

LETTER TO THE EDITOR

Haptoglobin genotypic distribution (including Hp⁰ allele) and associated serum haptoglobin concentrations in Koreans

K U Park, J Song, J Q Kim

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Background: Haptoglobin polymorphism is associated with the prevalence of infections, autoimmune diseases, cardiovascular diseases, and other disorders. Congenital haptoglobin deficiency is associated with anaphylactic transfusion reactions in anaphylactoid patients with antihaptoglobin antibody.

Aims: To investigate haptoglobin genotypic distribution (including the Hp⁰ allele) and associated serum haptoglobin concentrations in Koreans.

Methods: Five hundred and nine healthy Korean adults were randomly selected. Two methods were used: haptoglobin genotyping based on a polymerase chain reaction (PCR) system that exploited the structural difference of the Hp¹ and Hp² alleles, and another PCR method that detected haptoglobin gene deletion by amplification of the junctional region of the Hp⁰ allele. Serum haptoglobin concentrations were measured by nephelometry.

Results: The haptoglobin genotypes of 509 subjects were as follows: Hp¹Hp¹, 7.1%; Hp²Hp¹, 37.7%; Hp²Hp², 49.3%; Hp⁰Hp¹, 2.2%; Hp⁰Hp², 3.5%; Hp⁰Hp⁰, 0.2%. The gene frequency of Hp⁰ in Koreans was calculated to be 0.031. Significant differences were seen among the concentrations of each haptoglobin genotype (Kruskal-Wallis test). Hp⁰Hp², but not Hp⁰Hp¹, was associated with hypohaptoglobinaemia.

Conclusions: PCR methods for differentiating between haptoglobin genotypes, including the Hp⁰ allele, may be useful in a broad spectrum of basic studies and clinical examinations.

Haptoglobin is genetically determined by two autosomal codominant alleles, Hp¹ and Hp². Recently, the Hp⁰ allele, which is an allelic deletion in the haptoglobin gene cluster, has been identified.¹ Because haptoglobin polymorphism has an effect on a broad range of diseases, a rapid and practical method for the distinction between haptoglobin variants is needed for large scale routine laboratory use. In our present study, we have adopted a haptoglobin genotyping method based on the polymerase chain reaction (PCR) and a simple method to detect haptoglobin deletion by PCR. Using these methods, we investigated haptoglobin genotypic distribution (including the Hp⁰ allele) and associated serum haptoglobin concentrations in Koreans.

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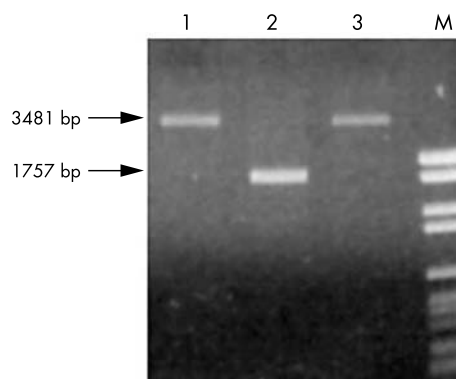


Figure 1 Determination of Hp¹ and Hp² alleles with primers A and B, respectively. In a polymerase chain reaction with primers A and B, alleles Hp¹ and Hp² were characterised by amplification bands of 1757 and 3481 bp, respectively. Lanes 1 and 3, DNA from an individual with the Hp²Hp² genotype; lane 2, DNA from an individual with the Hp¹Hp¹ genotype; lane M, DNA size marker.

MATERIALS AND METHODS

Healthy Korean adults (509 subjects) were randomly selected. Primers A and B were used for amplification of the Hp¹ and Hp² specific sequences, respectively, and primers C and D were used to amplify the Hp² specific sequence. Primers Del-U and Del-L were used to amplify the Hp⁰ allele, and exon 1 of the haptoglobin gene was coamplified in the same tube, as an amplification control. The primers and the amplification protocols have been described previously.^{1–3} PCR products underwent electrophoresis in a 1.8% agarose gel. The serum haptoglobin concentration was measured by nephelometry.

RESULTS

Figures 1–3 show representative electrophoresis patterns. The haptoglobin genotypes of 509 subjects were as follows: Hp¹Hp¹, 7.1%; Hp²Hp¹, 37.7%; Hp²Hp², 49.3%; Hp⁰Hp¹, 2.2%; Hp⁰Hp², 3.5%; Hp⁰Hp⁰, 0.2%. The gene frequency of Hp⁰ in Koreans was calculated to be 0.031, according to the Hardy-Weinberg law. Table 1 shows the serum haptoglobin concentrations. Significant differences were seen among the concentrations of each haptoglobin genotype. In addition, Hp⁰Hp², but not Hp⁰Hp¹, was shown to be associated with hypohaptoglobinaemia.

Abbreviations: Hp, haptoglobin; PCR, polymerase chain reaction

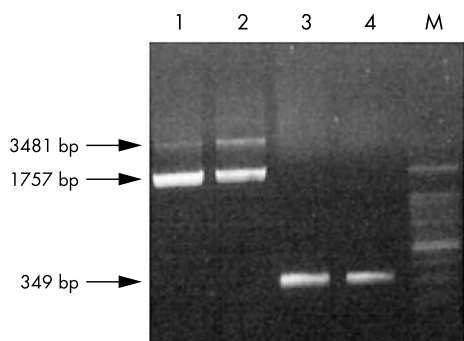


Figure 2 Determination of Hp¹ and Hp² alleles with primers C and D, respectively. With primers A and B, it was not possible to determine conclusively whether the 3481 bp band was also present when the 1757 bp band was detected (lanes 1 and 2, DNA from an individual with the Hp²Hp¹ genotype). Therefore, polymerase chain reaction (PCR) analysis using primers C and D was also performed for the complete genotyping. In a PCR with primers C and D, a 349 bp product was generated from genomic DNA of individuals homozygous or heterozygous for the Hp² allele, whereas no product was formed in the presence of the Hp¹ allele (lanes 3 and 4, DNA from the individual with the Hp²Hp¹ genotype).

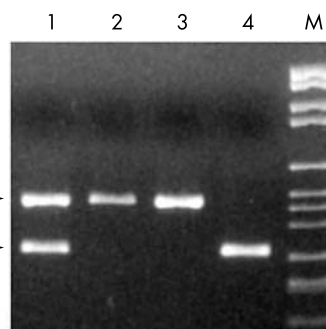


Figure 3 Detection of haptoglobin gene deletion (Hp⁰ allele). In a polymerase chain reaction for the detection of the Hp⁰ allele and the Hp allele (exon 1), both 315 bp and 476 bp bands were amplified from individuals heterozygous for the Hp⁰ allele. Only a 476 bp band was amplified from genomic DNA of a control individual, whereas only a 315 bp band was amplified from an individual homozygous for the Hp⁰ allele. Lane 1, heterozygote for Hp⁰ allele; lanes 2 and 3, control individuals; lane 4, homozygote for Hp⁰ allele; lane M, DNA size marker.

Table 1 Serum haptoglobin concentrations according to haptoglobin genotype (including the Hp⁰ allele) in Koreans

Genotype	Number	Mean (SD) (g/l)
Hp ¹ Hp ¹	26	1.216 (0.418)
Hp ² Hp ¹	168	1.189 (0.521)
Hp ² Hp ²	211	0.829 (0.509)
Hp ⁰ Hp ¹	8	0.426 (0.200)
Hp ⁰ Hp ²	16	0.178 (0.119)
Hp ⁰ Hp ⁰	1	0.001
	430	p=0.000

The p value was obtained using the Kruskal-Wallis test among the six haptoglobin genotypes.

DISCUSSION

Using PCR analysis with primers A and B, the heterozygous genotype Hp²Hp¹ could not easily be detected because, in the presence of the 1757 bp band, it was not possible to determine conclusively whether the Hp² specific 3481 bp band was also present. With the Hp²Hp¹ genotype, the 1757 bp band was considerably more intense than the 3481 bp band (fig 2; lane 1 and 2). Such a problem can occur in cases in which the PCR is run under suboptimal conditions, with extensively degraded DNA, or with limited quantities of DNA.² Therefore, PCR using primers C and D was also performed for the complete genotyping of the common haptoglobin polymorphism.

Haptoglobin genotype frequencies and their associated serum haptoglobin concentrations were similar to the haptoglobin phenotyping results in Koreans using sodium dodecyl sulfate–polyacrylamide gel electrophoresis.⁴ However, electrophoresis phenotyping could not discriminate between hypohaptoglobinaemia and true anhaptoglobinaemia. In our study, Hp⁰Hp², but not Hp⁰Hp¹, was shown to be associated with hypohaptoglobinaemia. These results signify a gene–dosage effect, similar to that reported by Koda *et al.*¹

In conclusion, PCR methods for differentiating between haptoglobin genotypes, including the Hp⁰ allele, may be useful in a broad spectrum of basic studies and clinical examinations.

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