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Allelic variance among *ABO* blood group genotypes in a population from the western region of Saudi Arabia

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Background
Characterization of the ABO blood group at the phenotype and genotype levels is clinically essential for transfusion, forensics, and population studies. This study elucidated ABO phenotypes and genotypes, and performed an evaluation of their distribution in individuals from the western region of Saudi Arabia.

Methods
One-hundred and seven samples underwent standard serological techniques for ABO blood group phenotype analysis. *ABO* alleles and genotypes were identified using multiplex polymerase chain reaction, and electrophoretic analysis was performed to evaluate the highly polymorphic *ABO* locus.

Results
A phenotype distribution of 37.4%, 30.8%, 24.3%, and 7.5% was found for blood groups O, A, B, and AB respectively in our study cohort. Genotype analysis identified 10 genotype combinations with the *O01*/*O02* and *A102*/*O02* genotypes being the most frequent with frequencies of 33.6% and 14.95%, respectively. Common genotypes such as *A101*/*A101*, *A101*/*A102*, *A101*/*B101*, *B101*/*B101*, and *O01*/*O01* were not detected. Similarly, the rare genotypes, *cis-AB01*/*O02*, *cis-AB01*/*O01*, and *cis-AB01*/*A102* were not found in our cohort. The most frequently observed allele was O02 (35.98%) followed by the *A102* allele (17.76%). Furthermore, our findings are discussed in reference to *ABO* allele and genotype frequencies found in other ethnic groups.

Conclusion
The study has a significant implication on the management of blood bank and transfusion services in Saudi Arabian patients.

Key Words ABO blood-group system, Alleles, Genotype, Saudi Arabia

INTRODUCTION

The *ABO* gene, which is located on chromosome 9, has seven exons and is over 18 kilobases (kb) in length. The major coding region of *ABO* is found in exons 6 and 7 [1], and many *ABO* alleles have been described [2]. These alleles have minor differences in their genomic sequences, and most have emerged because of hybrid alleles, base insertions, deletions, or substitutions, or by splice site mutations [2, 3]. Furthermore, the frequencies of these alleles differ among different populations and ethnicities. For example, the *A101*, *O01*, *B101*, *A102*, and *O02* alleles are common in Asian populations [1, 4]. Alleles such as *O04* and *A205* are found in the Han Chinese, and allele *O06* is found in the Japanese [5, 6]. Rare alleles such as *cis-AB01*, *cis-AB02*, and *cis-AB06* are more frequently encountered in the Japanese, Korean, and Chinese populations [5, 7, 8].

The *ABO* locus has three main phenotypes, A, B, and O, with the combination of glucosyltransferases encoded by different alleles determining the A, B, AB, or O blood group phenotype [9, 10]. Each phenotype can be dis-

tinguished serologically using anti-A and anti-B antisera generated by polyclonal or monoclonal techniques. These serological tests are important for matching the blood groups of acceptors and donors and not only enable the determination of additional subgroups, but also facilitate differentiation between weak A and B antigens [11, 12]. From an application perspective, allele and genotype studies of *ABO* are of primary importance in paternal discrepancy, forensics, organ transplantation, and population studies [11]. A number of strategies based on polymerase chain reaction (PCR) have been developed for *ABO* genotyping including restriction fragment-length polymorphism (RFLP) analysis and allele-specific PCR [13], single-strand conformation polymorphism analysis [14], amplified product length polymorphism (APLP), and sequencing [15]. To date, there has been no study in the western region of Saudi Arabia using these more current genotyping approaches. Therefore, the purpose of this study was to determine and evaluate the frequency and distribution of ABO phenotypes and their alleles using specific primers by multiplex PCR.

MATERIALS AND METHODS

Collection of blood samples

This prospective study was approved by the ethical committee of the Faculty of Medicine, King Abdulaziz University Hospital at Jeddah (Saudi Arabia). Peripheral blood was obtained with informed consent from 107 participants. ABO phenotypes were serologically determined with anti-A and anti-B antibodies using an AutoVue Innova (Ortho Clinical Diagnostics, Raritan, NJ, USA).

Genomic DNA extraction

Genomic deoxyribonucleic acid (DNA) from blood was extracted using the QIAamp DNA kit (Qiagen, Hilden, Germany). Briefly, 200 μL of sample was mixed with 20 μL of Qiagen protease in a microcentrifuge tube; 200 μL of buffer AL was added followed by thorough mixing and incubation at 56°C for 10 min. Afterwards, 200 μL of absolute ethanol was added to the lysate, followed by gentle vortexing for proper mixing. Next, the mixture was transferred to a mini spin column for centrifugation and washed three time using buffer AWI before a final elution with 50 μL of AE buffer. Extracted DNA was quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA). The method followed the procedure used by Muro *et al.* [16].

Multiplex PCR and agarose gel electrophoresis

Primers used for PCR are listed in Table 1. The genotyping approach for this study was used previously in several studies [17, 18]. Four separate PCR reactions were performed using different sets of primers in each sample. Each 20 μL reaction contained 2 μL DNA mixed with 1 μL of each allele-specific primer (10 pmol), 10 μL master mix (2X), and 7 μL nuclease free water. PCR was performed in a thermal cycler (Applied Biosystems, Wilmington, DE, USA). The cycling conditions followed an initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 40 s, annealing at 56°C for 40 s, and elongation at 72°C for 40 s, followed with a final elongation at 72°C for 5 min. PCR products were separated by electrophoresis on 3% agarose gels containing ethidium bromide at 40 mV for 20 min followed by 70 mV for 30–40 min. A 100 base pair (bp) DNA ladder was used as a molecular marker (Invitrogen, Wilmington, DE, USA). The gel was visualized using a G-BOX UV transilluminator (Syngene, Cambridge, UK).

RESULTS

Phenotype distribution

We found that serological *ABO* typing and phenotypes

Table 1. Primers used for *ABO* genotyping of a Saudi Arabian population.

PCR reaction	Primer pair	Fragment size (bp)	Allele specificity
1	PG-ABO1: 5′-GCAGTAGGAAGGATGTCCTCGTGTTG-3′ PG-ABO9: 5′-AGACCTCAATGTCCACAGTCACTCG-3′	205	*A101, A102, B101, cis-AB01*
	PG-ABO5: 5′-CCACTACTATGTCTTCACCGACCATCC-3′ PGABO8: 5′-CACCGACCCCCCCGAAGATCC-3′	381	*A101, O01, O02*
2	PG-ABO3: 5′-CCATTGTCTGGGAGGGCCCA-3′ PG-ABO9: 5′-AGACCTCAATGTCCACAGTCACTCG-3′	164	*A101, A102, O01, cis-AB01*
	PG-ABO5: 5′-CCACTACTATGTCTTCACCGACCATCC-3′ PG-ABO7: 5′-CACCGACCCCCCCGAAGATCG-3′	381	*B101*
3	PG-ABO2: 5′-GCAGTAGGAAGGATGTCCTCGTGTTA-3′ PG-ABO9: 5′-AGACCTCAATGTCCACAGTCACTCG-3′	205	*O01,O02*
	PG-ABO6: 5′-CCACTACTATGTCTTCACCGACCATCT-3′ PG-ABO8: 5′-CACCGACCCCCCCGAAGATCC-3′	381	*A102*
4	PG-ABO4: 5′-CCATTGTCTGGGAGGGCCCG-3′ PG-ABO9: 5′-AGACCTCAATGTCCACAGTCACTCG-3′	164	*B101, O02*
	PG-ABO6: 5′-CCACTACTATGTCTTCACCGACCATCT-3′ PG-ABO7: 5′-CACCGACCCCCCCGAAGATCG-3′	381	*cis-AB01*

Table 2. Frequency distribution of *ABO* phenotype and genotypes in a cohort from the western region of Saudi Arabia (N=107).

Phenotype	Frequency	Percentage	Genotype	Frequency	Percentage
A	33	30.8	*A101/A101*	0	0
			A101/102	0	0
			A101/O01	4	3.7
			A101/O02	3	2.8
			A102/A102	4	3.7
			A102/O01	6	5.6
			A102/O02	16	15.0
B	26	24.3	*B101/O01*	12	11.0
			B101/O02	14	13.0
			B101/B101	0	0
AB	8	7.5	*A101/B101*	0	0
			A102/B101	8	7.5
O	40	37.4	*O01/O01*	0	0
			O01/O02	36	33.7
			O02/O02	4	3.7

of study participants inferred from genotypes were concordant with an expected distribution between the A (N=33; 30.84%), B (N=26; 24.30%), AB (N=8; 7.48%), and O (N=40; 37.38%) blood groups.

Genotype distribution

Out of 21 possible *ABO* genotypes, 10 genotypes were observed in our study group (Table 2). The *O01/O02* genotype was the most frequent accounting for 33.6% of participants followed by the *A102/O02* genotype that was found in 14.9% of participants. In addition, we found that frequencies of the *B101/O02*, *B101/O01*, *A102/B101*, and *A102/O01* genotypes were 13.1%, 11.2%, 7.5%, and 5.6%, respectively. Genotypes such as *A101/A101*, *A101/A102*, *A101/B101*, *O01/O01*, and *B101/B101* as well as rare genotypes including *cis-AB01/O02*, *cis-AB01/O01*, and *cis-AB01/A102*, were not be detected in our cohort. We found that eight individuals were homozygous for either *A102/A102* (N=4) or *O02/O02* (N=4), while the remaining 99 individuals were heterozygous.

Allele variation

Primers used to amplify the six alleles of *ABO* are shown in Table 1. The frequency distributions of *ABO* phenotypes, genotypes, and alleles are summarized in Table 2 and Table 3. Our allele distribution consisted of *A101* (N=7), *A102* (N=38), *B101* (N=34), *O01* (N=58), and *O02* (N=77). We found that *O02* was the most abundant allele in our study cohort accounting for 36.0% of all alleles, while the *A102* allele had a frequency of 17.8%. The frequencies of the *B101* and *O01* alleles were 15.9% and 27.1% respectively, and the *A101* allele had the lowest frequency at 3.27%. We found that our western Saudi population had an increased frequency of the *O02* allele compare to the *O01* allele. Fig. 1 shows the electrophoretic patterns of the 10 recognized genotypes, while Table 4 provides a comparison of allele frequencies among different populations.

Table 3. *ABO* allele frequencies in a Saudi population.

Allele	Number detected	Percentage
A101	7	3.27
A102	38	17.76
B101	34	15.89
O01	58	27.10
O02	77	35.98
Total	214	100.0

DISCUSSION

We found in our cohort from the western region of Saudi Arabia phenotype frequencies of 30.8%, 24.3%, 7.5%, and 37.4% for the A, B, AB, and O blood groups, respectively. Previous studies performed in other Saudi populations found different frequencies of 34.2%, 24.8%, 2.5%, and 38.5% for the A, B, AB, and O blood groups, respectively [19]. For all studies including the present one, blood group O has the highest frequency followed by A, B, and AB. This variation in ABO phenotype found between different Saudi populations is expected among ethnic groups [20]. For example, in the United Kingdom, blood group B has a low frequency (10%), whereas in India it is 18.8% [4].

We found 10 *ABO* genotypes in our cohort, of which *O01/O02* was the most predominant found in 33.6% of individuals. These findings corroborate similar findings in a Han Chinese population where *O01/O02* is the most frequently observed genotype (16.8%). In addition, we found that the frequency of the *O02/O02* genotype in our Saudi population (3.7%) was comparable to that found in a Han Chinese population. Furthermore, and similar to our findings, another study did not find the *A101/A101* genotype in their cohort [5]. However, our findings differed from those found

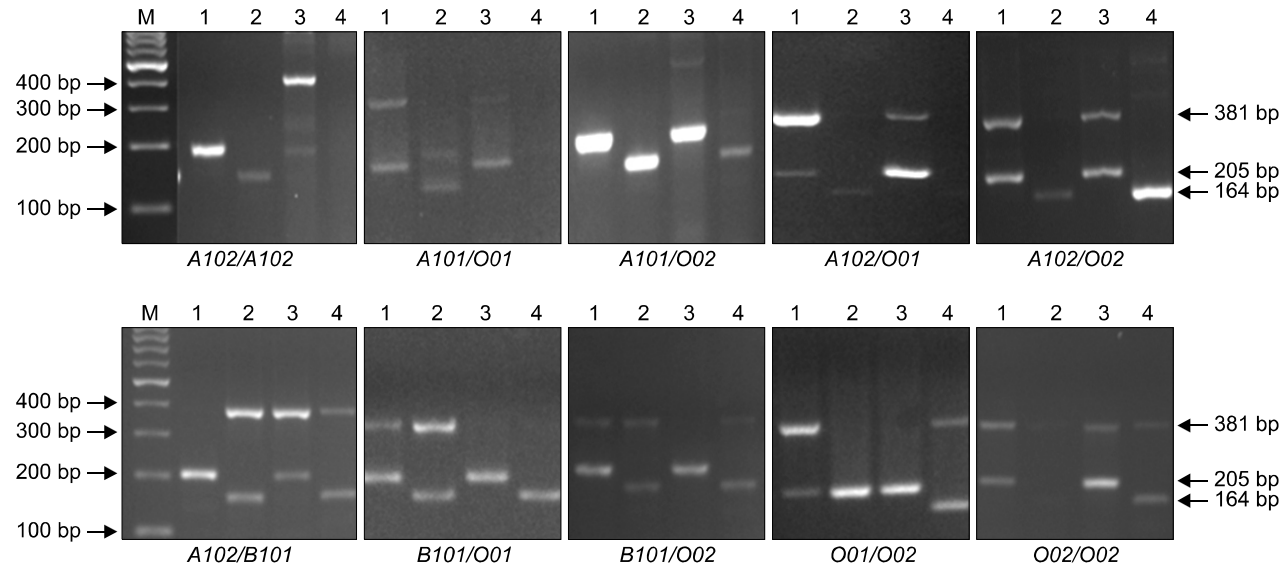

Fig. 1. Electrophoretic pattern of the 10 identified *ABO* group genotypes in a Saudi population using allele-specific multiplex polymerase chain reaction (PCR). The numbers on top of each gel are the PCR reaction tube number. Column M shows a 100 base pair (bp) DNA ladder.

Table 4. Comparisons of *ABO* allele frequencies in Saudi Arabian and other populations.

Allele	Relative frequency							
	Chinese [5]	Kuwaiti [21]	Korean [8]	Japanese [16]	Azari [22]	European [23]	Palestinian [24]	Saudi Arabian (current study)
A101	0.0156	0.0746	0.2094	0.0565	0.1169	0.1378	0.1741	0.0327
A102	0.1978	0.0592	0.0217	0.222	0.1236	0.0561	0.0671	0.1776
A205	0.0024	NA	NA	NA	NA	NA	NA	NA
B101	0.2062	0.1676	0.2094	0.163	0.1754	0.1276	0.1617	0.1589
O01	0.3369	0.6831	0.5592	0.282	0.2641	0.4082	0.3756	0.2710
O02	0.223	0.0155	NA	0.257	0.3185	0.0102	0.2214	0.3598
O04	0.0036	NA	NA	NA	NA	NA	NA	NA
O05	0.0024	NA	NA	0.001	NA	NA	NA	NA
Cis-AB06	0.0012	NA	NA	NA	NA	NA	NA	NA

Abbreviation: NA, not available.

in a Kuwaiti population where the homozygous *O01*/*O01* genotype was detected in 47.0% of blood samples [21] whereas it has not been reported in a Saudi population [19]. In contrast, we found that the *A102*/*O02* genotype was present in 14.9% of blood samples which was absent in the Kuwaiti population. Rare genotypes such as *cis-AB01*/*O02*, *cis-AB01*/*O01*, and *cis-AB01*/*A102* were conspicuously absent in the current study with these latter alleles being detected among 13 different genotypes in the Korean population [8]. In addition, the *cis-AB01* genotype has been reported in Japanese and Chinese populations [5, 6]. The absence of these genotypes *cis-AB01*/*O02*, *cis-AB01*/*O01* and *cis-AB01*/ *A102* in studies from the Middle East indicates that the Middle Eastern population differs considerably from the Chinese, Korean, and Japanese populations in respect to these rare genotypes.

Of the six possible alleles, we found five alleles of which the most frequent allele was *O02* with a frequency of 0.3598 followed by the *O01* allele with a frequency of 0.271. A comparable study on ABO blood group genotypes in a Chinese population found that the *O01* allele was the most frequent with a frequency of 0.3369 followed by the *O02* allele. In addition, alleles *A102* and *B101* with frequencies of 0.1978 and 0.2062 in our cohort were comparable to frequencies of 0.1776 and 0.1589 respectively, in the Chinese cohort [5].

As anticipated, the frequency of various alleles in different populations around the world can differ widely because of ethnicity (demonstrated in Table 4). This variation has been widely observed in many studies [5, 6, 8, 21-24]. In the majority of populations examined, the *A101* and *A102* alleles are common, whereas the *O02* allele is predominant in the

Saudi population. In comparison, the *O01* and *O02* alleles are frequent in Han Chinese, Kuwaiti, and other populations with varying frequencies [5, 21]. Similar differences in allele frequencies are found in the European, Japanese, German, Kuwaiti, and Indian populations [4, 6, 21, 23].

In conclusion, the present study reports the distribution of ABO phenotypes and genotypes in a group of individuals from the western region of Saudi Arabia. Using allele-specific multiplex PCR, 10 *ABO* genotypes were identified. In addition, this pilot study identified five alleles *A101*, *A102*, *B101*, *O01*, and *O02*, which were found at different frequencies in our cohort, and we performed a comparative assessment to other studies. Future plans include analyzing a larger number of samples to obtain a more comprehensive understanding of the *ABO* genotype pattern in Saudi Arabia.

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ABOM and SIH performed the research; ABOM and QA designed the research study; MZA, ABOM, SIH, and AH analyzed the data; WA, MZA, ABOM, AH, and GAD authenticated the data and prepared the manuscript.

Authors' Disclosures of Potential Conflicts of Interest

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

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