한국인에서 O형 대립유전자의 분자유전학 및 혈청학적 분석

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Molecular Genetic and Serologic Analysis of the O allele in the Korean Population

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Background: The recent expansion of knowledge about various *ABO* alleles has led to the need for a comprehensive measure to cover the numerous polymorphisms dispersed in the *ABO* gene. A few studies have examined the diversity of the *O* allele compared to *A* or *B* subgroup alleles, resulting in antigenic changes. This study investigated the relationship between the serologic and molecular genetic characteristics of the *O* alleles in the Korean population.

Methods: One hundred and five samples from healthy blood group O subjects were selected randomly. The isoagglutinin titer was measured using a tube agglutination and gel microcolumn assay. The *ABO* alleles were analyzed by sequencing exons 6 and 7 of the *ABO* gene. When the origin of a heterozygous nucleotide sequence was ambiguous, it was separated into a single allele using mono-allele amplification or cloning.

Results: The median IgM isoagglutinin titer was eight. In contrast, the median IgG anti-A and anti-B isoagglutinin titers were 64 and 32, respectively. The IgG isoagglutinin titer showed a significant increase with age ($P < 0.0001$). Six *O* alleles were observed in 105 blood group O populations by sequencing. The *O01* and *O02* alleles were common (0.57, 0.36). Three rare *O* alleles (*O04*, *O05*, and *O06*) and one novel non-deletional *O* allele were found.

Conclusion: The distribution of isoagglutinin titers of blood group O and the genetic frequency of *O* alleles in this study would form the basis of the development and interpretation of ABO genotyping and serologic workup in the Korean population. (Korean J Blood Transfus 2019;30:124-137)

Key words: Isoagglutinin titer, *O* allele, non-deletional *O* allele, Sequence analysis

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Introduction

As ABO incompatible organ transplantation increases, the ABO antibody titers play a critical role in determining the pretreatment regimen as well as monitoring organ engraftment after transplantation. A study on the distribution of isoagglutinin titers in the normal population is needed. Although the IgM antibody is related to the immunologic reaction against transfusion and transplantation, the IgG antibody also plays an important role in the immunologic response [1]. Currently, in most laboratories at tertiary hospitals, isoagglutinin titration for pre-transplant testing is done routinely by distinguishing between the IgM and IgG antibodies. Measurements of the isoagglutinin titer using a gel microcolumn assay have recently been adopted [2,3]. Only one study on the IgM isoagglutinin titer distribution using the immediate spin method in the general Korean population is reported [4]. Therefore, an examination of the IgM and IgG isoagglutinin titer distribution among healthy subjects in the Korean population is needed.

The *O* phenotype is common in Korea and the incidence of *O* alleles is 28 % [4]. Blood type *O* has no antigens but contains both anti-A and anti-B antibodies, whereas IgM is the predominant antibody found in blood group A and B individuals. On the other hand, IgG is the major antibody for anti-A and anti-B in blood group *O* serum. The *O* allele has a single-base deletion at nucleotide position 261, which results in an entirely different transferase protein with different amino acids encoded after this nucleotide. When the 261delG single nucleotide polymorphism (SNP) is present in an allele, the encoded protein lacks glycosyl transferase activity. The most

common *O* allele, *O01*, is virtually identical to *A101* with the exception of a deletion of a G nucleotide at residue 261 [5]. A second common *O* allele, *O02*, also contains the 261delG SNP as well as nine other SNPs throughout exons 3~7 that differentiate it from *A101* [6]. The two common *O* alleles make up at least 95 % of the overall frequency of the *O* allele, but at least 58 different *O* alleles have been characterized thus far [7]. The less common *O03* allele lacks the G261 deletion but has a mutation, such as 802G>A, which is responsible for greatly reducing or eliminating its enzymatic activity [8]. A nondeletional *O* allele has the same features of the *O03* allele, which lacks the 261delG SNP but contains different SNPs. The absence of the G261 deletion in an *O* allele complicates most genotype screening methods targeting the universal SNPs of the *ABO* gene because the non-deletional *O* allele is similar to *A101* [9]. If the specific SNP that defines each of these alleles is not detected, then it would be identified as a normal *A* allele. Further study is needed by an analysis of the sequences in introns as well as exons 6 and 7 of the *ABO* gene. Lastly, two novel non-deletional *O* alleles were reported in Japanese blood donors with the *O* phenotype [10]. Moreover, one of the novel *O* alleles without a 261G deletion but with a 467C>T SNP was found among one hundred of the Chinese Han population [11]. Different ethnic groups have their own *O* allele genetic characteristics [12-15]. Won et al. [16], who examined ABO discrepancies, reported that various kinds of *O* alleles, such as *O02var*, *O04*, *O04var*, and *O07* existed in the Korean population. Therefore, a study of the distribution of various *O* alleles in the Korean population is needed and it is expected

that this study will lead to the detection of novel *O* alleles by sequence analysis.

This study investigated the relationship between the serologic and molecular genetic characteristics of randomized selected *O* phenotype subjects by measuring the IgM and IgG isoagglutinin titers and identifying various *O* alleles by sequence analysis of exons 6 and 7 of the *ABO* gene.

Materials and Methods

1. Study subjects

The Institutional Review Board of Inje University Busan Paik Hospital (IRB no. 10-174) approved this study. One hundred and five samples from blood group *O* healthy subjects were selected randomly and their sera were separated from clotted blood samples and frozen at -20°C . EDTA blood samples were also collected from the same subjects of with the phenotype. For antibody titration ($N=100$), four samples were excluded because their remnant sera were insufficient or some subjects were diagnosed later with malignant conditions. The antibody titers of one non-deletional *O* allele subject were also measured, but not included in the normal population's antibody titration data. For genotyping ($N=105$), the total genomic DNA was extracted from the blood using a QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany).

2. Antibody titration of blood group *O*

1) Conventional tube agglutination (immediate spin method, IS)

Serial dilutions, ranging from 2 to 1024, were

prepared using a clean pipette to mix and transfer each dilution. Anti-A and anti-B antibody titrations were performed according to the American Association of Blood Banks Technical Manual [17]. Briefly, 100 μL of each serum dilution and the same volume of 2~4% red blood cell reagent (Immucor Gamma, Norcross, GA, USA) were mixed well and the tubes were centrifuged for 15 seconds at $3400\times g$. After centrifugation, the reactions were examined macroscopically for any agglutination. The titer endpoint was reported as the reciprocal of the highest dilution that produced 1+ macroscopic agglutination.

2) Gel microcolumn assay method with dithiothreitol treatment (gel-DTT)

Before the gel microcolumn assay, the serially diluted sera were incubated for 30 minutes at 37°C by adding 0.01M dithiothreitol (DTT) to destroy the IgM antibody. A 50 μL sample of 0.8% red blood cell reagent and the same volume of each serum dilution was added to the microtube of a LISS/Coombs card (DiaMed Ag, Cressier, Morat, Switzerland). After 15 minutes incubation at 37°C , the card was centrifuged for 10 minutes. After centrifugation, the reactions were examined macroscopically for agglutination on an illuminated view box. The titer endpoint was reported as the reciprocal of the highest dilution grading 1+ according to the manufacturer's interpretation guidelines. The same person performed both methods of antibody titration to avoid variations in the technique and interpretation. Precision testing was performed on one sample selected randomly per day by the same person to evaluate replicates of the antibody titers on a sequential day.

3. Molecular analysis of *ABO* gene

1) Direct sequencing of exons 6 and 7

PCR-based gene analyses with four primer pairs (Table 1) were performed as follows. The PCR reaction mixture (20 μ L) contained PCR buffer, 10 pmol of each primer, 2.5 μ M dNTP mixture, 25 μ M $MgCl_2$, 2.5 U of Taq DNA polymerase (Intron Biotechnology, Seongnam, Korea) and 20 ng of template DNA. The PCR reactions were performed using the following conditions: 95°C for 10 minutes, followed by 32 cycles of 94°C for 1 minute, 69°C for 1 minute except for 60°C in the case of primer ABO-1F, and 72°C for 2.5 minutes, plus a final extension at 72°C for 10 minutes. The purified PCR products were sequenced bidirectionally with four primer sets and analyzed using Applied Biosystems SeqScape software (Applied Biosystems, Foster City, CA, USA).

2) Separation of single allele using mono-allele amplification or cloning

The PCR products that showed ambiguity or the novel heterozygous nucleotide sequence in sequence analysis were selected. To separate the alleles with and without c.261delG, the extracted DNA was amplified using the forward primer, 261G-F and 261X-F with the 261N-R reverse primer, respectively. The amplified products were cloned using a TOPO TA cloning sequencing kit (Invitrogen, Carlsbad, CA, USA) to split the two alleles apart. The plasmid DNAs from the chosen colonies were then sequenced bi-directionally with the primers M13F and M13R (Table 1).

3) Classification and nomenclature of *ABO* alleles

The *ABO* allele was named according to the nomenclature used in the Blood Group Antigen Gene Mutation database [7].

Table 1. Primers used for amplification and direct sequencing of *ABO* gene

Primer	Sequence	Purpose
ABO-1F	GTGCCAGAGGCGCATGTGGG	Amplification of exon 6
ABO-1R	TCGCCACTGCCTGGGTCTCTACC	Amplification of exon 6
ABO-2F	attCCCCCGTCCGCCTGCCTTGC	Amplification of exon 7
ABO-2R	attCGTAGAAGCCGGGGTGCAGGGTG	Amplification of exon 7
ABO-3F	AGCGAGGTGGATTACCTGGTGTGCG	Amplification of exon 7
ABO-3R	TGGTGGCAGGCCCTGGTGAGC	Amplification of exon 7
ABO-4F	GGGTCTCTCGGGGGTTCGGTGCAAG	Amplification of exon 7
ABO-4R	CTGCTAAAACCAAGGGCGGGAGGGGGA	Amplification of exon 7
261G-F	GGATGTCCTCGTGGTG	Mono-allele amplification of exon 6, intron 6, and exon 7
261X-F	CCCTCGTGGTACCCCT	Mono-allele amplification of exon 6, intron 6, and exon 7
261N-R	TGGCAGCCGCTCACGGGTT	Mono-allele amplification of exon 6, intron 6, and exon 7
M13 F	GTAAAACGACGGCCAG	Sequencing of cloned product
M13 R	CAGGAAACAGCTATGAC	Sequencing of cloned product

4. Statistical analysis

Statistical analysis was conducted using MedCalc software (ver. 9.0, MedCalc Software, Mariakerke, Belgium). A Mann-Whitney *U* test was used to determine the significance of the differences between the IgM and IgG antibody titers. The correlations among the anti-A, anti-B titer, and age were analyzed using a Spearman rank test. Kruskal-Wallis analysis was performed to compare the median isoagglutinin titers among the various O genotypes. A *P* value < 0.05 was considered significant. The allele frequencies within the O subjects were calculated using Hardy-Weinberg equilibrium [18].

Results

1. Serologic characteristics of the O alleles

1) IgM isoagglutinin titers by immediate spin method

The antibody titer values were acceptable because duplicate titers were within two standard dilutions. Anti-A and anti-B titers by the conventional tube agglutination method were distributed between 2 to 256 (median 8, Table 2). No significant differences in the median IgM titers of anti-A and anti-B among the ages were observed. The Spearman's coefficient of the rank correlation (ρ) between the IgM titer of anti-A and ages was the -0.00937 (95% confi-

Table 2. Distribution of IgM anti-A and anti-B titer by the immediate spin method in blood group O healthy subjects

Age (yr)	IgM anti-A titer												Total (n)
	0 [†]	1 [‡]	2	4	8	16	32	64	128	256	512	1024	
1~19	-	-	-	4	10	10*	3	2	-	-	-	-	29
20~29	-	-	1	8	9*	5	2	1	-	-	-	-	26
30~39	-	-	1	2	5	5*	3	-	1	-	-	-	17
40~49	-	-	-	2	3	3*	2	1	-	-	-	-	11
50~59	-	-	1	2	1	3*	1	1	-	-	-	-	9
60~79	-	-	-	2	1	5*	-	-	-	-	-	-	8
Total (n)	0	0	3	20	29*	31	11	5	1	-	-	-	100

Age (yr)	IgM anti-B titer												Total (n)
	0 [†]	1 [‡]	2	4	8	16	32	64	128	256	512	1024	
1~19	-	-	-	7	9*	8	3	2	-	-	-	-	29
20~29	-	-	1	6	12*	5	2	-	-	-	-	-	26
30~39	-	-	-	5	3	4*	5	-	-	-	-	-	17
40~49	-	-	1	2	1	4*	-	1	1	1	-	-	11
50~59	-	-	-	5*	-	1	3	-	-	-	-	-	9
60~79	-	-	-	-	3	3*	2	-	-	-	-	-	8
Total (n)	0	0	2	25	28*	25	15	3	1	1	-	-	100

*The median antibody titer according to age group, [†]Negative reaction in undiluted serum, [‡]Positive reaction in undiluted serum.

dence interval (CI), $-0.205 \sim 0.187$, $P=0.9263$) and IgM titer of anti-B and ages was 0.0943 (95% CI, $-0.104 \sim 0.285$, $P=0.3508$) (Fig. 1A and B).

2) IgG isoagglutinin titers by gel microcolumn method with DTT treatment

The IgG anti-A and anti-B titers by the gel microcolumn assay were distributed between 2 to 1024 (median 64 and 32, respectively, Table 3). The median isoagglutinin titer determined by the gel micro-

column assay was higher than that observed with the tube agglutination method in all age groups ($P < 0.0001$). In addition, an increase in the IgG titers of anti-A and anti-B according to age was observed. The median of IgG titer of anti-A and anti-B from the ≤ 30 years age group was 32 and 16, respectively, whereas the one from the ≥ 50 years age group was 128 and 64, respectively. IgG anti-B was not detected in a 4-year-old person. The Spearman's co-

Fig. 1. Isoagglutinin titers according to the age in blood group O. (A) and (B) show the correlations between IgM anti-A and anti-B titers by immediate spin method and age ($P=0.9263$ and $P=0.3508$, respectively), (C) and (D) reveal the correlations between IgG anti-A and anti-B titers by gel microcolumn agglutination method with DTT treatment and age ($P=0.0001$ and $P < 0.0001$, respectively).

Table 3. Distribution of IgG anti-A and anti-B titer by the microcolumn method with DTT treatment in blood group O healthy subjects

Age (yr)	IgG anti-A titer												Total (n)
	0 [†]	1 [‡]	2	4	8	16	32	64	128	256	512	1024	
1~19	-	-	2	1	3	4	8*	10	1	-	-	-	29
20~29	-	-	1	-	4	3	5*	3*	7	2	1	-	26
30~39	-	-	-	2	-	1	2	3	4*	4	-	1	17
40~49	-	-	-	-	1	-	5*	-	1	2	-	2	11
50~59	-	-	-	-	-	1	-	2	3*	2	-	1	9
60~79	-	-	-	-	1	-	2	1*	2*	2	-	-	8
Total (n)	0	0	3	3	9	9	22	19*	18	12	1	4	100

Age (yr)	IgG anti-B titer												Total (n)
	0 [†]	1 [‡]	2	4	8	16	32	64	128	256	512	1024	
1~19	1	-	4	2	4	11*	4	1	2	-	-	-	29
20~29	-	-	1	4	4	6*	5	3	2	1	-	-	26
30~39	-	-	1	-	-	3	7*	4	2	-	-	-	17
40~49	-	-	-	-	1	1	2	1	2*	2	1	1	11
50~59	-	-	-	-	-	3	2*	2	2	-	-	-	9
60~79	-	-	-	-	-	1	2	-	2*	1	1	1	8
Total (n)	1	0	6	6	9	25	22*	11	12	4	2	2	100

*The median antibody titer according to age group, [†]Negative reaction in undiluted serum, [‡]Positive reaction in undiluted serum.

efficient of rank correlation (ρ) between the IgG titer of anti-A and age was 0.378 (95% CI, 0.196~0.534, $P=0.0001$) and IgG titer of anti-B and age was 0.505 (95% CI, 0.343~0.638, $P<0.0001$) (Fig. 1C and D). The isoagglutinin titers of the O subjects did not reveal significant gender differences (Fig. 2A).

2. Molecular characteristics of O alleles

1) Genotype distribution and frequency of O alleles

Sequence analysis of the 105 O phenotype individuals was classified into 10 genotypes (Table 4). The most predominant genotype was *O01/O02* with a frequency of 41.9% (44/105). Thirty three subjects

were homozygous for the *O01* allele. The *O01/O02* genotype was more prevalent in the *O01* allele than the homozygous case. A total of 48 individuals (45.7%) were homozygotes at the allelic level. The genotype frequencies within the O individuals were in Hardy-Weinberg equilibrium.

Six O alleles, including one novel allele were identified (Table 5). Ninety one subjects had two common *O01* or *O02* alleles. The allele frequency of *O01* and *O02* was 0.57 and 0.36, respectively. Four rare alleles except for *O01* and *O02* were found: *O04*, *O05*, *O06*, and novel non-deletional O allele. The total frequency of these alleles was 12.3% (13/105). *O04* had only an extra 579T>C and *O05*

Fig. 2. Isoagglutinin titers according to gender (A) and genotype (B). There were no significant differences in titers between genders. The error bars denote the median and ranges of 5~95 percentiles. The dashed lines demonstrate isoagglutinin titers of *O01/O^{var}* individual.

Table 4. Distribution of *O* genotypes in 105 Korean subjects by Hardy-Weinberg equilibrium

Genotype	No. of subjects	Genotype frequency (%)	Expected value	χ^2
<i>O01/O01</i>	33	31.43	33.72	0.0152
<i>O01/O02</i>	44	41.90	43.07	0.0202
<i>O02/O02</i>	14	13.33	13.75	0.0045
<i>O01/O04</i>	3	2.86	3.40	0.0471
<i>O02/O04</i>	3	2.86	2.17	0.3162
<i>O04/O04</i>	0	0.00	0.09	0.0857
<i>O01/O05</i>	3	2.86	1.70	0.9941
<i>O02/O05</i>	1	0.95	1.09	0.0068
<i>O04/O05</i>	0	0.00	0.09	0.0857
<i>O05/O05</i>	0	0.00	0.02	0.0214
<i>O01/O06</i>	2	1.90	2.27	0.0314
<i>O02/O06</i>	0	0.00	1.45	1.4476
<i>O04/O06</i>	0	0.00	0.11	0.1143
<i>O05/O06</i>	0	0.00	0.06	0.0571
<i>O06/O06</i>	1	0.95	0.04	24.2881
<i>O01/O^{var}</i>	1	0.95	1.01	0.0001

had a 297A>G SNP compared to *O01*. The *O04* allele was identified in six heterozygote individuals (three *O01/O04*, three *O02/O04*), with an allelic fre-

Table 5. *O* allele frequencies in this study

Genotype	Frequency
<i>O01</i>	0.57
<i>O02</i>	0.36
<i>O04</i>	0.03
<i>O05</i>	0.01
<i>O06</i>	0.02
<i>O^{var}</i>	0.01

quency of 0.03. The *O05* allele was identified in four heterozygote individuals, three of *O01/O05* and one *O02/O05*. *O06* had the same sequence as *O02* except for 297A>G SNP. The *O06* allele was identified in one heterozygote (*O01/O06*) and one homozygote (*O06/O06*). The calculated χ^2 value of genotype *O06/O06* was higher than one degree of freedom because the genotype frequency of the *O06* homozygote was higher than the value expected in this population.

2) Identification of novel non-deletional *O* allele

The novel non-deletional *O* allele lacking the

Fig. 3. Sequence analysis of *ABO* in an *O01/O^{var}* individual. (A) The top chromatogram demonstrates heterozygous peaks after nt. 261 due to heterozygous deletion G in the forward primer sequencing reaction. The lower chromatogram shows heterozygous peaks before nt. 261 due to heterozygous deletion G in the reverse primer sequencing reaction. (B, C, D, E) The chromatograms reveal heterozygous peaks of *O01/O^{var}* alleles at nt. 297, 526, 703, and 803, respectively.

261delG was found with the additional polymorphisms (297A > G, 526C > G, 703G > A, 803G > C) (Fig. 3, Table 6). This allele *O^{var}* was not identical to any known alleles. The O phenotype person with this variant allele was heterozygous with *O01*. The IgM anti-A and anti-B titers of the subject with this non-deletional *O^{var}* allele were eight and 32, whereas the IgG titers of anti-A and anti-B were 128 and 16, respectively (Fig. 2B). The IgM anti-A titer was lower than the IgM anti-B titer.

Discussion

The detection of rare or novel *O* alleles is more

difficult than that of A or B subgroup alleles because blood type O has no antigen and observing weak antibody activity by manual ABO serum typing is problematic. This study investigated the antibody titration as a tool to screen the antibody activity and study the relationship between the genotype and phenotype of the blood group O population. This is the first report of the serologic and genetic characteristics of blood group O in the Korean population obtained prospectively.

The isoagglutinin titers can differ according to age, gender, race and environmental factors [19]. The association between the isoagglutinin titer and age was unclear. The isoagglutinin titer was believed

Table 6. The nucleotide polymorphism in exons 6 and 7 of a novel allele in this study compared to common *A*, *B* and *O*

<i>ABO</i> alleles	Exon 6					Exon 7									
	261	297	467	526	646	657	681	703	721	771	796	802	803	829	930
<i>A101</i>	G	A	C	C	T	C	G	G	C	C	C	G	G	G	G
<i>A102</i>	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-
<i>B101</i>	-	G	-	G	-	T	-	A	-	-	A	-	C	-	A
<i>O01</i>	Del	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>O02</i>	Del	G	-	-	A	-	A	-	-	T	-	-	-	A	-
<i>O03</i>	-	G	-	G	-	-	-	-	-	-	-	A	-	-	-
<i>O04</i>	Del	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>O05</i>	Del	G	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>O06</i>	Del	-	-	-	A	-	A	-	-	T	-	-	-	A	-
<i>O^{var}</i> (non-deletional <i>O</i>)	-	G	-	G	-	-	-	A	-	-	-	-	C	-	-
Amino acid position	87	99	156	176	216	219	227	235	241	257	266	268	268	277	310
Consensus (<i>A101</i>)	Val	Thr	Pro	Arg	Phe	His	Pro	Gly	Arg	Pro	Leu	Gly	Gly	Val	Leu
Altered protein	fs	nc	Leu	Gly	Ile	nc	nc	Ser	Trp	nc	Met	Arg	Ala	Met	nc

Abbreviations: fs, frameshift; nc, no change.

to be highest in teenagers and decreased with age based on the statement in a famous transfusion medicine textbook [20]. This statement, however, was based on a study conducted by Thomsen and Kettel in 1929. In contrast to this statement, Auf der Maur et al. [21] demonstrated that the median IgG antibody titer was elevated in an elderly person of blood group O in 1993. Kim et al. [4] reported that in Korea, no decrease in the ABO isoagglutinin titers was observed with increasing age in adults (20~70 years old). In this study, the isoagglutinin titers of all age groups of blood group O ranging from one to 80 years old were measured.

Column agglutination techniques with and without DTT for determining the total and IgG ABO antibody titers have been suggested to be more sensitive and objective for a pre-transplant setting [3,22]. On the other hand, for comparison with previous studies

conducted in the general population using the conventional tube agglutination technique [2,4], the IgM isoagglutinin titer was measured using the conventional immediate spin method. No significant differences in IgM isoagglutinin titer according to age were observed. For a precise determination of the IgG titer, column agglutination techniques with DTT treatment were adopted. An increase in the IgG isoagglutinin titers was observed according to age, which is similar to that of the Japanese population [2]. Similar to a study of the Japanese blood group O, the IgG isoagglutinin titer was higher than the IgM isoagglutinin titer and there was a significant correlation between the IgG titers of anti-A and anti-B and age. Compared to Kim et al. [4], the median IgM titers of anti-A and anti-B appeared to decrease between 1997 and 2010 (64 vs 8). Furthermore, IgG anti-B was not detected in a 4-year-old

boy with the *O01/O02* alleles. The mechanism responsible for the reductions in the anti-A and anti-B titers is unknown, but environmental factors, such as the change in lifestyle, enteric bacteria, etc., might affect the isoagglutinin titers [2].

The frequency of *O* alleles varies among different ethnic groups. In previous reports, the distribution of *ABO* alleles in the Korean population was analyzed by a polymerase chain reaction (PCR)-restriction fragment length polymorphism or single-stranded conformational polymorphism targeted to limited SNPs [23,24]. In the case of the *O* allele, only two alleles (*O01* and *O02*) were found and their frequencies differed according to the targeted nucleotide of the *ABO* gene. In addition, an *ABO* genotyping study [16] using PCR-direct sequencing for *ABO* discrepancies reported more alleles, such as *O02var*, *O04*, *O04var*, and *O07*, in the Korean population. The present study also adopted direct sequencing of the *ABO* gene to determine the distribution of *O* alleles. The two *O* alleles, *O01* and *O02*, were common. The frequency of *O01* was much higher than that of *O02* (0.57 and 0.36, respectively). The distribution frequency of *O01* and *O02* was similar to that of 142 *O* phenotypes in the Chinese Han population (0.337 vs 0.223) [25]. The *O05* allele was first reported in the Korean population in this study. *O04* had an extra 579T>C and *O05* had a 297A>G substitution compared to *O01*. These rare alleles were also found at low frequencies in the Chinese Han population. *O06* had the same sequence as *O02* except for no 297A>G substitution, and this allele has not been reported in the Chinese Han population. Surprisingly, two individuals with the *O06* alleles (*O06/O06* and *O01/O06*) were found in this study. In previous

reports, no *O03* or non-deletional *O* alleles were found in the Korean population. A non-deletional *O* allele without a 261G deletion was found for the first time. This non-deletional *O* allele could have been misinterpreted as an *A* or *B* allele in most *ABO* genotype screening methods targeting limited SNPs.

Although the vast majority of *O* alleles produce the expected *O* phenotype, some uncommon exceptions appear occasionally, such as weak *A* antigen expression or weak anti-*A* activity. By screening the blood group *O* donor prospectively, a suspiciously weakened anti-*A* in reverse typing can be associated with the presence of the *O03* allele, a representative non-deletional *O* allele. In addition, some individuals with non-deletional *O* alleles express a weak blood group *A* phenotype and the *A* antigen is detectable by adsorption-elution [26]. The non-deletional *O* allele is the most frequent cause of isoagglutinin detection problems in blood group *O* donors [27]. In this study, an individual with a novel non-deletional *O* allele showed an uncomplicated group *O* phenotype according to routine forward and reverse typing. The expression of the *A* antigen could not be detected by the adsorption-elution study. Upon titration of IgM isoagglutinin, however, the anti-*A* titer was eight, which is lower than the anti-*B* titer, 32. A few cases of missing isoagglutinins with no apparent explanation in blood group *O* have been reported in Korea, but most cases showed weak expression or a loss of the anti-*B* antibody [28,29]. We could not demonstrate the difference in isoagglutinin titers and various *O* alleles because of the insufficient number of *O* phenotype subjects but detected the characteristic features of isoagglutinin titer in novel non-deletional *O* allele.

In the case of a non-deletional *O* allele, the reduction in anti-A titers might have been caused by factors other than low levels of A antigen modulating the immune response [30]. This rare *O* phenotype, such as weak anti-A is induced by the residual activity of glycosyl transferase in the non-deletional *O* allele. In particular, the *O03* and *Aw08* alleles are known for the non-deletional *O* allele associated with weak isoagglutinin activity [27].

The frequency of the *O03* allele varies between 2~7% according to the population [9,26]. The non-deletional *O* allele was first reported in Korea. Further investigation of the *ABO* gene including all exon and intron regions should be performed to establish the precise nomenclature. Although selective weakening of the anti-A activity rather than anti-B was the suspected serological finding of *O03* or other non-deletional *O* alleles, it was difficult to detect this serologic result in the routine manual method of ABO typing. Weak isoagglutinin activity can be detected easily using an automated blood group analyzer. Therefore, further study will be needed to define the molecular mechanism for the phenotypic variability of non-deletional *O* alleles.

Conclusion

This study provided information on the distribution of isoagglutinin titers and the frequency of *O* alleles in the Korean population. In healthy subjects with blood group O, the isoagglutinin titers revealed marked variability. The IgG isoagglutinin titer was higher than the IgM isoagglutinin titer in all age groups and increased with increasing age ($P < 0.0001$). In addition, the *O* allele frequency was di-

verse with a total of six alleles, including common *O01* and *O02*, as well as rare *O04*, *O05*, *O06*, and novel non-deletional *O* alleles. This serologic and genetic information would form the basis for the development and interpretation of ABO genotyping and serologic workup in the Korean population.

요약

배경: *ABO* 대립유전자에 대한 지식이 늘어나면서 *ABO* 유전자에 광범위하게 분포한 다양한 다형성을 전반적으로 검출할 필요성이 증가하고 있다. 항원성의 변화로 쉽게 발견하게 되는 A형이나 B형의 다양한 대립유전자에 비해, O형 대립유전자의 다양성에 대한 연구는 상대적으로 부족하다. 이에 한국인에서 O형 대립유전자가 갖는 혈청학적 및 분자유전학적 특성과 이들의 관계에 대해 연구하였다.

방법: 건강인에서 O형으로 확인된 105명을 무작위로 선정하였다. 시험관법과 미세원주응집법을 이용하여 동종응집소를 측정하였다. *ABO* 유전자의 엑손 6번, 7번 영역의 염기서열분석을 통해 대립유전자를 확인하였다. 이형접합 염기서열이 판정이 불분명할 때는, 단일대립유전자증폭이나 클로닝 방법을 이용하여 분리하여 분석하였다.

결과: 건강인에서 IgM 동종응집소 역가의 중간값은 8이었다. IgG 항-A 항-B 동종응집소 역가의 중간값은 각각 64와 32로, IgM 동종응집소보다 높았다($P < 0.0001$). IgG 동종응집소는 나이에 따라 유의하게 증가하였다($P < 0.0001$). 염기서열 분석으로 105명의 O형 혈액형에서 6종류의 O형 대립유전자를 확인하였다. *O01*과 *O02*가 흔한 대립유전자였고(빈도 0.57, 0.36), 세 개의 드문 대립유전자(*O04*, *O05*, *O06*)와 하나의 새로운 비결실

O형 대립유전자가 발견되었다.

결론: 한국인에서 O형 혈액형의 동종응집소 역가 분포와 O형 대립유전자의 빈도에 대한 정보를 파악하여, 이를 ABO 유전형 검사와 혈청학적 검사의 개발과 해석의 기초자료로 사용할 수 있을 것이다.

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