Journal of King Saud University - Science 36 (2024) 103571

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Journal of King Saud University - Science

journal homepage: www.sciencedirect.com

Genetic background of Diego blood group in Saudi Arabia

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ARTICLE INFO

Keywords:
Diego blood group
Blood group genotyping
Red cell alloimmunization
Blood transfusion

ABSTRACT

Background: Multiply-transfused patients are prevalent in Jazan region, Saudi Arabia. Alloimmunization may occur in case of red cell incompatibility. Therefore, extensive serotyping for other blood groups is essential. Two alleles of the Diego (DI) blood group system, *DI*A* and *DI*B* encode the main DI antigens Di(a + b–) and Di (a–b+), respectively. Anti-Di^a and anti-Di^b can be involved in transfusion reactions and pregnancy issues. This study aimed to investigate the allele and genotype frequencies of the DI blood groups in Jazan Province of Saudi Arabia.

Methods: One-hundred-fifty samples were collected from Saudi blood donors in Jazan Province. DNA was extracted and sequence-specific primers were designed to amplify the SNV region (rs2285644), which distinguishes the *DI*A* allele from the *DI*B* allele. The resulting PCR amplicons were sequenced.

Results: The frequency of *DI*B* alleles was 100 %, while the *DI*A* allele was not observed. Therefore, the only detected genotype was *DI*B/DI*B* at 100 %.

Conclusions: This study reported the allele and genotype frequencies of the DI blood group system in Saudi Arabia. This study may help establish a national database for blood groups in Jazan region. Moreover, it may help to reduce the risk of alloimmunization by providing matching blood units.

1. Introduction

In 1955, an anti-Di^a antibody, which was identified as being produced against the first antigen in the Diego (DI) system, by a Venezuelan patient suffering from serious hemolytic disease of the fetus and newborn (HDFN) (Layrisse et al., 1955). The Diego (DI) blood group system is considered the tenth system (system symbol: DI, System number: 010) according to the International Society of Blood Transfusion (ISBT) (International Society of Blood Transfusion, 2023).

The DI antigens are carried on a glycoprotein molecule that traverses the red cell membrane several times forming seven extracellular loops with both the carboxyl and amino termini located intracellularly. This glycoprotein is known as anion exchange 1 (AE1) and also denoted as band 3 (Schofield et al., 1994). Moreover, is composed of 911 amino acid residues. It acts as an anion transporter and crucially interacts with the cytoskeleton, allowing it to maintain the structural integrity of the red cell membrane (Telen, 1995).

Twenty-three antigens have been identified to date, which belong to the DI blood group system. Three high-prevalence antigens (Di^b, Wr^b, and DISK) have been identified, while the remaining antigens are low prevalence. Six of these antigens form antithetical pairs, including Di^a/Di^b, Wr^a/Wr^b, Wu/DISK, Mo(a+)/Hg(a+), BOW+/NFLD+ and Jn(a+)/KREP+ (International Society of Blood Transfusion. Red cell immunogenetics and blood group terminology [Online], 2023; Figueroa, 2013). These antigens are represented by a single gene, *SLC4A1* gene, which is located on chromosome 17q21.31. The gene is composed of 20 exons, which span approximately 20 Kbp of the human genome (Schofield et al., 1994). The most important antigens are Di^a and Di^b, which result from a single nucleotide variation (rs2285644). For example, in Di(a + b-), the nucleotide change c.2561C > T leads to amino acid substitution p.Pro854Leu (Bruce et al., 1994).

The antibodies of the DI antigens, i.e. anti-Di^a and anti-Di^b, can be involved in hemolytic transfusion reactions (HTR) as well as the HDFN. Anti-Di^a can cause mild to severe and fatal HDFN as well as severe

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https://doi.org/10.1016/j.jksus.2024.103571
Received 22 May 2024; Received in revised form 28 November 2024; Accepted 28 November 2024
Available online 4 December 2024

immediate/ delayed HTR (Issitt and Anstee, 1998). On the other hand, anti-Di^b may lead to mild HDFN with a positive direct antiglobulin test and may show no symptoms In terms of the blood transfusion, anti-Di^b can cause moderate and delayed HTR (Thompson et al., 1967). It has been reported that autoanti-Di^b has been observed in a few cases (Issitt and Anstee, 1998).

The prevalence of the Di^a and Di^b antigens varies from one population to another. For the Di^a antigen, it has been reported in 12 % of Japanese, 11 % of Chippewa Indians, 5 % of Chinese, 1 % of Hispanics, and 0.47 % of Poles (Reid et al., 2012). In addition, in South American Indians, the prevalence varies from 2 % in Caracas Indians to 54 % in Kainganges Indians. Concerning the Di^b antigen, it is a high prevalence antigen that has been observed as 99 % and 100 % in native Americans and most populations, respectively (Reid et al., 2012).

Given the importance of the DI blood group system and its clinical significance, this study aims to investigate the frequencies of the DI alleles and genotypes in the Saudi population living in Jazan Province of Saudi Arabia.

2. Materials and methods

2.1. Blood samples

This study was carried out between October 2023 and January 2024. Ethical approval was obtained from Jazan Health Ethics Committee (reference number: 2386) and was in compliance with the Declaration of Helsinki. Informed consent was obtained from all subjects involved in the present study. All participants who contributed to the current study provided informed consent.

The sample size was calculated as a total of 143 samples and was rounded to 150 samples as previously described by Halawani et al. (2022) (Halawani et al., 2022).

One-hundred-fifty blood samples were collected from the blood bank center at King Fahad Central Hospital, Jazan Province, Saudi Arabia. The samples were received in ethylene diamine tetraacetic acid tubes.

2.2. Inclusion criteria

Inclusion criteria included Saudi citizens living in Jazan Province who were healthy and donated blood voluntarily. The age of the blood donors was 18 years old or above and free from any infectious diseases such as hepatitis.

2.3. Exclusion criteria

Any blood donors from other nationalities or unsuccessful blood donations were excluded from this study.

2.4. DNA extraction

DNA extraction was carried out using a GeneJET Whole Blood Genomic DNA Purification Mini Kit (Thermo Fisher Scientific, Paisley, United Kingdom) according to the manufacturer's instructions. The DNA purity and quantification were assessed by NanoDrop 200 spectrophotometer (Thermo Fisher Scientific, Paisley, United Kingdom).

2.5. PCR primers

PCR preparation began with designing a primer pair using the National Center for Biotechnology Information primer BLAST tool to amplify a single nucleotide variation responsible for the *DI*A* and *DI*B* alleles (ID: rs2285644). Table 1 demonstrates the primer pair used to amplify the target of interest. The target size for the PCR product was 450 base pairs. The human growth hormone was used as an internal control, as described by Touinssi et al. (2008) (Touinssi et al., 2008). The primer pair was manufactured by Macrogen (Seoul, South Korea).

2.6. PCR setup

PCR experiments were conducted using 1X Phusion Green Hot Start II High-Fidelity PCR Master Mix (Thermo Fisher Scientific, Paisley, United Kingdom), 50 ng of DNA template, and 0.5 μM of each forward and reverse primer. The cycling conditions were optimized as follows: initial denaturation at 98 °C for 30, followed by 35 cycles of denaturation 98 °C for 10 s; annealing 60 °C for 30 s; and extension 72 °C for 30 s. Then, the final extension at 72 °C for 10 min followed by a 4 °C hold. Finally, the PCR amplicons were observed using 2 % agarose gel electrophoresis.

2.7. Sequencing reactions

The PCR amplicons were shipped abroad for sequencing services (Macrogen, Seoul, South Korea). MacVector Software (Version 12.7) was used to assess the sequencing electropherograms and evaluate the genotyping outcomes (MacVector, Inc., North Carolina, United States).

2.8. Statistical analysis

The data were analyzed to determine the rates of the alleles as well as the genotypes of the *DI*A* and *DI*B* and were demonstrated as percentages. Data comparison of the present study with other ethnic backgrounds was carried out by calculating the *p*-values using the chi-square test to demonstrate any statistically significant differences. The *p*-values < 0.05 and < 0.01 indicated significant and highly significant differences, respectively.

3. Results

One-hundred-fifty DNA samples were conducted to the PCR reactions followed by Sanger sequencing. The prevalence of the DI alleles is listed in Table 2. The only observed allele in the Saudi population living in Jazan Province was *DI*B* with a frequency of (n = 300, 100 %). Regarding the genotyping, according to the allelic outcomes, the only

Table 2
The frequencies of DI alleles among 150 Saudi Arabians living in Jazan Province.

Allele	Predicted antigen	Observation (n)	Frequency (%)
*DI*A*	Di^a	0	0
*DI*B*	Di^b	300	100

DI: Diego.

Table 1
The primer pairs used to amplify the *DI*A* and *DI*B* alleles.

Primer	5′ to 3′ sequence	Product size (bp)	Chromosomal location
DI- rs2285644-F	ACACAGAGAAACAAGGCCCC	450	chr17:44250982 + 44251431
DI- rs2285644-R	CTACGTCAAGCGGGTACAGG		
HGH-F*	GCCTTCCCAACCATTCCCTTA	429	chr17:61995373–61995801
HGH-R*	TCACGGATTTCTGTTGTGTTTC		

DI: Diego, Bp: base-pair, HGH: human growth hormone, F: forward, R: reverse.
*The HGH was used for internal control.

genotypes observed was *DI*B/DI*B* (n = 150, 100 %) as shown in Table 3. Data comparison of the present study with various ethnicities is shown in Table 4.

4. Discussion

Jazan Province of Saudi Arabia is endemic for hemoglobinopathies, such as thalassemia and sickle cell disease (Alsaeed et al., 2018). Some of these are transfusion-dependent patients, who require receiving blood transfusion regularly. Accordingly, extended phenotyping for such patients is crucial to preclude the risk of red cell alloimmunization (Halawani et al., 2022). Therefore, knowledge of the frequencies of various blood group antigens is essential and may assist in preventing such issues. Many research studies were conducted among the Saudi Arabian population who live in Jazan Province. These include frequencies of ABO, RH, KEL, MNS, JK, FY, LE, LU and DO blood groups (Saboor et al., 2020; Halawani et al., 2021; Halawani et al., 2022; Halawani et al., 2021; Halawani et al., 2023; Daniels, 2023).

In this study, we identified the frequencies of the DI blood group system. The frequency of the *DI*B* allele was determined to be 100 % as it is a high prevalence antigen. Accordingly, the only detected genotype was *DI*B/DI*B*. On the other hand, there was no observation regarding the *DI*A* allele. These findings of the present study are consistent with other reported studies investigating different ethnicities. For example, Italians in Naples (Belsito et al., 2015), Esan in Nigeria, Gambian in Western Division-Mandinka, British from England and Scotland, and Toscani in Italy have shown similar findings (1000 Genomes Project Consortium, 2015).

However, very few studies have reported the incidence of the *DI*A* allele, which demonstrates variations in the *DI*B* allele compared to the present study. For example, the frequencies of the *DI*A* allele were reported in other ethnicities and they range from 0.6 % in Native Alaska/Aleut to 6 % in Chinese (Chengdu) (Gong et al., 2014) as demonstrated in Table 4.

Our findings demonstrate that there were statistically significant differences ($p < 0.05$) between the Saudis living in Jazan Province and Han Chinese in Beijing, Han Chinese South, Japanese in Tokyo, Chinese (Shen-Zhen), Colombian in Medellín, Brazilian Japanese descendants, and Southern Brazilians (Nathalang et al., 2016; Wu et al., 2002; Flôres et al., 2014; da Costa et al., 2016). Interestingly, observations of high statistically significant differences ($p < 0.01$) were seen between the population of the current study and Han Chinese (Shanghai), Korean (Seoul), Korean, Chinese (Chengdu), Japanese, and Mexican Ancestry in Los Angeles, and Peruvian in Lima (Nathalang et al., 2016; Delaney et al., 2015; Ye et al., 2015; Kim et al., 1999; Gong et al., 2014).

Building an extended panel for the multiply transfused patients is crucial by investigating many alleles/antigens of different blood group systems. It is highly recommended for healthcare providers in Saudi Arabia to switch to blood group genotyping especially for such patients. This protocol investigates the DNA alleles accurately compared to the conventional serotyping, and avoids mistyping especially in patients receiving recent transfusions (Daniels, 2023). In other words, those patients may suffer from red cell alloimmunization due to the wrong typing of the donor red cells in their bloodstream.

The limitations of this study may include that only two alleles were investigated regarding the DI blood group system. However, these two alleles are the main ones among the system and cause the most clinically significant outcomes in cases of mismatched antigens during blood transfusion or pregnancies.

Table 3
The frequencies of the DI genotypes among Saudis in Jazan Province, Saudi Arabia.

Genotype	Predicted phenotype	Observation (n)	Frequency (%)
*DI*A/DI*A*	Di(a$^+$b$^-$)	0	0
*DI*A/DI*B*	Di(a$^+$b$^+$)	0	0
*DI*B/DI*B*	Di(a$^-$b$^+$)	150	100

DI: Diego.

Table 4
Comparison of the frequency of DI alleles between the Jazan population (present study) and various ethnic backgrounds.

Population	Count	Allele frequencies		*p*-values[¥]
		*DI*A*	*DI*B*	
Saudis (Current study)	150	0.0000	1.0000	–
Bengali in Bangladesh (Nathalang et al., 2016)	172	0.012	0.988	0.185
Chinese Dai in Xishuangbanna, China (Nathalang et al., 2016)	186	0.016	0.984	0.121
Han Chinese in Beijing (Nathalang et al., 2016)	206	0.029	0.971	0.035*
Han Chinese South (Nathalang et al., 2016)	210	0.029	0.971	0.036*
Japanese in Tokyo (Nathalang et al., 2016)	208	0.038	0.962	0.015*
Central Thais (Halawani et al., 2021)	1,011	0.0183	0.9817	0.090
Southeast Asian (Delaney et al., 2015)	942	0.0145	0.9855	0.132
Filipino (Delaney et al., 2015)	1,333	0.0075	0.9925	0.287
Chinese (Shen-Zhen) (Wu et al., 2002)	1,766	0.0357	0.9643	0.018*
Han Chinese (Shanghai) (Ye et al., 2015)	403	0.0447	0.9553	0.008**
Korean (Seoul) (Kim et al., 1999)	116	0.0474	0.9526	0.005**
Korean (Delaney et al., 2015)	1,033	0.0580	0.9420	0.002**
Chinese (Chengdu) (Gong et al., 2014)	300	0.0600	0.9400	0.002**
Japanese (Delaney et al., 2015)	1,022	0.0430	0.9570	0.009**
African ancestry in Southwest United States (Nathalang et al., 2016)	122	0.008	0.992	0.266
Esan in Nigeria (Nathalang et al., 2016)	198	0.0000	1.000	–
Gambian in Western Division–Mandinka (Nathalang et al., 2016)	226	0.0000	1.000	–
British From England and Scotland (Nathalang et al., 2016)	182	0.0000	1.000	–
Toscani in Italy (Nathalang et al., 2016)	214	0.0000	1.000	–
Italians (Naples) (Belsito et al., 2015)	225	0.0000	1.0000	–
American Native (Delaney et al., 2015)	970	0.0110	0.9890	0.189
Colombian in Medellín (Nathalang et al., 2016)	188	0.037	0.963	0.016*
Mexican Ancestry in Los Angeles (Nathalang et al., 2016)	128	0.047	0.953	0.007**
Peruvian in Lima (Nathalang et al., 2016)	170	0.106	0.894	0.000**
Puerto Rican in Puerto Rico (Nathalang et al., 2016)	208	0.024	0.976	0.056
Alaska Native/Aleut (Delaney et al., 2015)	621	0.0065	0.9935	0.324
Hawaiian/Pacific Islander (Delaney et al., 2015)	522	0.0060	0.9940	0.352
Brazilian Japanese descendants (Flôres et al., 2014)	209	0.0431	0.9569	0.010*
Brazilians (Novaretti et al., 2010)	4,326	0.0181	0.9819	0.097
Southern Brazilians (da Costa et al., 2016)	373	0.0282	0.9718	0.033*

[¥]Chi-Square test.
*Significant differences from Saudis,
**highly Significant differences from Saudis.

5. Conclusions

This study reported the frequencies of the DI alleles and genotypes in Saudi blood donors living in Jazan Province. This may help establish a national database and design an extended phenotyping panel to include blood groups. As a result, this will provide better transfusion practices and may assist in reducing the risk of the alloimmunization of the red cells.

6. Institutional review board statement

The study was approved by the Jazan Health Ethics Committee (H-10-Z-073, approval number: 2386). It was conducted following the guidelines of the Declaration of Helsinki.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

CRediT authorship contribution statement

Amr J. Halawani: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Saif Elden B. Abdalla:** Writing – original draft, Investigation, Formal analysis, Data curation. **Abdullah Meshi:** Writing – original draft, Investigation, Formal analysis, Data curation. **Ghalia Shamlan:** Writing – review & editing, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Mahmoud M. Habibullah:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank Researchers Supporting Project number (RSPD2024R631), King Saud University, Riyadh, Saudi Arabia for funding this project.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2024.103571.

References

Alsaeed, E.S., Farhat, G.N., Assiri, A.M., Memish, Z., Ahmed, E.M., Saeedi, M.Y., Al-Dossary, M.F., Bashawri, H., 2018. Distribution of hemoglobinopathy disorders in Saudi Arabia based on data from the premarital screening and genetic counseling program, 2011–2015. J. Epidemiol. Glob. Health 7 (Suppl 1), S41–S47.

Belsito, A., Costa, D., Fiorito, C., De Iorio, G., Casamassimi, A., Perrotta, S., Napoli, C., 2015. Erythrocyte genotyping for transfusion-dependent patients at the Azienda Universitaria Policlinico of Naples. Transfus. Apher. Sci. 52, 72–77. https://doi.org/10.1016/j.transci.2014.12.006.

Bruce, L.J., Anstee, D.J., Spring, F.A., Tanner, M.J.B., 1994. 3 Memphis variant II. Altered stilbene disulfonate binding and the Diego (Dia) blood group antigen are associated with the human erythrocyte band 3 mutation Pro854–>Leu. J. Biol. Chem. 10, 16155–16158.

da Costa, D.C., Schinaider, A.A., Santos, T.M., Schörner, E.J., Simon, D., Maluf, S.W., de Moraes, A.C.R., Silva, M.C.S., 2016. Frequencies of polymorphisms of the Rh, Kell, Kidd, Duffy and Diego systems of Santa Catarina, Southern Brazil. Rev. Bras. Hematol. Hemoter. 38, 199–205. https://doi.org/10.1016/j.bjhh.2016.04.005.

Daniels, G., 2023. An overview of blood group genotyping. Ann. Blood 8 (3), 1–11.

Delaney, M., Harris, S., Haile, A., Johnsen, J., Teramura, G., Nelson, K., 2015. Red blood cell antigen genotype analysis for 9087 Asian, Asian American, and Native American blood donors. Transfusion 55, 2369–2375. https://doi.org/10.1111/trf.13163.

Figueroa, D., 2013. The Diego blood group system: a review. Immunohematology 29, 73–81.

Flôres, M.A., Visentainer, J.E., Guelsin, G.A., Fracasso, A.S., de Melo, F.C., Hashimoto, M. N., Sell, A.M., 2014. Rh, Kell, Duffy, Kidd and Diego blood group system polymorphism in Brazilian Japanese descendants. Transfus. Apher. Sci. 50, 123–128. https://doi.org/10.1016/j.transci.2013.09.014.

1000 Genomes Project Consortium. A global reference for human genetic variation. Nature **2015**, 526(7571), 68–74, doi:10.1038/nature15393.

Gong, T., Hong, Y., Wang, N., Fu, X., Zhou, C., 2014. Validation of a blood group genotyping method based on high-resolution melting curve analysis. Immunohematology 30 (4), 161–165.

Halawani, A.J., Arjan, A.H.A.B.O., Rh,, 2021. and KEL1 antigens, phenotypes and haplotypes in Southwestern Saudi Arabia. Clin. Lab. 67 (2), 344–348. https://doi.org/10.7754/clin.lab.2020.200633.

Halawani, A.J.; Habibullah, M.M.; Dobie, G.; Alhazmi, A.; Bantun, F.; Nahari, Dawmary, I,; M.H.; Abu-Tawil, H.I. Frequencies of MNS blood group antigens and phenotypes in southwestern Saudi Arabia. Int J Gen Med 2021. 9315–9319, doi:10.2147/ijgm.s344826.

Halawani, A.J., Saboor, M., Abu-Tawil, H.I., Mahzari, A.A., Mansor, A.S., Bantun, F., 2021. Prevalence of Duffy blood group antigens and phenotypes among Saudi blood donors in Southwestern Saudi Arabia. Clin. Lab. 67 (1), 173–177. https://doi.org/10.7754/clin.lab.2020.200505.

Halawani, A.J., Saboor, M., Abu-Tawil, H.I., Alhazmy, A.Y., Mashlawi, W.Q., Bantun, F., Mansor, A.S., 2022. The frequencies of Kidd blood group antigens and phenotypes among Saudi blood donors in Southwestern Saudi Arabia. Saudi J Biol Sci 29 (1), 251–254. https://doi.org/10.1016/j.sjbs.2021.08.081.

Halawani, A.J., Mansor, A.S., Assaggaf, H.M., Almasmoum, H.A., Abu-Tawil, H.I., Dobie, G., Alsharif, K.F., Habibullah, M.M., 2022. Investigation of Dombrock Blood Group Alleles and Genotypes among Saudi Blood Donors in Southwestern Saudi Arabia. Genes 13, 1079. https://doi.org/10.3390/genes13061079.

Halawani, A.J., Mobarki, A.A., Arjan, A.H., Saboor, M., Hamali, H.A., Dobie, G., Alsharif, K.F., 2022. Red Cell Alloimmunization and Autoimmunization Among Sickle Cell Disease and Thalassemia Patients in Jazan Province. Saudi Arabia. Int J Gen Med 15, 4093–4100. https://doi.org/10.2147/IJGM.S360320.

Halawani, A.J., Abu-Tawil, H.I., Dawood, Y.M., Ageeli, M.A., Hamzi, E.A., Muyidi, F.I., Kabrah, S.M., Alnadawi, H., Almehmadi, M., Alnafai, A., Alzubaidi, A., Chandika, R. M., Alhaj, H.O., 2023. The Prevalence of Lewis, Lutheran, and P1 Antigens and Phenotypes in Southwestern Saudi Arabia. Clin. Lab. 69, 2496–2500. https://doi.org/10.7754/clin.lab.2023.230609.

International Society of Blood Transfusion. Red cell immunogenetics and blood group terminology [Online]. 2023. Available at: http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology/ [Accessed 19 December 2023].

Issitt, P.D., Anstee, D.J., 1998. Applied blood group serology, 4th ed.;. Montgomery Scientific Publications:, Durham, NC, p. 584.

Kim, D.A., Kim, T.Y., Choi, T.Y., 1999. Genotyping of Diego blood group system by use of polymerase chain reaction and Nae I restriction enzyme. Korean J Clin Pathol 19, 246–251.

Layrisse, M,; Arends, T., Dominguez, S.R., 1955. Nuevo grupo sanguíneo encontrado en descendientes de Indios. Acta Med Venez 3, 132–138.

Nathalang, O., Panichrum, P., Intharanut, K., Thattanon, P., Nathalang, S., 2016. Distribution of DI*A and DI*B Allele Frequencies and Comparisons among Central Thai and Other Populations. PLoS One 20. https://doi.org/10.1371/journal.pone.0165134.

Novaretti, M.C., Ruiz, A.S., Dorlhiac-Llacer, P.E., Chamone, D.A., 2010. Application of real-time PCR and melting curve analysis in rapid Diego blood group genotyping. Immunohematology 26, 66–70.

Reid, M.E., Lomas-Francis, C., Olsson, M.L., 2012. DI - Diego Blood Group System. In: The Blood Group Antigen FactsBook, 3rd ed.;. Academic Press, Oxford, UK, pp. 383–414. https://doi.org/10.1016/B978-0-12-415849-8.00012-0.

Saboor, M., Zehra, A., Hamali, H.A., Halawani, A.J., Mobarki, A.A., Madkhali, A.M., Abdullah, S., 2020. Prevalence of A2 and A2B subgroups and anti-A1 antibody in blood donors in Jazan, Saudi Arabia. Int. J. Gen. Med. 7, 787–790. https://doi.org/10.2147/IJGM.S272698.

Schofield, A.E., Martin, P.G., Spillett, D., Tanner, M.J., 1994. The structure of the human red blood cell anion exchanger (EPB3, AE1, band 3) gene. Blood 84, 2000–2012.

Telen, M.J., 1995. Erythrocyte blood group antigens: not so simple after all. Blood 85, 299–306.

Thompson, P.R., Childers, D.M., Hatcher, D.E., 1967. Anti-Dib—first and second examples. Vox Sang. 13, 314–318.

Touinssi, M., Chiaroni, J., Degioanni, A., Granier, T., Dutour, O., Bailly, P., Bauduer, F., 2008. DNA-based typing of Kell, Kidd, MNS, Dombrock, Colton, and Yt blood group systems in the French Basques. Am. J. Hum. Biol. 20 (3), 308–311.

Wu, G.G., Su, Y.Q., Yu, Q., Jin, S.Z., Zhao, T.M., 2002. Development of a DNA-based genotyping method for the Diego blood group system. Transfusion 42, 1553–1556.

Ye, L., Zhang, J., Wang, C., Yang, Q., Han, S., Zhu, Z., 2015. Performance of a microarray-based genotyping system for red cell and platelet antigens in China. Blood Transfus. 13, 690–693. https://doi.org/10.2450/2015.0?301-14.