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Investigation of Wright Blood Group Alleles and Genotypes in Malaria-Endemic Area in Southwestern Saudi Arabia

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Purpose: Inherited blood disorders as well as malaria are prevalent in southwestern Saudi Arabia. Patients with specific hemoglobinopathies may require frequent blood transfusions. Accordingly, alloimmunization may occur if donors and recipients are incompatible. Therefore, examination of various blood group antigens to provide compatible blood units is essential. Two alleles of the Diego (DI) blood group system, *DI*02.03* and *DI*02.04* encode the Wright antigens; Wr^a and Wr^b, respectively. Anti-Wr^a may lead to alloimmunization during transfusion and pregnancy. Furthermore, the Wr^b antigen may involve in interaction between protein receptors for *Plasmodium falciparum*. This study aimed to investigate the allele/genotype frequencies of the Wright blood groups in southwestern Saudi blood donors regarding the blood transfusion and assessed the population of Jazan Province for susceptibility of *Plasmodium falciparum* invasion.

Materials and Methods: One-hundred-fifty Saudi blood donors were enrolled to this study. DNA was extracted from the blood samples. Primer pairs were designed to capture a single nucleotide variation that distinguishes the Wright alleles. Polymerase chain reaction (PCR) was conducted and followed by standard sequencing.

Results: Among the 150 genotyped samples, the only observed allele was *DI*02.04* (n = 150, 100%). Accordingly, the genotype prevalence of *DI*02.04/DI*02.04* was accounted for (n = 150, 100%).

Conclusion: This study demonstrated the allele frequencies of *DI*02.03* and *DI*02.04* of the DI blood group system in Saudi blood donors. The *DI*02.04* allele was the only allele that was observed. Furthermore, the prevalence of the genotypes was determined and the only observed genotype was *DI*02.04/DI*02.04*. Interestingly, this study indicates that the Saudi Arabian population living in Jazan Province may be more susceptible to *Plasmodium falciparum* invasion. Moreover, adding the Wright alleles for the transfusion screening panel is not recommended.

Keywords: wright blood group, blood group genotyping, blood transfusion, malaria, Saudi Arabia

Introduction

In 1953, Holman described the Wra antigen, which has a prevalence of 1:1000 in the White population.[1] Countries, such as Australia and Papua New Guinea, lack this antigen.[2] In 1971, it was found in the serum of women with Wr(a+) red cells that she had an anti-Wrb antibody, which is an antibody to the highly prevalent antigen, Wrb.[3]

Diego (DI) is the tenth blood group system comprising 23 antigens according to the International Society of Blood Transfusion.[4] Two antithetical antigens, low-frequency (DI3, Wra) and high-frequency (DI4, Wrb).[5] The frequencies of these two antigens vary among the ethnic groups.

Amplicons-based sequencing of Band 3 cDNA revealed that a single nucleotide polymorphism c.1972G>A in the SLC4A1 gene differentiates the Wra antigen from the Wrb antigen. As a result, this mutation gives rise to amino acid substitution (p.Glu658Lys).[6]

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Anti-Wra antibodies are relatively common and can cause acute hemolytic transfusion reactions (HTRs).[7–9] In addition, these antibodies can lead to hemolytic disease of the fetus and newborn (HDFN).[1,10–12] The majority of reported classes of anti-Wra antibodies in healthy blood donors are IgM or IgM plus IgG. Furthermore, the most commonly observed subclasses regarding the anti-Wra antibodies in transfused patients and pregnant women were IgG1 and IgG3.[13,14] Interestingly, the anti-Wrb antibodies are relatively common autoantibodies.[15,16] Fetal intravascular hemolysis was observed in patients with a positive direct antiglobulin test (DAT) have caused by Anti-Wrb autoantibodies.[17,18]

In addition, Jazan region has patients with inherited hematological disorders, such as sickle cell disease.[19] These patients may be considered as polytransfused patients who regularly require transfusion units with extended phenotyping to preclude red cell alloimmunization.[20]

Interestingly, it has previously been demonstrated that the Wrb antigen may act as a receptor for malaria.[21] The formation of the Wrb antigen may involve both residues from Arg61 on glycophorin A (GPA) and Glu658 on Band 3.[6] The Band 3-GPA complex on the red cell surface is vital for invading malaria parasite.[22] Indeed, the Jazan Province of Saudi Arabia has been considered as a malaria-endemic area.[23]

Given the importance of blood groups in transfusion practices and their relationship to diseases such as malaria, this study aimed to explore the distribution of the Wright blood groups in Saudi Arabian population living in Jazan Province.

Materials and Methods

Ethical Considerations and Samples Preparation

Ethical approval was obtained from the Jazan Health Ethics Committee (H-10-Z-073, approval number: 2386) and the study was conducted in compliance with the Declaration of Helsinki. Blood samples were collected by phlebotomists at the blood bank center of the King Fahad Central Hospital, Jazan, Saudi Arabia. Samples were collected from volunteer Saudi blood donors. The samples were received in anticoagulated tubes containing ethylenediaminetetraacetate. Informed consent was obtained from all subjects involved in this study.

DNA samples were extracted using a GeneJET Whole Blood Genomic DNA Purification Mini Kit (Thermo Fisher Scientific, Paisley, United Kingdom) according to the manufacturer's instructions. The quality and quantity of the extracted DNA samples were analyzed using a NanoDrop 200 spectrophotometer (Thermo Fisher Scientific, Paisley, United Kingdom).

PCR Reactions and Sequencing

Primers were designed using the National Center for Biotechnology Information primer BLAST tool to amplify the target of interest containing a single nucleotide variant, ID: rs75731670 [Table 1]. The target size of the PCR product was 400 base pairs (bp). The primer pair was synthesized and purified by high-performance liquid chromatography (Macrogen, Seoul, South Korea).

The PCR reactions comprised the following ingredients: 50 ng of DNA template, 1X Phusion Green Host Start II High-Fidelity PCR Master Mix (Thermo Fisher Scientific, Paisley, United Kingdom), and forward and reverse primers (0.5 μM of each).

The PCR cycling conditions were set as follows: the first stage of initial denaturation at 98°C for 30s, followed by the second stage (35 cycles of denaturation at 98°C for 10s, annealing at 60°C for 30s, and extension at 72°C for 30s), and final extension at 72°C for 10 min, followed by a 4°C hold. Finally, the PCR products were purified on 2% agarose gel and run on electrophoresis.

Table 1 Wright Primers Used for Sequencing

Primer	5′ to 3′ Sequence	Product Size (bp)	Chromosomal Location
DI-rs75731670-F	CACACACCCGCAGGGACTA	400	chr17:44254381+44254780
DI-rs75731670-R	GTAAGTTCCCAAGTGCCTCCAA		

For sequencing, the PCR samples were sent to Macrogen (Seoul, South Korea). MacVector Software, Version 12.7 was used to visualize sequencing electropherograms (MacVector, Inc., North Carolina, United States).

Statistical Analysis

The sample size was calculated as described by Halawani et al.[24] This sample size calculation was carried out by G* Power Software Version 3.1.9.4. The total number of samples was estimated to 143 samples with a 81% confidence level and a 11% margin of errors.

The distributions of Wright alleles and their genotypes were identified and standardized as percentages. A chi-square test was used to compare the current data in the present study with various ethnic backgrounds.

Results

This study investigated the Wright alleles and genotypes of 150 blood donors in southwestern Saudi Arabians living in Jazan Province. Table 2 demonstrates the prevalence of the Wright alleles. There was no observation for the *DI*02.03* allele encoding the Wra antigen. In contrast, the *DI*02.04* allele, which encodes the Wrb antigen, was observed in 100% of the entire study population.

Table 3 lists the rates of the Wright genotypes and the predicted phenotypes in Jazan Province of Saudi Arabia. Of note, the *DI*02.04/DI*02.04* allele was observed in the entire study population (n = 150, 100%). However, the other genotypes, *DI*02.03/DI*02.04* and *DI*02.03/DI*02.03* were not reported. Table 4 compares the frequencies of Wright alleles and genotypes among the present study with the other reported ethnic backgrounds obtained from the 1000 Genome Project Consortium.[25]

Discussion

The Jazan Province of Saudi Arabia has a unique population. Previous studies have reported various antigens/alleles and phenotypes/genotypes in several blood groups, including ABO, MNS, RH, KEL, JK, FY, LE, LU, and DO.[24,26–32] Therefore, the distribution of the Wright alleles and genotypes was investigated.

The observation of the *DI*02.04 allele*, encoding the Wrb antigen, in Jazan Province was found in the entire population at 100%. Indeed, these outcomes were consistent with the other populations, including (African, European, East Asian, and South Asian),[25] as demonstrated in Table 3. On the other hand, the antithetical allele was reported as 0.001 and 0.005 in American and Mexican Ancestry in Los Angeles, respectively.[25] In addition, Muniz et al reported that the frequency of Wra antigen was 0.06% among 1662 Brazilian blood donors.[33]

Table 2 The Prevalence of Wright Alleles in 150 Saudi Arabian Blood Donors

Allele	Predicted Antigen	Observation (n)	Frequency (%)
*DI*02.03*	Wr^a	0	0
*DI*02.04*	Wr^b	300	100

Table 3 The Prevalence of the Wright Genotypes Among 150 Saudi Arabian Blood Donors

DI allele	Genotype	Predicted Phenotype	Observation (n)	Frequency (%)
*DI*02.03*	*DI*02.03/DI*02.03*	$Wr(a^+b^-)$	0	0
*DI*02.03* and *DI*02.04*	*DI*02.03/DI*02.04*	$Wr(a^+b^+)$	0	0
*DI*02.04*	*DI*02.04/DI*02.04*	$Wr(a^-b^+)$	150	100

Table 4 Comparison of Frequencies of Wright Allele and Genotypes Between the Saudi Arabians and Several Ethnic Backgrounds Reported in the 1000 Genomes Project Consortium

Population	Allele Frequency (Count)	Genotyping Frequency (Count)	P-values
Southwestern Saudi Arabian[a]	**G**: 1.000 (300)	**G|G**: 1.000 (150)	
African[25]	**G**: 1.000 (192)	**G|G**: 1.000 (96)	
Yoruba in Ibadan, Nigeria[25]	**G**: 0.999 (693)	**G|G**: 0.997 (346)	0.974
American[25]	**A**: 0.001 (1)	**G|A**: 0.003 (1)	0.317
Colombian in Medellín, Colombia[25]	**G**: 0.995 (187)	**G|G**: 0.989 (93)	0.924
Mexican Ancestry in Los Angeles, United States[25]	**A**: 0.005 (1)	**G|A**: 0.011 (1)	0.135
East Asian[25]	**G**: 1.000 (208)	**G|G**: 1.000 (104)	
European[25]	**G**: 1.000 (198)	**G|G**: 1.000 (99)	
South Asian[25]	**G**: 1.000 (214)	**G|G**: 1.000 (107)	

Note: [a](Current study).

The *DI*02.04/DI*02.04* was the only observed genotype in the present study and in populations other than Americans and MXL, where the C>T nucleotide encoding the *DI*02.03* allele, were 0.003 and 0.011, respectively. There were no statistically significant differences between this study and other reported ethnic backgrounds regarding the Wright alleles and genotypes. Accordingly, there was no need to add the Wright alleles to the blood screening panels for both donors and the transfusion-dependent patients.

Furthermore, this area is a subtropical region in which malaria is endemic. The Wrb antigen is located on the residues of two proteins that act as receptors for *Plasmodium falciparum*.[34] The observations of the current study could explain why the Saudi population of Jazan Province may be susceptible to invasion by *Plasmodium falciparum*.

A previous report on the Duffy blood group system indicated that the prevalence of Fynull Fy(a−b−) was 78.32%.[24] This highest frequency was among the Fynull reported worldwide. Furthermore, this phenotype was reported to be resistant to invasion by *Plasmodium vivax* and *Plasmodium knowlesi*.[35,36]

A limitation of the current study was that only two alleles, *DI*02.03* and *DI*02.04*, were investigated. More alleles need to be investigated in the unique Saudi Arabian population living in Jazan Province to preclude the risk of alloimmunization to incompatible red cells and to investigate the association of Malaria with blood groups. In addition, the current study lacks of comparative group especially from non-endemic area with malaria. Furthermore, this may be due to the specific geographical location, inherited hemoglobinopathies, disorders including SCD and thalassemia, and a malaria-endemic region.

Conclusion

In summary, we reported allele frequencies of Wright blood groups in the Saudi Arabian population living in Jazan Province. Remarkably, the *DI*02.04* allele was detected in the entire population. Genotype rates were also determined. Notably, this study indicates that the Jazan population could be more susceptible to *Plasmodium falciparum* invasion. In addition, it is not essential to add the Wright alleles in the transfusion screening panel for both donors and patients. To improve transfusion procedures, particularly for polytransfused patients, research on the variable alleles of different blood group systems with clinically significant outcomes in the Saudi Arabian population is strongly encouraged.

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Disclosure

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