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Frequency of Red Blood Cell Antigens According to Parent Ethnicity in Korea Using Molecular Typing

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Frequencies of red blood cell (RBC) blood group antigens differ by ethnicity. Since the number of immigrants is increasing in Korea, RBC antigens should be assessed in children/youths with parents of different ethnicities to ensure safe transfusions. We investigated the frequency of RBC antigens, except for ABO and RhD, in 382 children and youths with parents having Korean and non-Korean ethnicities. Subjects were divided into those with ethnically Korean parents (Korean group; N=252) and those with at least one parent of non-Korean ethnicity (non-Korean group; N=130). The 37 RBC antigens were genotyped using the ID CORE XT system (Progenika Biopharma-Grifols, Bizkaia, Spain). The frequencies of the Rh (E, C, e, hr^S, and hr^B), Duffy (Fy^a), MNS (Mi^a), and Cartwright (Yt^b) antigens differed significantly between the two groups. Eight and 11 subjects in the Korean and non-Korean groups, respectively, exhibited negative expression of high-frequency antigens, whereas 14 subjects in the non-Korean group showed positive expression of low-frequency antigens. The frequency of RBC antigens has altered alongside demographic changes in Korea and might lead to changes in distribution of RBC antibodies that cause acute or delayed hemolytic transfusion reaction.

Key Words: Antigen, Alloimmunization, Blood group system, RBCs, Frequency, Ethnicity, Korea

Red blood cell (RBC) alloimmunization is acquired when a person is exposed to an RBC antigen (except for the ABO blood group) through blood transfusion, transplantation, or during pregnancy. Alloimmunization potency differs by blood type and exposure source [1]. Of the >300 RBC antigens [2], clinically significant alloantibodies (those against the Rh, MNS, Kell, Duffy, and Kidd blood group antigens) can cause hemolytic transfusion reaction and hemolytic disease in the fetus and newborn [3].

The frequencies of RBC blood group antigens differ by ethnicity. Accordingly, a change in the ethnicity distribution of a population will lead to altered RBC antigen expression [1].

Transfusion laboratories utilize molecular testing of C/c, E/e, K/k, Kpa/ Kpb, Jsa/Jsb, Jka/Jkb, Fya/Fyb, MN, S/s, Lua/Lub, Dia/Dib, Coa/Cob, Doa/Dob, Joa, Hy, LWa/LWb, Sc1/Sc2, and other antigens for targeted blood donor recruitment to provide transfusion support for ethnically diverse patient populations [2]. In recent years, multiple molecular testing platforms have been used to predict phenotypes based on blood group genetics [4-6]. The ID CORE XT system (Progenika Biopharma-Grifols, Bizkaia, Spain) can be used to simultaneously identify multiple allelic variants encoding the most important RBC antigens. This system has received the Conformité Européenne label, and its ability to accurately identify RBC antigens in various ethnic

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groups has been well established [7-9].

Several studies have reported the phenotyping and genotyping of blood groups in Korean adults [10-12]. However, the number of immigrants to Korea has been increasing; the proportion of foreign residents among the total Korean population has risen from 1.9% in 2004 to 4.0% in 2016 [13]. The cumulative number of interethnic marriages has also been growing; 93,786 and 152,374 marriages involving immigrants occurred in Korea in 2006 and 2016, respectively [13]. Therefore, up-to-date information on RBC antigens in non-Korean adults, children, and youths is needed to reduce the risk of RBC alloimmunization.

This prospective and observational multi-center study investigated the frequency of RBC antigens, except for ABO and RhD, in children and youths with ethnically Korean and non-Korean parents, using molecular typing, and assessed the characteristics of the RBC antigens.

We recruited a total of 382 healthy volunteers and patients aged <30 years from September 2015 to August 2017 at seven training hospitals in Korea. The subjects or their parents identified parental ethnicities and were divided into the Korean group (both parents born in Korea and of Korean ethnicity; N=252) and non-Korean group (at least one parent born outside Korea and of non-Korean ethnicity; N=130). Of the latter group, 85.3% had one or both parents of Southeast or Chinese, Japanese, and Mongolian ethnicity. The subjects' general character-

istics are described in Table 1. No differences were observed between the two groups, except for age.

The study protocol was approved by the institutional review board of the Pusan National University Hospital (H-1509-001-033), and written informed consent was obtained from all subjects or their guardians.

Genomic DNA was extracted from whole blood and supplemented with EDTA, using the QuickGene DNA whole blood kit S (Kurabo Industries Ltd., Osaka, Japan), according to the manufacturer's protocol. The DNA samples were frozen at –80°C until analyzed using the ID CORE XT assay. The ID CORE XT system, by Luminex 100 Instrument (Luminex, Austin, TX, USA) based on the Luminex xMAP technology, was used to genotype 37 RBC antigens. The raw data were processed with the ID CORE XT Analysis Software to obtain the genotypes as well as the predicted blood group phenotypes based on the published literature. Antigens with a negative expression frequency <1.0% and >99.0% in the Korean group were defined as high- and low-frequency antigens, respectively [1].

Continuous variables were expressed as medians and ranges. Comparisons were performed using Pearson's chi-squared test and Fisher's exact test. Data were analyzed using IBM SPSS Statistics 22 (IBM Corp., Armonk, NY, USA). $P < 0.05$ was considered statistically significant.

RBC antigen genotype frequencies in Korean and non-Korean subjects are shown in Table 2. Nine "no calls" were observed in the Lutheran blood group (low signal or indeterminate genotype in LU:c.230A>G); these did not resolve following retesting.

The frequencies of the predicted phenotypes of the RBC antigens in Korean and non-Korean subjects are shown in Table 3. The frequency of most phenotypes was similar in Korean and non-Korean subjects; however, the positive predicted phenotype frequencies of the Rh (C, E, e, hr^S, and hr^B), Duffy (Fy^a), MNS (Mi^a), and Cartwright (Yt^b) antigens differed ($P < 0.05$). We observed a higher frequency of C, e, hr^S, hr^B, Mi^a, and Yt^b, as well as a lower frequency of E and Fy^a in non-Korean subjects (Table 3).

Regarding high-frequency antigens, the expression of Duffy (Fy^a), Diego (Di^b), and Dombrock (Do^b) was not detected in three (1.2%), one (0.4%), and four (1.6%) Korean subjects, respectively, and in seven (5.4%), one (0.8%), and one (0.8%) non-Korean subjects, respectively. Expression of MNS(s) was not observed in two (1.5%) non-Korean subjects but was observed in all Korean subjects (Table 3).

Regarding low-frequency antigens, Kell (K, Js^a), MNS (Mi^a), Cartwright (Yt^b), and Lutheran (Lu^b) were not expressed in Korean subjects, but were expressed in two (1.5%), one (0.8%),

Table 1. Subject characteristics

	Korean (N=252)	Non-Korean (N=130)	P*
Age			
Median, yr (min–max)	15.0 (0–30)	4.0 (0–28)	<0.001
Sex			
Male	138	67	0.549
Female	114	63	
Parents			
Both parents foreign-born	0	27	
One parent foreign-born	0	103	
Both parents Korean	252	0	
Region of birth of non-Korean parent(s)			
Southeast Asia	0	83	
China, Japan, Mongolia	0	28	
Central Asia	0	4	
South Asia	0	3	
Other than Asia	0	12	

*Calculated using Pearson's chi-squared test and Fisher's exact test.

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Table 2. Genotype frequencies by blood group in Korean and non-Korean subjects

Blood group	Genotype	Korean (N = 252) N (%)	Non-Korean (N = 130) N (%)	$P^{\dagger}$
Rh	RHCE*Ce/RHCE*Ce	92 (36.5)	56 (43.1)	0.018
	RHCE*Ce/RHCE*cE	93 (36.9)	46 (35.4)	
	RHCE*ce/RHCE*Ce	28 (11.1)	21 (16.1)	
	RHCE*cE/RHCE*cE	30 (11.9)	3 (2.3)	
	RHCE*ce/RHCE*ce	1 (0.4)	2 (1.5)	
	RHCE*ce/RHCE*cE	4 (1.5)	2 (1.5)	
	RHCE*Ce/RHCE*CE	4 (1.5)	0 (0)	
Kell	KEL*k_KPB_JSB/KEL*k_ KPB_JSB	252 (100)	127 (97.7)	0.053
	KEL*k_KPB_JSB, KEL*k_ KPB_JSA*	0 (0)	1 (0.8)	
	KEL*K_KPB_JSB/ KEL*k_ KPB_JSB*	0 (0)	2 (1.5)	
MNS	GYPA*M/GYPA*M	73 (29.0)	35 (26.9)	0.846
	GYPA*M/GYPA*N	124 (49.2)	68 (52.3)	
	GYPA*N/GYPA*N	55 (21.8)	27 (20.8)	
	GYPA*S/GYPA*S	0 (0)	2 (1.5)	0.141
	GYPA*S/GYPA*s	32 (12.7)	17 (13.1)	
	GYPA*s/GYPA*s	220 (87.3)	111 (85.4)	
Kidd	JK*A/JK*A	59 (23.4)	30 (23.1)	0.408
	JK*A/JK*B	124 (49.2)	72 (55.4)	
	JK*B/JK*B	69 (27.3)	28 (21.5)	
Duffy	FY*A/FY*A	222 (88.1)	102 (78.4)	0.018
	FY*A/FY*B	27 (10.7)	20 (15.4)	
	FY*B/FY*B	3 (1.2)	4 (3.1)	
	FY*A/FY*B_GATA	0 (0)	3 (2.3)	
	FY*B_GATA/FY*B_GATA	0 (0)	1 (0.8)	
Diego	DI*A/DI*A	1 (0.4)	1 (0.8)	0.791
	DI*A/DI*B	19 (7.5)	8 (6.2)	
	DI*B/DI*B	232 (92.1)	121 (93.1)	
Dombrock	DO*A/DO*A	4 (1.5)	1 (0.8)	0.393
	DO*A/DO*B	51 (20.2)	20 (15.4)	
	DO*B/DO*B	197 (78.2)	109 (83.8)	
Colton	CO*A/CO*A	252 (100)	130 (100)	
Cartwright	YT*A/YT*A	252 (100)	127 (97.7)	0.039
	YT*A/YT*B	0 (0)	3 (2.3)	
	YT*B/YT*B	0 (0)	0 (0)	
Lutheran*	LU*A/LU*B	0 (0	1 (0.8)	0.335
	LU*B/LU*B	248 (100)	124 (99.2)	

*There were a total of 125 Korean and 248 non-Korean subjects, because of "no calls" in Lutheran in nine subjects.
†Calculated using Pearson's chi-squared test and Fisher's exact test.

seven (5.3%), three (2.3%), and one (0.8%) non-Korean subjects, respectively (Table 3).

To the best of our knowledge, this study is the first to report the frequency of RBC antigens in children and youths with interethnic parents in Korea, using molecular typing. While the results for Korean subjects were similar to those reported in other studies [10-12], we identified significant differences in the frequency rates of Rh, Duffy, MNS, and Cartwright antigens between Korean and non-Korean subjects.

It is likely that some transfusion recipients produce antibodies thus far rarely identified in Korea. When children/youths with a non-Korean parent donate blood at an appropriate age, Korean recipients are at risk of producing anti-K, anti-Js[a], anti-Mi[a], anti-Yt[b], or anti-Lu[b] antibodies. When children/youths with a non-Korean parent receive blood products in Korea, they are at risk of producing anti-Fy[a], anti-s, anti-Di[b], or anti-Do[b] antibodies. There might also be changes in the frequencies of Rh antibodies, which are associated with changes in the expression frequencies of the E, C, and e antigens. Changes in the frequencies of antibodies against antigens of the Rh, Kell, and Duffy blood group systems are particularly important because they have high immunogenicity [14].

The number of transfusion recipients with negative expression of high-frequency antigens and of blood donors with positive expression of low-frequency antigens will likely increase in Korea because of demographic changes in the population. Our findings show that the frequency of the clinically significant alloantibodies anti-K, anti-E, anti-C, anti-e, and anti-Fy[a] will alter in cases of transfusions and transplantations and in pregnant women in Korea. As demographic changes will likely continue in Korea, the frequency of the major RBC antigens should be assessed continuously.

The limitation of this study is that the ethnic distribution of Korean nationals and foreign residents in Korea was not consistent. Because the effect on RBC antigens of each ethnic group may differ, it is necessary to conduct a large-scale study, in which the number of foreign residents from each ethnic group is consistent with the number of Korean nationals.

Authors' Disclosures of Potential Conflicts of Interest

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

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Table 3. Frequencies of predicted phenotypes by blood group in Korean (N=252) and non-Korean subjects (N=130)

Blood group	Predicted antigen phenotype	Korean (N=252)		Non-Korean (N=130)		$P^{\dagger}$
		Positive N (%)	Negativen N (%)	Positive N (%)	Negative N (%)	
	C (RH:2)	217 (86.1)	35 (13.89)	123 (94.6)	7 (5.4)	0.012
	E (RH:3)	131 (52.0)	121 (48.0)	51 (39.2)	79 (60.8)	0.018
	c (RH:4)	156 (61.9)	96 (38.1)	74 (56.9)	56 (43.1)	0.346
	e (RH:5)	222 (88.1)	30 (11.9)	127 (97.7)	3 (2.3)	0.002
	CW (RH:8)	0 (0)	252 (100)	0 (0)	130 (100)	
	V (RH:10)	0 (0)	252 (100)	0 (0)	130 (100)	
	hr^S (RH:19)	233 (92.5)	19 (7.54)	128 (98.5)	2 (1.5)	0.015
	V^S (RH:20)	0 (0)	252 (100)	0 (0)	130 (100)	
	hr^B (RH:31)	222 (88.1)	30 (11.9)	127 (97.7)	3 (2.3)	0.002
Kell	K (KEL:1)	0 (0)	252 (100)	2 (1.5)	128 (98.5)	0.115
	k (KEL:2)	252 (100)	0 (0)	130 (100)	0 (0)	
	Kp^a (KEL:3)	0 (0)	252 (100)	0 (0)	130 (100)	
	Kp^b (KEL:4)	252 (100)	0 (0)	130 (100)	0 (0)	
	Js^a (KEL:6)	0 (0)	252 (100)	1 (0.8)	129 (99.2)	0.340
	Js^b (KEL:7)	252 (100)	0 (0)	130 (100)	0 (0)	
Kidd	Jk^a (JK:1)	183 (72.6)	69 (27.4)	102 (78.5)	28 (21.5)	0.214
	Jk^b (JK:2)	193 (76.6)	59 (23.4)	100 (76.9)	30 (23.1)	0.941
Duffy	Fy^a (FY:1)	249 (98.8)	3 (1.2)	123 (94.6)	7 (5.4)	0.035
	Fy^b (FY:2)	30 (11.9)	222 (88.1)	24 (18.5)	106 (81.5)	0.081
MNS	M (MNS:1)	197 (78.2)	55 (21.8)	103 (79.2)	27 (20.8)	0.812
	N (MNS:2)	179 (71.0)	73 (29.0)	95 (73.1)	35 (26.9)	0.674
	S (MNS:3)	32 (12.7)	220 (87.3)	19 (14.6)	111 (85.4)	0.602
	s (MNS:4)	252 (100)	0 (0)	128 (98.5)	2 (1.5)	0.115
	U (MNS:5)	252 (100)	0 (0)	130 (100)	0 (0)	
	Mi^a (MNS:7)	0 (0)	252 (100)	7 (5.4)	123 (94.6)	<0.001
Diego	Di^a (DI:1)	20 (7.9)	232 (92.1)	9 (6.9)	121 (93.1)	0.723
	Di^b (DI:2)	251 (99.6)	1 (0.4)	129 (99.2)	1 (0.8)	1.000
Dombrock	Do^a (DO:1)	55 (21.8)	197 (78.2)	21 (16.2)	109 (83.8)	0.188
	Do^b (DO:2)	248 (98.4)	4 (1.6)	129 (99.2)	1 (0.8)	0.665
	Hy (DO:4)	252 (100)	0 (0)	130 (100)	0 (0)	
	Jo^a (DO:5)	252 (100)	0 (0)	130 (100)	0 (0)	
Colton	Co^a (CO:1)	252 (100)	0 (0)	130 (100)	0 (0)	
	Co^b (CO:2)	0 (0)	252 (100)	0 (0)	130 (100)	
Cartwright	Yt^a (YT:1)	252 (100)	0 (0)	130 (100)	0 (0)	
	Yt^b (YT:2)	0 (0)	252 (100)	3 (2.3)	127 (97.7)	0.039
Lutheran*	Lu^a (LU:1)	0 (0)	252 (100)	1 (0.8)	129 (99.2)	0.335
	Lu^b (LU:2)	248 (98.4)	4 (1.6)	125 (100)	5 (3.8)	

*There were a total of 125 Korean and 248 non-Korean subjects, because of "no calls" in Lutheran in nine subjects.
†Calculated using Pearson's chi-squared test and Fisher's exact test.

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