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Phenotypic and Allelic Distribution of the ABO and Rhesus Blood Groups among students at Hawassa University, Ethiopia

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ABSTRACT

A prior information on the distribution of ABO and Rh groups is important for management of blood bank and transfusion, genetic counseling, anthropological studies, to study the association of blood groups and diet; to investigate the association between blood and diseases. This study aimed to determine the frequency of ABO and Rh bloods and investigate gene diversity at both loci among students in Ethiopia. A descriptive cross-sectional survey was employed involving randomly selected two thousand thirty nine (2039) university students (1054 males and 985 females) with an age range of 18–29 years. Blood groups were determined based on agglutination reaction. The most common blood group was found to be O (42.47%), followed by A (27.86%), B (21.87%), and AB (7.80 %). The frequency of Rh+ and Rh- were 90.88% and 9.12 %, respectively. The combined blood types showed O+, A+, B+ and AB+ were: 38.60 %, 25.20%, 20.10% and 7.00%, respectively. Slightly different distribution pattern of ABO blood group was observed among females from Amhara region (O> B> A>AB). The distribution of ABO phenotypes from Addis Ababa and Amhara did not differ significantly from those expected under the Hardy Weinberg Equilibrium. A high level of gene diversity was observed for both loci. In general, the O blood type is most frequent and followed by A, B and AB. A similar pattern of distribution of the ABO and Rh blood groups was found in male and female study subjects. The present study will generate a baseline data that could be used in blood bank management and transfusion, genetic counseling, population genetic and anthropological studies, and for disease management.

INTRODUCTION

The knowledge on the distribution of ABO and Rh groups is important for the management of blood bank and transfusion, genetic counseling, population genetics, and anthropological studies (Liu *et al*., 2017; Canizalez-Román *et al*., 2018) and to study the association of blood groups and diet, to relate the association between blood and diseases (Puryear, 2017). Individuals with the O blood type are thought to be resistant to viral disease (Zhao *et al*., 2021) but susceptible to some bacterial infections (Harris *et al*., 2005) and Hepatitis B virus (Jing *et al*., 2020). People with the A blood group have a higher risk, whereas people with blood group O have a

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lower risk for SARS-Cov-2 infection and COVID-19 severity (Zhao *et al*., 2021).

The frequencies of ABO and Rh blood groups vary with ethnicity, geographical area, race, population movements, natural selection and genetic drift. In the USA, and Mexico the O type is the most frequent, followed by A, B and AB (Garratty*et al*., 2004;Canizalez-Román *et al*., 2018). Nevertheless, in China (Liu *et al*., 2017), type A is the most common, followed by O, B and AB. In southeast Asia, A and B blood groups were interchangeably taking the most common blood group place, while AB was the least common (Dewan, 2015). Type O is the most frequent whereas AB is less common in most African countries (Ndoula *et al*., 2014; Anifowoshe *et al*., 2017). The frequency of Rh- blood is less or rare in African and Asian countries (Anifowoshe *et al*., 2017; Liu *et al*., 2017). The available limited data indicate that the O blood is more frequent but AB is least common in Ethiopia (Golassa *et al*., 2017; Fufa and Debelo, 2019).

To date in Ethiopia, there are limited works done on the distribution of ABO and Rh blood groups and the most are small scale in terms of sample size and regional coverage (Golassa *et al*., 2017; Fufa and Debelo, 2019). In this work, however, the subjects were originated from almost all regions in the country and relatively larger sample size of the study subjects involved in the study. Therefore, this study aimed to determine the distribution of ABO and Rh bloods and investigate gene diversity of both loci among students in Ethiopia. It is anticipated that the data may contribute to strategies of supply and demand of blood products in transfusion services countrywide and could have implications in investigating susceptibility to various conditions known to be associated with blood groups.

MATERIALS AND METHODS

Study area and study subjects

The study was conducted in four campuses (College of Agriculture, Main Campus, Institute of Technology and; College of Medicine and Health Sciences) of Hawassa University. It is assumed that the regional and ethnic diversity of the Ethiopian population could be represented in the university student population. In Ethiopia students from different regions are randomly allocated to federal universities in the country, we therefore, believe that, student populations in Hawassa University could represent the regional and ethnic diversity in Ethiopia. Ethiopia has eleven regional States and two city administrations.

A descriptive cross-sectional survey was employed involving randomly selected two thousand thirty nine (2039) students (1054 males and 985 females) with an age range of 18–29 years. The selected study subjects originally came from eight regional states (Afar, Amhara, Benishangul Gumuz, Gambela, Oromia, Southern Nation Nationalities and People State (SNNPRS), Somali and Tigray) and two city administrations (Addis Ababa (AA) and Dire Dawa). The inclusion criteria were: Ethiopian students who are above 18 years old and willing to participate in this study.

Blood group determination

The ABO and Rh blood group testing was done in the Hawassa University students’ clinics, using a commercially available kit for blood grouping (Human Diagnostic, Germany).

Briefly, for ABO and Rh blood group tests, a drop of anti-A, anti-B and anti-D human sera was added into 5% red blood cell suspension in normal saline in test tubes and the mixture was stirred with glass rods. And blood groups were determined based on agglutination reaction.

Allelic frequency and gene diversity analysis

The frequencies of the I^A allele (p_1), I^B allele (p_2) and I^O allele (p_3) were calculated based on the extension of Hardy-Weinberg Equilibrium (HWE) for multiple alleles with two co-dominant allele and one recessive allele (Hamilton, 2009). Genotypic frequencies were calculated under HWE assumptions as $[(p_1 + p_2 + p_3)^2 = p_1^2 + p_2^2 + p_3^2 + 2\,p_1p_2 + 2\,p_1p_3 + 2\,p_2\,p_3 = 1]$as p_1^2 (I^AI^A) + 2 p_1p_3 (I^AI^O) + p_2^2 (I^BI^B) + 2 p_2 p_3 (I^BI^O) + 2 p_1p_2 (I^AI^B) + p_3^2 (I^OI^O). Nevertheless, the Rh system alleles "D" and "d" were assigned q1 and q2, respectively, and their frequencies were also calculated using HWE for two allele system $[(q_1 + q_2)^2 = q_1^2 + 2\,q_1q_2 + q_2^2 = 1]$ as q_1^2(DD), 2 q_1q_2 *(Dd)*, q_2^2 *(dd)*. Gene diversity (He) was analyzed according to Nei (1973). Blood group phenotypic frequencies were expressed in percentage and allele frequencies estimated under the assumption of Hardy–Weinberg Equilibrium (HWE). The Chi - square test was used to compare observed allelic and genotypic frequency distributions of the blood group and Rh antigens to that expected under the HWE (Hamilton, 2009). The level of statistical significance was at p<0.05.

Ethical Approval

The study was conducted after ethical approval of the Institutional Review Board (IRB) of Hawassa University (Ethiopia), College of Natural and Computational Sciences (Ref. No. IRB/203/11; Date: 05/03/2019). Accordingly, the study objectives were explained to students, and written consent for participation in the study was obtained.

RESULTS

Distribution of ABO and Rh blood groups

The frequencies of O, A, B and AB blood types among the participants were: 42.47%, 27.86, 21.87% and 7.80, respectively. The O blood group had the highest frequency while blood group AB had the least frequency (Table1). The study revealed that the ABO blood group pattern was in the order of O > A > B > AB. Statistically, no significant variation was observed in the proportions of the A, B, O and AB blood groups among regions ($\chi^2_{0.05,\,12}$ = 15.055; p < 0.05). But there were slight differences in the frequencies of ABO blood types. In terms of each blood type the highest proportion of the A phenotype (30.12%), the B phenotype (25.47%) and O phenotype (44.04%) was observed in SNNPRS, Amhara and Addis Ababa, respectively (Table1). The proportion of ABO blood antigens significantly different from those expected under Hardy–Weinberg Equilibrium (HWE) ($\chi^2_{0.05,\,1}$ = 10.498; p < 0.05) for the combined data set, Oromia region ($\chi^2_{0.05,\,1}$ = 7.304; p < 0.05), SNNPRS ($\chi^2_{0.05,\,1}$ = 6.027; p < 0.05) and 'Others' ($\chi^2_{0.05,\,1}$ = 4.248; p < 0.05), respectively. However, the distributions in Addis Ababa ($\chi^2_{0.05,\,1}$ = 1.063; p < 0.05) and Amhara ($\chi^2_{0.05,\,1}$= 0.004, p < 0.05) did not deviate from HWE.

Table- 1: Phenotypic frequency of the ABO Blood groups and Rh system based on regions/ towns

Regions	ABO Blood					Rh System	
	N**	Type A (%)	TypeB (%)	TypeAB (%)	TypeO (%)	Rh $^{+}$ (%)	R $^{-}$ (%)
Addis Ababa	234	62(24.50)	52(22.22)	17(7.26)	103(44.04)	202(86.32)	32(13.68)
Amhara	691	185(26.77)	176(25.47)	44(6.37)	286(41.39)	635(91.90)	56(8.10)
Oromia	603	167(27.69)	128(21.23)	53(8.79)	255(42.29)	554(91.87)	49(8.13)
SNNPRS	437	132(30.21)	77(17.62)	36(8.22)	192(43.94)	393(89.93)	44(10.07)
Others *	74	22(28.57)	13(17.57)	9(12.16)	30(40.54)	69(93.24)	5(6.76)
Total	2039	568 (27.86)	446(21.87)	159(7.80)	866(42.47)	1853(90.88)	186(9.12)

*Afar(1,1,0,2/3,1), BenishangulGumz (1,1,1,5/8,0), Dire Dewa (1,2,0,1/4,0), Gambela (1,1,0,1/3,0), Somali (3,2,1,4/7,3)and Tigray(15,6,7,17/44,1). Numbers in the parenthesis are the numbers of individuals with ABO/Rh system, respectively. **sample size.

The frequency of allele O was larger as compared to alleles A or B (p_3>p_1>p_2). A comparable higher level of gene diversity was found in each region for both loci (Table2).

Table- 2: Allelic frequencies and Gene diversity of ABO blood group and Rh systems

Region/City	*ABO allele*			Rh allele		*ABO*	*Rh*	*ABO**				*Rh**			
	P_1	P_2	P_3	q_1	q_2	H_e	H_e	H_S	H_T	D_{ST}	G_{ST}	H_S	H_T	D_{ST}	G_{ST}
AA	0.186	0.16	0.65	0.63	0.37	0.51	0.47								
Amhara	0.182	0.17	0.64	0.76	0.24	0.52	0.37								
Oromia	0.203	0.16	0.63	0.72	0.29	0.53	0.41								
SNNPRS	0.215	0.14	0.65	0.68	0.32	0.52	0.43								
Others	0.238	0.14	0.62	0.74	0.26	0.54	0.39	0.54	0.51	0.04	0.07	0.41	0.42	0.004	0.01

*The figures are for the entire population. H_e:gene diversity in a subpopulation; H_S: average gene diversity within subpopulation; H_T: gene diversity for the entire population; D_{ST}: gene diversity among subpopulation; G_{ST}: Gene differentiation among subpopulation; AA; Addis Ababa; SNNPRS: Southern Nation Nationalities People Regional State

The Rh+ blood group comprised 90.88% for the overall data set (Table 1). The highest frequency of Rh+ (93.00%) was observed for 'other' group and least was for Addis Ababa (86.32%). In Addis Ababa the frequencies of Rh+ and Rh- were 86.32 % and 16.68%, respectively. The largest frequency of q_1 allele and lowest level of gene diversity was observed in Amhara (Table2). The expected gene diversity was highest in Addis Ababa and the lowest from 'others' (Table 2). For the ABO locus, the highest frequency of gene diversity was observed in the 'others' groups (H_e= 0.535) but the lowest was in Addis Ababa (H_e = 0.512). The sex specific allele frequency is similar in male and female participants (Table3). A unique phenotypic (O>B>A>AB) was observed for females in Amhara.

Table- 3: Phenotypic, allelic frequencies and gene diversity of ABO blood based on sexes [Male (M); Female (F)]

Regions	Sexes		ABO blood type				Allelic frequencies*			
		N	A (%)	B (%)	AB (%)	O (%)	p_1	P_2	p_3	H_e
Addis Ababa	M	119	31(26.05)	27(22.68)	7(5.88)	54(45.37)	0.175	0.155	0.670	0.496
	F	115	31(26.95)	25(21.73)	10(8.69)	39(33.91)	0.254	0.220	0.526	0.610
Amhara	M	325	89(27.38)	77(23.69)	22(6.76)	137(42.15)	0.189	0.166	0.645	0.521
	F	366	96(26.22)	99(27.04)	22(6.01)	149(40.71)	0.177	0.182	0.641	0525
Oromia	M	320	86(26.87)	70(21.87)	23(7.18)	141(44.06)	0.188	0.158	0.654	0.512
	F	283	81(28.62)	58(20.49)	30(10.60)	114(40.28)	0.220	0.170	0.610	0.551
SNNPRS	M	249	75(30.12)	37(14.85)	21(8.43)	116(46.58)	0.216	0.124	0.660	0502
	F	188	57(30.31)	40(21.27)	15(7.97)	76(40.42)	0.214	0.159	0.627	0.536
Others	M	39	11(28.20)	7(17.94)	4(10.25)	17(43.58)	0.215	0.153	0.632	0.531
	F	35	12(34.28)	7(20.00)	4(11.42)	12(34.28)	0.263	0.172	0.565	0.585
Total	M	1054	292(27.70)	218(20.68)	78(7.40)	466(44.21)	0.194	0.152	0.654	0.512
	F	985	276(28.02)	228(23.14)	81(8.22)	400(40.60)	0.201	0.190	0.609	0.553

*Sex specific allelic frequencies

AB negative case was not observed from Addis Ababa. The highest frequency for B+ was from Amhara while O+ was highest for Oromia. The O+ was observed more than one third of the population, while AB- was recorded in less than 1% (Table 4).

Table- 4: Phenotypic frequencies of ABO blood types based on the Rh system

Regions/town		Phenotype (%)							
	N*	A+	A-	B+	B-	AB+	AB-	O+	O-
Addis Ababa	234	52(22.22)	10(4.27)	50(21.4)	2(0.85)	17(7.30)	0(0.00)	83(35.47)	20(8.54)
Amhara	691	171(24.70)	14(2.00)	159(23.00)	17(2.5)	40(5.80)	4(0.60)	265(38.40)	21(3.00)
Oromia	603	150(24.9)	17(2.8)	117(19.4)	11(1.80)	48(8.00)	5(0.80)	239(39.40)	16(2.70)
SNNPRS	437	120(27.50)	12(2.70)	71(16.20)	6(1.40)	30(6.90)	6(1.40)	172(39.40)	20(4.60)
Others	74	21(28.37)	1(1.35)	12(16.23)	1(1.35)	8(10.81)	1(1.35)	28(37.33)	2(2.70)
Total	2039	514(25.20)	54(2.60)	409(20.10)	37(1.80)	143(7.00)	16(.80)	787(38.60)	79(3.90)

*N: Sample size (number of students examined)

The proportions of the ABO/Rh blood groups were significantly different among the five regions ($\chi^2_{0.05,\ 28} = 43.033$; $p < 0.05$). There was a clear difference in the frequency distribution of the blood types between males and female subjects among the regions. For instance, the frequency of the A+ and O+ blood type showed a difference between the male and female from Addis Ababa (Table5).

Table- 5: Phenotypic frequencies of ABO blood types based on the Rh system for male (M) and female (F) subjects

Regions/city	Sex	N*	Phenotype (%)							
			A+ (%)	A-(%)	B+ (%)	B-(%)	AB+ (%)	AB-(%)	O+ (%)	O-(%)
Addis Ababa	M	119	25(10.70)	6(2.60)	26(21.84)	1(.84)	7(5.88)	0(0.00)	46(38.65)	8(6.72)
	F	115	27(24.35)	4(3.47)	24(20.86)	1(0.86)	10(8.69)	0(0.00)	37(32.17)	12(10.43)
Amhara	M	325	82(25.23)	7(2.15)	68(20.92)	9(2.76)	21(6.46)	1(0.31)	131(40.31)	6(1.84)
	F	366	89(24.32)	7(1.91)	91(24.86)	8(2.18)	19(5.19)	3(0.81)	134(36.61)	15(4.10)
Oromia	M	320	80(25.00)	6(1.87)	65(20.31)	5(1.56)	20(6.25)	3(0.93)	131(40.93)	10(3.12)
	F	283	70(24.73)	11(3.88)	52(18.37)	6(2.12)	28(9.82)	2(0.63)	108(38.16)	6(2.12)
SNNPRS	M	249	70(28.11)	5(2.01)	35(14.05)	2(0.80)	18(6.12)	3(1.20)	104(41.76)	12(4.81)
	F	188	50(26.59)	7(3.72)	36(19.15)	4(2.13)	12(6.38)	3(1.59)	68(36.17)	8(2.25)
Others	M	41	11(26.82)	0(0.00)	7(17.07)	0(0.00)	4(9.75)	1(2.43)	17(41.46)	1(2.43)
	F	33	10(30.30)	1(3.03)	5(15.15)	1(3.03)	4(12.12)	0(0.00)	11(33.33)	1(3.03)
Total	M	1054	268(25.42)	24(2.27)	201(19.07)	17(1.61)	70(6.64)	8(0.76)	428(40.60)	37(3.51)
	F	985	246(24.97)	30(3.05)	208(21.11)	20(2.03)	73(7.41)	8(0.81)	358(36.34)	42(4.26)

*N: Sample size (number of students examined)

DISCUSSION AND CONCLUSIONS

Distribution of ABO and Rh blood groups

The frequency distribution of ABO blood group varies from race to race, population to population and differs in different geographical areas. This study could serve as an initial countrywide report, as participants from a national university receiving students from all over the country and representing the Ethiopian population. Ethiopia has a rich cultural, linguistic, and ethnic diversity and is home to over 70 different ethnic groups and over 80 living languages (Pagani *et al*., 2012, 2015). Therefore, Ethiopia is an important region for studying how genetic diversity and differentiation correlate with linguistic and cultural diversity. Furthermore, the knowledge on the distribution of ABO and Rh groups is important for management of blood bank and transfusion, genetic counseling, population genetics and anthropological studies and to study the association of blood groups and diet, to relate the association between blood and diseases (Dewan, 2015; Liu *et al*., 2017; Puryear, 2017; Canizalez-Román *et al*., 2018).

The proportion of AB blood was relatively larger compared to previous studies in Ethiopia

(Tesfaye*et al*., 2015; Fufa and Debelo, 2019), this could be due to the wider sampling regions covered in this study (Table1). The distribution pattern of ABO blood group of females from Amhara seems similar to that of the Bengali population in Bangladesh (Dewan, 2015). Golassa *et al*. (2017) found two patterns of ABO Phenotypes O>A>B>AB [Nilotic people] and A>O>B>AB [‘Highlanders] in Gambela, southwestern Ethiopia. The results of this study are similar to that of previous studies in Cameroon (Ndoula *et al*., 2014), Nigeria (Anifowoshe *et al*., 2017), and Mexico (Canizalez-Román *et al*., 2018). However, the distribution differs from reports made in Egypt (Abdelmonem *et al*., 2019), China (Liu *et al*., 2017), and Bangladesh (Dewan, 2015).

Most of the participants in the current study were Rh+ (90.88%), while the rest were Rh- (9.12%). In general, the frequency of Rh- blood is less or rare in African and Asian countries (Liu *et al*., 2017; Abdelmonem *et al*., 2019). Golassa *et al*. (2017), however, reported a relatively higher frequency of Rh- (19.37%) in Gambela, southwestern Ethiopia. The overall frequency of Rh+ in the current study is comparable to Egypt (Abdelmonem *et al*., 2019), whereas lower compared to Cameroon (Ndoula *et al*., 2014), and Nigeria (Anifowoshe *et al*., 2017). The proportion of Rh+ in Addis Ababa is similar to white non-Hispanic in USA (Garratty *et al*., 2004), but large relative to Gambela (Golassa *et al*., 2017). The proportion of Rh- ranged from 7-14%, which is wider relative to studies done in Ethiopia (Tesfaye *et al*., 2015; Fufa and Debelo, 2019). Such a large range in the proportion of Rh- in this study could reflect wider regional coverage – the participants are almost from all the regions of the country.

The proportions of O+, A+, B+ and AB+ were: 38.60%, 25.20%, 20.10% and 7.00%, respectively. There was variation in the distribution of ABO/Rh between regions. The proportion of B+ was higher in the Amhara but O+ was higher in the Oromia (Table 4). The O+ frequency was found to be over one third of the entire population, while AB- was recorded in less than 1% of the study population. Similarly, in Cameroon (Ndoula *et al*., 2014), the O+ blood group is highly predominant, representing about half of the entire population, while AB- is very infrequent.

Allelic and genotypic diversity

The order of the ABO allele frequency was $I^O>I^A> I^B$, and similar to earlier studies (Ndoula *et al*., 2014; Anifowoshe *et al*., 2017),but differs from Bangladesh (Dewan, 2015), and in Egypt (Abdelmonem *et al*., 2019).The frequency of q_1 allele in this study is lower compared to that of Nigeria (Anifowoshe *et al*., 2017). However, a higher level of among subpopulation gene diversity (D_{ST}) and gene differentiation (G_{ST}) was found to be comparable to Mexican populations at both loci (Canizalez-Román *et al*., 2018). The higher level of gene diversity supports the hypothesis that Ethiopia is an important region for studying how genetic diversity and differentiation correlate with linguistic and cultural diversity (Pagani *et al*., 2012, 2015). The distribution of ABO phenotypes for Addis Ababa city and Amhara are not significantly different from those expected under the HWE. If a population is in a HWE the genotypic frequency will remain stable unless the equilibrium is perturbed. That would be a good opportunity for the management of blood banks as the frequencies of the blood groups will be stable generation after generation (Ndoula *et al*., 2014; Canizalez-Román *et al*., 2018).

Participants from different regions showed a similar pattern of distribution for both loci which may reflect the complex processes, population admixing among the regions (Hamilton, 2009; Dewan, 2015; Canizalez-Román *et al*., 2018). Although the general pattern of distribution of the ABO and Rh blood type was similar among different populations, there are also slight differences in the frequencies of different blood types, genotypes and allele frequencies. Such differences of in the phenotypic, genotypic and allelic frequencies could be due to differences in the culture, geography, endemic diseases or population admixture (Hamilton, 2009; Dewan, 2015; Canizalez-Román *et al*., 2018). Some of these factors could put a selective pressure in favoring one allele over the other and could shape the genetic structure of the ABO and Rh loci of populations in the respective areas in a long term. Furthermore, the low level of gene differentiation observed among subpopulations, could be due to complex population admixture among regions (Dewan, 2015;Canizalez-Román *et al*., 2018).

Association between blood groups and diseases

The higher frequency of O blood group observed in this study could have an evolutionary advantage in conferring resistance to disease like malaria. In malaria prone countries of Africa group O are dominant with the distribution ranging from 40.0% to 80.0 % (Cserti and Dzik, 2007). As Ethiopia is a malaria endemic area in the sub-Saharan Africa (Anstee, 2010; Golassa *et al*. (2017), the dominance of O blood type in the present study, could be advantageous for protection against protozoan diseases (Harris *et al*., 2005; Panda *et al*., 2011). Tekeste and Petros (2010) reported a strong association between ABO blood group distribution and the prevalence of malaria in three malaria endemic areas in Ethiopia. The same authors have found among study participants with severe malaria the most frequent blood is type A, whereas among the healthy control groups the most common blood type is O. Similarly, a study done by Panda *et al*. (2011) in India found that the most common blood type is B among participant with severe malaria in while blood type O is the most frequent blood type among the healthy control groups. Both of these studies support the hypothesis that O blood confers protection to severe malaria albeit the exact mechanism of protection is not well understood and needs further investigation. A review made by Rowe *et al*. (2009) on the association between falciparum malaria and ABO blood group support the hypothesis that non-O blood groups emerging as significant risk factors for life-threatening malaria, through the mechanism of enhanced rosette formation. Although the O blood is hypothesized to give protection against malaria, it makes people susceptible *Vibrio cholera* (Harris *et al*., 2005). Therefore, this interplay between the different diseases on ABO blood type (e.g., cholera vs. malaria) could contribute to the phenomenon of a balanced polymorphism in the human population genetic structure (Hamilton, 2009).

In a nut shell, the current study established that among the various ABO and Rh blood groups, blood group O is the most common, followed by blood groups A, B, and AB with a predominance of Rh positivity. This work will provide useful information for health institutions in the establishment of regional and national programs that speed up blood transfusions and tissue transplants needed in clinical practice. Additionally, this work is expected to generate interest in population geneticists and anthropologists to study genetic variation at ABO and Rh loci, as well as for physicians interested in the application of immunogenetics in diagnosis and clinical practice.

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