Journal of Oncology Research and Therapy
Osman AE, et al. Oncol Res Ther 9: 10201.
www.doi.org/10.29011/2574-710X.10201
www.gavinpublishers.com

OPEN ACCESS
GAVIN PUBLISHERS

Opinion article

Molecular Genotype of the ABO Blood Group System among Saudi Population

Awad Elsid Osman[1*], Abdullah Aljurryyan[1], Ahmed Alyami[1], Haya Aljurayb[1], Alyazeed Alsaif[1] and Asim Ali Elbagir[2]

[1]Pathology and Clinical Laboratory Management Department, King Fahad Medical City, Riyadh, Saudi Arabia [2]King Salman Specialist Hospital, Hail, Saudi Arabia

***Corresponding Author**: Awad Elsid Osman, Pathology and Clinical Laboratory Management Department, King, Fahad Medical City, Riyadh, Saudi Arabia

Citation: Osman AE, Aljurryyan A, Alyami A, Aljurayb H, Alsaif H, et al. (2024) Molecular Genotype of the ABO Blood Group System among Saudi Population. J Oncol Res Ther 9: 10201. DOI: 10.29011/2574-710X.10201

Received Date: 21 February, 2024; **Accepted Date:** 26 February, 2024; **Published Date**: 29 February, 2024

Summary

Introduction: The ABO is still the most important blood group system in transfusion and transplantation therapy. ABH antigens are the product of gene combinations at three different loci (ABO, Hh, and Se) and produce certain glycosyltransferases for oligosaccharide precursor chain with monosaccharides at specified linkages, where the terminal sugar influences antigen specificity. Exons 6 and 7 are the longest coding region providing the catalytic domain for the ABO glycosyltransferases. The substitution of amino acid within exons 6 and 7 coding regions of ABO glycosyltransferases is responsible for ABO groups/ subgroups determination.

Objectives: our objective is to identify the presence of different ABO alleles in a sample of the Saudi population, establish a reference for ABO alleles data utilizing sequence-based typing (SBT), and identify possible linkage with specific ABO genotypes.

Materials & Methods: We recruited 96 healthy normal Saudi individuals with known A, B, AB and O blood groups. Using the SBT Sanger sequencing method for genotyping, we focused on 13 locations within the coding regions in exon six and exon seven. DNA sequences for these exons were amplified using polymerase chain reaction (PCR), implementing specific primers covering interested parts.

Results: we tested each polymorphic site for HWE conformation (p-value <0.05). No conformation was observed in 261 and 526 locations in the A blood group; 297, 526, 703, 796 and 803 in the B blood group; 261, 657 and 703 in the AB blood; and only one site in O blood group 803. Also, we identified the polymorphic sequence that defined the A, B, AB and O blood groups.

Conclusion: we identified the DNA sequence variations for exons 6 and 7 in this Saudi sample. Our genetic result showed correspondence with the initial phenotype result. Also, we demonstrated the HWE among the tested DNA sequence.

Introduction

The ABO blood group has been described as one of the most important systems in transfusion and transplantation practice. Antibodies in the ABO system are produced as naturally occurring antibodies to non-self ABO antigens due to a result of individual exposure to substances present in nature [1], individuals greater than six months old have clinically significant anti-A /B antibodies or both. They may result in an immediate haemolytic transfusion reaction if they receive an ABO-incompatible blood. The frequency distribution for ABO groups depend on the population's ethnic background; however, O and A groups are the most frequent in human, whereas the lowest reported group was AB [2].

ABH antigens are generated by the combination of three different genes (ABO, Hh, and Se) that create glycosyltransferases to add certain monosaccharides at precise linkages to oligosaccharide precursor chains, with the terminal sugar determining antigen specificity [3]. The antigens A, B, and H are all formed from the same basic precursor material (paragloboside or glycan), to which sugars are linked in response to a transferase enzyme elicited by an inherited gene [4].

The H antigen is a product of inheritance from the H gene and is characterized as an O blood group in which the precursor for A and B antigens is made. The H(FUT1) and Se (FUT2) genes are both found on chromosome 19q13.3, while the ABO gene is located on chromosome 9q34.2 [5,6]. The fucosyltransferase gene H(FUT1) encodes a fucosyltransferase expressed on red blood cells (RBCs) and adds fucose to type 2 glycoproteins in a (1,2) link to create the H antigen. Se (FUT2) is a fucosyltransferase that is expressed on epithelial cells and adds fucose to type 1 glycoprotein chains in the ratio (1,2) [7]. The ABH antigens are synthesized in low quantities on the surfaces of RBCs during early fetal life and subsequently reach the maximum development capacity by 2 to 4 years of age. Race, genetic interaction, and illness conditions may influence the expression of ABH.

The ABO gene is divided into seven exons ranging between 28 and 688 bp, exons 6 and 7 are the longest and contain the catalytic domain for ABO glycosyltransferases and predominantly affect the amino acid changes [8-11]

ABO blood group antigens are expressed on other body cells such as platelets, vascular endothelial cells, mucus secretions, and epithelial tissues contributing to elevating the risk of diseases [12]. ABO blood group studies have shown great interest in health and disease, significant association between SNPs present within the glycosyltransferase gene and the risk of pancreatic cancer was confirmed [13,14]. Also, Blood group antigens modify the innate immune response by enhancing the intracellular uptake, adhesion molecules, or signal transduction that affects the infection course in the host body [15]

A Saudi study by Mohamed et al. used a pre-designed multiplex polymerase chain reaction and agarose gel electrophoresis for to detect ABO blood groups. A cohort study by Alzahrani, et al. used in-house molecular techniques to genotype the ABO and Rhesus blood for potential Saudi stem cell donors.

In this study, we aimed to characterize the ABO blood groups in a Saudi sample utilizing the sequence-based typing (SBT) method. The purpose was to identify the single nucleotide polymorphisms (SNPs) within exons 6 and 7 of the ABO gene and constitute reference control data for future genetic disease association studies.

Material and methods

We recruited 96 healthy Saudi individuals for this genetics experimental study with known ABO group phenotypes, as shown in Table 1. IRB clearance is approved by the IRB committee at King Fahad Medical City (KFMC). Whole blood samples were collected in EDTA and the informed consent was obtained. We used the MagNa Pure Compact instrument (Roche Diagnostics) for DNA extraction. DNA quality and quantity were measured using Nanodrop® 2000c spectrophotometer (Thermo Fisher Scientific). Primer pairs mol-46/mol-57 and mol-71/mol-101, as described by Yu et al. [18], were utilized to amplify the sequences of interest in exons 6 (251 bp) and 7 (843 bp) and the PRC products were visualized in 3% gel electrophoresis. For the PCR reaction, we used 5 µL of AmpliTaq Gold™ 360 Master Mix (Thermofisher), 1 µL RNase-Free Distilled Water, 3 µL pooled PCR primers (forward and reverse) and 1 µL DNA template (10ng). Amplification is carried out in Veriti™ 96-Well Fast Thermal Cycler using the following program: 1 cycle of 95°C for 10 min; then 10 cycles of 94°C for 60 s, 63°C for 90 s, and 72°C for 60 s and more 25 cycles composed of 94°C for 60 s, 61°C for 90 s and 72°C for 60 s followed by a final cycle of 72°C for 10 min. We treated amplicons with ExoSAP-IT and proceeded into the second stage for the genotyping reaction (PCR sequencing reaction) using 2 µl of BigTerminatorator v3.1, 1 µl of 5x sequencing buffer, 3 µl of deionized water, and 1 µl forward primer (3.2 µM) or reverse primer (3.2 µM) and 3 µl of clean purified PCR product (PCR product+ExoSAP-IT). The following program: 96°C for 7 min, 25 cycles composed of 96°C for 10 s, 58°C for 5 s and 72°C for 4 min, then hold at 4°C was used. SAM/BigDyeX terminator to purify sequencing reactions was used. The Genetic Analyser 3130xl was used for data collection.

We implemented SNPstat software ((https://www.snpstats.net/start.htm) to detect Hardy-Weinberg equilibrium (HWE) for the expected and observed distribution of polymorphic sites and the Linkage disequilibrium (LD) tests, including D statistic, D' statistic, r statistic was also calculated.

Citation: Osman AE, Aljurryyan A, Alyami A, Aljurayb H, Alsaif H, et al. (2024) Molecular Genotype of the ABO Blood Group System among Saudi Population. J Oncol Res Ther 9: 10201. DOI: 10.29011/2574-710X.10201

Results

Based on the serological phenotypes, we have chosen A, B, AB and O blood groups considering male and female representation, as shown in Table 1. Thirteen different locations, including 261 (A>AG) and 267 (C>CT) in exon 6 and 526 (C>CG), 646 (T>TA), 657 (C>CT), 681(G>GA), 703 (G>GA), (771 (C>CT), 796 (C>CA), 803 (G>GC), 829 (G>GA), 1023 (C>AC) and 1061 (del/C) in exon 7 were demonstrated as shown in Table 2.

For the A blood group has shown conformation to HWE (p-value <0.05) except for 261 and 526 locations. The number of polymorphisms not conforming to HWE was increased in the B blood group individuals including 297, 526, 703, 796 and 803 sites. Additionally, three polymorphisms among the AB blood group individuals 261, 657 and 703 also were not observed conforming to HWE and only 803 (G>GC) site in the O blood group not conforming to HWE.

Among the A blood group, we observed more than 90% of individuals were homozygous within the sites of the protein-coding region at exon 6 and exon 7 including 297A, 657C, 703G, 796C, 803G, 1023C, and 1061C. The 261(A>AG) position at exon 6, demonstrated 100% heterozygous (wt/G) in the female group, while only 75% were heterozygous in males and 25% were homozygous (G/G). At positions 526C<CG, 646T>TA, 681 G>GA, 771 C>CT and 829 G>GA, we observed multiple polymorphisms in both groups but no significant differences between the male and female in the genotype frequencies.

For the B blood group people, we observed monomorphic genotypes among the male group at 297 (A/A), 526 (C/G), 703 (G/A), 796 (C/A), 803 (G/C) and 1023 (C/C) locations and only 1061 (C/C) in the female group. Variable genotypes (homozygous/heterozygous) in other locations for exon 6 and exon 7 were detected, but there were no significant differences between males and females when frequency distributions were compared. For the AB blood group, 10/13 locations showed monomorphic genotypes in the female group, including 297 (G/A), 526 (G/G), 646 (T/T), 657 (C/C), 681(G/G), 703 (G/A), 771(C/C), 829 (G/G), 1023(C/C) and 1061(C/C), whereas, only 3/13 location have shown monomorphic genotypes in male. Other polymorphic sites were identified in both male and female groups, but there were no significant differences between the two groups in terms of genotype frequency distributions.

O blood group genotypes

For the O blood group, similar genotypes in males and females were demonstrated in three genetic sites, including 261(del/del), 1023(C/C) and 1061(C/C), except in the 261 position, one male was observed with del/G genotype. Four more monomorphic sites were detected among the male group but not in females including 526 (C/C), 657 (C/C), 703 (G/G) and 796 (C/C). No statistical significance was observed between males and females in regional sites with polymorphisms.

Blood Group	Male n (%)	Female n (%)	Total
A	11 (48)	12 (52)	23
B	13 (54)	11 (46)	24
AB	14 (58%)	10 (42)	24
O	12 (50)	12 (50)	24
Total	50	45	95

Table 1: shows the number and phenotypes on the gender base.

ISSN: 2574-710X

Table 2, genotype for A, B, AB and O blood groups in Saudi

Location	Genotype	A blood group		B blood group		AB blood group		O blood group	
		Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
261	Del/Del							11 (91.7)	12 (100)
	G/G	3 (25)		10 (77)	10 (90.9)	4 (33.3)	3 (33.3)		
	Del/G	9 (75)	12 (100)	3 (23)	1 (9.1)	8 (66.7)	6 (66.7)	1 (8.3)	
297	A/A	12 (100)	12 (100)					3 (25)	1 (8.3)
	G/A			13 (100)	8 (72.7)	12 (100)	9 (100)	7 (58.3)	8 (66.7)
	G/G				3 (27.3)			2 (16.7)	3 (25)
526	G/G	3 (25)	2 (16.7)						
	C/G		1 (8.3)	13 (100)	9 (81.8)	4 (33.3)			1 (9.1)
	C/C	9 (75)	9 (75)		2 (18.2)	8 (66.7)	9 (100)	12 (100)	11 (90.9)
646	T/T	5 (41.6)	9 (75)	7 (53.8)	6 (54.5)	7 (58.3)	9 (100)	2 (16.7)	
	T/A	7 (58.4)	3 (25)	6 (46.2)	5 (45.5)	5 (41.7)		7 (58.3)	7 (58.3)
	A/A							3 (25)	5 (41.7)
657	C/C	12 (100)	11 (91.6)	1 (7.7)		7 (58.3)	9 (100)	12 (100)	11 (91.7)
	C/T		1 (8.4)	12 (92.3)	9 (81.8)	5 (41.7)			1 (8.3)
	T/T				2 (18.2)				
681	G/A	7 (58.3)	3 (25)	5 (41.7)	4 (36.4)	3 (25)		3 (25)	8 (66.7)
	G/G	5 (41.7)	9 (75)	8 (58.3)	7 (63.6)	7 (58.3)	9 (100)		
	A/A					2 (16.7)		9 (75)	4 (33.3)
703	G/A		1 (8.4)	13 (100)	9 (81.8)	5 (41.7)	9 (100)		1 (9.1)
	G/G	12 (100)	11 (91.6)			7 (58.3)		12 (100)	11 (90.9)
	A/A				2 (18.2)				
771	C/C	5 (41.6)	9 (75)	6 (46.2)	6 (54.5)	8 (72.7)	9 (100)	3 (25)	
	C/T	7 (58.4)	3 (25)	7 (53.8)	5 (45.5)	3 (27.3)		7 (58.3)	8 (66.7)
	T/T							2 (16.7)	4 (33.3)
796	C/C	12 (100)	11 (91.6)			7 (58.3)		12 (100)	11 (91.9)
	C/A		1 (8.4)	13 (100)	10 (90.9)	5 (41.7)	6 (66.7)		1 (8.3)
	A/A				1 (9.1)		3 (33.3)		
803	G/G	12 (100)	11 (91.6)				1 (11.1)	3 (25)	4 (33.3)
	G/C		1 (8.4)	13 (100)	10 (90.9)	5 (41.7)	8 (88.9)		1 (8.3)
	C/C				1 (9.1)	7 (58.3)		9 (75)	7 (58.4)
829	G/G	6 (50)	5 (41.6)	8 (61.5)	5 (50)	8 (66.7)	9 (100)	3 (25)	
	G/A	6 (50)	7 (58.4)	4 (30.8)	5 (50)	3 (25)		6 (50)	7 (58.3)
	A/A			1 (7.7)	1 (8.3)	1 (8.3)		3 (25)	5 (41.7)
1023	C/C	11 (91.6)	12 (100)	13 (100)	12 (100)	12 (100)	9 (100)	12 (100)	12 (100)
	C/A	1 (8.4)							
	A/A								
1061	C/C	12 (100)	12 (100)	12 (92.3)	11 (100)	12 (100)	9 (100)	12 (100)	12 (100)
	C/G			1 (7.7)					

ISSN: 2574-710X

Discussion

There are three main alleles for the ABO blood group genotype, A(A101), B (B101) and O (O101). One single base deletion at exon 6 causes single nucleotide substitutions that determine the variations among these main different blood groups [19], Yamamoto et al. were the first to utilize allele-specific variation and restriction fragment length polymorphism (RFLP) methods for molecular ABO genotype, and accordingly, they were able to differentiation between the homozygous AA and the heterozygous AO and between the homozygous BB and the heterozygous BO blood groups [20].

Currently, we studied known serological blood groups phenotype (A, B, AB and O) to characterize and identify the genetic variations within exons 6 and 7 utilizing SBT (Sanger method). The A1 blood group allele can be associated with the O allele in a heterozygous form or with the other alleles of A blood groups, including A101, A102, A103, A104 or A112. The A101 allele varies from the O101 allele at position 261(G) that corresponds to codon number 87 of the A transferase enzyme. The deletion of 261G is found in the O blood group and can cause the downstream codon's premature ending and consequently restrict the protein's elongation to only 116 amino acids length that deficient in the C-terminal catalytic domain [20,21].

The nucleotides 297A, 526C, 646T, 657C, 681G, 703G, 771C, 796C, 803G and 829G are associated with the A101 allele found in the homozygous individuals for the A101 genotype [22]. Based on our result, we identified multiple polymorphic regions in each blood group, and we observed 41.7% of females and 46.2% of males are heterozygous for the 646T/A genotype; this may indicate the presence of A and O blood groups in the same individuals. The heterozygosity could be confirmed by the presence of the genotypes in other locations that are significant to the O allele, including 681 (A), 771 (T) and 829 (A) and also by the presence of the del/261 genotype at 261 locations. On the other hand, individuals having G/G genotype at 261 positions were not observed carrying the 681 (A), 771 (T) and 829 (A) genotypes [22].

The A blood group of alleles containing 297G, 526G, 657T, 703A, 796A and 803C SNPs, are associated with B blood group characteristics; our data have shown at least one allele in each tested individual positive for this group and the homozygosity of 261 (G/G) corresponds with the possibility of a B/B genotype in those tested individuals. The presence of heterozygosity (Del/G) genotype at 261 locations was found in three people within the B blood group, indicating the possibility of BO heterogeneity in these individuals. Additionally, the nucleotides 297, 526, 657, 703, 796 and 803 resulted in 4 amino acids changes (Arginine176Glycine, Glycine235Serine, Leucine266Methionine, Glycine268Alanine) during the polypeptide formation are used to differentiate between A101 and B101 blood groups [23,24]. Accordingly, our observation demonstrated clear variations in A and B blood group genotypes in this study. 297G, 526G, 796A and 803C are the main characteristic of B(A)01 blood group while the presence of 297A, 526C, 796C and 803C found to characterize the cis-AB01 blood group. Cis-AB contains A and weak B antigens and with a high presence of H material on the red blood cells surface [25].

Both alleles in the O blood group in this study had deleted nucleotide (Del/Del) at position 261 except in one person who showed a heterozygous genotype of Del/G at 261 sites that correspond with Hosseini-Maaf et al. finding [26]. The predominance of Del/Del genotypes among the O blood group confirms the serological result for this group. Non-deleted 261DelG is found to be associated with the O303, O304, O301 and O302 alleles in the O blood group, which is one of the essential criteria utilized to differentiate these alleles from the O101 and O201 ones. So, the heterozygous Del/G genotype observed in one person in the O blood group may indicate the presence of one of these alleles (O303, O304, O301 or O302).

Conclusion

In this study, we identified the genetic sequence for exons 6 and 7 in known serological ABO blood group samples of Saudi individuals. Our genetic result showed correspondence with the initial phenotype result. Also, we demonstrated the HWE among the tested DNA sequence. This data could be used as a control group for potential genetic disease association studies related to infection, cancer or immunological diseases in the Saudi population.

References

1. C. M. Westhoff, J. R. Storry, and B. H. Shaz (2018) "Human Blood Group Antigens and Antibodies," in Hematology: Basic Principles and Practice.
2. D. D. Farhud and M. Z. Yeganeh (2023) "A brief history of human blood groups," Iran. J. Public Health.
3. L. C. de Mattos (2016) "Structural diversity and biological importance of ABO, H, Lewis and secretor histo-blood group carbohydrates," Revista Brasileira de Hematologia e Hemoterapia.
4. P. Stanley and R. D. Cummings (2015) Structures Common to Different Glycans.
5. B. Michalewska et al., (2018) "FUT1 mutations responsible for the H-deficient phenotype in the Polish population, including the first example of an abolished start Codon," Blood Transfus.
6. W. Liang, F. Cai, L. Yang, Z. Zhang, and Z. Wang (2018) "Four non-functional FUT1 alleles were identified in seven Chinese individuals with para-Bombay phenotypes," Iran. J. Public Health.
7. D. O. Donghaile et al., (2009) "Defining the Molecular Mechanisms Responsible for the ABO High Expresser Phenotype.," Blood.
8. M. A. Chester and M. L. Olsson (2001) "The ABO blood group gene: A locus of considerable genetic diversity," Transfusion Medicine Reviews.

9. J. M. Zhang and J. An, (2007) "Cytokines, inflammation, and pain," International Anesthesiology Clinics, vol. 45.

10. M. Yamamoto, E. Cid, and F. Yamamoto (2017) "ABO blood group A transferases catalyze the biosynthesis of FORS blood group FORS1 antigen upon deletion of exon 3 or 4," Blood Adv.

11. "Genomic analysis of clinical samples with serologic ABO blood grouping discrepancies: Identification of 15 novel A and B subgroup allelesM.L. Olsson, N.M. Irshaid, B. Hosseini-Maaf, et al. Blood 98:1585–1593, 2001," Transfus. Med. Rev., 2002,

12. L. Cooling (2015) "Blood groups in infection and host susceptibility," Clin. Microbiol. Rev.

13. H. E. Groot, L. E. V. Sierra, M. A. Said, E. Lipsic, J. C. Karper, et al. (2020) "Genetically determined ABO blood group and its associations with health and disease," Arterioscler. Thromb. Vasc. Biol.

14. Y. Li, L. Liu, Y. Huang, H. Zheng, and L. Li (2020) "Association of ABO polymorphisms and pancreatic Cancer/ Cardiocerebrovascular disease: A meta-Analysis," BMC Med. Genet.

15. J. Liu, S. Zhang, M. Liu, Q. Wang, H. Shen, and Y. Zhang (2018) "Distribution of ABO/Rh blood groups and their association with hepatitis B virus infection in 3.8 million Chinese adults: A population-based cross-sectional study," J. Viral Hepat.

16. A. B. O. Mohamed et al. (2016) "Allelic variance among ABO blood group genotypes in a population from the western region of Saudi Arabia," Blood Res.

17. M. Alzahrani, D. Jawdat, A. Alaskar, N. Cereb, and A. H. Hajeer (2018) "ABO and Rh blood group genotypes in a cohort of Saudi stem cell donors," Int. J. Immunogenet.

18. Q. Yu, Z. H. Deng, G. G. Wu, Y. L. Lian, and Y. Q. Su (2005) "Molecular genetic analysis for a novel Ael allele of the ABO blood group system," J. Hum. Genet.

19. G. Daniels and I. Bromilow (2010) Essential Guide to Blood Groups.

20. M. Cohen, N. Hurtado-Ziola, and A. Varki (2009) "ABO blood group glycans modulate sialic acid recognition on erythrocytes," Blood.

21. M. Yamamoto, M. C. Tarasco, E. Cid, H. Kobayashi, and F. Yamamoto (2019) "ABO blood group A transferase and its codon 69 substitution enzymes synthesize FORS1 antigen of FORS blood group system," Sci. Rep: 9.

22. F. Yamamoto (2022) "A historical overview of advances in molecular genetic/genomic studies of the ABO blood group system," Glycoconjugate Journal 39.

23. B. R. Curtis, J. T. Edwards, M. J. Hessner, J. P. Klein, and R. H. Aster (2000) "Blood group A and B antigens are strongly expressed on platelets of some individuals," Blood.

24. B. Hosseini-Maaf, Å. Hellberg, M. A. Chester, and M. L. Olsson (2007) "An extensive polymerase chain reaction-allele-specific polymorphism strategy for clinical ABO blood group genotyping that avoids potential errors caused by null, subgroup, and hybrid alleles," Transfusion.

25. M. H. Yazer, M. L. Olsson, and M. M. Palcic (2006) "The cis-AB Blood Group Phenotype: Fundamental Lessons in Glycobiology," Transfusion Medicine Reviews: 20.

26. B. Hosseini-Maaf, Å. Hellberg, M. J. Rodrigues, M. A. Chester, and M. L. Olsson (2003) "ABO exon and intron analysis in individuals with the AweakB phenotype reveals a novel Olv-A2 hybrid allele that causes four missense mutations in the A transferase," BMC Genet: 4.