Observational Study

Medicine®

OPEN

Association of rs2000999 in the haptoglobin gene with total cholesterol, HDL-C, and LDL-C levels in Mexican type 2 diabetes patients

Fernando Suarez-Sanchez, PhD[a], Miguel Vazquez-Moreno, MS[a], Ema Herrera-Lopez, MS[a], Jaime H. Gomez-Zamudio, PhD[a], José J. Peralta-Romero, PhD[a], Osvaldo D. Castelan-Martinez, PhD[b], Miguel Cruz, PhD[a], Esteban J. Parra, PhD[c], Adan Valladares-Salgado, PhD[a,*]

Abstract

Recently, studies have shown significant association between the rs2000999 polymorphism in the *haptoglobin*-encoding gene (*HP*) and low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) levels, which are important risk factors for cardiovascular diseases. However, the association of rs2000999 with serum lipids in Latin American diabetic populations is still uncharacterized. Here, we analyzed the association of rs2000999 with TC, high-density lipoprotein cholesterol (HDL-C), and LDL-C levels in 546 Mexican adults with type 2 diabetes (T2D) and in 654 controls without T2D. In this observational case-control study we included adults from 4 centers of the Mexican Social Security Institute in Mexico City recruited from 2012 to 2015. TC, HDL-C, LDL-C, triglycerides (TG), and glucose levels were measured by an enzymatic colorimetric method. The variant rs2000999 was genotyped using TaqMan real time polymerase chain reaction. The percentage of Native-American ancestry showed a negative association with the rs2000999 A allele. In contrast, the rs2000999 A allele had a strong positive association with European ancestry, and to a lesser extent, with African ancestry. Linear regression was used to estimate the association between the variant rs2000999 and lipid concentrations, using different genetic models. Under codominant and recessive models, rs2000999 was significantly associated with TC and LDL-C levels in the T2D group and in controls without T2D. In addition, the group with T2D showed a significant association between the variant and HDL-C levels. In summary, the rs2000999 A allele in Mexican population is positively associated with the percentage of European and negatively associated with Native American ancestry. Carriers of the A allele have increased levels of TC and LDL-C, independently of T2D diagnosis, and also increased concentrations of HDL-C in the T2D sample.

Abbreviations: GWAS = genome-wide association study, HDL-C = high-density lipoprotein cholesterol, *HP* = haptoglobin-encoding gene, LDL-C = low-density lipoprotein cholesterol, SE = standard error, T2D = type 2 diabetes, TC = total cholesterol, TG = triglycerides.

Keywords: haptoglobin, HDL-C, LDL-C, Mexican population, rs2000999, total cholesterol, type 2 diabetes

1. Introduction

The National Institute of Statistics and Geography (INEGI) reported in 2018 that cardiovascular diseases are the primary cause of death in Mexico.[1] The most important risk factors for the onset of these diseases are high levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides, and low levels of high-density lipoprotein cholesterol (HDL-C). Genetic studies of the loci associated with lipid levels have enabled the identification of polymorphisms that may serve to develop new approaches for the prevention and treatment of cardiovascular diseases. In particular, our research group has collaborated with other research institutions with the objective of characterizing the loci associated with dyslipidemia in the Mexican and Hispanic populations. In a genome-wide association study (GWAS), we found that the genes, *CELSR2*, *ZNF259/APOA5*, *KANK2/DOCK6* and *NCAN/MAU2*, were associated with TC levels; *CELSR2*, *APOB*, and *NCAN/MAU2* with LDL-C levels[2]; and *DAGLB* with HDL-C levels.[3] The same GWAS study failed to find an association between the haptoglobin-encoding (*HP*) gene (located at chromosome 16q22.2) with lipid levels. However, previous studies have showed that the A allele of the rs2000999 polymorphism in *HP* is associated with high levels of TC and LDL-C. It has been proposed that the rs2000999 A allele, which is associated with reduced *HP* expression, has decreased antioxidant protection for APOE, contributing to elevated cholesterol levels.[4,5]

Editor: Leonardo Roever.

Suarez-Sanchez Fernando and Vazquez-Moreno Miguel Equal contribution.

Financial support: This work was supported by the Fondo Sectorial de Investigación en Salud y Seguridad Social (SSA/IMSS/ISSSTE-CONACYT) project 150352.

The authors have no conflicts of interests to disclose.

[a] Unidad de Investigación Médica en Bioquímica, Hospital de Especialidades, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, [b] Facultad de Estudios Superiores Zaragoza, Universidad Nacional Autónoma de México, Mexico City, Mexico, [c] Department of Anthropology, University of Toronto at Mississauga, Mississauga, ON, Canada.

[*] Correspondence: Adan Valladares-Salgado, Unidad de Investigación Médica en Bioquímica, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, Av. Cuauhtémoc 330, Col. Doctores, Delegación Cuauhtémoc, Ciudad de México, CP 06720, México (e-mail: adanval@gmail.com).

How to cite this article: Fernando SS, Miguel VM, Herrera-Lopez E, Gomez-Zamudio JH, Peralta-Romero JJ, Castelan-Martinez OD, Cruz M, Parra EJ, Adan VS. Association of rs2000999 in the haptoglobin gene with total cholesterol, HDL-C and LDL-C levels in Mexican type 2 diabetes patients. Medicine 2019;98:39(e17298).

Received: 22 March 2019 / Received in final form: 22 August 2019 / Accepted: 27 August 2019

http://dx.doi.org/10.1097/MD.0000000000017298

Hp is an alpha-glucoprotein commonly found in plasma; it is composed of 2 light chains (α) and 2 heavy chains (β) that are covalently bound. It is mainly produced in the liver and has been reported to play a role in cholesterol esterification, particularly with respect to the binding of apolipoprotein A–I to HDL-C, which promotes cholesterol efflux from cells and stimulates the enzyme lecithincholesterol acyltransferase to esterify cholesterol.[6] Hp has been proposed to target and protect the ApoA–I effector domain of lecithin-cholesterol acyltransferase from oxidative stress; Hp can also bind to ApoE. ApoA-I and ApoE contain similar sequences, which are able to stimulate LCAT to achieve cholesterol esterification in reverse cholesterol transport.[7–10] Recently, Boettger et al. (2016) identified a strong association between the rs2000999 (G/A) polymorphism in *HP* with TC and LDL-C levels. The rs2000999 A allele decreases Hp expression, consequently reducing its antioxidant capacity, which in turn leads to higher cholesterol levels.[4] The human *HP* gene has 2 common alleles, Hp1 and Hp2, which are the result of a small intragenic duplication of the *HP* gene.[11] The Hp1 allele exhibits enhanced antioxidant activity compared to Hp2. The frequency of the alleles Hp1 and Hp2 is quite variable in world populations. In general, the Hp1 allele is less frequent in East Asian populations than in other population groups, including European, African, and Native American groups.[12–17]

Studies in patients with diabetes revealed that the Hp2-1 or Hp2-2 genotypes are associated with increased risk of vascular injury compared to the Hp1-1 genotype.[18,19] In addition, the rs2000999 A allele is almost exclusively associated with the Hp2 isoform and decrease in expression of Hp; on the other hand, rs2000999 (G) is associated with the Hp1 isoform.[4] A study performed in Chinese diabetic patients reported that the variant rs2000999 is not associated with diabetic macrovascular diseases, although, the A allele is associated with higher levels of LDL-C.[20]

To date, the association of rs2000999 of *HP* with serum lipids in the Latin American diabetic population is uncharacterized. Therefore, the aim of this study was to analyze the association of rs2000999 with TC, HDL-C, and LDL-C and in the Mexican population with type 2 diabetes (T2D).

2. Material and methods

2.1. Study population

We investigated 1,200 Mexican adults of both sexes (546 adults with T2D, the T2D group; and 654 controls without T2D, the No T2D group) from 4 centers of social security in Mexico City from 2012 to 2015. The T2D group was selected based on fasting glucose levels, in accordance with American Diabetes Association (ADA) guidelines.[21] The present work was designed as an observational case-control study with a convenience sample size.

The study protocol complies with the ethical guidelines of the 1975 Declaration of Helsinki. The study was authorized by the Instituto Mexicano del Seguro Social ethics committee and informed consent was obtained from all participants.

2.2. Procedure

All participants were weighed using a digital scale (Seca, Hamburg, Germany). Height was measured with a portable stadiometer (Seca 225, Hamburg, Germany). Body mass index (BMI), calculated as weight (kg)/height $(m)^2$. Waist circumference was measured taken after expiration at the midpoint between the low rib margin and iliac crest. Systolic and diastolic blood pressure (SBP and DBP) were measured using a mercurial sphygmomanometer (ALPK2, Tokyo, Japan). Data regarding age and smoking status (current smokers/non-smokers) was determined by self-report.

Two blood samples were collected from each participant to estimate the biochemical parameters and for DNA extraction. The IL-650 equipment (Instrument Laboratory, Bedford, MA, USA) was used to quantify TC, HDL-C, LDL-C, triglycerides (TG), and glucose levels measured by enzymatic colorimetric method (kit numbers 0018250540, 0018255740, 0018256040, 0018480500 and 0018250740 of Werfen Czech s.r.o, respectively). According with Grundy et al,[22] metabolic syndrome was defined with the presence of 3 or more risk factors (waist circumference ≥102 cm in men or ≥88 cm in women, triglycerides ≥150 mg/dl, HDL-C < 40 mg/dl in men or <50 in women, systolic blood pressure ≥ 130 mm Hg or diastolic blood pressure ≥ 85 mm Hg and fasting glucose ≥ 100 mg/dl).

DNA isolation was performed in the AutoGen Flex Star (AutoGen, MA, USA) following the manufacturer's recommendations. DNA quantity (260 nm) and purity (260/280 nm ratio) were evaluated in the Epoch Microplate Spectrophotometer using Gen5 Microplate Data Analysis (BioCell, VT, USA). DNA integrity was also assessed by electrophoresis in agarose gels (.8%).

Genotyping of rs2000999 was performed by real time polymerase chain reaction using the TaqMan allelic discrimination assay C__11439054_10 on the 7900HT Fast Real-Time PCR system (Applied Biosystems, CA, USA), following standard protocols. Duplicates were performed in 10% of the samples. Genotype discrimination was evaluated using the SDS software (Applied Biosystems, CA, USA).

Estimation of individual ancestral proportions: Given that the individuals in this sample are of mixed ancestry, the Axiom LAT microarray (Affymetrix, CA, USA) was used to determine the Native-American (NAM), European and African ancestry proportions of the participants using the program ADMIXTURE.[23] We added individual ancestral proportions as a covariate in the association analyses.

2.3. Statistical analysis

The normal distribution of continuous variables was tested using the Kolmogorov-Smirnov test. For the traits that significantly deviate from normality, rank based inverse normal transformations were applied. Differences between the cases and controls for continuous and categorical traits were evaluated using the Student *t* test and Chi-Squared test, respectively. We tested the association of rs200099 with individual ancestral proportions, using an additive model, with adjustments for age, sex and T2D diagnosis. The association of rs2000999 with lipid concentrations was assessed using linear regression under different genetic models (codominant, dominant and recessive). All statistical analyses were performed using SPSS software (version 22.0, IBM, Armonk, NY, USA). Two-sided *P* values $< .05$ were considered significant.

3. Results

3.1. Characteristics of the study population

The characteristics of the 1200 participants included in the sample are presented in Table 1. Compared with non-diabetic

Table 1

General characteristics of cases and controls of type 2 diabetes Mexican adults.

	Without T2D N = 654	T2D N = 546	*P*
Woman n (%)	307 (46.9)	322 (58.9)	2.0×10^{-4}
Age (years)	49.615 ± 9.281	57.077 ± 10.006	1.3×10^{-13}
Current smokers n (%)	65 (9.9)	142 (26.0)	1.8×10^{-10}
Waist circumference (cm)	90.8 ± 13.2	97.3 ± 12.4	8.0×10^{-11}
BMI (kg/m^2)	27.944 ± 4.712	29.427 ± 5.140	5.5×10^{-6}
TC (mg/dL)	195.751 ± 39.605	188.659 ± 43.873	.009
HDL-C (mg/dL)	49.418 ± 13.595	43.977 ± 13.099	4.8×10^{-7}
LDL-C (mg/dL)	135.582 ± 33.539	127.957 ± 35.479	.001
TC/HDL-C	4.2 ± 1.3	4.5 ± 1.4	.002
LDL-C/HDL-C	2.9 ± 0.9	3.1 ± 1.0	.60
NonHDL-C	146.3 ± 36.2	145.1 ± 40.1	.616
NonHDL-C/HDL	3.2 ± 1.3	3.5 ± 1.4	.002
TG (mg/dL)	151.5 ± 74.9	184.36 ± 93.4	2.5×10^{-4}
TG/HDL-C	3.6 ± 2.8	4.7 ± 3.4	4.5×10^{-4}
Systolic blood pressure (mmHg)	118.0 ± 13.7	123.7 ± 16.0	.002
Diastolic blood pressure (mmHg)	73.2 ± 9.2	82.3 ± 9.6	4.2×10^{-13}
Glucose (mg/dL)	92.2 ± 26.8	148.6 ± 16.0	6.4×10^{-28}
With metabolic syndrome n (%)	55 (8.4)	336 (61.5)	1.2×10^{-36}
Variant rs2000999 (A) (%)	18.8	19.9	.858
African (%)	3.5 ± 2.3	3.4 ± 2.4	.308
European (%)	32.8 ± 17.9	32.2 ± 17.0	.628
Amerindian (%)	63.7 ± 19.3	64.3 ± 18.2	.673

Analysis by Student *t* and Chi-Squared.

Data are represented as n (%) and mean ± standard deviation. BMI = body mass index, HDL-C = high density lipoprotein cholesterol, LDL-C/HDL-C = ratio of LDL-C and HDL-C, LDL-C = low density lipoprotein cholesterol, NonHDL-C/HDL = ratio of nonHDL-C and HDL-C, T2D = type 2 diabetes, TC/HDL-C = C/HDL = ratio of TC and HDL-C, TC = total cholesterol, TG/HDL-C = ratio of TG and HDL-C, TG = triglycerides.

individuals, the T2D group exhibited significantly higher frequency of women, age, waist circumference, BMI, TG, ratios of TC/HDL-C, NonHDL-C/HDL and TG/HDL-C, blood pressure, glucose, and frequency of current smokers and individuals with metabolic syndrome. However, the non-diabetic individuals showed significantly higher levels of TC, HDL-C and LDL-C than the T2D group. LDL-C/HDL-C ratio and NonHDL-C did not display significant difference between the non-diabetic and T2D groups. Similarly, the non-diabetic and T2D groups had similar frequencies of the rs2000999 A. The polymorphism was in Hardy–Weinberg equilibrium in both groups ($P_{NoT2D} = .530$ and $P_{T2D} = .991$).

3.2. Association of rs2000999 A allele with individual ancestry proportions

The rs2000999 A allele had a strong positive association with the percentage of European ancestry (β ± SE [standard error] = 0.028 ± 0.011, $P = .013$) and to a lesser extent African ancestry (β ± SE = 0.003 ± 0.002, $P = .041$). On the other hand, the percentage of Native-American ancestry showed a strong negative association with the A allele of rs2000999 of *HP* (β ± SE = −0.031 ± 0.012, $P = .012$).

3.3. Association of rs2000999 with serum lipids

We tested the association of the variant rs2000999 with serum lipids under different genetic models, using the A allele as the effect allele. Under the codominant model (AA vs AG vs GG) the rs200099 A allele was associated with higher TC and LDL-C levels in the No T2D group (TC: β ± SE = 9.102 ± 3.677, $P = .014$; LDL-C: β ± SE = 6.425 ± 3.144, $P = .042$) and the T2D group (TC: β ± SE = 8.918 ± 3.955, $P = .025$; LDL-C: β ± SE = 8.222 ± 3.240, $P = .012$) (Table 2). Under the recessive model (AA vs AG + GG), AA homozygotes are significantly associated with higher TC and LDL-C levels in the No T2D group (TC: β ± SE = 10.102 ± 4.038, $P = .013$; LDL-C: β ± SE = 7.287 ± 3.453, $P = .035$) and T2D group (TC: β ± SE = 10.196 ± 5.555, $P = .026$; LDL-C: β ± SE = 8.662 ± 3.736, $P = .021$) (Table 2). For HDL-C, there are also significant associations under the codominant and recessive models in the T2D group (codominant: β ± SE = 2.402 ± 1.126, $P = .034$; recessive: β ± SE = 3.283 ± 1.295, $P = .012$), but not in the non-diabetic group (Table 2). Under the dominant model, rs20009999 was not significantly associated with lipid levels, although we saw similar trends indicating higher lipid levels in carriers of the A allele, particularly for TC and LDL-C (Table 2).

4. Discussion

This is the first study to describe an association of the rs2000999 variant of *HP* with serum lipids in a Latin American diabetic population. The allelic frequency of the variant was quite similar in the No T2D and T2D groups. In this work, we show that the percentage of Native-American ancestry is negatively associated

Table 2

Association of variant rs2000999 of *haptoglobin* gene with serum lipids in 654 non diabetic and 546 diabetic Mexican adults.

T2D	AA/AG/GG	*P*	AA/AG+GG	*P*	AA+AG/GG	*P*
TC (mg/dL)						
NO	9.102 ± 3.677	.014	10.102 ± 4.038	.013	10.680 ± 14.162	.451
YES	8.918 ± 3.955	.025	10.196 ± 5.555	.026	12.339 ± 12.580	.327
HDL (mg/dL)						
NO	1.279 ± 1.160	.271	1.156 ± 1.275	.325	3.494 ± 4.444	.432
YES	2.402 ± 1.126	.034	3.283 ± 1.295	.012	-0.738 ± 3.585	.837
LDL (mg/dL)						
NO	6.425 ± 3.144	.042	7.287 ± 3.453	.035	5.650 ± 12.093	.641
YES	8.222 ± 3.240	.012	8.662 ± 3.736	.021	16.961 ± 10.301	.100

Analysis by linear regression adjusted for age, sex, obesity diagnosis, smoke status and individual ancestry proportions.

Data as β ± SE. HDL-C = high density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol, SE = standard error, T2D = Type 2 diabetes, TC = total cholesterol.

with the rs2000999 A allele. In contrast, the rs2000999 A allele has a strong positive association with European ancestry, and to a lesser extent, with African ancestry (it is important to note that the average African ancestry in the Mexican sample is quite low, around 3.5%). Our results are quite consistent with data from different world populations. In the allele frequency database ALFRED (https://alfred.med.yale.edu/alfred/index.asp), rs2000999 A allele frequencies in Southern European populations are higher than 20%, and in Native American groups from Mexico (Pima, Maya) do not surpass 10%. The frequencies of the A allele in African populations range from 0% to 12.8%. Given that the rs2000999 A allele is present almost exclusively on Hp2 haplotypes,[4] our results indicate that that frequency of the Hp2 allele is lower in the Native American ancestral population than in the European or African ancestral populations. This is consistent with studies that have reported that the frequencies of the Hp1 allele are quite high in some Native American groups, including indigenous groups from Mexico.[16,17] Further studies are needed to describe in detail the linkage disequilibrium patterns between rs200099 and the Hp1 and Hp2 alleles in different population groups, including indigenous groups from the Americas.

We observed that under codominant and recessive models, rs2000999 is associated with TC and LDL-C levels in both the No T2D and T2D groups. Interestingly, under codominant and recessive models, rs2000999 was also significantly associated with HDL-C levels in the T2D group, but not in the non-diabetic group. Our results indicate that carriers of the A allele have higher lipid concentrations than non-carriers, although dominant models did not reach significance. These findings are in agreement with previous studies showing significant positive associations of the A allele with lipid concentrations in other population groups.[20,24] Given the associations reported between the rs2000999 A allele and the Hp2 allele, our results are also consistent with previous studies that have reported that Hp2 is associated with 5-times higher risk of developing cardiovascular diseases, when compared to the Hp1 allele, in the T2D population.[25]

The present study also has some limitations. Patients with T2D included in the study are undergoing a healthy eating program and physical activity. These interventions may improve HDL-C levels of the T2D group. The sample size is relatively small, and we did not determine the Hp1 and Hp2 alleles of *HP*, so we could not evaluate the extent to which the effect of rs2000999 and Hp1/Hp2 on lipid levels is independent or not. Of note, a recent meta-analysis have indicated that the effect of rs2000999 on Haptoglobin levels is independent from the effect of the Hp1/Hp2 alleles.[26] This suggests that these 2 polymorphisms may have independent effects on lipid levels. Our results suggest that rs2000999 could be important in evaluating the genetic predisposition to high levels of lipids in patients with T2D and people without T2D. However, as future directions, longitudinal studies in Latin American population, through the evaluation of the response of carriers of rs2000999 A allele to pharmacological intervention, could offer evidence to improve the treatment lipids and the programs to prevent or delay the onset of cardiovascular diseases.

In conclusion, our results evidence that in the Mexican population, the rs2000999 A allele is positively associated with the percentage of European (and to a lesser extent African ancestry), and negatively associated with Native American ancestry. We also show that carriers of the A allele have increased levels of TC and LDL-C, independently of T2D diagnosis, and also increased concentrations of HDL-C in the T2D sample, but not in the non-diabetic group.

Acknowledgments

We thank all the individuals who volunteered to participate in the study.

Author contributions

Conceptualization: Adan Valladares-Salgado.
Data curation: José J Peralta-Romero.
Formal analysis: Fernando Suarez-Sanchez¥.
Funding acquisition: Adan Valladares-Salgado.
Methodology: Miguel Vazquez-Moreno¥, Ema Herrera-Lopez, Jaime H Gomez-Zamudio, José J Peralta-Romero, Osvaldo D Castelan-Martinez.
Project administration: Adan Valladares-Salgado.
Supervision: Osvaldo D Castelan-Martinez, Miguel Cruz, Esteban J Parra, Adan Valladares-Salgado.
Writing – original draft: Fernando Suarez-Sanchez¥, Miguel Vazquez-Moreno¥, Ema Herrera-Lopez.
Writing – review & editing: Jaime H Gomez-Zamudio, Miguel Cruz, Esteban J Parra, Adan Valladares-Salgado.

References

[1] INEGI. Dirección General de Estadísticas Sociodemográficas. Instituto Nacional de Estadística y Geografía de México. 2018. Accessed July 23, 2018.
[2] Below JE, Parra EJ, Gamazon ER, et al. Meta-analysis of lipid-traits in Hispanics identifies novel loci, population-specific effects, and tissue-specific enrichment of eQTLs. Sci Rep 2016;6:19429.
[3] Graff M, Emery LS, Justice AE, et al. Genetic architecture of lipid traits in the Hispanic community health study/study of Latinos. Lipids Health Dis 2017;16:200.
[4] Boettger LM, Salem RM, Handsaker RE, et al. Recurring exon deletions in the HP (haptoglobin) gene contribute to lower blood cholesterol levels. Nat Genet 2016;48:359–66.
[5] Zheng NS, Bastarache LA, Bastarache JA, et al. A common deletion in the haptoglobin gene associated with blood cholesterol levels among Chinese women. J Hum Genet 2017;62:911–4.
[6] MacKellar M, Vigerust DJ. Role of haptoglobin in health and disease: a focus on diabetes. Clin Diabetes 2016;34:148–57.
[7] Nielsen MJ, Moestrup SK. Receptor targeting of hemoglobin mediated by the haptoglobins: roles beyond heme scavenging. Blood 2009;114:764–71.
[8] Braeckman L, De Bacquer D, Delanghe J, et al. Associations between haptoglobin polymorphism, lipids, lipoproteins and inflammatory variables. Atherosclerosis 1999;143:383–8.
[9] Balestrieri M, Cigliano L, Simone ML, et al. Haptoglobin inhibits lecithin-cholesterol acyltransferase in human ovarian follicular fluid. Mol Reprod Dev 2001;59:186–91.
[10] Salvatore A, Cigliano L, Carlucci A, et al. Haptoglobin binds apolipoprotein E and influences cholesterol esterification in the cerebrospinal fluid. J Neurochem 2009;110:255–63.
[11] 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.
[12] Su YC, Chen YC, Li SC, et al. Detection of Hpdel in healthy individuals and cancer patients in Taiwan. Clin Chem Lab Med 2009; 47:745–9.
[13] Park KU, Song J, Kim JQ. Haptoglobin genotypic distribution (including Hp0 allele) and associated serum haptoglobin concentrations in Koreans. J Clin Pathol 2004;57:1094–5.
[14] Shimada E, Odagiri M, Chaiwong K, et al. Detection of Hpdel among Thais, a deleted allele of the haptoglobin gene that causes congenital haptoglobin deficiency. Transfusion 2007;47:2315–21.

[15] Wu J, Province MA, Coon H, et al. An investigation of the effects of lipid-lowering medications: genome-wide linkage analysis of lipids in the HyperGEN study. BMC Genet 2007;8:60.
[16] Wobeto VPdA, Zaccariotto TR, Sonati MdF. Polymorphism of human haptoglobin and its clinical importance. Genet Molec Biol 2008;31: 602–20.
[17] Hardwick RJ, Menard A, Sironi M, et al. Haptoglobin (HP) and Haptoglobin-related protein (HPR) copy number variation, natural selection, and trypanosomiasis. Hum Genet 2014;133:69–83.
[18] Levy AP, Roguin A, Hochberg I, et al. Haptoglobin phenotype and vascular complications in patients with diabetes. N Engl J Med 2000;343:969–70.
[19] Levy AP, Hochberg I, Jablonski K, et al. Haptoglobin phenotype is an independent risk factor for cardiovascular disease in individuals with diabetes: the strong heart study. J Am Coll Cardiol 2002;40:1984–90.
[20] Wang S, Zhang R, Wang T, et al. Association of the genetic variant rs2000999 with haptoglobin and diabetic macrovascular diseases in Chinese patients with type 2 diabetes. J Diabetes Complications 2019;33: 178–81.
[21] American Diabetes A. Standards of medical care in diabetes–2012. Diabetes Care 2012;35(Suppl 1):S11–63.
[22] Grundy SM, Cleeman JI, Daniels SR, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 2005;112:2735–52.
[23] McKeigue PM, Carpenter JR, Parra EJ, et al. Estimation of admixture and detection of linkage in admixed populations by a Bayesian approach: application to African-American populations. Ann Hum Genet 2000;64 (Pt 2):171–86.
[24] Froguel P, Ndiaye NC, Bonnefond A, et al. A genome-wide association study identifies rs2000999 as a strong genetic determinant of circulating haptoglobin levels. PLoS One 2012;7:e32327.
[25] Lioupis C, Barbatis C, Drougou A, et al. Association of haptoglobin genotype and common cardiovascular risk factors with the amount of iron in atherosclerotic carotid plaques. Atherosclerosis 2011;216: 131–8.
[26] Kazmi N, Koda Y, Ndiaye NC, et al. Genetic determinants of circulating haptoglobin concentration. Clin Chim Acta 2019;494:138–42.