



# Application of Multiplex Ligation-Dependent Probe Amplification Assay for Genotyping Major Blood Group Systems Including DEL Variants in the D-Negative Korean Population

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**Background:** The DEL blood type, a very weak D variant, is a major concern in the field of transfusion medicine because of its potential to cause anti-D alloimmunization. We investigated the molecular basis of serologically D-negative phenotypes, including the DEL type, and the distribution of other blood group systems in the Korean population using the recently developed multiplex ligation-dependent probe amplification (MLPA) assay.

**Methods:** Blood group genotyping using the MLPA assay and RhCE phenotyping were performed on randomly selected 95 D-negative red blood cell products. The MLPA results were verified by multiplex PCR for the *RHD* promoter, exons 4, 7, and 10 and by direct sequencing of *RHD* exon 9.

**Results:** Out of 95 cases, total deletion of the *RHD* was observed in 74 cases (77.9%) and four cases (4.2%) had an *RHD-CE-D* hybrid allele. The other 17 cases (17.9%) had an *RHD(1227G>A)* allele, which was further confirmed by sequencing analysis. The RhCE phenotypes of *RHD(1227G>A)* alleles were composed of 14 Cce and 3 CcEe, and all 60 cases of the ce phenotype were revealed to have a total deletion of the *RHD*. Genotyping results and allele distribution of the other 17 blood group systems were consistent with previous reports on the East Asian population.

**Conclusions:** MLPA assay correctly determined *RHD* genotype, including *RHD-CE-D* hybrid alleles or *RHD(1227G>A)* allele, and other clinically relevant blood group genotypes in D-negative Koreans. The use of MLPA assay on serologically D-negative individuals may help improve transfusion safety by preventing anti-D alloimmunization.

**Key Words:** Rh-Hr blood-group system, Genotype, Multiplex ligation-dependent probe amplification

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## INTRODUCTION

The Rh blood group D antigen is highly immunogenic in the human blood group system, and anti-D alloimmunization can cause severe hemolytic transfusion reactions and hemolytic disease in

the fetus and newborn [1]. DEL type is a very weak D variant that cannot be detected by routine serological tests and presents as a D-negative phenotype unless adsorption and elution studies are performed [2, 3]. In addition, DEL type can cause anti-D alloimmunization despite small amounts of D antigen on

red blood cell (RBC) surfaces. Several cases of anti-D alloimmunization resulting from RBC transfusion of DEL type have been reported [4-7]. Currently, about 30 *RHD* alleles associated with DEL type have been reported, showing differences in distribution based on ethnicity [2]. In Caucasians, the frequency of DEL type among serologically D-negative individuals is 1/1,000–1/3,000, and *RHD*(885G>T) and *RHD*(IVS3+1G>A) are the most common DEL alleles. In East Asians, 15–20% of serologically D-negative individuals are DEL type, and the *RHD*(1227G>A) allele is the most common [8-11].

Owing to the very low frequency of the D-negative phenotype (0.15%) in Korea, ‘RhD-Negative Blood Club’ was organized to assist the supply of D-negative blood products since 1973 [8, 12]. However, Seo *et al* [12] reported that 20 individuals with DEL type (n=16, 14.5%), weak D (n=2, 1.8%), and partial D (n=2, 1.8%) were classified as D-negative among 110 RhD-Negative Blood Club members. Therefore, the accurate determination of D variant (including DEL type) is important to protect D-negative individuals from inadvertent alloimmunization to the D antigen. Several *RHD* genotyping strategies have been proposed specifically for East Asians. The proposed methods use *RHD*-specific multiplex PCR for the detection of total- or partial deletion and are followed by PCR with sequence-specific priming (PCR-SSP) or exon 9 sequencing to detect DEL alleles [8, 12, 13].

Recently, a multiple ligation-dependent probe amplification (MLPA) assay has been developed to detect *RHD* variants and zygosity, as well as the 17 other blood group systems [14, 15]. Compared with previously proposed strategies, the MLPA assay has more advantages, as the presence, absence, and copy number of 48 blood group alleles and 112 variant alleles can be determined simultaneously [14, 15]. Furthermore, unlike the most widely used comprehensive blood group genotyping platforms such as the PreciseType HEA Molecular Beadchip (ImmuCor, Warren, NJ, USA) and the Progenika ID CORE XT (Progenika Biopharma-Grifols, Bizkaia, Spain), the MLPA assay can detect four frequent DEL alleles [*RHD*(1227G>A), *RHD*(885G>T), *RHD*(3G>A), and *RHD*(IVS3+1G>A)] [16, 17].

The performance of the MLPA assay has not been validated in the East Asian population with serologically D-negative phenotype. In this study, we investigated the molecular basis of the D-negative phenotype, including the DEL type, and determined the distribution and prevalence of other blood group genotypes in the Korean population.

## METHODS

### 1. Samples

We investigated 95 randomly selected samples of D-negative RBC products that were supplied by the Korea Red Cross Blood Service and the Hanmaum Blood Center in Korea between August 2015 and October 2015. The D antigen status of the RBC products was confirmed by D typing using two different anti-D reagents [Bioclone (Ortho-Clinical Diagnostics, Raritan, NJ, USA) and Anti-D (RH1) IGM1 (Diagast, Loos, France) in the Korean Red Cross Blood Service; Bioclone and Bioscot (Millipore, Livingston, UK) in the Hanmaum Blood Center], and the indirect antiglobulin technique (IAT) in each Blood Center. An adsorption-elution test was not performed routinely by blood centers.

All blood samples were serotyped for C/c and E/e antigens using monoclonal anti-C, -c, -E, and -e reagents (Ortho-Clinical Diagnostics) with the standard tube method according to the manufacturer’s recommendations. This study was approved by the Institutional Review Board of Severance Hospital, Yonsei University Health System (4-2015-0882).

### 2. Genomic DNA extraction

Genomic DNA was isolated using the QIAamp DSP DNA Blood Mini kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol, and genomic DNA concentration and purity were assessed using a spectrophotometer (NanoDrop 2000, Thermo Fischer Scientific, Waltham, MA, USA).

### 3. MLPA assay

We used commercial SALSA MLPA Probemixes P401-A1, P402-A1, and P403-A1 Blood Group Genotyping kits (MRC-Holland, Amsterdam, the Netherlands) for the MLPA assay. The three probe mixes contained a total of 129 probes, which included 104 wild type and/or mutation probes to determine the presence, absence, and copy number of the major blood group alleles and variant alleles. The other 25 reference probes included were selected from chromosomal regions supposed to be chromosomally stable in diseases. All procedures were carried out according the manufacturer’s protocol. In brief, ligation was performed using 100 ng of genomic DNA in the following steps: denaturation at 98°C for 5 minutes, hybridization with each SALSA Probemix at 60°C for 16 hours, ligation reaction by Ligase-65 (MRC-Holland) at 54°C for 15 minutes, and ligase inactivation by incubation at 98°C for 5 minutes. Finally, multiplex PCR was performed using fluorescence-labeled universal primers, dNTPs, PCR buffer, and polymerase for 35 cycles (95°C for

30 seconds, 60°C for 30 seconds, 72°C for 1 minute) using a C1000 thermal Cycler (BioRad, Cressier, Switzerland). The fragments were analyzed using an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster, CA, USA) and GeneMarker 2.2 software (SoftGenetics, State College, PA, USA). Zygosity was determined as the signal ratio between tested samples and control samples.

#### 4. Multiplex PCR and sequencing

Multiplex PCR was performed to confirm the *RHD* genotype of MLPA results classified as total *RHD* gene deletion, *RHD-CE-D* hybrid, and DEL type. Three exons (exon 4, 7, and 10) and the promoter region were selected for amplification to detect the *RHD* gene (Table 1). In each multiplex PCR reaction, a 434-bp fragment of the human growth hormone gene was included as the internal control. The PCR reactions were performed with 50–100 ng of genomic DNA containing AccuPower PCR Premix (Bioneer, Daejeon, Korea) and 0.5 μM of each primer in a 20 μL total volume. PCR amplification was performed using a C1000 thermal cycler (Bio-Rad) with an initial denaturation at 95°C for

10 minutes, followed by 35 cycles of 30 seconds at 95°C, 30 seconds at 50°C for set A or 30 seconds at 60°C for set B, and 30 seconds at 72°C. The presence or absence of *RHD* exons and the internal control were determined by electrophoresis in a 2% agarose gel.

Direct sequencing of the *RHD* exon 9, including the exon 9-intron 9 boundary, was performed in MLPA assay *RHD*(1227G>A)-positive samples to verify the results. The PCR reaction was performed in a 20 μL reaction mixture containing AccuPower PCR Premix (Bioneer), 50 ng genomic DNA, and 0.5 μM of each primer (Table 1). PCR amplification was performed at 37°C for 2 minutes for the uracil-DNA glycosylase reaction by incubating at 95°C for 10 minutes, followed by 35 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 60 seconds. Sequencing reactions were performed using a PRISM BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems). The DNA samples containing amplified products were added to Hi-Di formamide (Applied Biosystems), and the mixture was incubated at 95°C for 5 minutes, cooled for 5 minutes on ice, and then analyzed using an ABI Prism 3730 DNA analyzer (Applied Biosystems).

**Table 1.** Primers used for multiplex PCR and sequencing

Method	Genomic target	Nucleotide sequence	Product size (bp)	References
Multiplex PCR (set A)	<i>RHD</i> exon 4	F: CCACATGAACATGATGCACA R: CAAACTGGGTATCGTTGCTG	127	[18]
	<i>RHD</i> exon 10	F: TAAGCAAAGCATCAA R: ATGGTGAGATTCTCCT	186	[6]
Multiplex PCR (set B)	<i>RHD</i> exon 7	F: GTTGTAACCGAGTGCTGGGATTC R: TGCCGGCTCCGACGGTATC	123	[19]
	<i>RHD</i> promoter	F: TCCACTTTCCACCTCCCTGC R: GCAGCCAACCTCCCTGTG	256	[19]
Multiplex PCR (internal control)	<i>HGH</i>	F: TGCCTTCCCAACCATTCCCTTA R: CCACTCACGGATTCTGTTGTGTTTC	434	[13]
Sequencing	<i>RHD</i> exon 9	F: AGATACTGTCGTTTTGACACACAAT R: GCTGAGGACTGCAGATAGGG	463	In this study

**Table 2.** RhCE phenotype and *RHD* analysis using the MLPA assay

Type of <i>RHD</i> variation	<i>RHD</i> allele	RhCE phenotype	No. (%) of samples	Subtotal (%)
<i>RHD</i> deletion	Total deletion	ce	60 (63.2)	74 (77.9)
		cEe	9 (9.5)	
		Cce	5 (5.3)	
<i>RHD-CE-D</i> hybrid allele	<i>RHD-CE-D/RHD-</i>	Cce	4 (4.2)	4 (4.2)
<i>RHD</i> allele with SNP	<i>RHD</i> (1227G>A)	Cce	14 (14.7)	17 (17.9)
		CcEe	3 (3.2)	
Total				95 (100)

Abbreviations: MLPA, multiplex ligation-dependent probe amplification; SNP, single nucleotide polymorphism.

## RESULTS

### 1. *RHD* and *RHCE* genotypes

Three *RHD* genotypic patterns were apparent in the MLPA analysis, and these patterns were confirmed using multiplex PCR for the promoter, and exons 4, 7, and 10 of *RHD* gene, as summa-

rized in Table 2. Among the 95 cases, total deletion of the *RHD* gene was observed in 74 cases (77.9%) where all *RHD* wild-type probe combination signals were absent. Four cases (4.2%) contained the *RHD-CE-D* hybrid allele showing only the 5'-UTR and exon 10 of *RHD* gene. The remaining 17 cases (17.9%) showed positive signals at the 5'-UTR and exons 3, 4, 5, 6, 7, 9,

**Table 3.** Genotype frequency of RBC antigens in the 95 RhD-negative samples

Blood group system	Expected phenotypes	Genotype	Genotype frequency (%), n = 95	Blood group system	Expected phenotypes	Genotype	Genotype frequency (%), n = 95		
002 MNS	M+N-S+s-	<i>GYP A*01/01; GYP B*03/03</i>	0 (0)	011 Yt	Yt(a+b-)	<i>YT*01/01</i>	95 (100)		
	M+N-S+s+	<i>GYP A*01/01; GYP B*03/04</i>	7 (7.4)		Yt(a+b+)	<i>YT*01/02</i>	0 (0)		
	M+N-S-s+	<i>GYP A*01/01; GYP B*04/04</i>	21 (22.1)		Yt(a-b+)	<i>YT*02/02</i>	0 (0)		
	005 Lutheran	M+N+S+s-	<i>GYP A*01/02; GYP B*03/03</i>	0 (0)	013 Scianna	Sc:1,-2	<i>SC*01/01</i>	95 (100)	
		M+N+S+s+	<i>GYP A*01/02; GYP B*03/04</i>	6 (6.3)		Sc:1,2	<i>SC*01/02</i>	0 (0)	
		M+N+S-s+	<i>GYP A*01/02; GYP B*04/04</i>	37 (38.9)		Sc:-1,2	<i>SC*02/02</i>	0 (0)	
		006 Kell	M-N+S+s-	<i>GYP A*02/02; GYP B*03/03</i>	0 (0)	014 Dombrock	Do(a+b-)	<i>DO*01/01</i>	4 (4.2)
			M-N+S+s+	<i>GYP A*02/02; GYP B*03/04</i>	0 (0)		Do(a+b+)	<i>DO*01/02</i>	15 (15.8)
			M-N+S-s+	<i>GYP A*02/02; GYP B*04/04</i>	24 (25.3)		Do(a-b+)	<i>DO*02/02</i>	78 (82.1)
008 Duffy			Lu(a+b-)	<i>LU*01/01</i>	0 (0)	015 Colton	Jo(a-)	<i>DO*01.-05</i>	0 (0)
			Lu(a+b+)	<i>LU*01/02</i>	0 (0)		Gy(a-)	<i>DO*02N.01</i>	0 (0)
			Lu(a-b+)	<i>LU*02/02</i>	95 (100)		Hy(-)	<i>DO*02.-04</i>	0 (0)
009 Kidd	K+k-		<i>KEL*01/01</i>	0 (0)	016 LW	Co(a+b-)	<i>CO*01/01</i>	95 (100)	
	K+k+		<i>KEL*01/02</i>	0 (0)		Co(a+b+)	<i>CO*01/02</i>	0 (0)	
	K-k+		<i>KEL*02/02</i>	95 (100)		Co(a-b+)	<i>CO*02/02</i>	0 (0)	
	010 Diego	Kp(a+b-)	<i>KEL*03/03</i>	0 (0)	020 Gerbich	Ge:2,3,4	<i>GE*01/01</i>	95 (100)	
		Kp(a+b+)	<i>KEL*03/04</i>	0 (0)		Ge:-2,3,4	<i>GE*01.-02/01.-02</i>	0 (0)	
		Kp(a-b+)	<i>KEL*04/04</i>	95 (100)		Ge:-2,-3,4	<i>GE*01.-03/01.-03</i>	0 (0)	
		011 Yt	Js(a+b-)	<i>KEL*06/06</i>	0 (0)	021 Cromer	Cr(a+)	<i>CROM*01/01</i>	95 (100)
			Js(a+b+)	<i>KEL*06/07</i>	0 (0)		Cr(a-)	<i>CROM*-01/-01</i>	0 (0)
			Js(a-b+)	<i>KEL*07/07</i>	95 (100)		022 Knops	Kn(a+b-)	<i>KN*01/01</i>
008 Duffy		Fy(a+b-)	<i>FY*01/01</i>	80 (84.2)	Kn(a+b+)	<i>KN*01/02</i>		0 (0)	
		Fy(a+b+)	<i>FY*01/02</i>	13 (13.7)	Kn(a-b+)	<i>KN*02/02</i>		0 (0)	
		Fy(a-b+)	<i>FY*02/02</i>	2 (2.1)	023 Indian	In(a+b-)	<i>IN*01/01</i>	0 (0)	
009 Kidd	Jk(a+b-)	<i>JK*01/01</i>	25 (26.3)	In(a+b+)		<i>IN*01/02</i>	0 (0)		
	Jk(a+b+)	<i>JK*01/02</i>	48 (50.5)	In(a-b+)		<i>IN*02/02</i>	95 (100)		
	Jk(a-b+)	<i>JK*02/02</i>	22 (23.2)	024 Ok	Ok(a+)	<i>OK*01/01</i>	95 (100)		
	010 Diego	Di(a+b-)	<i>DI*01/01</i>		0 (0)	Ok(a-)	<i>OK*01.-01/01.-01</i>	0 (0)	
		Di(a+b+)	<i>DI*01/02</i>		12 (12.6)				
		Di(a-b+)	<i>DI*02/02</i>	83 (87.4)					
		Wr(a+b-)	<i>DI*03/03</i>	0 (0)					
		Wr(a+b+)	<i>DI*03/04</i>	0 (0)					
		Wr(a-b+)	<i>DI*04/04</i>	95 (100)					

and 10 of the *RHD* gene and were assumed to be the non-deletion type. For all the non-deletion types, only the *RHD*(1227G>A) mutation probe showed a positive signal in MLPA analysis. The *RHD*(1227G>A) mutation was further confirmed by sequencing analysis.

Among the 95 total cases, *RHCE*\*C/*RHCE*\*c heterozygotes and *RHCE*\*c/*RHCE*\*c homozygotes of C/c alleles comprised 26 (27.4%) and 69 (72.6%) cases, respectively, and *RHCE*\*E/*RHCE*\*e heterozygotes and *RHCE*\*e/*RHCE*\*e homozygotes of E/e alleles numbered 11 (11.6%) and 84 (88.4%), respectively. The most commonly predicted RhCE phenotype from the MLPA assay was ce (60, 63.2%), followed by Cce (24, 25.3%), cEe (9, 9.5%), and CcEe (2, 2.1%).

## 2. Other blood group genotypes

The prevalence of the other 16 blood group genotypes and expected phenotypes in the 95 RhD-negative donors are described in Table 3. For the MNS blood group, the numbers of cases with *GYP*A\*01/01, *GYP*A\*01/02, and *GYP*A\*02/02 genotypes were 28 (29.5%), 43 (45.3%), and 24 (25.3%), respectively. The numbers of cases with *GYP*B\*03/04 and *GYP*B\*04/04 genotypes were 13 (13.7%) and 82 (86.3%), respectively. Variants of the MNS blood group such as Gp.Mur or Gp.Dane were not detected. The most common phenotypic combination of the MNS blood group was M+N+S-s+ (38.9%), followed by M-N+S-s+ (25.3%) and M+N-S-s+ (22.1%). In the Duffy blood group, the numbers of cases with *FY*\*01/01, *FY*\*01/02, and *FY*\*02/02 genotypes were 80 (84.2%), 13 (13.7%), and 2 (2.1%), respectively. In the Kidd blood group, the numbers of cases with *JK*\*01/01, *JK*\*01/02, and *JK*\*02/02 genotypes were 25 (26.3%), 48 (50.5%), and 22 (23.2%), respectively. The numbers of cases with *DI*\*01/02 and *DI*\*02/02 genotypes in the Diego blood group were 12 (12.6%) and 83 (87.4%), respectively. In the Dombrock blood group, the numbers of cases with *DO*\*01/01, *DO*\*01/02, and *DO*\*02/02 genotypes were 4 (4.2%), 15 (15.8%), and 78 (82.1%), respectively. All 95 cases were homozygous for *LU*\*02/02, *KEL*\*02/02, *KEL*\*04/04, *KEL*\*07/07, *DI*\*04/04, *YT*\*01/01, *SC*\*01/01, *CO*\*01/01, *LW*\*05/05, *GE*\*01/01, *CROM*\*01/01, *KN*\*01/01, *IN*\*02/02, and *OK*\*01/01.

## DISCUSSION

The frequency of the D-negative phenotype varies from 0.5% in East Asians to 3-7% in Africans and 12-18% in Caucasians [20, 21]. In Caucasians, the most common cause of the D-negative phenotype is a homozygous total deletion of the *RHD* gene, and

other causes are rare [1]. In Africans, 67% of D-negative individuals have at least a single copy of *RHD*\* $\Psi$ , inactivated by a 37-bp insertion in intron 3 and exon 4. This 37-bp duplication generates a reading frameshift and a premature stop codon [20]. Additionally, *RHD*-*CE*-*D* hybrid genes are relatively common in D-negative Africans. These hybrid genes consist of *RHD* exons 1, 2, 9, and 10 and exons 4-8 from the *RHCE* gene, producing no D antigen [1, 20].

In Koreans, about 75% of D-negative individuals have a total deletion of the *RHD* gene, and 9-10% have *RHD*-*CE*-*D* hybrid genes. In addition, 13-16% of D-negative Koreans have the *RHD* (1227G>A) allele, which is the most common cause of the DEL type in East Asians [8, 12, 13]. In this study, total deletion of the *RHD* gene (74/95, 77.9%) was most commonly observed using the MLPA assay. Other genotypes were *RHD*-*CE*-*D* hybrid gene (4/95, 4.2%) and the *RHD*(1227G>A) allele (17/95, 17.9%). All DEL cases with the *RHD*(1227G>A) allele were further confirmed by sequencing *RHD* exon 9. This result indicating the molecular cause of the D-negative phenotype in Koreans is consistent with previous reports [8, 21].

The DEL type is phenotypically determined by the absence of agglutination in the indirect antiglobulin test and a positive result in the adsorption/elution technique [3, 22]. As the sensitivity of the indirect antiglobulin test may vary across laboratories and false-positive results in adsorption/elution technique may also occur, it is difficult to accurately define the borderline between the DEL type and a true D-negative phenotype [2]. This leads to the necessity of *RHD* genotyping in blood donors, because it can exclude D variant (including DEL type) donors among serologically D-negative blood donors [2, 20, 23, 24].

DEL type arises from several molecular mechanisms, including splice site mutations, missense mutations, frame shift mutations, *RHD*-*CE*-*D* hybrid, and partial deletion of the *RHD* gene [2, 9, 11, 25]. The most common DEL type in the East Asian population is caused by the *RHD*(1227G>A) allele. Although this substitution is a silent single nucleotide polymorphism, the substitution results in aberrant frame shift or exon deletion, and generates D protein with a weak antigen-presenting function [11]. Additionally, other alleles such as *RHD*(3G>A), *RHD*(28C>T), *RHD*(53T>C), *RHD*(251T>C), *RHD*(410C>A), and *RHD*(1222T>C) have also been identified in the East Asian population [8, 12, 25]. However, the typical alleles that were identified in Caucasians, *RHD*(885G>T), *RHD*(IVS3+1G>A), *RHD*(1252insT), and *RHD*(IVS5-38del4) have not been found in East Asians [11]. In this study, all non-deletion type MLPA results were identified as the *RHD*(1227G>A) allele. Although the current MLPA assay

can detect only four frequent DEL alleles [*RHD*(1227G>A), *RHD*(885G>T), *RHD*(3G>A), and *RHD*(IVS3+1G>A)], other mutation probes can be easily added according to the population-specific data on molecular causes of the DEL type (i.e. a probe to detect *RHD*(1222T>C) allele in Koreans) [8, 12, 26, 27].

Previous reports showed that the DEL type is highly associated with the RhC+ phenotype (Cc or CC) [2, 12, 19, 28-30]. Among 126 DEL cases in Taiwan, Wang *et al* [28] reported that 107 (84.9%) cases were Cc and 19 (15.1%) cases were CC. Srijinda *et al* [29] also reported that 42 (84.0%) cases were Cc and eight (16.0%) cases were CC among 50 DEL cases in Thailand. This association between DEL type and RhC phenotype might be explained by the suppressive effect of C, where D antigen density is suppressed by the C antigen [19]. Recently published reports showed a similar distribution pattern in Korea, and the cc phenotype was exclusively found in D-negative individuals with total *RHD* deletion. Therefore, this study proposed the addition of RhCE phenotyping in the first step of a diagnostic approach to discriminate the D-negative phenotype from total *RHD* deletion inferred by 'C-E-c+e+' phenotype [12]. In this study, the RhC phenotype was identified as the Cc phenotype in all DEL cases, and the DEL allele with the cc phenotype was not found.

Since numerous blood group antigens can be accurately determined by a single test, several comprehensive blood group genotyping platforms have been developed and are currently used in diverse situations [31]. The most important clinical utility of this test is to identify donors lacking high-prevalence antigens or multiple common antigens like those for rare donor programs. The frequency of the D-negative phenotype is 0.15% in Koreans, about 1/100th of the frequency in the Caucasian population [8]. Considering that the DEL type is relatively common in D-negative Koreans, the true D-negative individuals fall into the rare blood donor category. Comprehensive blood group genotyping methods could be valuable tools for accurately determining the *RHD* genotype (including DEL type) and other blood group antigens in serologically D-negative Korean donors.

In this study, we further evaluated the performance of an MLPA assay in 17 blood group systems other than the *RHD* gene, and the distribution data for major alleles from 17 blood group systems were similar to the previous data in the East Asian population [27, 32]. In the MNS blood group system, the Ss genotype frequency showed ethnic variation. The frequencies of S/S, S/s, and s/s genotypes were 0.14, 0.40, and 0.46 among the Europeans and 0.08, 0.12, and 0.80 among the Asians, respectively [1, 33]. Our findings showed similar results in that 0.14 of S/S

genotypes, 0.86 of s/s genotypes, and no cases of SS genotype were identified in D-negative cases. In the Duffy blood group system, two Fy<sup>a</sup>-negative cases were encountered, similar to previous results [33]. The frequency of the Fy<sup>a</sup>-negative phenotype in the Korean population is very low compared with that in the European population, and it is extremely difficult to find Fy<sup>a</sup>-negative RBCs for anti-Fy<sup>a</sup> alloimmunized patients [34, 35]. Other relevant antigens, including Lutheran, Kell, Yt, Scianna, Colton, LW, Gerbich, Cromer, Knops, Indian, and Ok blood group systems, are not polymorphic in the Korean D-negative individuals.

In conclusion, the MLPA assay correctly determined *RHD* genotype including *RHD-CE-D* hybrid alleles or *RHD*(1227G>A) allele, and other clinically relevant blood group genotypes in D-negative Koreans. The use of MLPA assay in serologically D-negative individuals may help improve transfusion safety by preventing anti-D alloimmunization.

### Author's Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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