Genomic analysis of clinical samples with serologic ABO blood grouping discrepancies: identification of 15 novel A and B subgroup alleles

Martin L. Olsson, Nidal M. Irshaid, Bahram Hosseini-Maaf, Åsa Hellberg, Marilyn K. Moulds, Hannele Sareneva, and M. Alan Chester

Since the cloning in 1990 of complementary DNA corresponding to messenger RNA transcribed at the blood group ABO locus, polymorphisms and phenotype-genotype correlations have been reported by several investigators. Exons 6 and 7, constituting 77% of the gene, have been analyzed previously in samples with variant phenotypes but for many subgroups the molecular basis remains unknown. This study analyzed 324 blood samples involved in ABO grouping discrepancies and determined their *ABO* genotype. Samples from individuals found to have known subgroup alleles (n = 53), acquired ABO phenotypes associated with different medical conditions (n = 65), probable chimerism (n = 3), and common red blood cell phenotypes (n = 109) were evaluated by *ABO* genotype screening only. Other samples (n = 94) from apparently healthy donors with weak expression of A or B antigens were considered potential subgroup samples without known molecular background. The full coding region (exons 1-7) and 2 proposed regulatory regions of the *ABO* gene were sequenced in selected A (n = 22) or B (n = 12) subgroup samples. Fifteen novel *ABO* subgroup alleles were identified, 2 of which are the first examples of mutations outside exon 7 associated with weak subgroups. Each allele was characterized by a missense or nonsense mutation for which screening by allele-specific primer polymerase chain reaction was performed. The novel mutations were encountered in 28 of the remaining 60 A and B subgroup samples but not among normal donors. As a result of this study, the number of definable alleles associated with weak ABO subgroups has increased from the 14 previously published to 29. (Blood. 2001;98:1585-1593)

Introduction

In clinical transfusion medicine, ABO is the most important blood group system. Depending on a person's ABO blood group, preformed IgM anti-A and/or anti-B can be present in serum, constituting a major barrier against ABO-incompatible blood transfusions and organ transplantation. The ABO haptens are terminally located carbohydrate residues on the cell surface and in secretions that are biosynthesised by blood group-specific glycosyltransferases (reviewed by Watkins[1]) encoded by the *ABO* locus on the long arm of chromosome 9.[2,3] Ninety years after the discovery of the ABO blood groups[4] the molecular genetic basis of the system was defined[5] and the polymorphisms of the common alleles at this blood group locus established.[6] However, for many of the variant phenotypes of the ABO system our knowledge of the molecular basis for altered antigen expression is sparse.

In 1993, molecular alterations at the *ABO* locus in individuals with inherited subgroup phenotypes were reported.[7-10] Only one or 2 individuals with each phenotypic variant were investigated by sequencing of exons 6 and 7 in the *ABO* gene. Investigation of other subgroup alleles followed,[11-15] but several examples exist in which no deviation from the consensus sequence was found in exons 6 and 7.[7,15,16] In total, 8 A (including *cis*-AB) and 6 B subgroup [including 2 B(A)] alleles defined by missense mutations in exon 7 have been described to date. However, no investigation of exons 1 to 5 in samples with unusual ABO phenotypes has been published despite mutations located in the transmembrane or stem regions of other glycosyltransferases being shown to affect the resulting carbohydrate structures.[17,18]

ABO genotyping is a valuable complement to serology for correct determination of donor and patient ABO blood group status.[19-24] As an independent tool in the clinical laboratory it can confirm a weakly expressed A or B antigen in inherited subgroups[14] and also exclude *B* allele markers in the acquired B antigen phenotype.[25] Although monoclonal antibodies recognizing and agglutinating most weak subgroups exist, the risk for mistyping (eg, A_x donors as blood group O) remains when using commercially available routine reagents. This may have adverse consequences when transfusing such blood to group O recipients.[26]

ABO allele nomenclature poses significant problems that are still under consideration by the International Society of Blood Transfusion. In this paper alleles are referred to by their serologic activity. Different alleles associated with the same serology have been numbered sequentially.

From the Blood Centre, University Hospital, Lund, Sweden; Department of Transfusion Medicine, Institute of Laboratory Medicine, Lund University, Sweden; Immucor/Gamma Reference Laboratories, Atlanta, GA, and Houston, TX; and Red Cross Blood Transfusion Service, Helsinki, Finland.

Submitted January 2, 2001; accepted April 26, 2001.

Supported in part by the Claes Högman SAGMAN Stipendium, Georg Danielssons Fond för Blodsjukdomar, Tore Nilsons Stiftelse för Medicinsk Forskning, Universitetssjukhusets donationsfonder, the Medical Faculty at Lund University, Sweden (M.L.O.), and the World Health Organization (N.M.I.).

Part of this work was presented at the Annual Meeting of the American Association of Blood Banks held in San Francisco, CA, November 8, 1999, and published in abstract form.[54]

M.K.M. is employed by Immucor/Gamma Reference Laboratories, whose products were used in the present work.

Reprints: Martin L. Olsson, Blood Centre, University Hospital, S-221 85 Lund, Sweden; e-mail: martin_l.olsson@transfumed.lu.se.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

We evaluated the molecular genetic basis for ABO discrepancies in clinical samples including suspected inherited subgroups and acquired variant phenotypes sent to our laboratory during the past 7 years. The full *ABO* coding region (exons 1-7) and 2 potential regulatory 5′-untranslated regions (UTRs)[27,28] were analyzed by direct DNA sequencing in selected samples for which the reason for the ABO discrepancy was still unexplained after *ABO* genotype screening and evaluation of medical records. In this way, 15 novel A and B subgroup alleles were identified.

Materials and methods

Blood samples and DNA preparation

Blood was drawn by venipuncture at the referring centers. DNA was prepared in Lund, Sweden from EDTA or acid-citrate-dextrose blood with a simple salting-out method.[29] Blood from apparently healthy random donors was used to screen for mutations associated with the novel subgroup alleles.

Blood group serology

ABO serology was performed with commercially available monoclonal, polyclonal, and lectin anti-A, anti-B, anti-AB, anti-A_1, and anti-H reagents according to the routine of the referring laboratories. Serologic investigations were performed in Lund, Sweden as previously described[14] if the red blood cell (RBC) condition allowed it. Saliva inhibition studies were performed according to modern blood grouping practice[30] when required for subgroup diagnosis, although in many cases either saliva samples were unavailable or the Lewis phenotype indicated nonsecretor status of the propositus.

ABO genotyping

A duplex polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method[19] was used for the initial *ABO* genotype screening. The presence of mutations associated with known alleles was confirmed by methods described earlier (Table 1). A method[36] linking mutations in exons 6 and 7 by allele-specific primer PCR (PCR-ASP) across intron 6 was used when exclusion of recombinant hybrid alleles was required. Primers in Table 2 (labeled ‡) were used under the PCR conditions described below for primer mixes W2/W3.

Amplification of the *ABO* gene for DNA sequencing

Oligonucleotide primers were synthesized by DNA Technology ApS (Aarhus, Denmark). Primers used to amplify DNA fragments for direct sequencing are shown in Table 2. PCR with primer pair 1 was described elsewhere[37]; PCR with primer pairs 2 to 5 was performed as follows: For one reaction 2 pmol of each primer (Table 2) was mixed with 200 ng genomic DNA, 4 nmol of each dNTP, and 1 U AmpliTaq Gold (Perkin-Elmer/Roche molecular system, Branchburg, NJ) in the buffer supplied. The final volume was 20 μL. Thermocycling was undertaken in GeneAmp PCR system 2400 (Perkin-Elmer/Cetus, Norwalk, CT): Initial denaturation at 96°C for 10 minutes was followed by 10 cycles at 94°C for 20 seconds, 63°C for 30 seconds, 72°C for 1 minute, then 25 cycles at 94°C for 20 seconds, 60°C for 30 seconds, and 72°C for 1 minute. PCR products were excised from 1.5% to 3% agarose gels (Seakem, FMC Bioproducts, Rockland, ME), stained with ethidium bromide (0.56 mg/L gel, Sigma Chemicals, St Louis, MO), and purified using Qiaquick gel extraction kit (Qiagen, Hilden, Germany). Primer pairs 6 and 7a-c (depending on the *ABO* genotype of the sample, Table 2) were used to amplify allele-specific fragments covering exon 6, intron 6, and exon 7 of the *ABO* gene under the following cycling conditions: Initial denaturation at 96°C for 10 minutes followed by 15 cycles at 94°C for 20 seconds, 65°C for 2.5 minutes, then 25 cycles at 94°C for 20 seconds, 61°C for 30 seconds, 72°C for 2.5 minutes, and a final extension for 5 minutes. PCR products obtained with primer pair 6 from samples heterozygous for O^1 or O^{1v} alleles were digested by addition of 2 U of the restriction endonuclease *Kpn*I (Life Technology, Gaithersburg, MD) in the appropriate buffer and incubated at 37°C for 6 hours. Fragments were separated in 1% agarose and the undigested fragments (lacking the 261delG of O^1/O^{1v} alleles) subjected to sequencing after purification from gel.

The Big Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA) and an ABI PRISM 310 Genetic Analyzer (Applied Biosystems) were used for direct DNA sequencing according to the manufacturer's instructions. Besides the PCR primers in Table 2, the following internal primers were used as sequencing primers: mo-46,[19] mo-75,[11] EPB79,[3] and fy-47[6] for sequencing of exons 6 and 7. In addition, allele-specific primers (Table 2) were used as sequencing primers to ensure that the sequencing results obtained for exons 6 and 7 reflected only the relevant allele. Sequence analysis was performed with SeqEd software 1.03 (Applied Biosystems).

Screening for subgroup-specific mutations by PCR-ASP

Three different PCR mixes (W1, W2, W3) containing the primers in Table 3 were used for subgroup mutation screening. Each primer (2 pmol) was mixed with 100 ng genomic DNA, 2 nmol dNTP, and 0.5 U of AmpliTaq Gold in the buffer supplied under the following cycling conditions: initial denaturation at 96°C for 10 minutes, followed by either 30 cycles at 94°C for 20 seconds, 65°C for 30 seconds, and extension at 72°C for 40 seconds (W1), or 32 cycles at 94°C for 30 seconds, 65°C for 30 seconds, and extension at 72°C for 90 seconds with a final extension at 72°C for 2 minutes (W2/W3). PCR products were separated for 30 minutes at 150 V using 2% ethidium bromide-stained agarose gels and visualized as described.[19]

Table 1. Polymorphic nucleotides detectable by restriction endonuclease digestion or sequence-specific primer PCR as used for *ABO* genotyping in this study

Polymorphism detected by PCR-		Nucleotide position	Mutation	Alleles detectable*	Allele reference	Method reference
RFLP	ASP					
*BstU*I	−	188-189	GC → AT	O^{1v}	31	31
*Kpn*I	−	261	G → −	O^1, O^{1v}, O^1 hybrids	6,32	19
*Hpa*II	−	467	C → T	Asian A^1, A^2, O^3, *cis-AB*, O^1-A^2 hybrid	8,32-35	19
*Nhe*I	+	526	C → G	*B*, O^1-*B* hybrids	6,32	14,36
*Mbo*I	−	646	T → A	A^x, A^x hybrids, O^{1v}	9,15,31	14
*Hpa*II	−	703	G → A	*B*, O^1-*B* hybrids	6,32	19
*Dde*I	−	771	C → T	A^x hybrids, O^{1v}	31	15,31
—	+	796	C → A	*B*	6	36
—	+	802	G → A	O^2	10	36
—	+	803	G → C	*B*	6	36
—	+	798-804	+G	A^{el}, O^3	11,35	11
*Sal*I	−	871	G → A	A^3, B^x	7,13	14
—	+	1059-1061	−C	A^2, O^3	34,35	14
*Hpa*II	−	1096	G → A	*B*, O^2	19	19

*This list of alleles is not complete.

Table 2. Oligonucleotide primer pairs used for amplification and direct sequencing of *ABO* gene fragments

Primer designation	Primer pair	Primer sequence (5′ → 3′)	Primer location	*ABO* region amplified	Fragment size* (bp)
Pro-F	1[37]	ggaaacaaatcctacccctac	5′-UTR	Enhancer (CBF-NFY)	215/344
Pro-R		gtgctgcctgtgcctgttac	5′-UTR		
mo-12F	2	ggcgccgtcccttcctag	5′-UTR	Promoter, exon 1	267
mo-12R		cctgcggtagcggctcct	Intron 1		
mo-21F	3	ggtgagagaaggagggtgag	Intron 1	Exon 2-3	935
mo-31R		ccagcaccccggccagca	Intron 3		
mo-41F	4[31]	taaatcctgctcctagactaaac	Intron 3	Exon 4	148
mo-42R		ggacaattctgtgacatgggag	Intron 4		
mo-51F	5	tgcatcccacgctttccatgc	Intron 4	Exon 5-6	823
mo-46R[19]		actcgccactgcctgggtctc	Intron 6		
mo-57a	6	gggtttgttcctatctctttgc	Intron 5	Exon 6-7	2273
mo-71R[19]		gggcctaggcttcagttactc	3′-UTR		
ABO-261F-C	7a	AGGAAGGATGTCCTCGTGGTG	Exon 6	Exon 7 (non-O^1/O^{1v})	2002
mo-71R[19]		gggcctaggcttcagttactc	3′-UTR		
ABO-261F-C	7b	AGGAAGGATGTCCTCGTGGTG	Exon 6	Exon 7 (A^2-specific)	1888
ABO-1060R-A2		CCTGGCAGCCGCTCACGGT	Exon 7		
ABO-261F-C	7c	AGGAAGGATGTCCTCGTGGTG	Exon 6	Exon 7 (*B*-specific)	1923
ABO-1096R-B		GAGGGGGACGGGGCTGCT	3′-UTR		
ABO-297F-B†	—	CCATTGTCTGGGAGGGCACG	Exon 6	—	—
ABO-467F-A2†	—	CTATGTCTTCACCGACCAGCT	Exon 7	—	—
ABO-467R-A2‡		CGGGGCACCGCGGCCAG	Exon 7		
ABO-526R-B‡		GCCAGCGCTTGTAGGCGCC	Exon 7		
ABO-796R-B‡		CCCCGAAGAACGCCCCCAT	Exon 7		
ABO-802R-O2‡		CCGACCCCCCGAAGAACCT	Exon 7		
ABO-803R-B‡		ACCGACCCCCCGAAGAACG	Exon 7		

Primer sequences corresponding to untranslated sequences are shown in lower case.
*The exact number of nucleotides may vary slightly between different alleles.
†Used as sequencing primers only.
‡Each primer was used with ABO-261F-C for coupling of mutations in exons 6 and 7.[36] The nucleotide- and allele-specificity is indicated by the name of the primers.

Results

We investigated 324 clinical samples referred to our laboratory due to ABO grouping discrepancies since 1994. The samples were divided into different categories based on the medical condition of the donor, the serologic discrepancies, and the genomic typing results as described below. In addition, approximately 1500 bp of *ABO* gene sequence covering exons 1 to 7 and splice sites, the promoter region (from nucleotide [nt] −118 to −1) and a 215- or 344-bp enhancer region (around nt −3800) from each of 36 selected donors was evaluated.

Table 3. Oligonucleotide primers used to screen for novel *ABO* gene mutations found in this study

Primer designation	Reaction ID	Primer sequence (5′ → 3′)	Primer location in *ABO* gene
JK-F3	W1	CATGCTGCCATAGGATCATTGC	*
JK-R3	W1	GAGCCAGGAGGTGGGTTTGC	*
ABO-in3F	W1	gctggccgttacagggtctg	Intron 3
ABO-203CR	W1	AGGGAGGCACTGACATTATAC**G**	Exon 4
mo-57a	W1	gggtttgttcctatctctttgc	Intron 5
ABO-350CR	W1	CTTGATGGCAAACACAGTTAAC**G**	Exon 6
mo-101s	W2-3	ccgtccgcctgccttgcag	Intron 6
ABO-407R-Aw	W2	CCATGAAGTGCTTCTCCGCC**A**	Exon 7
ABO-965R-Aw	W2	GCTGCTGGTCCCACAAGTAC**C**	Exon 7
ABO-996R-Aw	W2	TCCTCAGGACGGCGGG**T**C	Exon 7
ABO-539R-Bw	W3	CATGGACACGTCCTGCCAG**T**	Exon 7
ABO-548R-Bw	W3	ATGCGGCGCATGGACACG**C**	Exon 7
ABO-721R-ABw	W3	CTCGTAGGTGAAGGCCTCCC**A**	Exon 7
ABO-863R-Bw	W3	GTTGGCCTGGTCGACCATC**C**	Exon 7
ABO-873R-Bw	W3	CTCGATGCCGTTGGCCTG**C**	Exon 7
ABO-1036R-Bw	W3	CGGACCGCCTGGTGGTTC**C**	Exon 7
ABO-1055R-Bw	W3	GCAGCCGCTCACGGGTTC**T**	Exon 7

Polymorphic nucleotides in primer sequences are shown in bold, primer sequences targeting intron sequences in lower case. In addition to the primers in this table, mo-21F and mo-31R (Table 2) were used as the internal control primer pair in primer mixes W2 and W3.
*These primer sequences correspond to exons 8 and 9 sequences in the JK blood group gene.[38]

Acquired variant ABO phenotypes

Sixty-five samples were included in this group based on serologic or medical factors in the presence of which acquired phenotype changes have often been described.[26,39] Although the simultaneous presence of such factors and an unusual ABO subgroup allele is theoretically possible, no further analysis beyond *ABO* genotype screening was done in this category.

Pregnancy. Variable weak A expression was observed serologically during pregnancy (n = 13). Based on standard agglutination tests using repeat samples drawn 1 to 6 months after delivery, A antigen expression returned to normal levels in 5 women available for repeat testing. However, the other women were unavailable for repeat sampling or became pregnant again before new samples were taken. In 3 of the unavailable women normal blood grouping results had been obtained before pregnancy. All samples showed heterozygosity for the *A* allele (Table 4).

Hematologic disorders. Weak but variable A (or in 2 cases B) antigen expression was also noted in nontransfused patients (n = 16) with a variety of hematologic diseases including acute and chronic myelogenous leukemia, multiple myeloma, non-Hodgkin lymphoma, and myelodysplastic syndrome. In these patients transfusion of high-demand, group O blood components

Table 4. *ABO* genotype screening results obtained in samples with ABO blood grouping discrepancies and known risk factors for noninherited RBC phenotype variants

Phenotype/condition	No.	*ABO* genotype											
		A^1A^1	A^1O^1	A^1O^{1v}	A^1A^2	A^2O^1	A^2O^{1v}	A^1B	A^2B	$B\,O^1$	BO^{1v}	O^1O^{1v}	$O^{1v}O^{1v}$
Weak A expression in:													
Pregnancy	13		5	3		1			4				
Hematologic disorders	14	1	3	2	2	3	2		1				
Miscellaneous diagnoses	13		3	3		3	2	2					
Weak B expression in hematologic disorders	2								1	1			
Polyagglutination													
Acquired B syndrome	19	2	5	6	4	1	1						
Acquired A-like antigen	1												1
Unspecified T-activation	3		1								1	1	

was avoided, partly due to the *ABO* genotyping results confirming their non-O status (Table 4).

A patient with myelodysplastic syndrome showed serologic agglutination patterns compatible with an acquired A antigen. The historical blood grouping was uncomplicated (blood group O) and *ABO* genotyping indicated the $O^{1v}O^{1v}$ genotype. A patient with non-Hodgkin lymphoma had polyagglutinable RBCs, but was shown to have the O^1O^{1v} genotype.

Various medical conditions associated with ABO discrepancies. Nineteen patients with nonhematologic malignancies (mainly adenocarcinomas of the colon, rectum, or uterus but also 3 cases with brain tumors) and gastrointestinal infections showed serologic results compatible with a form of polyagglutination often referred to as acquired B.[30,39] Seventeen of the samples had *ABO* genotypes compatible with the A_1 phenotype. However, a patient with advanced gynecologic carcinoma and another with malignant glioma had A^2O^1 and A^2O^{1v} genotypes, respectively. The presence of *B* allele markers was investigated by *ABO* genotyping, and 4 missense mutations (526C>G, 703G>A, 796C>A, and 803G>C) differentiating the *B* from the *A* allele were excluded (Table 1). Erroneous transfusion of AB blood to this type of blood group A patient has been documented[40] but could be avoided.

A patient undergoing orthopedic surgery and another with systemic lupus erythematosus had other forms of polyagglutinable red cells but had BO^{1v} and A^1O^1 genotypes, respectively.

Thirteen patients expressed weak A antigen, similar to the situation among patients with hematologic disorders above. Their records revealed a wide variety of medical conditions including septicemia, orthopedic, or abdominal surgery and autoimmune disease.

The genotyping results obtained in samples with known risk factors for noninherited erythrocyte phenotype variants are summarized in Table 4.

Inherited ABO phenotypes due to known subgroup alleles

Genotype screening of 53 samples with suspected weak A subgroup phenotypes revealed heterozygosity for previously recognized variant alleles associated with variant A expression, namely A^{el},[11] *B(A)*,[9] and different variants of A^x.[9,15] Each of these samples showed the expected serologic reactions according to the subgroup allele found. Two of the A^x alleles had exon 1 to 7 sequences identical to earlier reported A^x hybrid alleles[15] but different crossing-over regions shown by intron 6 sequencing. These 2 novel alleles are dealt with in a later section.

The B(A) phenotype sample had initially been interpreted serologically as $A_{weak}B$ but was genotyped as *B(A)* O^1, that is, heterozygous for a *B* allele lacking the *B*-specific mutation at nt 703.

A sample defined serologically as A_x by a major European reference laboratory was genotyped as A^3O^2 where the A^3 allele (A^1 with the 871G>A mutation) is that earlier described in 2 individuals with the A_3B phenotype.[7] These genotypes were confirmed by sequencing exons 1 to 7 in the *B(A)* and A^3 alleles. In agreement with the original A^3 and *B(A)* reports consensus *A* and *B* sequences were found with the exception of 871G>A and 703A>G, respectively. The number of known subgroup alleles encountered in this study is summarized in Tables 5 and 6.

Common ABO phenotypes

Relatives of subgroup individuals. Samples from parents, siblings, or children of the suspected subgroup propositus fell into 2 groups: samples with common ABO phenotypes (n = 81) and those with the same weak A or B subgroup as the propositus. The former group was not studied further following *ABO* genotyping when the expected inheritance and phenotype/genotype correlation was observed. In 2 cases the serologic consequences of the A subgroup allele were masked by a normal A^1 allele (A^1A^{el} or A^1A^x).

Unexpected absence or very low titer of anti-A or anti-B despite common RBC phenotype. Twenty-eight samples lacking (or having very low titers of) anti-A and/or anti-B despite the absence of serologic proof of B and/or A antigen, respectively, were genotyped. Adsorption/elution tests had not usually been performed or else gave negative or inconclusive results. This group included patients undergoing immunosuppressive therapy and also apparently healthy blood donors lacking one of their isoagglutinins (in most cases anti-A). The genotypes found included only well-known *ABO* alleles compatible with common red cell phenotypes, thus not confirming the suspicion of weak A or B expression.

Suspected chimerism

Two samples showed signs of more than 2 alleles following *ABO* genotyping. In both cases the third allele gave weak reactions in PCR-ASP tests and represented an *A* or *B* allele compatible with the weak A or B expression also observed serologically. A third suspected chimeric sample was referred due to mixed-field reactions in agglutination tests involving antigenic markers for several blood group systems. Unfortunately, *ABO* genotyping was uninformative because only 2 *ABO* alleles were detected. Re-examination of the clinical records revealed that the first 2 individuals had a twin sibling, thus making chimerism due to intrauterine exchange of hematopoietic precursor cells the most likely explanation for their discrepant ABO typing result. Twin status could not be confirmed for the third patient. Because chimerism was not the main topic of this study, these samples were not examined further.

Table 5. Previously published A subgroup alleles and the 8 novel A subgroup alleles reported here

Allele description	Nucleotide change(s)	Amino acid change(s)*	Individuals (families)†	Geographic origin‡	Reference/GenBank accession no.
A^3-1	871G>A	**D**291N	1§	France (United States)	A3[7]
A^3-2	829G>A	V277M	—	(Brazil)	A3[12]
	1060C−	P354 frameshift			
A^x-1	646T>A	F216I	12 (6)	Various	Ax[9], A^x(1)[15], A108[13]
A^x-2‖	646T>A	F216I	6 (2)	Sweden	A^x(2), AF016625[15]
	681G>A				
	771C>T				
	829G>A	V277M			
A^x-3	297A>G		3 (1)	Sweden	A^x(3), AF016624[15]
	646T>A	F216I			
	681G>A				
	771C>T				
	829G>A	V277M			
►A^x-4‖	646T>A	F216I	1	Poland	AF324006
	681G>A				
	771C>T				
	829G>A	V277M			
►A^x-5‖	646T>A	F216I	1	United States	AF324007
	681G>A				
	771C>T				
	829G>A	V277M			
►A^x-6	996G>A	W332 stop	1	New Zealand	AF324013
A^{el}-1	798-804 (+G)	F269 frameshift	28 (16)	Various	A^{el}[11], A109[13]
A^{el}-2	467C>T	P156L	—	(Japan)	A110[13]
	646T>A	F216I			
	681G>A				
►A^w-1	407C>T	T136M	2 (2)	United Kingdom	AF324010
	467C>T	P156L			
	1060C−	P354 frameshift			
►A^w-2	350G>C	G117A	6 (6)	New Zealand, United Kingdom, Australia, United States	AF324009
	467C>T	P156L			
	1060C−	P354 frameshift			
►A^w-3	203G>C	**R**68T	28 (> 5)	Scandinavia	AF324008
	467C>T	P156L			
	1060C−	P354 frameshift			
►A^w-4	721C>T	**R**241W	2 (2)	Belgium, Germany	AF324011
►A^w-5	965A>G	**E**322G	2 (1)	Finland	AF324012
cis-AB	467C>T	P156L	—	(mainly Japan)	*cis-AB*[8,41]
	803G>C	G268A			

Nucleotide and amino acid changes are given in comparison to the consensus A^1 allele.

► indicates novel A subgroup allele reported in this paper.

*Charged residues involved in amino acid changes are shown in bold.

†The number of samples identified by sequencing or PCR-ASP in this study is given for each allele (the number of apparently unrelated families investigated is given in brackets).

‡Country from which the samples were referred (countries in parentheses refer to samples reported elsewhere).

§The phenotype of this sample was reported to be A_x, not A_3.

‖These alleles differ by their intron 6 polymorphism showing 3 different cross-over regions between *A* and O^{1v}, namely, nt 189-225 in intron 6 for A^x-2, nt 236-445 in intron 6 for A^x-4, and nt 298 in exon 6 to nt 41 in intron 6 for A^x-5.

Variant ABO phenotypes potentially due to unknown subgroup alleles

The remaining samples came from apparently healthy donors without identifiable risk factors for acquired ABO phenotype changes to explain their variant phenotypes. Mutations associated with known subgroup alleles were excluded by *ABO* genotype screening as described above (Table 1). These samples were potentially A (n = 72) or B (n = 22) subgroups with an unknown molecular basis. Inheritance of the phenotype was shown in 17 and 18 cases, respectively (data not shown).

Serologic classification. Several of the donors had been blood grouped repeatedly over a number of years in different laboratories and always gave the expected reactions for the subgroup assigned.[26,30] Subgroup status was confirmed with samples from relatives, if available. Frequently, however, no assignment was possible, usually due to lack of saliva or the donor's nonsecretor status.

ABO genotyping. All A and B subgroup samples were heterozygous for *A*- and *B*-like alleles, respectively. For the A subgroup samples, A^1- or A^2-like alleles were predicted. In the presence of the 721C>T and 1055G>A mutations (see below) anomalous bands appeared using the PCR-RFLP method with simultaneous digestion with *Kpn*I and *Hpa*II endonucleases,[19] thus preventing the erroneous prediction of normal *A* and *B* alleles in these cases. The anomalous bands were the result of allele-specific mutations removing *Hpa*II sites present in the consensus A^1 and *B* sequences.

Sequencing of novel ABO subgroup alleles. The full coding region (exons 1-7) and 2 proposed regulatory motifs of the *ABO*

Table 6. Previously published B/B(A) subgroup alleles and the 7 novel B subgroup alleles reported here

Allele description	Nucleotide change	Amino acid change*	Individuals (families)†	Geographic origin‡	Reference/GenBank accession no.
B^{3}-1	1054C>T	**R**352W	—	(United States)	B3[7]
B^{el}-1	641T>G	M214**R**	—	(Japan)	B105[13]
B^{el}-2	669G>T	**E**223**D**	—	(Japan)	B106[13]
B^{w}-1	871G>A	**D**291N	—	(Japan)	B104[13]
►*B*^{w}-2	873C>G	**D**291**E**	1	France	AF324018
►*B*^{w}-3	721C>T	**R**241W	8 (2)	Sweden	AF324014
►*B*^{w}-4	548A>G	**D**183G	5 (2)	Sweden	AF324016
►*B*^{w}-5	539G>A	**R**180**H**	1	United States	AF324015
►*B*^{w}-6	1036A>G	**K**346**E**	3 (1)	Finland	AF324019
►*B*^{w}-7	1055G>A	**R**352**Q**	2 (1)	United States	AF324020
►*B*^{w}-8	863T>G	M288**R**	1	Turkey	AF324017
B(A)-1	703A>G	S235G	1	Germany	[9]
B(A)-2	700C>G	P234A	—	(Asia)	[42]

Nucleotide and amino acid changes are given in comparison to the consensus *B* allele.

► indicates novel B subgroup allele reported in this paper.

*Charged residues involved in amino acid changes are shown in bold.

†The number of samples identified by sequencing or PCR-ASP in this study is given for each allele (the number of apparently unrelated families investigated is given in parentheses).

‡Country from which the samples were referred (countries in parentheses refer to samples reported elsewhere).

gene were sequenced in genomic DNA from selected A (n = 22) or B (n = 12) subgroup samples representing different serologic patterns and varying geographic origin. Priority was given to samples shown to have inherited ABO discrepancies.

Except for the mutations stated below, all analyzed A and B subgroup alleles had consensus A^1/A^2 and *B* sequences, respectively, including regulatory regions. Novel sequences have been deposited in GenBank (accession numbers AF324006-AF324020). The 15 novel *A* and *B* variant alleles reported here are summarized and compared to previously published subgroup alleles in Table 5 and Table 6, respectively.

A subgroup alleles. Ten samples classified as A_3 had apparently A^1 alleles. Two fully sequenced samples had exons and regulatory regions identical to the consensus A^1 allele. The same was true for 2 Norwegian sisters with the A_{el} phenotype reported to have consensus exons 6 and 7.[16]

The majority of Scandinavian A_{end}, A_{weak}, and A_x samples lacking the A^x mutation at nt 646[9,15] had an A^2 allele. Two A_{end} and 2 A_x samples were fully sequenced and had the same missense mutation, 203G>C, in exon 4 causing R68T (single-letter amino acid codes). Because these samples had been assigned to different serologic categories, this allele was designated A^w-3.

A donor of Polish origin had the $A_{weak}B$ phenotype and an American donor was suspected to have the B(A) phenotype. Both were heterozygous for A^x alleles and sequencing showed 2 novel variants of the A^x hybrid allele including the missense mutations at nt 646 (F216I) and 829 (V277M) (A^x-4 and A^x-5 in Table 5). As in the previously described recombinant A^x alleles (A^x-2 and A^x-3) the 5′ end of common alleles were fused with the 3′ end of O^{1v} alleles. In both cases exon 7 was derived from O^{1v}, but by sequencing of intron 6, 2 novel crossing-over regions were defined (Table 5 and GenBank accession numbers therein). Another A_x sample from New Zealand had a nonsense mutation, 996G>A, predicting loss of 23 amino acids due to a premature stop codon (A^x-6).

Samples from 2 unrelated British donors categorized as A_{weak} or weak A_x phenotypes were sequenced and had A^2-like alleles with an additional 407C>T (T136M) (A^w-1), while 2 unrelated American donors with $A_{x?}$ or A_{weak} phenotype and one weak $A_{x?}$ sample from New Zealand had A^2-like alleles with an additional 350G>C (G117A) (A^w-2).

Two samples from Belgium and Germany were only very weakly positive in agglutination and adsorption/elution tests using anti-A, B and had only anti-B in their sera. Both were heterozygous for an A^1-like allele with the additional presence of 721C>T (R241W) (A^w-4) also noted in some B_{weak} families (see below).

A Finnish family with very weak erythrocyte A expression (differing from the A_{finn} phenotype according to the Finnish Red Cross Transfusion Service) had an A^1-like allele (A^w-5) with a 965A>G mutation predicting E322G.

B subgroup samples. The B_{weak} samples had weak anti-B in plasma and very similar mixed field agglutination patterns with a low proportion of agglutinated red cells. Saliva studies were performed on the Swedish samples but were uninformative, because one family in each genetic variant had low amounts of secreted B substance, and the other 2 families lacked B substance. No further attempts were made to categorize samples into different subgroups, a decision supported by the vague or sometimes contradictory criteria for classification published in current textbooks. However, a Finnish family (B^w-6) had been classified[26] as B_v by the Finnish Red Cross Transfusion Service.

The *B* alleles studied showed remarkable genetic heterogeneity and were geographically dispersed. Seven novel sequences were found despite the serologic similarity. All were *B* consensus sequences with the additional presence of a single missense mutation (Table 6).

Earlier work by Professor Ch. Salmon (oral communication, Dr LePennec, Paris, July 2000) and Professor W. M. Watkins (written report, London, 1983) showed decreased B-transferase activity in samples now shown to have the D291E and D183G substitutions, respectively (Table 6).

Screening for subgroup-specific mutations by PCR-ASP

Primers specific in their 3′ ends for all the novel mutations detected in this study were synthesized (Table 3). Primer mix W1 was designed to detect mutations outside exon 7, whereas W2 and W3 detect mutations in exon 7 of weak A and B subgroup alleles, respectively. Sixty random blood donor samples were screened with 3 different multiplex primer mixes to see if the novel mutations were found in samples from normal ABO phenotypes. None were found except for a donor with the A^1A^2 genotype, who also had the 203G>C mutation in the A^2 allele (according to a family study). His phenotype was A_1 as expected. Some

of his relatives had the A_{end} phenotype due to *A*^{w}*-3 O*^{1} or *A*^{w}*-3 O*^{1v} genotypes.

The 60 remaining variant samples (not previously sequenced) were screened by PCR-ASP and 28 were positive for one of the new subgroup markers. The total number of samples found positive for each mutation is given in Tables 5 and 6, as is their geographic distribution. Representative gel electrophoretograms corresponding to primer mixes W1 and W3 are shown in Figure 1. The remaining 32 samples not associated with a definable allele were not further evaluated, in most cases because of unsatisfactory DNA quality or insufficient amounts of available DNA.

Discussion

The clinical use of genomic blood grouping in transfusion medicine practice has evolved during the past decade as a result of the cloning of most blood group genes. A recent review[43] outlined different situations in the clinical laboratory where blood group genotyping may be valuable and *ABO* genotyping has been used for many of them, although acquired and inherited variant phenotypes are probably the most important.

In this study we evaluated serologic ABO discrepancies encountered in a consecutive series of clinical samples referred to us for *ABO* genotyping. Two acquired variant phenotypes are of particular interest: weakened A antigen expression (eg, in leukemia and pregnancy) and acquired B antigen. Concern is motivated due to their frequency and the implications in clinical transfusion. The underlying reason for the observed alterations is unknown. Erythrocyte A or B antigen activity decreased in a number of patients while being treated for leukemia or other hematologic disorders. The correlation between leukemia and erythrocyte ABO typing irregularities was first described in 1957[44] and has been amply confirmed.[45] A similar phenomenon has also been reported in patients of blood group B. Why leukemia affects the expression of ABO system antigens is unknown.

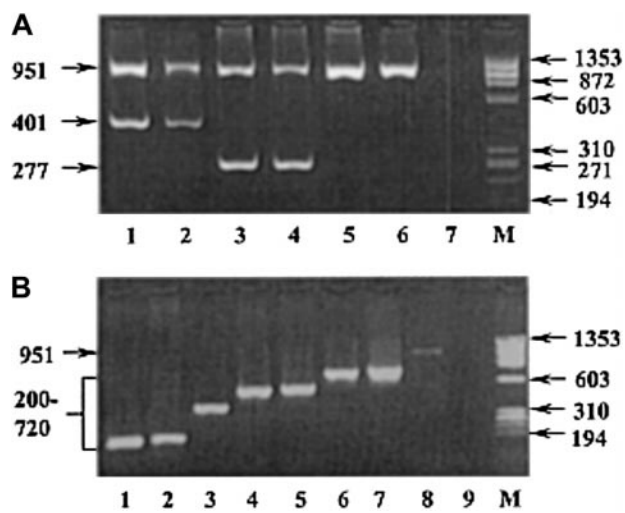

Figure 1. Representative gel electrophoretograms showing PCR-ASP screening for subgroup alleles. PCR-ASP amplification products for A and B subgroup alleles with the W1 (A) and W3 (B) primer mixes, respectively, as separated on 2% agarose gels. The W2 primer mix gel is not shown because it works according to the same principle and looks virtually identical to the W3 gel. Samples from individuals with the following weak A and B subgroup mutations are shown. (A) Lanes 1-2, donors with the *A*^{w}-2 (350G>C) allele; lanes 3-4, donors with the *A*^{w}-3 (203G>C) allele; lanes 5-6, *A*^{1}*O*^{1} and *A*^{2}*O*^{1v} random donors; and lane 7, H_2O contamination control. (B) Lane 1, *B*^{w}-5 (539G>A) allele; lane 2, *B*^{w}-4 (548A>G); lane 3, *B*^{w}-3 (721C>T); lane 4, *B*^{w}-8 (863T>G); lane 5, *B*^{w}-2 (873C>G); lane 6, *B*^{w}-6 (1036A>G); lane 7, *B*^{w}-7 (1055G>A); lane 8, *BO*^{1} random donor; and lane 9, H_2O contamination control. M is the molecular size marker ϕX174RF DNA *Hae*III (Life Technologies). Arrows indicate the size of DNA fragments in base pairs.

An elevated frequency of the Le(a−b−) phenotype has been noted in pregnant women,[46] where A_1 women were the most likely to lose their Le^{b} antigen on RBCs. Changes during pregnancy and lactation suggest this to be influenced by hormonal factors.[39] The decreased A activity in the 13 women is interesting and a 10% decrease in antibody binding to RBCs in pregnant women has been described.[47] This is not due to abnormal *A* alleles because none were found and A expression often reverted to normal. No correlation to *A*^{1} (n = 8) or *A*^{2} (n = 5) alleles was obvious.

Acquisition of B-mimicking activity on RBCs usually occurs in A_1 patients (occasionally A_2) with diseases of the digestive tract, in particular colon cancer but also other adenocarcinoma of the female reproductive system and in septic states.[39] This phenomenon is found, but rarely, in apparently normal individuals. Interestingly, 3 patients with brain tumors were among the acquired B patients in this study. The connection between bacterial enzyme activity and infection/gastrointestinal disease is more obvious than why erythrocytes would be affected in patients with tumors behind the blood–brain barrier. Two thirds of the acquired B referrals occurred as a result of unexpected RBC agglutination with the monoclonal ES-4 anti-B reagent, which has a high affinity for the deacetylated A antigen.[48] Several samples were referred even after the pH adjustment of this reagent, as required by the Food and Drug Administration, was made by the manufacturer, suggesting that the measure taken was inadequate. Subsequent cases (after withdrawal of ES-4) have all occurred as a result of testing with polyclonal human reagents. All samples examined with a serologically suspected acquired variant A or B phenotype were genotyped unambiguously and no unusual or unexpected (eg, *B*^{w}) alleles were found.

A striking feature of the genotypes of individuals with weak A and B phenotypes is the diversity of alleles giving the same phenotype, and to some extent the assignment of the same allele to more than one phenotype. A number of practical problems arise as interpretation of incomplete hemagglutination can vary between laboratories and even between individuals in the same laboratory. Furthermore, different clones (monoclonal) or batches (polyclonal) of anti-A, anti-B and anti-A, B reagents show a high grade of interreagent variation. As an example, many of the true A subgroups reported here were initially detected by the referring centers as single weak reactivities against commercially available monoclonal anti-A (eg, A003 or BRIC186) or anti-A, B blend reagents (most often containing the ES-15 clone). Over time, blood grouping reagents may vary and availability changes continually. As a result, the delineation between some of the weak subgroups is diffuse. This is exemplified by the *A*^{w}-3 allele (Table 5) that was present in numerous samples submitted to us and described as both A_{end}, A_x, and unspecified A_{weak}. The relevance of categorical subgroup classification based on serologic phenomena alone is becoming questionable; so use of the more general terms A_{weak} and B_{weak} is not unfounded.

In 28 cases submitted due to an anti-A or anti-B deficiency there was no evidence for the presence of a weak *A* or *B* allele. Following genotyping blood units donated by such donors could be issued as regular units labeled either blood group A, B, or O.

Not surprisingly, genotyping of many samples from blood donors and patients throughout the world selected due to irregular serologic findings resulted in detection of several new subgroup alleles. Until recently, *ABO* genotyping examined exclusively exons 6 and 7 of the

gene, representing 77% of the expressed enzyme protein. Very few alleles were actually sequenced in the early reports. Currently, we analyze all 7 exons, intron 6, and promoter and enhancer regions. The 8 new *A* alleles described here comprised 3 serologically classified as A^x and 5 that we prefer to call $A^{weak}(A^w)$ because the serologic results did not allow further classification. Two of the A^x alleles (A^x-4 and A^x-5) were similar to A^x-2 described previously.[15] All are hybrid alleles having similar but not identical recombination sites in intron 6 fusing *A* and O^{1v} alleles.

Of the 5 new A^w alleles, 3 were normal A^2 alleles with additional missense mutations, 2 of which are the first mutations (203G>C in exon 4 and 350G>C in exon 6) outside exon 7 to be associated with weak subgroup phenotypes. The remaining 2 alleles were A^1 but with a missense mutation in exon 7.

As shown in Table 6 each of the 7 new B^w alleles resulted from a single amino acid-changing mutation in the original *B* allele. The heterogeneity is striking and it is also interesting to note that the point mutations in all B^w alleles described involve charged amino acids. The latter also applies to 4 of the A^w alleles reported.

The 721C>T mutation weakens both A and B activity (A^w-4 and B^w-3) through an R241W substitution. A similar effect has also been observed with 871G>A (D291N) that differentiates A^3-1 from the consensus *A* allele and B^w-1 from a normal *B* allele. Amino acid substitutions spread along the length of the enzyme protein (Figure 2) have varying effects on enzyme activity resulting in the different ABO subgroup phenotypes. It is worth noting how most of the mutations reported to date cluster in certain regions of the *A* and *B* alleles, most predominantly between nt 539 to 548 (2 alleles) and nt 641 to 721 (6 alleles), nt 829 to 873 (5 alleles), and nt 965 to 1060 (8 alleles). Amino acid residues located in these regions may be directly involved in the enzyme's active site or affect protein conformation and could make interesting targets for site-directed mutagenesis.

Of all defined *ABO* alleles to date, only a few [A^1, A^2, *B*, *B(A)*, O^2 and possibly A^3-1][50-52] have been expressed in synthetic systems to evaluate the causative relationship between the mutations and phenotypes observed. As shown by discrepancies between the initially reported in vitro experiments using *BABB* plasmid constructs (resulting in pure B antigenicity detected by flow cytometry in HeLa cells[50]) and the same sequence in genomic DNA [resulting in the B(A) phenotype in vivo[9]] limited sensitivity or other difficulties may lead to results not necessarily reflecting subgroup phenotypes. Nevertheless, a future aim is to express subgroup alleles to show how they affect the enzymic activities of these subgroup glycosyltransferases.

ABO genotyping is a powerful, independent tool in the reference laboratory for resolution of clinical ABO blood grouping discrepancies. A major use of this technique in transfusion medicine is the unequivocal discrimination between acquired and inherited variant phenotypes. Subgroup alleles can be differentiated from normal alleles temporarily resulting in weak agglutination, for example, during pregnancy or in patients with hematologic disorders. In this way the safety of the ABO grouping is further enhanced so that all donors and patients can be correctly grouped to ensure the safest possible use of the blood supply. The rapidly increasing number of alleles being found enhances the usefulness in paternity testing and other applications where individual-specific genetic markers are of interest.[43] In addition, amniotic fluid DNA has been referred to this center for fetal *ABO* genotyping to optimize the care of pregnant women with a history of severe hemolytic disease of the newborn or neonatal alloimmune thrombocytopenia due to high-titer IgG anti-A in earlier pregnancies.

The frequent occurrence of hybrid *ABO* alleles, at least in some ethnic groups,[32,53] has emphasized the need for better *ABO* genotyping protocols to achieve safe phenotype prediction. In addition, none of the hitherto published methods for genomic typing has included means to define subgroup alleles.[49] Following this study, the number of A and B subgroup alleles has more than doubled, a fact that further accentuates the need for improvement.

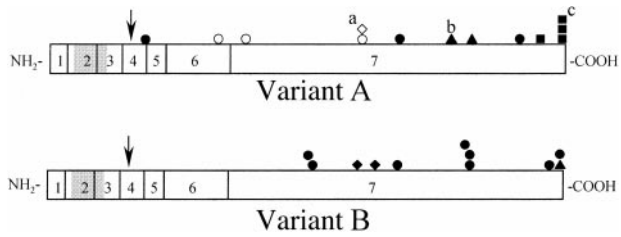

Figure 2. Schematic representation of single missense amino acid substitutions in A and B glycosyltransferases associated with decreased expression of A and B antigens, respectively. The 2 horizontal bars represent 20 of the variant A (upper) and B (lower) glycosyltransferases in Tables 5 and 6 with the addition of 4 A_2 transferases.[49] Cis*AB* and *B(A)* alleles were not included. Vertical lines separate regions coded for by different exons (numbered 1-7) in the *ABO* gene. The putative transmembrane domain (amino acid 15-39 of the glycosyltransferase) is shown in gray.[5] The arrows indicate the stem region susceptible to proteolytic cleavage for generation of soluble enzyme. All amino acid changes published to date are shown as geometric symbols above the bars (except for P156L, which does not affect glycosyltransferase activity). Filled symbols represent substitutions involving a charged amino acid and open symbols involve noncharged residues. Different ABO phenotypes are represented by the symbols as follows: ■ and □, A_2; ▲, A_3/B_3; ● and ○, A_x, A_w/B_w; ◆ and ◇, A_{el}/B_{el}. The A^x alleles other than A^x-1 (hybrids A^x-2 to A^x-5 and A^x-6 with a nonsense mutation at nt 996, Table 5) have been excluded from this figure, as has the frame-shifting A^{el}-1 allele. Three alleles each having 2 mutations resulting in amino acid changes were included: a, this diamond represents A^{el}-2 that also has the neutral P156L change; b, this triangle represents a V277M substitution in A^3-2 that also has the common A_2-related mutation (1060C−); c, one of the 3 squares represents the common A^2-1 allele (a nonsense, not missense, mutation).

Acknowledgments

We express our gratitude to the following persons for performing serologic investigations on samples referred to our laboratory and obtaining samples from relatives: Ms Imelda Bromilow (Diamed, Switzerland), Ms Rida Chiu (Oakland, CA), Dr Hans-Erik Heier (Oslo, Norway), Dr Stephen Henry (Auckland, New Zealand), Dr France Noizat-Pirenne and Dr PierreYves LePennec (Paris, France), Mr Steven Pierce (Kansas City, MO), Ms Anna Pirkola (Helsinki, Finland), Ms Joyce Poole (Bristol, United Kingdom), Dr Hans Gerhard Ruhl (Dresden, Germany), Dr Karin Schneider and Dr Jan Säfwenberg (Uppsala, Sweden), Dr Hans Sonnenborn (Biotest, Germany), Dr Rudi Steffensen (Aalborg, Denmark), Dr Jill Storry (New York, NY), and Dr Vered Yahalom (Tel Aviv, Israel).

References

1. Watkins WM. Biochemistry and genetics of the ABO, Lewis and P blood group systems. In: Harris H, Hirschhorn K, eds. Advances in Human Genetics. Vol. 10. New York: Plenum Press; 1980:1-136.
2. Ferguson-Smith MA, Aitken DA, Turleau C, de Grouchy J. Localisation of the human ABO: Np-1: AK-1 linkage group by regional assignment of AK-1 to 9q34. Hum Genet. 1976;34:35-43.
3. Bennett EP, Steffensen R, Clausen H, Weghuis DO, van Kessel AG. Genomic cloning of the human histo-blood group ABO locus. Biochem Biophys Res Commun. 1995;206:318-325.
4. Landsteiner K. Zur Kenntnis der antifermentativen, lytischen und agglutinierenden Wirkungen des Blutserums und der Lymphe. Zbl Bakt. 1900; 27:357-362.
5. Yamamoto F, Marken J, Tsuji T, White T, Clausen H, Hakomori S. Cloning and characterization of

DNA complementary to human UDP-GalNAc: Fuc-alpha1–2Gal alpha1–3GalNAc transferase (histo-blood group A transferase) mRNA. J Biol Chem. 1990;265:1146-1151.

6. Yamamoto F, Clausen H, White T, Marken J, Hakomori S. Molecular genetic basis of the histo-blood group ABO system. Nature. 1990;345:229-233.
7. Yamamoto F, McNeill PD, Yamamoto M, et al. Molecular genetic analysis of the ABO blood group system, 1: weak subgroups: A3 and B3 alleles. Vox Sang. 1993;64:116-119.
8. Yamamoto F, McNeill PD, Kominato Y, et al. Molecular genetic analysis of the ABO blood group system, 2: cis-AB alleles. Vox Sang. 1993;64: 120-123.
9. Yamamoto F, McNeill PD, Yamamoto M, Hakomori S, Harris T. Molecular genetic analysis of the ABO blood group system, 3: A^x and $B^{(A)}$ alleles. Vox Sang. 1993;64:171-174.
10. Yamamoto F, McNeill PD, Yamamoto M, Hakomori S, Bromilow IM, Duguid JK. Molecular genetic analysis of the ABO blood group system, 4: another type of O allele. Vox Sang. 1993;64:175-178.
11. Olsson ML, Thuresson B, Chester MA. An A^{el} allele-specific nucleotide insertion at the blood group ABO locus and its detection using a sequence-specific polymerase chain reaction. Biochem Biophys Res Commun. 1995;216:642-647.
12. Barjas-Castro ML, Carvalho MH, Locatelli MF, Bordin S, Saad ST. Molecular heterogeneity of the A3 subgroup. Clin Lab Haematol. 2000;22:73-78.
13. Ogasawara K, Yabe R, Uchikawa M, et al. Molecular genetic analysis of variant phenotypes of the ABO blood group system. Blood. 1996;88: 2732-2737.
14. Olsson ML, Chester MA. Polymorphisms at the ABO locus in subgroup A individuals. Transfusion. 1996;36:309-313.
15. Olsson ML, Chester MA. Heterogeneity of the blood group A^x allele: genetic recombination of common alleles can result in the A_x phenotype. Transfus Med. 1998;8:231-238.
16. Hansen T, Namork E, Olsson ML, Chester MA, Heier HE. Different genotypes causing indiscernible patterns of A expression on A_{el} red blood cells as visualized by scanning immunogold electron microscopy. Vox Sang. 1998;75:47-51.
17. Mollicone R, Reguigne I, Kelly RJ, et al. Molecular basis for Lewis alpha(1,3/1,4)-fucosyltransferase gene deficiency (FUT3) found in Lewis-negative Indonesian pedigrees. J Biol Chem. 1994;269:20987-20994.
18. Dupuy F, Petit JM, Mollicone R, Oriol R, Julien R, Maftah A. A single amino acid in the hypervariable stem domain of vertebrate alpha1,3/1,4-fucosyltransferases determines the type 1/type 2 transfer: characterization of acceptor substrate specificity of the Lewis enzyme by site-directed mutagenesis. J Biol Chem. 1999;274:12257-12262.
19. Olsson ML, Chester MA. A rapid and simple ABO genotype screening method using a novel B/O^2 versus A/O^1 discriminating nucleotide substitution at the ABO locus. Vox Sang. 1995;69:242-247.
20. Fukumori Y, Ohnoki S, Shibata H, Yamaguchi H, Nishimukai H. Genotyping of ABO blood groups by PCR and RFLP analysis of 5 nucleotide positions. Int J Legal Med. 1995;107:179-182.
21. Gassner C, Schmarda A, Nussbaumer W, Schonitzer D. ABO glycosyltransferase genotyping by polymerase chain reaction using sequence-specific primers. Blood. 1996;88:1852-1856.
22. Pearson SL, Hessner MJ. A(1,2)BO(1,2) genotyping by multiplexed allele-specific PCR. Br J Haematol. 1998;100:229-234.
23. Ogasawara K, Bannai M, Saitou N, et al. Extensive polymorphism of ABO blood group gene: three major lineages of the alleles for the common ABO phenotypes. Hum Genet. 1996;97:777-783.
24. Yip SP. Single-tube multiplex PCR-SSCP analysis distinguishes 7 common ABO alleles and readily identifies new alleles. Blood. 2000;95: 1487-1492.
25. Fischer GF, Fae I, Dub E, Pickl WF. Analysis of the gene polymorphism of ABO blood group specific transferases helps diagnosis of acquired B status. Vox Sang. 1992;62:113-116.
26. Anstee DJ, Issitt P. Applied Blood Group Serology. Miami, FL: Montgomery Scientific Publications; 1998.
27. Kominato Y, Tsuchiya T, Hata N, Takizawa H, Yamamoto F. Transcription of human ABO histo-blood group genes is dependent upon binding of transcription factor CBF/NF-Y to minisatellite sequence. J Biol Chem. 1997;272:25890-25898.
28. Kominato Y, Hata Y, Takizawa H, Tsuchiya T, Tsukada J, Yamamoto F. Expression of human histo-blood group ABO genes is dependent upon DNA methylation of the promoter region. J Biol Chem. 1999;274:37240-37250.
29. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16:1215.
30. Vengelen-Tyler V. Technical Manual. Bethesda, MD: American Association of Blood Banks; 1999.
31. Olsson ML, Chester MA. Frequent occurrence of a variant O^1 gene at the blood group ABO locus. Vox Sang. 1996;70:26-30.
32. Olsson ML, Guerreiro JF, Zago MA, Chester MA. Molecular analysis of the O alleles at the blood group ABO locus in populations of different ethnic origin reveals novel crossing-over events and point mutations. Biochem Biophys Res Commun. 1997;234:779-782.
33. Fukumori Y, Ohnoki S, Shibata H, Nishimukai H. Suballeles of the ABO blood group system in a Japanese population. Hum Hered. 1996;46:85-91.
34. Yamamoto F, McNeill PD, Hakomori S. Human histo-blood group A2 transferase coded by A2 allele, one of the A subtypes, is characterized by a single base deletion in the coding sequence, which results in an additional domain at the carboxyl terminal. Biochem Biophys Res Commun. 1992;187:366-374.
35. Olsson ML, Chester MA. Evidence for a new type of O allele at the ABO locus, due to a combination of the A^2 nucleotide deletion and the A^{el} nucleotide insertion. Vox Sang. 1996;71:113-117.
36. Olsson ML, Hosseini-Maaf B, Hellberg Å, Chester MA. Allele-specific primer PCR across Exon 6 resolves potential genotyping errors caused by recombinant hybrid alleles at the *ABO* locus. Transfusion. 1998;38(suppl.):3S.
37. Irshaid NM, Chester MA, Olsson ML. Allele-related variation in minisatellite repeats involved in the transcription of the blood group ABO gene. Transfus Med. 1999;9:219-226.
38. Irshaid NM, Thuresson B, Olsson ML. Genomic typing of the Kidd blood group locus by a single-tube allele-specific primer PCR technique. Br J Haematol. 1998;102:1010-1014.
39. Daniels G. Human Blood Groups. Oxford, United Kingdom: Blackwell Scientific; 1995.
40. Garratty G, Arndt P, Co A, Rodberg K, Furmanski M. Fatal hemolytic transfusion reaction resulting from ABO mistyping of a patient with acquired B antigen detectable only by some monoclonal anti-B reagents. Transfusion. 1996;36:351-357.
41. Fukumori Y, Ohnoki S, Yoshimura K, et al. Rapid detection of the cisAB allele consisting of a chimera of normal A and B alleles by PCR-RFLPs. Transfus Med. 1996;6:337-344.
42. Yu LC, Lee HL, Chan YS, Lin M. The molecular basis for the B(A) allele: an amino acid alteration in the human histoblood group B alpha-(1,3)-galactosyltransferase increases its intrinsic alpha-(1,3)-N-acetylgalactosaminyltransferase activity. Biochem Biophys Res Commun. 1999;262: 487-493.
43. Reid ME, Yazdanbakhsh K. Molecular insights into blood groups and implications for blood transfusion. Curr Opin Hematol. 1998;5:93-102.
44. van Loghem JJ, Dorfmeier H, van der Hart M. Two A antigens with abnormal serologic properties. Vox Sang. 1957;2:16-24.
45. Race RR, Sanger R. Blood Groups in Man. Oxford, United Kingdom: Blackwell Scientific; 1975.
46. Hammar L, Månsson S, Rohr T, et al. Lewis phenotype of erythrocytes and Leb-active glycolipid in serum of pregnant women. Vox Sang. 1981;40: 27-33.
47. Gupte SC, Thiel E, Majdic O, Thierfelder S, Bhatia HM. Quantitation of antibody uptake on A group erythrocytes using immunoautoradiography and monoclonal IgM anti-A. Blut. 1985;51: 251-258.
48. Beck ML, Kowalski M, Kirkegaard JR, Korth JL. Unexpected activity with monoclonal anti-B reagents. Immunohematology. 1992;8:22.
49. Chester MA, Olsson ML. The ABO blood group gene: a locus of considerable genetic diversity. Transfus Med Rev. 2001;15:177-200.
50. Yamamoto F, Hakomori S. Sugar-nucleotide donor specificity of histo-blood group A and B transferases is based on amino acid substitutions. J Biol Chem. 1990;265:19257-19262.
51. Yamamoto F. Molecular genetics of the ABO histo-blood group system. Vox Sang. 1995;69: 1-7.
52. Yamamoto F, McNeill PD. Amino acid residue at codon 268 determines both activity and nucleotide-sugar donor substrate specificity of human histo-blood group A and B transferases. In vitro mutagenesis study. J Biol Chem. 1996;271: 10515-10520.
53. Suzuki K, Iwata M, Tsuji H, et al. A de novo recombination in the ABO blood group gene and evidence for the occurrence of recombination products. Hum Genet. 1997;99:454-461.
54. Olsson ML, Hosseini-Maaf B, Irshaid NM, et al. Identification of novel A and B subgroup alleles at the ABO locus. Transfusion. 1999;39(suppl.):91S.