozygous Hp2 allele were also hypertensive and overweight. There was no difference ($p>0.05$) in the levels of triglycerides, total cholesterol, LDL and HDL between diabetic subjects with different haptoglobin phenotypes.

We conclude that hypertensive and overweight individuals with the Hp2-2 phenotype in Ghana are at a high risk of developing type 2 diabetes and may require a more aggressive management.

J Atheroscler Thromb, 2006; 13:90-94.

Key words; Diabetes, Haptoglobin, Ghana

Introduction

Diabetes mellitus is a disorder of carbohydrate, lipid and protein metabolism characterized by hyperglycemia^{1,2}. Type 2 diabetes accounts for the greater percentage of diabetic cases worldwide, due to an increasing prevalence of obesity, hypertension and sedentary lifestyles³. In the Greater Accra Region of Ghana, where the prevalence of diabetes is 6.3%, type 2 diabetes constitutes 90% of reported cases⁴. Anecdotal reports indicate that the condition is on the increase in Ghana, as is the trend worldwide³⁻⁵.

Type 2 diabetes is characterized by insulin resistance, which is caused by a collection of metabolic disorders: hypertension, inflammation, abnormal lipid

metabolism and a hypercoagulable state⁶⁻⁹. The early stages of endothelial dysfunction and arterial intima thickening occur in individuals at risk, long before the appearance of clinical symptoms¹⁰. Underlying the endothelial dysfunction is a low-level chronic inflammatory stimulus that upregulates the expression of acute phase proteins, cytokines and oxidized LDL^{11,12}. These events eventually lead to vascular damage, with the severity differing among ethnic groups and races^{9,13}.

The impact of ethnic and racial differences on diabetes underscores the need for the identification of genetic factors that contribute to differences in susceptibility to and the pathology of the disease. Recent studies in animals and humans have shown that the haptoglobin (Hp) gene is a major risk factor for diabetes vascular disease^{14,15}, however, the association between the Hp gene and diabetes varies between populations¹⁶⁻¹⁸. In African populations, no risk association data for haptoglobin and diabetes has been reported to date.

Haptoglobin is involved in all the inflammatory

Address for correspondence: Isaac K. Quaye, Department of Medical Biochemistry, University of Ghana Medical School, Korle-Bu, Ghana.

E-mail: dr.quaye@gmail.com

Received: September 14, 2005

Accepted for publication: January 6, 2006

Table 1. Haptoglobin (Hp) phenotypes and Hp1 allelic frequencies in the reference and diabetic population

Population	Hp1-1	Hp2-1	Hp2-2	Hp2-1M	Hp1 allelic freq
Reference Population	30	21	3	22	0.75
Type 2 Diabetics	32	42	24	21	0.54

stimuli associated with diabetes, due to its role as a scavenger of hemoglobin^{19, 20}. It is involved in IL-6 expression, inhibits LDL-oxidation, and limits hemoglobin-related oxidative stress²¹⁻²³. All populations have three major haptoglobin phenotypes, Hp1-1 homozygotes, Hp2-1 heterozygotes, and Hp2-2 homozygotes. A modified form of Hp2-1 resulting from a single nucleotide polymorphism in the Hp2 gene promoter, (Hp2-1M), is prevalent in African populations²⁴. Individuals with this phenotype express less Hp2 polypeptide than their Hp2-1 heterozygous counterparts²⁴. Here we show that the Hp2-2 phenotype is a risk factor for type 2 diabetes in Ghana while Hp1-1 and Hp2-1M phenotypes are protective.

Materials and Methods

Subjects

Diabetic patients were recruited consecutively from the National Diabetes Management and Research Center of the Korle-Bu Teaching Hospital, Accra, Ghana. A total of 129 type 2 diabetic subjects and 87 healthy non-diabetics were enrolled after providing informed written consent. All subjects underwent a standardized medical examination including measurements of blood pressure (BP) and anthropometry. Subjects were classified as diabetic or non-diabetic using the diagnostic criteria of the American Diabetes Association (ADA)².

Blood Sampling and Anthropometric Measurements

A fasting blood sample was withdrawn from a forearm vein into fluoride-oxalate or heparin. The samples were kept on ice and centrifuged within 15 minutes of their collection. The plasma was aliquoted into storage vials and stored at -70°C until analyzed. Fasting plasma glucose (FPG) levels were determined by the glucose oxidase method. Triglyceride levels were determined by a colorimetric procedure based on glycerol-3-phosphate oxidase. Total cholesterol levels were determined by the CHOD-PAP method using a kit from Randox. HDL cholesterol levels were estimated, following precipitation of LDL and VLDL, using phosphotungstic acid and Magnesium chloride, according to the manufacturer's instructions (Randox). LDL cholesterol levels were estimated by calculation.

Weight was measured to the nearest 0.1 kg and height was measured to the nearest 0.1 centimeter for the determination of body mass index (BMI). Waist girth at the umbilicus was measured to the nearest 0.1 centimeter.

Haptoglobin Phenotyping

Haptoglobin phenotyping was achieved by polyacrylamide gel electrophoresis of hemoglobin-supplemented sera followed by benzidine staining.

Ethics

The study was approved by the Ethical and Protocol Review Committee of the University of Ghana Medical School, and complied with the Helsinki Declaration of 1975 (revised in 1983 and 1989) on human experimentation.

Statistics

The significance of the association of diabetes with the haptoglobin allele was determined by the χ^2 test as were evaluations for agreement with Hardy Weinberg equilibrium. The ODDs ratio was computed using Vassar Stats (<http://faculty.vassar.edu/lowry/odds2x2.html>) software. Student's *t*-test was used to determine differences between means after performing an ANOVA test.

Results

The haptoglobin phenotypic frequencies for the control and diabetic subjects are presented in **Table 1**. The allelic frequencies were consistent with Hardy Weinberg equilibrium. The frequency of the Hp2 allele was higher in the diabetic population (84%) as opposed to the Hp1 allele with a frequency of 39%. The ODDs ratio indicates that the Hp2 allele is a risk factor for type 2 diabetes in homozygous individuals in Ghana (**Table 2**). The Hp1 allele appears protective as was the Hp2-1M phenotype. The non-diabetics with the Hp2-2 phenotype were leaner within the group whereas diabetic subjects with this phenotype were hypertensive and overweight ($p < .05$) (**Table 3**). The fasting plasma glucose level tended to be lower in Hp2-2 subjects with or without diabetes but this difference was not statistically significant. The mean se-

Table 2. ODDs ratio for developing type 2 diabetes with respect to haptoglobin (Hp) gene polymorphisms

Type 2 diabetics/non diabetics	Hp1-1	Hp2-1	Hp2-2	Hp2-1M
ODD Ratio (95% CI)	0.56 (0.31-1.0)	1.4 (0.8-2.0)	6.1 (1.8-21.2)	0.52 (.27-1.0)
P value	0.06	0.26	0.001	0.06

Table 3. Clinical variables in the control and type 2 diabetic subjects according to haptoglobin (Hp) phenotypes

Clinical variables	Hp1-1	Hp2-1	Hp2-2	Hp2-1M
Control subjects				
Age	43.8 ± 11.9 ^a	46.3 ± 12.3	44.5 ± 9.4	42.3 ± 9.8
FPG ^b	3.3 ± 1.6	3.9 ± 1.5	2.9 ± 1.7	3.6 ± 2.1
BMI ^c	26.3 ± 6.3	27.5 ± 5.5	20.5 ± 1.4	25.8 ± 6.1
SBP ^d	122 ± 28	132 ± 26	105 ± 10.1	125 ± 16
DBP ^e	86 ± 18	86 ± 11.8	74 ± 8.8	82 ± 13.1
Waist girth	79.5 ± 12.6	79.8 ± 9.2	63.2 ± 9.0	78.9 ± 9.0
Type 2 Diabetics				
Age	59.5 ± 11.2	54.0 ± 12.2	57.3 ± 11.7	49.7 ± 12.3
FPG	6.3 ± 3.8	7.5 ± 4.8	5.6 ± 3.6	9.9 ± 2.8
BMI	24.0 ± 0.3	24.0 ± 4.5	26.2 ± 6.8	21.8 ± 3.2
SBP	135.7 ± 18	135.0 ± 15.5	145.9 ± 16	134.7 ± 23.2
DBP	87.4 ± 12.9	83.6 ± 7.9	89.1 ± 13.4	83.9 ± 9.8
Waist girth	81.0 ± 12.1	83.1 ± 10.6	82.8 ± 10.6	77.6 ± 9.7

^aMean ± S.D., ^bFPG, fasting plasma glucose, ^cBMI, body mass index, ^dSystolic blood pressure, ^eDiastolic blood pressure

Table 4. Lipid profiles of control and diabetic subjects according to their haptoglobin phenotypes (Values are the mean ± SD, and the units are mmol/l)

Clinical variables	Hp1-1	Hp2-1	Hp2-2	Hp2-1M
Control Subjects				
Cholesterol	4.80 ± 1.19	4.8 ± 1.07	4.77 ± 0.68	5.12 ± 0.94
Triglycerides	0.84 ± 0.21	0.92 ± 0.29	0.77 ± 0.20	0.8 ± 0.11
HDL*	1.95 ± 0.41	1.25 ± 0.57	1.87 ± 0.40	2.13 ± 0.52
LDL**	2.86 ± 0.88	3.3 ± 1.03	2.5 ± 0.29	2.92 ± 0.90
Diabetic Subjects				
Cholesterol	5.04 ± 1.35	5.6 ± 1.25	5.51 ± 1.03	5.75 ± 1.15
Triglycerides	1.90 ± 0.44	2.38 ± 0.87	2.0 ± 0.30	1.95 ± 0.89
HDL	1.79 ± 0.78	1.96 ± 0.94	2.06 ± 1.21	2.14 ± 1.24
LDL	2.46 ± 1.48	2.55 ± 1.6	2.44 ± 1.43	2.66 ± 1.64

*LDL- Low Density Lipoprotein, **HDL- High Density Lipoprotein

rum cholesterol, triglycerides and LDL cholesterol levels did not differ between subjects with different haptoglobin phenotypes in either the control group or diabetic group. However, HDL cholesterol levels were significantly lower in control subjects with the Hp2-1 phenotype, compared to the rest of the subject categories (**Table 4**). Between the diabetic and control subjects, the major difference was in the serum triglyceride level, which was two times higher in the diabetic subjects ($p < 0.001$).

Discussion

This study has shown that the Hp2 allele is a high risk factor for type 2 diabetes in Ghana and that the Hp1 allele is protective. The finding contrasts with previous observations in Amerindians and Mexican Americans^{18, 25}, where the Hp1 allele was observed to be a risk factor. These differences highlight the role of genetic and ethnic variation in determining susceptibility to type 2 diabetes.

The risk associated with the Hp2 allele in the Ghanaian population appears to be directly associated with the amount of Hp2 polypeptide produced. Patients with the Hp2-1M allele, who express less Hp2 protein than Hp2-1 individuals²⁶⁾, exhibited protection equivalent to that for the Hp1-1 homozygotes. This result is significant as it represents the first example of a beneficial mutation with regards to the risk of developing diabetes.

Diabetic subjects with the Hp2 phenotype were also hypertensive and overweight. This contrasts with the non-diabetics of the same phenotype, who were leaner than their counterparts with other phenotypes. It is generally recognized that diabetes is a risk factor for hypertension and vice versa²⁷⁾. Together, these results suggest that the Hp2 allele increases the risk of type 2 diabetes in patients who are overweight and have hypertension but not in those who have normal weight and blood pressure.

We propose that the role of haptoglobin in diabetes risk is directly related to its function as an antioxidant. The hemoglobin-binding capacity and antioxidative function of Hp2 is less than that of Hp1^{19, 28-30)}. Mechanistically, this is attributed to a more efficient scavenging of the Hp1-1-Hb complex by CD163 than of the Hp2-2-Hb complex^{31, 32)}. Free hemoglobin promotes damage to LDL within the blood vessel through the Fenton reaction^{23, 33)}, leading to the early stages of endothelial dysfunction characteristic of type 2 diabetes. The situation is aggravated as the diabetes progresses and the turnover of blood cells is increased, making hemoglobin available for glycosylation³⁴⁾. Glycosylated hemoglobin (GlycHb) is even more difficult for CD163 to scavenge, reducing the antioxidative function of the complex further³¹⁾. The lack of difference in the lipoprotein levels of diabetic subjects with different haptoglobin phenotypes also points to the fact that homozygosity for the Hp2 allele is an independent risk factor for diabetes in the population studied. Further studies into the precise role of the Hp2-2 phenotype in diabetes would be informative.

We conclude that the Hp2 allele is a risk factor for type 2 diabetes in Ghana, whereas the Hp1 allele appears protective. This observation is strongly supported by the protection that is also afforded by the Hp2-1M phenotype. Diabetic subjects with the Hp2-2 phenotype in Ghana may require more aggressive management.

Acknowledgements

The authors appreciate the support from the University Temple United Methodist Church, Seattle

USA, Professor Carol Sibley of the University of Washington, Genome Sciences, and The National Diabetic and Management Center, University of Ghana Medical School, Accra, Ghana. Dr Eleanor Hankins is acknowledged for helpful criticism of the manuscript and Farida Quaye for technical support.

References

- 1) Alberti KG, and Zimmet PZ: Definition, diagnosis and classification of diabetes mellitus and its complications: diagnosis and classification of diabetes mellitus: provisional report of a WHO consultation. *Diabet Med*, 1998; 15:539-553.
- 2) The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care*, 1997; 20:1183.
- 3) Zimmet P, Alberti KG, and Shaw J: Global and societal implications of the diabetes epidemic. *Nature*, 2001; 414:782.
- 4) Amoah A, Owusu S, and Adjei S: Diabetes in Ghana: a community based prevalence study in Greater Accra. *Diabetes Res and Clin Pract*, 2002; 56:197.
- 5) King H, Aubert RE, and Herman WH: Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. *Diabetes Care*, 1998; 21:1414.
- 6) Abuissa H, Bell DSH, and O'Keefe Jr JH: Strategies to prevent type 2 diabetes. *Curr Med Res and Opinion*, 2005; 21:1107.
- 7) Haffner SM, Lehto S, Ronnema T, Pyorala K, and Laakso M: Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without myocardial infarction. *N Engl J Med*, 1998; 339:229.
- 8) Coutinho M, Gerstein H, Wang Y, and Yusuf S: The relationship between glucose and incident cardiovascular events: a meta-regression analysis of published data from 20 studies of 95,783 individuals followed for 12.4 years. *Diabetes Care*, 1999; 22:233.
- 9) Varughese GI, Tomson J, and Lip GYH: Type 2 diabetes mellitus: a cardiovascular perspective. *Int J Clin Prac*, 2005; 59:798.
- 10) Sjoholm S, and Nystrom T: Endothelial inflammation in insulin resistance. *Lancet*, 2005; 365:610.
- 11) Libby P: Inflammation in atherosclerosis. *Nature*, 2002; 420:868.
- 12) Fernandez-Real JM, and Ricart W: Insulin resistance and chronic cardiovascular inflammatory syndrome. *Endocr Rev*, 2003; 24:278.
- 13) UK Prospective Diabetes Study Group: Ethnicity and cardiovascular disease: the incidence of myocardial infarction in white, south Asian and afro-Caribbean patients with type 2 diabetes. *Diabetes Care*, 1998; 21:1271.
- 14) Miller-Lotan R, Herskowitz Y, Kalet-Litman S, Nakhoul F, Aronson D, Zoabi R, Asaf R, Ben-Izhak O, Sabo E, Lim SK, Baumann H, Berger FG, and Levy AP: Increased renal hypertrophy in diabetic mice genetically modified at the haptoglobin locus. *Diabetes Metab Res Rev*, 2005; 21:332.

- 15) Levy A, Roguin A, and Hochberg I: Haptoglobin phenotypes and vascular complications in diabetes. *N Engl J Med*, 2003; 343:969.
- 16) Awadallah SM, and Hamad H: The prevalence of type II diabetes is haptoglobin phenotype independent. *Cytobios*, 2000; 101:145.
- 17) Schmidt MI, Duncan BB, Sharrett AR, Lindberg G, Savage PJ, Offenbacher S, Azambuja MI, Tracy RP, and Heiss G: Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Community Study): a cohort study. *Lancet*, 1999; 353:1649.
- 18) Stern MP, Ferrell RE, Rosenthal M, Haffner SM, and Hazuda HP: Association between NIDDM, RH blood group, and haptoglobin phenotype. Results from San Antonio Heart Study. *Diabetes*, 1986; 35:387.
- 19) Melamed-Frank M, Lache O, Enav B, Szafrank T, Levy N, Ricklis R, and Levy A: Structure-function analysis of the antioxidant properties of haptoglobin. *Blood*, 2001; 98:3693.
- 20) Philippidis P, Mason JC, Evans BJ, Nadra I, Taylor KM, Haskard DO, and Landis RC: Hemoglobin scavenger receptor CD163 mediates interleukin-10 release and hem oxygenase-1 synthesis: antiinflammatory monocyte-macrophage responses in vitro, in resolving skin blisters in vivo, and after cardiopulmonary bypass surgery. *Circ Res*, 2004; 94:119.
- 21) Wang Y, Kinzie E, Berger F, Lim S, and Baumann H: Haptoglobin, an inflammation-inducible plasma protein. 2001.
- 22) Tseng CF, Lin CC, Huang HY, Liu HC, and Mao SJ: Antioxidant role of human haptoglobin. *Proteomics*, 2004; 4:2221.
- 23) Lim SK, Ferraro B, Moore K, and Halliwell B: Role of haptoglobin in free hemoglobin metabolism. *Redox Rep*, 2001; 6:219.
- 24) Maeda N: DNA polymorphisms in the controlling region of the human haptoglobin genes: a molecular explanation for the haptoglobin 2-1 modified phenotype. *Am J Hum Genet*, 1991; 49:158.
- 25) Chakraborty R, Ferrell RE, Stern MP, Haffner SM, Hazuda HP, and 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.
- 26) Connell G, and Smithies O: Human haptoglobins: estimation and purification. *Biochem J*, 1959; 72:115.
- 27) Mancina G: The association of hypertension and diabetes: prevalence, cardiovascular risk and protection by blood pressure reduction. *Acta Diabetol*, 2005; 42:S17.
- 28) Langlois MR, and Delanghe JR: Difference in hemoglobin-binding ability of polymers among haptoglobin phenotypes. *Clin Chem*, 1997; 43:2012.
- 29) Levy AP, Hochber I, Jablonski K, Resnick HE, Lee ET, Best L, and Howard BV: Haptoglobin phenotype is an independent risk factor for cardiovascular disease in individuals with diabetes: the strong heart study. *J AM Coll Cardiol*, 2002; 40:1984.
- 30) Halliwell B: Antioxidant defence mechanisms: from the beginning to the end (of the beginning). *Free Radic Res*, 1999; 31:261.
- 31) Asleh R, Marsh S, Shilkrut M, Binah O, Guetta J, Lejbkowitz F, Enav B, Shehadeh N, Kanter Y, Lache O, Cohen O, Levy NS, and Levy AP: Genetically determined heterogeneity in hemoglobin scavenging and susceptibility to diabetic cardiovascular disease. *Circulation Res*, 2003; 92:1193.
- 32) Kristiansen M, Graversen J, and Jacobsen C, et al: Identification of the haemoglobin scavenger receptor. *Nature*, 2001; 409:198.
- 33) Sadrzadeh SM, Graf E, Panter SS, Hallaway PE, and Eaton JW: Hemoglobin: a biologic Fenton reagent. *J Biol Chem*, 1984; 259:14354.
- 34) Venerando B, Fiorilli A, Croci G, Tringali C, Goi G, Mazzanti L, Curatola G, Segalini G, Massaccesi L, Lombardo A, and Tettamanti G: Acidic and neutral sialidase in the erythrocyte membrane of type 2 diabetic patients. *Blood*, 2002; 99:1064.