Review

Identification and Diagnosis of Complete Haptoglobin Gene Deletion, the Gene Responsible for Adverse Posttransfusion Reactions

Mikiko Soejima and Yoshiro Koda *

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Review

Identification and Diagnosis of Complete Haptoglobin Gene Deletion, the Gene Responsible for Adverse Posttransfusion Reactions

Mikiko Soejima and Yoshiro Koda *

Department of Forensic Medicine, Kurume University School of Medicine, Kurume 830-0011, Japan
* Correspondence: ykoda@med.kurume-u.ac.jp; Tel.: +81-942-31-7554

Abstract: Allergic reactions are the most frequent adverse effects of blood transfusion, and anaphylactic shock, although less frequent, is systemic and serious. The causes of allergic reactions to blood transfusions are largely unknown, but deficiencies in serum proteins such as haptoglobin (Hp) can lead to anaphylactic shock. A complete deletion of the haptoglobin gene (HP^{del}) was first identified in families with anomalous inheritance and then verified as a genetic variant that can cause anaphylactic shock because homozygotes of HP^{del} have an Hp deficiency. Thereby, they may produce antibodies against Hp from blood transfusions. HP^{del} is found in East and Southeast Asian populations, but not in other populations, with a frequency of approximately 0.9% to 4%. Diagnosis of Hp deficiency due to HP^{del} prior to transfusion is desirable because severe adverse reactions can be prevented by washing the red blood cells and/or platelets with saline or by administrating plasma products obtained from an Hp deficient donor pool. This review outlines the background of the identification of HP^{del} and several genetic and immunological methods developed for diagnosing Hp deficiency caused by HP^{del}.

Keywords: adverse reactions; haptoglobin deficiency; haptoglobin gene deletion; serum protein; transfusion

1. Introduction

Blood transfusion and the administration of blood products are relatively safe medical procedures, but they carry the risk of adverse reactions [1]. Therefore, investigation of the causes of transfusion adverse reactions and their prevention is an important issue for transfusion medicine.

Adverse reactions to blood transfusion are mainly classified as hemolytic adverse reactions, non-hemolytic adverse reactions, transfusion-associated graft versus host disease, and infections [2]. In Japan, the frequency of non-hemolytic adverse reactions is the highest among these, with 2,532 cases reported in a in a survey by the Japanese Red Cross Society in 2020, accounting for 96.1% of 2,634 cases of all blood transfusion adverse reactions (Haemovigilance by JRCS 2020, https://www.jrc.or.jp/mr/relate/info/pdf/Haemovigilance%20by%20JRCS%202020_JP.pdf). Among non-hemolytic adverse reactions, allergies were the most frequently reported, at 1,692 cases, accounting for approximately two-thirds of all reported adverse reactions, of which 332 cases were severe allergic reactions such as anaphylaxis, which can be life-threatening. The causes of allergic reactions to blood transfusions are largely unknown. However, the causes that have been identified include deficiencies of plasma proteins such as immunoglobulin (Ig) A, haptoglobin (Hp), and complement component 9 (C9), and patients with these deficiencies have received blood transfusions in the past, causing severe allergic reactions due to the production of antibodies to these proteins [3–9]. It is known that IgA deficiency is more common in Europe and the United States, whereas Hp deficiency is more common than IgA and C9 deficiency in Japan [10,11]. In order to prevent severe allergic reactions due to transfusions of red blood cell and platelet products, transfusions of products in which plasma and other components have been removed by washing (e.g., washed red blood cell products) have been reported to be effective [12]. In addition, when plasma products are transfused

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to patients whose allergy is identified as being caused by a plasma protein deficiency, plasma products derived from the donor of the deficient protein concerned may be required [13]. Therefore, the diagnosis of plasma protein deficiency prior to transfusion is very important for safe blood transfusion.

In this review, we will outline the background of identification of complete haptoglobin gene deletion (HP^{del}), its geographical distribution, and several diagnostic methods for congenital Hp deficiency (congenital anhaptoglobinemia) caused by HP^{del} [14,15].

2. Characteristics and Polymorphism of Haptoglobin

Hp is an acute phase serum protein synthesized primarily in the liver [16]. It binds to the highly toxic free hemoglobin (Hb) to form a stable Hp-Hb complex [17]. This complex is rapidly cleared by binding to the macrophage scavenger receptor CD163 and being digested in lysosomes to release heme, thereby preventing Hb-induced oxidative stress [18]. Because Hp is one of the positive acute phase reactants, its serum concentration increases in various clinical conditions such as infection and inflammation [19]. On the other hand, during hemolysis, the Hp concentration decreases dramatically due to the rapid clearance of the Hp-Hb complex [20]. In addition, Hp can bind apolipoprotein A1 and E, which may affect lipid metabolism through its ability to reverse transport of cholesterol from peripheral cells to the liver [21–23].

As shown in Figure 1, the haptoglobin gene (*HP*) has an extra copy called the haptoglobin-related protein gene (*HPR*). These two genes share a high degree of nucleotide sequence similarity, and *HPR* is located on chromosome 16, 2.2 kb downstream of *HP* [24]. Hp was the first serum protein found to be polymorphic, with two co-dominant alleles, HP^1 and HP^2, giving rise to three distinct common phenotypes: Hp1-1, Hp2-1, and Hp2-2 [25]. DNA sequence analysis suggested that HP^2 is generated by a 1.7 kb intragenic DNA duplication of a tandem two-exon (exons 3 and 4) segment of HP^1 (Figure 1) [24,26–28]. Hp is composed of an α chain and a β chain, Hp 1 is a tetramer of two α chains and two β chains, and Hp 2 and Hp 2-1 are multimeric proteins with more than a tetramer [16]. The α chain is encoded by exons 1 to 4 (Hp 1) or exons 1 to 6 (Hp 2), and the β chain is encoded by exon 5 (Hp 1) or exon 7 (Hp 2) [24]. It has been reported that Hp2-2 has lower antioxidant activity than Hp1-1 because Hp2-2 binds less efficiently to Hb than Hp1-1 [29]. It is also known that the serum concentration of Hp2-2 is lower than that of Hp1-1 and Hp2-1 [30–32].

In addition to common polymorphisms, two single nucleotide polymorphisms (SNPs), rs5471 and rs2000999, were reported to be associated with the blood concentration of Hp. Rs5471 (-61A>C) in the promoter region of *HP* is characteristic of African populations, and rs5471 C allele has been reported to be responsible for the Hp2-1 modified phenotype in which fewer Hp2 polypeptides are synthesized than Hp1 polypeptides [33]. Subsequently, it was reported that this variation was correlated with low serum Hp levels [34]. On the other hand, rs2000999 (G>A) in intron 2 of *HPR* was first identified as a genetic variation affecting serum cholesterol levels through a genome-wide association study [35], and the rs2000999 A allele was subsequently reported to be correlated with the low serum Hp levels [36]. The relative positions of rs5471 and rs2000999 are shown in Figure 1.

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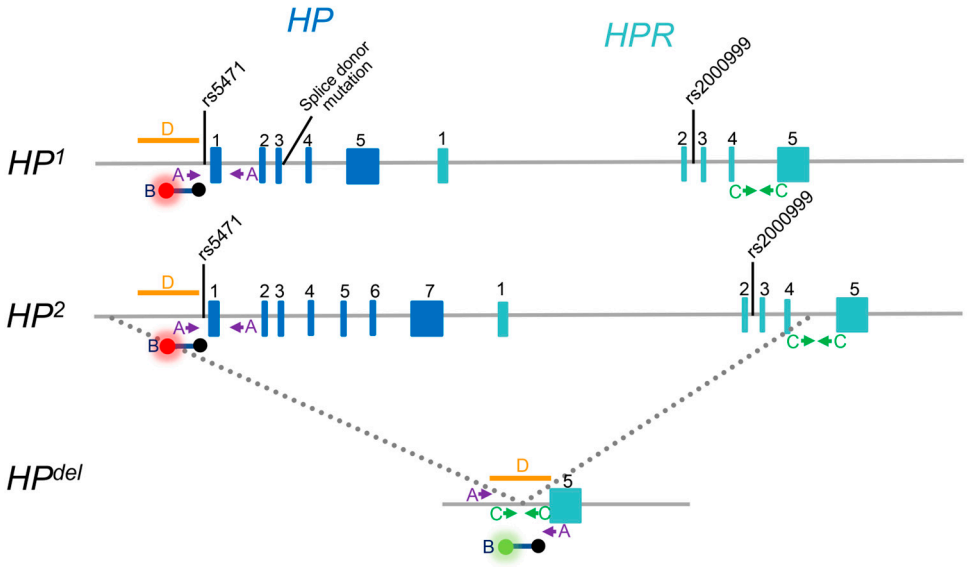

Figure 1. Gene structures of HP (*HP1*, *HP2*, and *HP*^{del}) and HPR. The exons of *HP* and *HPR* are indicated by gray and black boxes, respectively. Relative positions of rs5471, rs2000999, and splice donor mutation are indicated. In addition, approximate locations of PCR primers used for conventional PCR (A), SYBR-green I-based real-time PCR (C), and LAMP (D) and probes for TaqMan real-time PCR (B) for detection of *HP*^{del} are indicated.

3. Identification of Complete Haptoglobin Gene Deletion, *HP*^{del}

In the field of forensic medicine, Hp phenotyping by electrophoresis using starch or polyacrylamide gels was used to determine paternity until DNA testing was introduced in the late 1980s. Among the paternity test cases, there were occasionally so-called single locus exclusion cases in which the paternity was excluded based only on the Hp result (anomalous inheritance of Hp). An example is shown in Figure 2. The father has a child with Hp1, although he is determined to have phenotype of Hp2 because very faint bands equivalent to Hp2 can be seen, and this combination is not possible. The father and another child had low serum haptoglobin levels of 0.12 and 0.006 mg/ml, respectively, indicating hypohaptoglobinemia. In this way, because hypohatoglobinemic (low serum Hp) and/or anhaptoglobinemic (no serum Hp) individuals are often observed in such families, *HP*^{0}, a silent allele of Hp locus had been suggested to be responsible for such low or no serum Hp [14]. Furthermore, Yoshioka et al. screened serum Hp in 9,771 Japanese individuals and found one case of anhaptoglobinemia in which serum Hp was not detected even by the highly sensitive enzyme-linked immunosorbent assay (ELISA) method [37]. Southern blot analysis and PCR analysis of the promoter region of HP suggested that this individual was homozygous for complete deletion of the haptoglobin gene (*HP*^{del}). This deletion was estimated to extend from the upstream promoter region of *HP* to intron 4 of the *HPR* [14]. Furthermore, as shown in Figure 2, *HP*^{del} was found to be heterozygous in families with hypohaptoglobinemia, which further explained the anomalous inheritance of Hp [14]. Therefore, *HP*^{del} was first identified as an *HP*^{0} allele.

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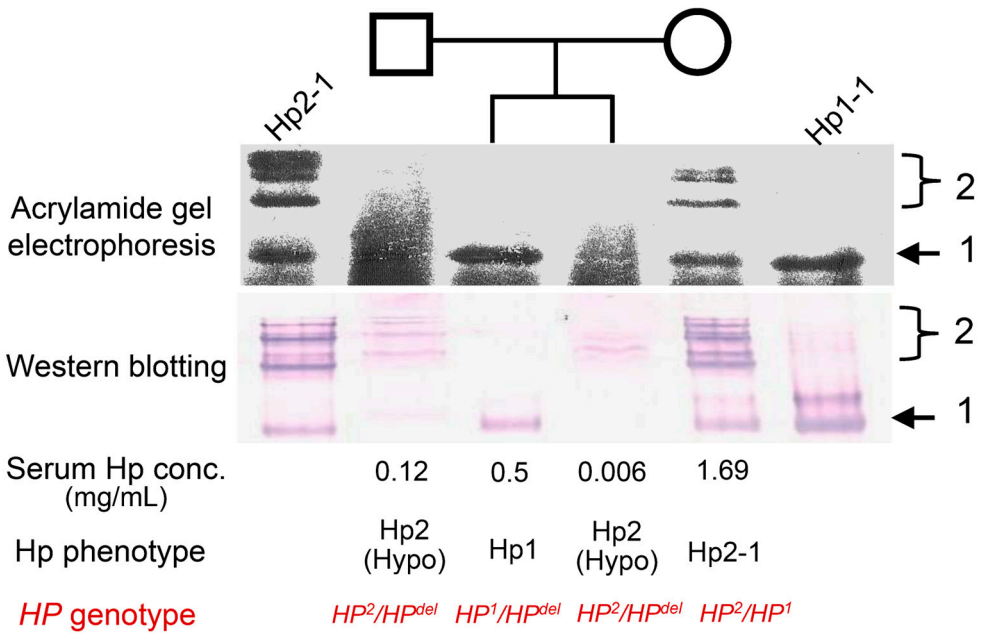

Figure 2. A family with anomalous inheritance of Hp that led to the identification of *HP*^{del}. The results obtained by acrylamide gel electrophoresis, Western blotting, serum Hp levels determined by ELISA, and resultant phenotypes are indicated. Finally, the genotypes deduced by semiquantitative Southern blotting are shown in red.

4. Anaphylactic Shock Following Blood Transfusion Due to HP Deficiency

There have been reports of patients suffering from anaphylactic shock due to the production of IgE-type anti-Hp antibodies in addition to IgG-type antibodies after blood transfusions or infusions of plasma components [9]. First, two Hp-deficient patients with anti-Hp antibodies who developed severe anaphylactic shock after infusion of blood products were reported [7,8]. One of them was a woman in her 30s who was 31 weeks into her first pregnancy. She was admitted to the hospital with threatened preterm labor due to chronic polyhydramnios. Lower limb edema with increased amniotic fluid and decreased serum albumin occurred, so a 25% albumin solution was injected. After administering a few drops, she had a severe anaphylactic reaction. The second case is a woman in her 90s with myelodysplastic syndrome. She received three transfusions of red blood cells and one transfusion of platelet concentrate over a 7-month period but had no adverse reaction. However, a month later, when she received a transfusion of platelet concentrates, she suffered an anaphylactic reaction. Both patients' symptoms improved with corticosteroid treatment. They both had anti-Hp antibodies and no Hp was detected in their serum [7,8].

Genomic DNA form B lymphocytes transformed by Epstein-Barr virus were extracted from two patients, and these two patients were found to be homozygous for *HP*^{del} by southern blot analysis [15]. However, at that time, the exact region of the deletion was not identified and the human genome had not yet been completely sequenced, so diagnosis of *HP*^{del} required laborious Southern blot analysis. Therefore, to determine the exact breakpoints of the *HP*^{del} allele, cassette-mediated PCR was performed as shown in Figure 3. Genomic DNA of an *HP*^{del} homozygote was digested with five restriction enzymes of *Alu*I, *Dra*I, *Eco*RV, *Hae*III, and *Ssp*I, and cassette DNA was ligated to both ends of the DNA fragment. Then cassette-mediated PCR (nested PCR) was performed using cassette primers and HPR exon 5 (the region present in *HP*^{del}) primers. The DNA sequence of the longest PCR product of 2.5 kb, which was obtained from the *Dra*I library, was determined to identify the breakpoints [15]. As shown in Figure 1, the 5′ breakpoint of the deletion was found to be located 5,170 bp upstream of the 5′ end of exon 1 of *HP*, and the 3′ breakpoint was located between 52 and 53 bp upstream of exon 5 of *HPR*. Therefore, the size of the deletion is estimated to be about 28 kb. The DNA sequences flanking the 5′ and 3′ breakpoints showed no significant DNA sequence similarity with the junction region of the deletion, except for two bases (TG). Identification of deletion

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breakpoints has made it possible to perform simple genetic diagnosis of *HP*^{del} using methods such as the PCR described below.

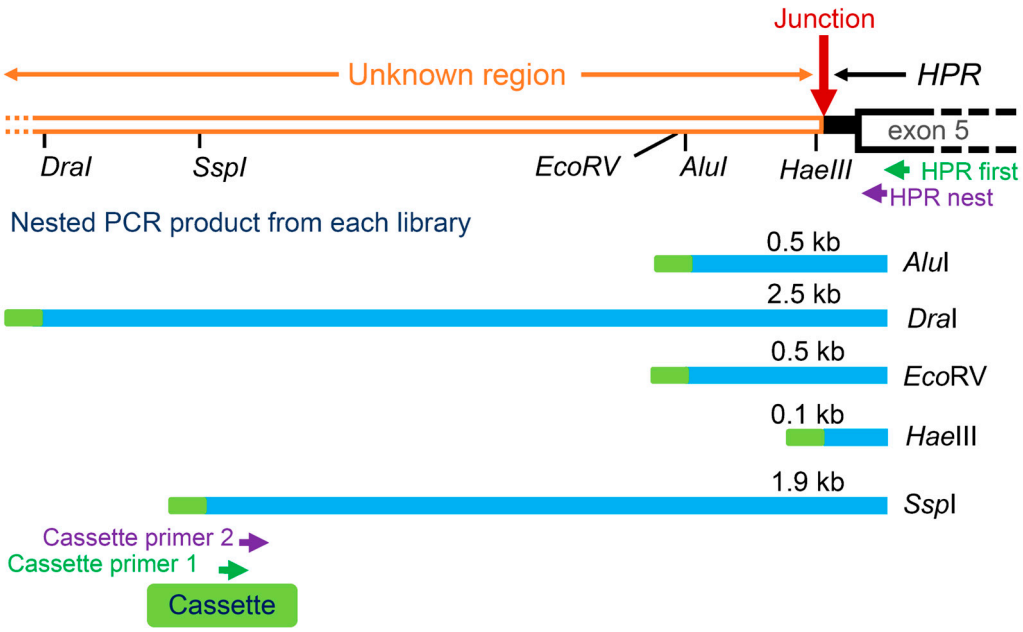

Figure 3. Strategy for cloning of deletion break points of *HP*^{del}. Restriction sites near the junction of *HP*^{del}, fragments obtained from each enzyme library, and relative positions of primers used are indicated. DNA of a homozygote of *HP*^{del} was digested with five restriction enzymes, ligated with a cassette, and libraries obtained. Nested PCR was performed using two sets of HPR-specific and cassette primers. The sequence of the longest product of 2.5 kb, obtained from the *Dra*I library, was determined.

In addition to these two cases, several cases of anaphylactic shock due to *HP*^{del} in Japanese, Korean, and Chinese patients have been reported [38–42].

5. Development of Several Methods for Genetic Diagnosis of *HP*^{del}

Several types of diagnostic methods depending on the site have been developed so far. Here we will outline those methods. Of course, genetic diagnosis of *HP*^{del} can be performed by Southern blot analysis, but it is a laborious and complicated method that is rarely performed at present, so we will not describe it here.

5.1. Conventional PCR Method for Detection of HP^{del}

PCR that spans the deletion junction region can determine the presence or absence of *HP*^{del}, but it cannot determine the zygosity of *HP*^{del}, that is, whether it is a homozygous (null zygote, *HP*^{del}/*HP*^{del}) or heterozygous (hemizygote, *HP*/*HP*^{del}) deletion. On the other hand, exon 1 of *HP* cannot be amplified in *HP*^{del}/*HP*^{del} because this region is deleted in *HP*^{del} but can in *HP*/*HP*^{del} and *HP*/*HP*. Therefore, a duplex conventional PCR method was developed to simultaneously amplify the region spanning the deletion junction (315 bp) and the region flanking exon 1 (476 bp) [15]. Using this method, the presence or absence of the deletion and its zygosity can be determined simultaneously by a single PCR (Figure 4A).

5.2. TaqMan Real-Time PCR for Detection of HP^{del}

Detection of *HP*^{del} by conventional PCR is an adequate method for genetic diagnosis of a small number of specimens. It also has the advantage of low initial cost. However, it is not suitable for genetic diagnosis of large numbers of samples because it takes 3 to 4 hours from extraction of genomic DNA to PCR amplification, followed by determination by agarose gel electrophoresis. Based on these

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limitations, a genetic diagnosis method using TaqMan PCR was developed with the aim of introducing it to relatively large medical institutions, because it eliminates the need for DNA extraction, is simpler and faster, and avoids carry-over of the amplified product because it can be completed in a closed tube [43].

In this method, similar to the conventional PCR method, a region spanning the deletion junction (129 bp) and a region adjacent to exon 1 (84 bp) are simultaneously amplified in a 20 μl reaction system, while using 1 μl of blood diluted 100-fold with 50 mM NaOH as a template instead of purified DNA (Figure 1). As shown in Figure 4B, amplification is detected using TaqMan (hydrolysis) probes labeled with two different fluorescent dyes [44]. Using this method, *HP*^{del} zygosity can be diagnosed within 1 hour 30 minutes after blood collection, and although real-time PCR equipment is required, the cost per sample is estimated to be around USD 1. Compared to conventional PCR, this method is a more rapid and inexpensive genetic testing method that can be used on a large number of samples.

5.3. SYBR Green I-Based Real-Time PCR Method for Detection of HP^{del}

A duplex SYBR Green I-based real-time PCR assay was developed to determine *HP*^{del} zygosity in a single tube [45]. Three primers consisting of two forward primers spanning the deletion junction (for *HP*^{del}) and *HPR* intron 4 (for *HP*) and a common reverse primer located in *HPR* intron 4 were used for PCR amplification, and then melting curve analysis was performed (Figure 1). Two distinct melting peaks corresponding to *HP*^{del} (amplification size = 124 bp and Tm = 80.3℃) and *HPR* intron 4, which is deleted in *HP*^{del} (amplification size = 148 bp and Tm = 84.5℃) were clearly discriminated (Figure 4C).

We can also use 1 μl of blood samples diluted 64- to 1024-fold with 50 mM NaOH solution as a template. The results obtained with this method were in complete agreement with those obtained by the TaqMan-based real-time PCR method. This method is easy to apply compared to TaqMan-based real-time PCR methods because it has a lower initial cost and can be analyzed using economical single color real-time PCR equipment.

5.4. Loop-Mediated Isothermal Amplification Reaction Method for Detection of HP^{del}

Loop-mediated isothermal amplification (LAMP)-based screening [46] for *HP*^{del} was performed using genomic DNA as a template and primer sets optimal for amplification of the spanning region of *HP*^{del} and the 5' region of *HP* in two different tubes [47]. This method also works well using blood samples diluted 100-fold in 50 mM NaOH or blood samples diluted 2-fold in water and then boiled as templates [47]. The advantage of this method is that the reaction is isothermal, and the amplification can be determined by the turbidity of the solution and diluted blood can be used as a template, so no special equipment is required (Figure 4D), and the reaction time is short (about 30 min) [46]. On the other hand, the disadvantages include that two reaction tubes are required to determine the zygosity of *HP*^{del}, and four to six different primers were required for amplification in one reaction.

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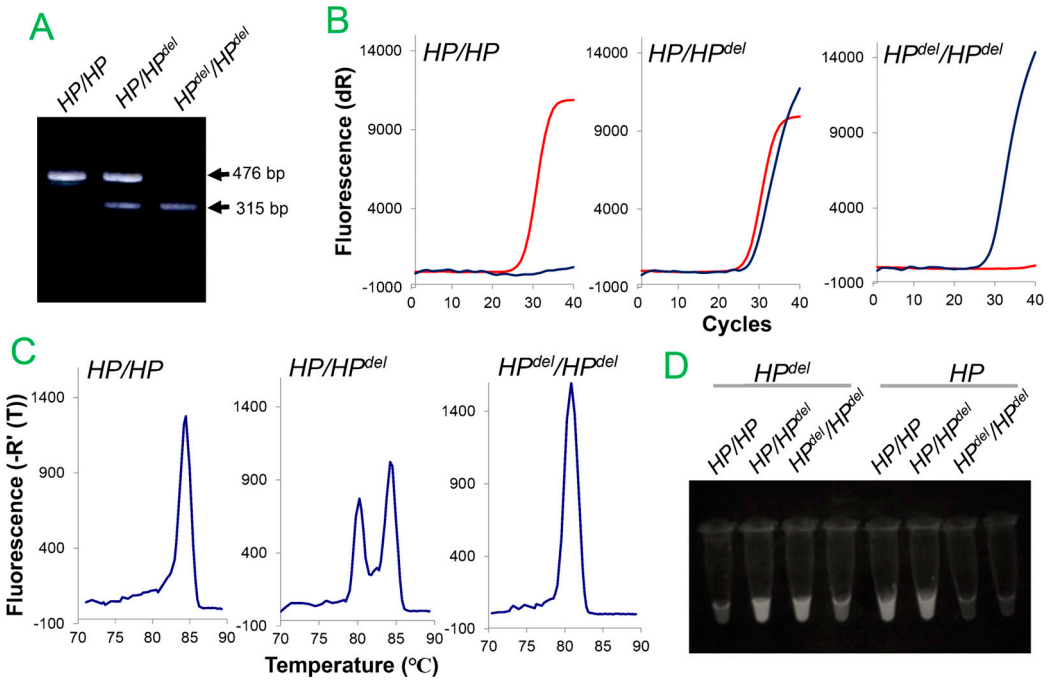

Figure 3. Representative results obtained using each test method for detection of *HP*^{del}. The results of conventional PCR (A), TaqMan real-time PCR (B), SYBR-green I-based real-time PCR (C), and LAMP (D) are shown. Purified DNA or diluted blood samples of individuals without (*HP*/*HP*), heterozygote (*HP*/*HPdel*), or homozygote (*HP*^{del}/*HP*^{del}) of *HP*^{del} were used as templates. Immunological methods for screening of *HP*$^{del.}$

6. Immunological Methods for Screening of HP^{del}

6.1. Enzyme-Linked Immunosorbent Assay for Detection of HP^{del}

In addition to genetic diagnosis, methods for detecting *HP*^{del} include methods for measuring serum Hp, such as measuring peroxidase activity by complex formation of Hp and Hb, simple radioimmunoassay, immunoturbidimetry, and immunonephelometry methods. Among these, the immunoturbidimetry and immunonephelometry methods are easy to perform and are widely used in clinical tests. However, since the detection limit of these methods is several mg/ml, it is difficult to differentiate between hypohaptoglobiemia and anhaptoglobiemia. The ELISA method, which has a detection sensitivity of several μg/ml, can be used as a highly sensitive detection method to compensate for these drawbacks. The ELISA method using an anti-HP antibody developed by Shimada et al. has a quick performance time of 40 min, and is a useful testing method because it can handle a relatively large number of specimens [48].

6.2. Latex Agglutination Method for Detection of HP^{del}

Recently, a new method for Hp concentration measurements by the latex agglutination method using an automatic analyzer was developed [13]. This method used a mouse monoclonal antibody conjugated with carboxylate-modified polystyrene latex beads. In this method, a linear absorbance curve was not obtained for the normal haptoglobin range (19–170 mg/dl) but was in the low Hp concentration range and Hp deficient ranges with a detection limit of 75 μg/dl. To confirm the results, samples with low protein concentrations detected by this method were re-examined by ELISA and *HP*^{del} was detected by PCR. Compared to ELISA, the advantages of this method are that it is automatable and inexpensive, which would make it useful for large-scale screening of blood donors. In addition, two anhaptoglobinemic individuals and 21 hypohaptoglobinemic individuals (Hp concentration of below 300 μg/dl) were detected using this method in the screening of 7,476 samples. Two anhaptoglobinemic individuals were homozygous for *HP*^{del}, while 19 of 21 hypohaptoglobinemic ones were heterozygous for *HP*^{del} [13].

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7. Protein-Based Methods for Detection of Anti-Hp Antibodies in Hp Deficient Patients

In several case reports, anti-Hp antibodies in Hp deficient patients were examined. This method differs from the two protein-based detection methods mentioned in the previous two terms in that it detects anti-Hp antibodies rather than Hp itself and, it is an effective method for investigating the cause rather than prevention of anaphylactic shock. In many cases, ELISA and western blotting are used to detect ant-HP antibodies [8,9,12,38–40]. Recently, instead of ELISA and western blotting methods, a surface plasmon resonance (SPR) method for detection of anti-Hp antibodies in serum was developed [42]. Using this method, IgG type anti-Hp antibodies were detected in the serum of an Hp-deficient Chinese patient living in the United States who developed anaphylactic shock after a blood transfusion. Although SPR appears to be less sensitive than ELISA or western blot methods, its advantages are that fewer biological reagents are required, it is easy to operate, and measurements can be performed in real time, resulting in faster results. Therefore, SPR method provides a rapid and easily available method for detecting clinically significant anti-HP antibodies, making it a potentially useful test for preventing in addition to investigating the cause of adverse post-transfusion effects such as anaphylactic shock.

8. Fluorescent Probe Based Real-Time PCR Method for Simultaneous Detection of *HP*^{del} and Other *HP* Polymorphisms

Two fluorescent probe-based real-time PCR methods for simultaneous detection of *HP*^{del} and other *HP* polymorphisms were developed. One was detection of *HP*^{del} and common *HP* polymorphisms by adding *HP*^{2}-specific TaqMan probe and primers to *HP*^{del} TaqMan probe and primers and *HP5′* TaqMan probe and primers [49]. Copy numbers of *HP*^{2} (0, 1, 2) were determined by ΔΔCt method using copy numbers of *HP5′* as a control and whether or not there was an *HP*^{del} by endpoint genotyping assays [49,50]. The other was the simultaneous detection of *HP*^{del} and rs2000999 G>A variation by endpoint genotyping assays and fluorescence melting curve analysis (FMCA) [51]. With this method, the rs2000999 G>A variation was detected by FMCA, one of the most robust SNPs detection methods [52]. Unlike the TaqMan assay, no degradation of the fluorescent probe would be preferable in the FMCA, but both *Taq* polymerases with and without 5'-3' exonuclease activity have been reported to be available [52].

These two methods are useful not only for screening *HP*^{del} but also for association studies of these *HP* polymorphisms on a relatively large scale, especially in East and Southeast Asian populations.

9. Geographic Distribution of *HP*^{del}

The distribution of *HP*^{del} in various populations was determined using genetic diagnostic methods. As shown in Table 1, *HP*^{del} has so far been detected in East Asian (Japanese, Korean, Chinese, Taiwanese, Mongolian) and Southeast Asian (Thai, Indonesian, Vietnamese) populations with a frequency of approximately 0.9% to 4%, and therefore the frequency of *HP*^{del} homozygotes was estimated to be approximately 1 in 640 to 12,600 people [15,30,32,43,53–58]. On the other hand, it was not present in Tibetans, Africans, Europeans, West Asians, or people from the Americas [15,30,49,55,59,60]. Notably, *HP*^{del} has not been detected in Africa, where anhaptoglobinemia was first reported 65 years ago [61] Although the rs5471 C allele is thought to be significantly associated with low serum Hp concentrations [34], no genetic variant causing anhaptoglobinemia has yet been found in African populations. Therefore, in especially malaria endemic areas in Africa, many cases of anhaptoglobinemia are thought to be acquired secondarily, probably due to malaria-induced hemolysis or the like [62].

On the other hand, among populations in East Asia and Southeast Asia, the highest frequency of *HP*^{del} is observed in the Chinese population, so it is assumed that this genetic variation originated in China and spread to surrounding regions. Surprisingly, *HP*^{del} was not observed in Tibetan populations, where a large-scale gene flow from lowland southern China is thought to have occurred

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[63]. Although the reason for this is not certain and requires further detailed analysis of East and Southeast Asian populations, it may suggest that *HP*^{del} arose relatively recently [60].

Table 1. Frequency of *HP*^{del} in various populations.

Populations	Number of chromosomes	*HP*^{del} frequency (%)	Estimated homozygotes	References
East Asia				
Mongolians	2130	0.9	1/12,600	[56]
Han Chinese	11942	4.0	1/640	[15,32]
Koreans	1332	2.9	1/1,200	[15,53]
Japanese	12404	1.7	1/3,500	[15,30]
Taiwanase	1962	2.9	1/1,200	[57]
Central Asia				
Tibetans	364	0	0	[55]
Tamangs	106	0	0	[55]
Uygurs	112	0	0	[55]
Southeast Asia				
Indonesians	210	1.0	1/11,000	[43]
Thais	400	1.5	1/4,400	[54]
Vietnamese	588	2.0	1/2,400	[58]
South Asia				
Bangladeshi	102	0	0	[55]
Tamils	104	0	0	[55]
Sinhalese	102	0	0	[55]
West Asia				
Turks	214	0	0	[55]
Europe				
Europeans	400	0	0	[15,30]
Africa				
Xhosans	202	0	0	[15]
Ghanaians	246	0	0	[49]
Gambians	1196	0	0	[59]
America				
Mexicans	372	0	0	[60]
Puerto Ricans	160	0	0	[60]
Colombians	140	0	0	[60]
Peruvians	140	0	0	[60]

10. HP-Deficient Alleles Other than *HP*^{del}

In addition to *HP*^{del}, a gene variation in the splice donor site of the *HP* that is specific to Irish people has been reported as a causative gene for HP deficiency [64]. This variation (NM_001126102.1:c.190 + 1G > C) causes the first two bases of intron 3 of *HP* to change from GT to CT, which prevents normal splicing and causes nonsense mutations to appear at an early stage (Figure 1). It is thought that not only in abnormal mRNA quality but also nonsense-mediated mRNA degradation occur. This is a genetic variation that is presumed to result in an extremely low expression of *HP* mRNA, resulting in Hp deficiency. Data from whole-genome sequencing of 8,453 Irish individuals gives an estimated frequency of this variation of 0.56%. Furthermore, in an analysis of 150,656 people, six people who were homozygous for this variation were detected, five of whom lived to between 47 and 69 years of age, and one person who lived until 91 years of age. Therefore, as also shown in the case of *HP*^{del}, it was further suggested that Hp is not essential for human survival, or at least does not shorten the lifespan, while it has the physiologically important function of

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removing harmful free Hb. However, it is estimated that such patients, like homozygotes of *HP*^{del}, are at risk of developing severe adverse reactions after blood transfusion due to anti-Hp antibodies.

11. Conclusions

Many of the causes of posttransfusion anaphylaxis are unknown, but among those caused by serum protein deficiency, particularly Hp deficiency is the most common cause in Japan and probably in East and Southeast Asian [10,11]. *HP*^{del} is a genetic variation discovered by chance through analysis of cases of discrepancies in parent-child relationships in forensic practice. It is also thought to have played a role in determining the existence of congenital HP defects, which had long been questioned by some researchers [62,65]. Also, considering the variations in various public databases, there are probably no alleles that have a similar frequency as the *HP*^{del} in other human populations.

HP^{del} is a germline variation, and serious blood transfusion adverse effects caused by this variation can be prevented by performing genetic testing once in a lifetime, and the cost is low at about 1 USD per sample. Conventional PCR and detection of anti-Hp antibodies are considered sufficient to determine whether adverse reactions caused by administration of blood transfusion products are due to Hp deficiency caused by *HP*^{del}. However, for screening homozygotes of *HP*^{del} with a high potential risk of adverse reactions after blood transfusion, the TaqMan method and SYBR green I method using real-time PCR, which can test a large number of samples, are more useful. In addition, among protein-based methods, the latex agglutination method using an automatic analyzer may be the most suitable for screening large numbers of samples. Similarly, in order to administer plasma products to such specimens or establish a donor pool for Hp-deficient individuals, it may be desirable to use real-time PCR based tests to find homozygotes for *HP*^{del}.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the ethical committee of Kurume University (approval no. 22158, approved date: 31 October 2022).

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Conflicts of Interest: The authors declare no conflict of interest.

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