Rapid Real-Time PCR Detection of HP<sup>del</sup> Directly from Diluted Blood Samples

To the Editor:

Anhaptoglobinemic patients have been reported to experience severe anaphylactic reactions to transfusions due to the production of anti-haptoglobin (anti-HP) antibodies (1, 2). Anhaptoglobinemia in patients homozygous for HP<sup>del</sup> which is a deletion of an approximately 28-kb segment of chromosone 16 extending from the promoter region of the HP (haptoglobin) gene to exon 5 of HPR (haptoglobin-related protein), has been adequately characterized only recently (1). Use of a simple duplex PCR method has detected the HP<sup>del</sup> allele in East and Southeast Asian populations at frequencies of 1%–3% but this allele has not been detected in African, West and South Asian, and European populations (1, 3, 4). Thus, diagnosing HP<sup>del</sup> homozygosity prior to blood transfusion or the infusion of blood components into individuals from East and Southeast Asian populations is effective for preventing anaphylactoid shock due to anti-HP antibodies. We have developed a simple method that uses a 5’ nuclease real-time PCR assay (TaqMan; Applied Biosystems) to detect the HP<sup>del</sup> allele without having to isolate genomic DNA. The ethics committee of Kurume University School of Medicine approved this study protocol.

To distinguish alleles, we performed real-time PCR assays that detect the 2 regions that encompass the HP<sup>del</sup> breakpoint and the 5’ region of HP exon 1, which is deleted in HP<sup>del</sup>. The 20-μL PCR reaction contained 200 μmol/L deoxyribonucleoside triphosphates, 1 μL of template (diluted blood or genomic DNA), 0.5 U of Ex TaqHS with its buffer (Takara), and the following primers and TaqMan probes (see Fig. 1 legend for sequences): Hp5–’-F and -R primers (450 nmol/L); Hp5–’-TaqMan probe (125 nmol/L) for detecting the 5’ region of HP; Hpdel-F and -R primers (900 nmol/L); and Hpdel–TaqMan probe (250 nmol/L) for detecting HP<sup>del</sup>. The PCR temperature profile was 95°C for 30 s, followed by 50 cycles of denaturing at 95°C for 5 s and annealing and extension at 60°C for 30 s. All oligonucleotides were synthesized by Biosearch Technologies. We monitored amplification progress by monitoring the fluorescence at the end of each cycle with an Mx3000P instrument (Stratagene) with excitation and emission wavelengths of 492 and 516 nm (FAM), and 585 and 610 nm (CAL Fluor Red 610).

With genomic DNA (5 ng/μL) as a template, we used dual-color scatter plots to distinguish individuals previously determined to have the HP/HP, HP/HP<sup>del</sup>, and HP<sup>del</sup>/HP<sup>del</sup> genotypes. Samples with the HP/HP genotype had little FAM fluorescence and plotted along the x-axis, HP<sup>del</sup>/HP<sup>del</sup> samples had little CAL Fluor Red 610 fluorescence and plotted along the y-axis, and HP/HP<sup>del</sup> samples were located between the homozygote samples in the plot (Fig. 1). To the TaqMan real-time PCR mixture, we directly added 1 μL of samples diluted 100-fold with PCR-grade water (previously frozen samples of buffy coat from 47 Indonesians from Surabaya or a freshly drawn blood sample from 1 Japanese individual from Fukuoka). Blood was collected in EDTA-containing tubes (Indonesian and Japanese samples) and in a heparin-containing tube (the Japanese sample). The results from 2 independent experiments showed no discrepancies. In addition, the results obtained with the present TaqMan real-time PCR method were fully concordant with those obtained with a previously described PCR method for the same individuals (i.e., 46 HP/HP individuals and 2 Indonesians with HP/HP<sup>del</sup>, Fig. 1) (1). We previously had collected blood samples from 105 Indonesian individuals from Surabaya and had not found the HP<sup>del</sup> allele in 58 of these individuals (3); however, in the present study we did find 2 HP<sup>del</sup> alleles among the remaining 47 individuals in this population sample. Thus, the HP<sup>del</sup> allele is also present in Southeast Asian popula-
A previous study demonstrated that TaqMan real-time PCR methods can distinguish alleles from samples of dried whole blood (5). In the present study, we instead diluted samples of whole blood 100-fold to decrease the concentrations of substances that could inhibit the PCR reaction or quench the fluorescence signal. This procedure is simple, but a proper assessment of its reliability requires further evaluation with many more samples. Compared with the conventional PCR approach, the present TaqMan real-time PCR assay is simple and rapid (<1.5 h). Moreover, this automated method is cost-effective and amenable to high throughput, eliminates preparation of genomic DNA and post-PCR handling, and prevents contamination via PCR product carryover. Thus, application of this method for routine clinical diagnosis prior to blood transfusions or the infusion of human blood products may prevent anaphylactoid shock caused by anti-HP antibodies. This method may also be useful for detecting $HP_{del}/HP_{del}$ homozygotes among donors of blood samples used for preparing blood products, such as albumin for anhaptoglobinemic recipients, and for large-scale screening of $HP_{del}$ in various populations.

**Fig. 1.** Real-time PCR results for distinguishing $HP_{del}$ and $HP$ alleles.

The results of a dual-color scatter plot of fluorescence signals (dR Last) from genomic DNA from 3 Japanese individuals (A, $HP/HP$; B, $HP/HP_{del}$; C, $HP_{del}/HP_{del}$) and blood samples from 47 Indonesians (D, 45 $HP/HP$ samples; E, 2 $HP/HP_{del}$ samples) and 1 Japanese individual (D, $HP/HP$). Results for no added template (F) are also indicated. We used the following primers and TaqMan probes [labeled at the 5' end with fluorescent substances and at the 3' end with Black Hole Quenchers (BHQ) (Biosearch Technologies)]: HP5'-F, 5'-CACATTACTGATTTCAGGCTGGA-3' (sequence from 513–536 bp, GenBank no. M10935); HP5'-R, 5'-CCTTTTACAGTAATTTCACCT-3' (reverse sequence from 571–596 bp, GenBank no. M10935); HP5'-TaqMan probe, 5'-CAL Fluor Red 610-AGCTTTTAAGCAATAGGGAGATGGCCACA-BHQ2–3' (sequence from 538–566 bp, GenBank no. M10935); HPdel-F, 5'-CTTTATGGCACTGGGGAACA-3' (sequence from 694–714 bp, GenBank no. AB025320); HPdel-R, 5'-AGCAAGACACTCGTGAAGTGGGAACA-3' (reverse sequence from 772–799 bp, GenBank no. AB025320); and HPdel-TaqMan probe, 5'-FAM-TGTGCAAGAGCCTTTCCAATTTTGATCA-CCTTTTCACAGTAATTTTCTCCACCT-3' (reverse sequence from 822–801 bp, GenBank no. AB025320). The $HP_{del}$ breakpoint is between base pairs 782 and 783 (GenBank accession no. AB025320).

**References**


