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The haptoglobin promoter polymorphism rs5471 is the most definitive genetic determinant of serum haptoglobin level in a Ghanaian population

Mikiko Soejima, Kwesi Teye[1], Yoshiro Koda*

Department of Forensic Medicine, Kurume University School of Medicine, Kurume 830-0011, Japan

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ABSTRACT

Background: The serum haptoglobin (HP) level varies in various clinical conditions and among individuals. Recently, the common HP alleles, rs5472, and rs2000999 have been reported to associate with serum HP level, but no studies have been done on Africans. Here, we explored the relationship of not only these polymorphisms but also rs5470 and rs5471 to the serum HP level in 121 Ghanaians.

Methods: Genotyping of rs2000999 was performed by PCR using hydrolysis probes, while the other polymorphisms have been already genotyped. Serum HP level was measured by a sandwich ELISA.

Results: We observed a significant association between rs5471 and the serum HP level ($p = 0.026$). It was also observed within the subgroups of HP^2/HP^2 and HP^2/HP^1. In addition, we detected a trend toward lower HP levels for individuals with the A allele of rs2000999 than those without A, but it was not statistically significant ($p = 0.156$). However, we did not observe the clear associations between other polymorphisms and serum HP level that were observed for Europeans and Asians because of the small sample size and the complexity of SNPs affecting the HP level.

Conclusions: We suggest that rs5471 is a strong genetic determinant of HP levels in Ghanaians, and this seems to be characteristic of Africans. Further investigation using large scale samples will help in understanding the genetic background of individual variability of the serum HP level.

1. Introduction

Haptoglobin (HP) is an acute-phase protein that functions to capture free hemoglobin and mediate its clearance from the circulation to prevent oxidative damage [1]. The three major subtypes, HP1-1, HP2-1, and HP2-2, are products of two co-dominant "common alleles", HP^1 and HP^2, while their frequencies vary among worldwide populations with HP^1 frequencies from 0.07 in parts of India to over 0.7 in parts of West Africa and South America [1]. HP^1 and HP^2 are an intragenic copy number variation (CNV) of a 1.7 kb segment encompassing two exons. HP^1 and HP^2 consist of 5 and 7 exons, respectively. A recent study suggested that modern HP^1 alleles have been generated through recurring deletions of HP^2 [2]. The major HP subtypes have been reported to be associated with many diseases. Such associations may be due to functional differences between HP1 and HP2 in the binding of hemoglobin and its rate of clearance from serum [1].

The serum level of HP reflects various clinical states. It increases rapidly and dramatically in the presence of acute inflammatory conditions such as infectious diseases, malignancy, autoimmune disease, and tissue necrosis. On the other hand, levels decrease during hemolysis, ineffective erythropoiesis, liver disease, and late pregnancy [1,3].

In addition to clinical condition-dependent variations, inter-individual variability of the circulating HP level was also reported. Sex, age, smoking, and plasma hemoglobin levels were shown to be associated with the HP concentrations in plasma [4]. Additionally, several genetic polymorphisms have been reported to affect the HP serum level so far. First, serum HP concentrations are dependent on the three common HP subtypes, although they seem to differ among different populations [1]. In addition, a haptoglobin gene deletion allele (HP^{del}) that we characterized as a causal mutation of anhaptoglobinemia also affects the concentration in heterozygotes especially in HP^2/HP^{del} [5,6]. HP^{del}, which lacks an approximately 28-kb segment that extends from the promoter region to exon 5 of the haptoglobin-related gene (HPR), is found only in East and Southeast Asian populations with a frequency of 0.009–0.040 [7–12]. And rs2000999, which resides in intron 2 of the neighboring HPR, was identified as a strong genetic determinant of the serum HP level in Europeans [4,13] and Asians [6,12]. In a public database (http://www.ncbi.nlm.nih.gov/projects/

* Corresponding author.
E-mail address: ykoda@med.kurume-u.ac.jp (Y. Koda).
[1] Current address: Kurume University Institute of Cutaneous Cell Biology, Fukuoka, Japan.

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SNP), this SNP is distributed unequally throughout the global population with a minor allele frequency (MAF) of 0.034–0.476. In addition, Bjornsson et al. recently identified a splice donor mutation at the first base of intron 3 in an Icelandic population at a frequency of 0.0056 [14]. They also showed the status of rs35283911, a 1-bp deletion located 9 kb upstream of HP, which correlated highly with that of rs2000999. These two SNPs were shown to be associated with the serum HP level [14].

Proximal promoter SNPs have also been reported to be associated with serum HP levels. A base substitution at position −61 from A to C (rs5471) was identified as a causal polymorphism of HP 2–1 modified phenotypes due to a decreased amount of HP2 polypeptide relative to that of HP1 [15]. The prevalence of the C allele of rs5471 seems to be restricted to Africans (0.100–0.142) and Hispanics (0.02) (http://www.ncbi.nlm.nih.gov/projects/SNP/). In addition, a promoter polymorphism at position −55 (rs5472) was one of the determinants of the serum HP level, and it was found to be in almost absolute linkage disequilibrium with rs2000999 in a Japanese population, while the linkage is weaker in Europeans [6]. The frequency of the G allele of rs5472 seems to be relatively high (0.15–0.52) in many populations (http://www.ncbi.nlm.nih.gov/projects/SNP/). The gene structures of HP^1, HP^2, and the relative positions of SNPs that are targets in this study are indicated in Fig. 1.

As observed above, recent studies have revealed that the genetic background affects the inter-individual variability of the circulating HP level. However, an association study in an African population had not been done yet. Previously, we determined the HP phenotype of the same Ghanaian samples used in the present study by polyacrylamide gel electrophoresis (PAGE) and subsequent staining and then performed double immunodiffusion and Western blotting for samples negative for HP in PAGE [16]. Because there is no definition of "hypohaptoglobinemia", we categorized the samples that were negative for HP in PAGE and positive in immunodiffusion and Western blotting as "hypohaptoglobinemia" and negative for HP in all three experiments in the previous study [16] as "anhaptoglobinemia". In addition, we determined common HP alleles, HP1F, 1S polymorphism, and sequence variation, in the promoter region and found that −61A > C (rs5471) and −101C > G (rs5470) were overrepresented in hypohaptoglobinemia and anhaptoglobinemia, respectively [16].

In this study, we determined the concentration of HP and defined an HP level < 5 mg/dl as a "sample with quite low HP" because the detection limit of nephelometry is 5 mg/dl of HP. We then evaluated the correlation between HP common alleles, rs2000999, and three promoter SNPs, rs5470, rs5471, and rs5472, and the serum HP level in a Ghanaian population. Further, the ratio of samples with quite low HP was also compared among genotypes.

2. Materials and methods

This study protocol was approved by the ethics committee of Kurume University School of Medicine.

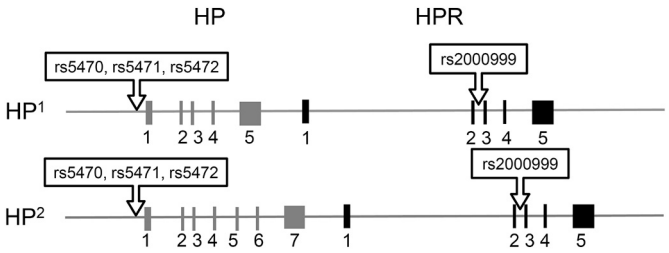

Fig. 1. Structures of HP^1 and HP^2. The exons of HP and haptoglobin-related gene (HRP) are indicated by grey and black boxes, respectively. Relative positions of rs5470, rs5471, rs5472, and rs2000999 are also indicated.

2.1. Blood and DNA samples

Genomic DNA from 121 randomly selected Ghanaians was isolated as described in a previous study [16].

2.2. Genotyping of HP

TaqMan-based real-time PCR was performed to determine genotypes of rs2000999 using methods described previously [6]. Briefly, the PCR procedure was carried out in a 96-well plate with 10 µl volume composed of 5 µl of universal probe master (FastStart, Roche Diagnostics, Tokyo, Japan), 0.08 µl probe-primer mix, and approximately 5–30 ng of DNA. PCR conditions included 95 °C for 10 min, followed by 45 cycles of 95 °C for 15 s and 60 °C for 45 s. To monitor the progress of amplification, we measured the fluorescence at the end of each cycle, and data were collected and analyzed using a LightCycler 480 instrument II (Roche Diagnostics). Allele and genotype frequencies were calculated by the counting method, and Hardy-Weinberg equilibrium (HWE) was tested using an online HWE calculator (http://www.oege.org/software/hwe-mr-calc.shtml).

2.3. Quantification of HP in sera

The level of HP was determined by the sandwich ELISA described in a previous study [6]. ELISA was performed in triplicate with 100-fold, 5000-fold, and 20,000-fold diluted serum samples. The detection limit of this system is 0.05 mg/dl.

2.4. Statistical analyses

To reveal an association between HP polymorphisms and the serum HP level, samples were classified into groups according to genotypes of HP common alleles, rs5470, ars5471, rs5472, and rs2000999. In addition, samples were classified into the genotype of each SNP within the groups of HP^2/HP^2, HP^2/HP^1 and HP^1/HP^1 to evaluate an association between each SNP and HP level. Serum HP levels (mg/dl) are shown by median and the first and third quartiles. Normality of distribution was tested by the chi-square fitness test. Mann-Whitney and Kruskal-Wallis tests were employed for comparison of two and three independent groups of data, respectively. Fisher's exact test was performed to compare the ratio of samples with quite low HP between or among genotypes by using VassarStats: Website for Statistical Computation (http://vassarstats.net/index.html). Other statistical analyses were performed using Statcel4 add-in software in Excel (OMS Publishing Inc., Saitama, Japan). $P < 0.05$ was considered statistically significant.

3. Results

We analyzed the association between HP common alleles, rs2000999, and three promoter SNPs, rs5470, rs5471, rs5472, and the serum HP level in 121 Ghanaian samples. No deviations from HWE were detected for HP common alleles, rs5471, rs5472, and rs2000999 in this population except for rs5470 ($p = 0.049$).

3.1. Association between HP common alleles and serum HP level

First, the samples were divided into three groups according to the genotypes of HP common alleles. As shown in Table 1, the medians were similar among the three genotypes. On the other hand, the ratio of samples with quite low HP (< 5 mg/dl) was significantly different among the three groups. The ratio increased in proportion to the number of HP^2 alleles (follows the additive model, $p = 0.012$).

3.2. Association between rs5470 and serum HP level

Next, we focused on rs5470, which we previously suggested to be