A novel *cis-AB* allele derived from a unique 796C>A mutation in exon 7 of *ABO* gene

Cheng-Hwai Tzeng, Ying-Ju Chen, Jau-Yi Lyou, Pei-Shan Chen, Hsueng-Mei Liu, Hui-Yu Hu, Jeong-Shi Lin, and Lung-Chih Yu

BACKGROUND: The *cis*-AB phenotype is very rare, and only three genotypes that correspond to specific *ABO* allele changes have been reported. *Cis-AB01* involves the *A102* allele with a nonsynonymous substitution G803C in exon 7, whereas *cis-AB02 and cis-AB03* involve different nonsynonymous substitutions A796C and C700T, respectively, on the *B101* allele background. The nucleotide substitutions give rise to a change of the respective glycosyltransferase, resulting in varying bifunctional AB transferase activities.

STUDY DESIGN AND METHODS: Two *cis*-AB phenotypes were identified in a Taiwanese C. family and two unrelated individuals, respectively. Serologic studies, molecular cloning, and sequencing of exon 6 and exon 7 were carried out to determine their respective phenotypic characteristics and *cis-AB* alleles. A cohort of 300 AB-phenotype, healthy random individuals served as controls.

RESULTS: A novel *cis-AB* allele is uncovered out of the three family members, of which a 796C>A substitution occurs predicting an amino acid change at residue 266 of leucine to methionine on the background of *A102* allele. It is serologically like *cis-AB03*, an A_2B phenotype, but molecularly different. Both of the two unrelated individuals are of *cis-AB01* allele, and all of the 300 AB blood group controls are excluded *cis*-AB phenotype.

CONCLUSION: The C. family described carries a novel *cis-AB* allele that differs molecularly from all previously reported *cis-AB* alleles.

The ABO blood group system was discovered by Karl Landsteiner[1] over a century ago. It is the most important blood group system in transfusion medicine. Antigens in the ABO blood group were the first human characters for which a Mendelian monofactorial mode of inheritance was demonstrated. In 1924, Bernstein et al.[2] first proposed that the inheritance of the ABO blood group was mediated by two codominant alleles, *A* and *B*, and one null, silent recessive allele, *O*. To the exception of this one gene locus, three allelic mode of transmission, Seyfield et al.[3] in 1964 first described *cis*-AB in a family where the ABO blood groups of the father and the mother were O and A_2B, respectively, and those of their two children were A_2B. Furthermore, the mother's mother had an O phenotype. The following year, Yamaguchi et al.[4] reported an A_2B_3 phenotype, which showed weak activity of both A and B antigens. The family study of this propositus suggested that both A_2 and B_3 genes were inherited simultaneously on one chromosome. In 1996, Yamaguchi et al.[5] reported another family where the three A_2B_3 children were born to the parents with O and A_2B_3 phenotype, respectively, and concluded that their A_2B_3 phenotype was inherited in the *cis* manner in contradiction to the ordinary *trans*-AB phenotype. Therefore, in addition to the classic mode of inheritance of A and B antigens expressed

ABBREVIATIONS: nt = nucleotide; aa = amino acid.

From the Section of Transfusion Medicine, Department of Medicine, Veterans General Hospital; and the College of Medicine, National Yang-Ming University; and the Institute of Biochemical Sciences, National Taiwan University, Taipei, Taiwan.

Address reprint requests to: Cheng-Hwai Tzeng, MD, Section of Transfusion Medicine, Department of Medicine, Taipei Veterans General Hospital and College of Medicine, National Yang-Ming University, 201 Shih-Pai Road, Sec 2, Taipei, Taiwan, 112; e-mail: chtzeng@vghtpe.gov.tw.

Received for publication April 5, 2004; revision received June 11, 2004, and accepted June 12, 2004.

TRANSFUSION 2004;44:50-55.

as two independent alleles, individuals with the *cis*-AB phenotype can be transmitted as a single allele.

The incidence of *cis*-AB phenotype is very low and has only been reported in a few well-documented family studies.[6-14] In 1990, Yamamoto et al.[15,16] elucidated the molecular genetic basis of three major alleles: A^1, classical *B*, and *O* (now known as *A101* or A^1; *B101*; and *O101*, O^1, or *O01*, respectively) at the blood group *ABO* gene locus by cloning A^1 transferase complementary deoxyribonucleic acid (cDNA) and cloning *B* and *O* alleles followed by nucleotide sequencing. The *ABO* gene spans over 18 kb and consists of seven exons, which range from 26 to 688 bp in size, with most of the coding sequence in exon 7. Single-base deletions and single-base substitutions account for the differences among these three major alleles, which occur mostly in the two largest exons (6 and 7) of the *ABO* gene. The *A101* allele is usually used as the reference against which all other alleles are compared, although the cDNA sequences of the *A102* allele were reported first.[15] The *O101* allele differs from the *A101* allele by a single base (G) deletion at nucleotide (nt) 261 corresponding to amino acid (aa) 87 of the A transferase.[17] This shifts the reading frame of the coding sequence and generates a premature termination codon downstream from the deletion, producing an altered and shortened polypeptide of 116 aa that lacks the C-terminal catalytic domain and hence is enzymatically inactive. The *B101* allele, on the other hand, differs from the *A101* allele by seven single-base substitutions within the coding sequence at nt 297, 526, 657, 703, 796, 803, and 930. Four of these base substitutions (nt 526, 703, 796, 803) result in aa substitutions (residues 176, 235, 266, and 268), explaining all the differences in the activity and the nucleotide–sugar donor specificity of the A and B transferases. The respective aa residues at these four positions are arginine, glycine, leucine, and glycine in A transferase, and glycine, serine, methionine, and alanine in B transferase.

Yamamoto and Hakomori[17] studied the functional role of these four aa by constructing artificial recombinant cDNA from A and B cDNA in a plasmid expression vector via transient transfection and expression in HeLa cells. Transfection experiments with these chimeric constructs established that only A or B transferase activity was demonstrated when the aa residues at the third and the fourth positions (266 and 268) were leucine and glycine (i.e., AA) or methionine and alanine (i.e., BB), respectively. For the chimeric constructs with AB, the status at the second locus seemed intriguingly influential; only A transferase activity was shown in constructs with AAB at the last three positions, whereas those with BAB showed weak B activity in addition to A activity. However, a chimeric A/B transferase activity was reported later by the same group for people with *cis-AB01* allele (AAAB).[18] In contrast, all constructs with BA at the last two positions showed both A and B transferase activities. They concluded that the third and the fourth aa substitutions were crucial, whereas the first one was not important in determining the nucleotide–sugar specificity.

As aforementioned, *cis*-AB is due to the inheritance of a single chromosome encoding an enzyme with both A and B transferase activity. *Cis-AB01* has the nonsynonymous substitution G803C (Gly268Ala) on the *A102* background and accounts for the only type found in Japan.[18-21] The alanine at codon 268 is specific to the B transferase. The encoded enzyme has the compositions of AAAB for the four characteristic aa residues with a chimeric A/B transferase activity, in contradiction to the original interpretation of the construct pAAAB by Yamamoto et al.[17] *Cis-AB02* or *cis-AB*^{var} with a single nonsynonymous substitution for *B101* at A796C (Met266Leu) in exon 7 was reported in a 37-year-old Vietnamese man, serologically typed as A_2B_{weak} with elevated H antigen on his RBCs, with a nonautoanti-B and a weak anti-A_1 in his serum.[12] The polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) study demonstrated this variant was of type BBAB for the four characteristic aa residues with a *B*-allele specificity at nt positions 526, 657, 703, and 803, but it also revealed a cytosine residue at nt 796, which is indicative of an *A*-allele. In the first series of transfection experiments, Yamamoto et al.[17] also demonstrated that a transferase with the composition of BBAB at the four characteristic aa residues exhibited chimeric A/B transferase activities. Recently, Roubinet et al.[14] described a third *cis-AB* allele in a French family. Molecular cloning and sequencing of this newly defined *cis-AB* allele named *cis-AB.tlse*01 (cis-AB03)* is identical to *B101*, except for a single point mutation at nt position 700, where a T replaces a C (BBBB), implying a change of aa (Pro234Ser).

We report here the identification of a novel *cis-AB* allele that appears to have arisen from a single nt substitution for *A102* at position 796, the third position of the four aa substitutions (AABA), which discriminate A^1 and B transferases, where an A replaces a C, implying a change of leucine being replaced by a methionine. The nomenclature *cis-AB*^{Taipei} is proposed to distinguish this new variant from the previously reported genetic configurations.

MATERIALS AND METHODS

Individuals studied

All the assessed individuals are ethnic Chinese and a written consent was obtained from all participants in consistence with the regulations of the Medical Ethics Review Board of our institution. The propositus Mrs C., a 38-year-old Taiwanese woman, was serologically typed group A_2B, gave birth to an O phenotype baby. Her husband was also typed group O. Parentage testing was performed for the trio, which confirmed their kinship by PCR-amplified short-tandem repeat analysis.[22] Her mother and one sister were also of A_2B phenotype. The three family members

and two other archived individuals without consanguinity typed as A_2B_3 were subject to genotyping by molecular cloning and sequencing both alleles for exon 6 and exon 7. The rest of the C. family with ordinary ABO blood groups received only PCR-RFLP genotyping[23] without sequencing study. A PCR/single-strand conformation polymorphism study[24] was conducted for a cohort of 300 AB-phenotype, healthy random donors referred by the Taipei Blood Center and served as controls.

Serology for ABO grouping

ABO phenotypes for RBCs were determined with commercial antisera according to the manufacturer's instructions by agglutination using murine monoclonal anti-A, anti-B, and anti-A,B immunoglobulin M antibodies (Gamma-clone, Gamma Biologicals, Houston, TX), and human polyclonal anti-A, anti-B (CLB, Amsterdam, the Netherlands), and plant lectins (*Dolichos biflorus*) for anti-A_1 (CLB), and *Ulex europaeus* for anti-H (CLB). Saliva testing was performed according to the latest edition of the AABB Technical Manual.

Molecular cloning and sequencing of the *cis-AB* gene

Genomic DNA was extracted from whole blood collected in ethylenediaminetetraacetate-coated vacuum tubes using a DNA isolation kit (Puregene, Gentra Systems, Minneapolis, MN) and processed according to the manufacturer's instructions. The exon 6 to 7 region of the *ABO* genes was PCR-amplified with ABOFh3 as forward primer (GGG TGG TCA GAG GAG GCA GAA GCT GAG TGG, 91 bp upstream to exon 6) and ABORz as reverse primer (GTT GTG AGT AAC TGA AGC CTA GGC CCC GTC, 99 bp downstream to the stop codon),[25] which yielded a PCR product with a size around 2.1 kb. Each PCR reaction was carried out with a final volume of 12.5 µL containing 1.25 µL 10× Pfu buffer, 2.5 mmol/L deoxynucleoside triphosphates, 0.625 U Pfu Turbo DNA Polymerase (Strategene, La Jolla, CA), 5 µmol/L of primers, and 30 ng genomic DNA as a template. Cycling conditions were 95°C for 2 minutes before incubation, which was followed by 35 cycles of 94°C for 30 seconds, 67°C for 30 seconds, and 72°C for 2 minutes; and a final extension at 72°C for 7 minutes, then soaked at 4°C. The PCR products were then cloned into the Zero Blunt TOPO vectors by a cloning kit (TOPO TA, Invitrogen, Groningen, the Netherlands). DNA sequences were determined with a sequence kit (BigDye Terminator Cycle Sequencing Ready Reaction Kit, Applied Biosystems, Foster City, CA) and analyzed by software (BioEdit Sequence Alignment Editor software, http://www.mbio.ncsu.edu/BioEdit/bioedit.html). To prevent any PCR-induced errors from actual sequence polymorphisms, sequencing was carried out using multiple clones from different batches of PCR products.

RESULTS

Table 1 illustrates the rare ABO phenotypes for the propositus (LJ), her sister (LL), and mother (MJ) of the C. family (A_2B) and the two individuals without consanguinity (A_2B_3). Their respective deduced genotypes of exons 6 and 7 of the *ABO* gene are shown in Table 2 with those of the well-documented *cis-AB01*, *cis-AB02*, and *cis-AB03* for comparison. It is obvious that the allelic mutation sites of the two archived unrelated individuals are identical to each other and are similar to those of *cis-AB01*, originally reported by Yamamoto et al.[18] For the trio of the C. family, however, a 796C>A substitution was noted predicting an aa change at residue 266 of leucine to methionine (Fig. 1), which is completely different as compared to the three subtypes already published. The substitution happens to

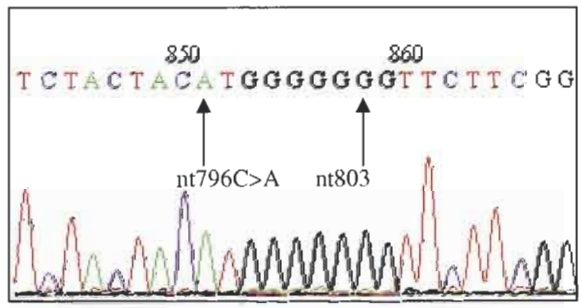

Fig. 1. The nucleotide sequence analysis at and around nt 796 of exon 7 of the *cis-AB*^{Taipei} allele. Adenine (A) residue at nt 796 is indicative of a *B* allele.

TABLE 1. Rare ABO phenotypes for the C. family and two unrelated individuals

	C. family			Unrelated individuals	
	C-LJ	C-LL	C-MJ	L-HW	H-MY
Anti-A	4+	4+	4+	4+	4+
Anti-A_1	—	—	—	—	—
Anti-B	4+	4+	4+	2+^{mf*}	2+^{mf}
Anti-A,B	4+	4+	4+	4+	4+
Anti-H	4+	4+	1+^{w}	4+	4+
A_1 cell	1+	3+^{s}	2+	—	—
Anti-B in serum	No	No	No	Yes	Yes
B secretion	NA	NA	NA	Weak	NA
pH effect†	NA	NA	NA	No	No
Serology typing	A_2B	A_2B	A_2B	A_2B_3	A_2B_3

* mf = mixed field.
† RBCs were tested with human anti-B serum at pH 6.0. Acquired B antigens do not react with the acidified antiserum, whereas normal B antigens do.

TABLE 2. The mutation sites on exons 6 and 7 of *cis-AB*^{Taipei} and other ABO/*cis-ABs*

Nucleotide position	Exon 6		Exon 7																
	261	297	467	526	579	646	657	681	700	703	771	796	802	803	829	871	930	1054	1059-61
A^{1} *(A101)*	G	A	C	C	T	T	C	G	C	G	C	C	G	G	G	G	G	C	CCC
A^{1v} *(A102)*	—	—	T	C	—	—	—	—	—	G	—	C	—	G	—	—	—	—	—
B (B101)	—	G	—	G	—	—	T	—	—	A	—	A	—	C	—	—	A	—	—
L-HY (AAAB)	—	—	T	C	—	—	—	—	—	G	—	C	—	C	—	—	—	—	—
H-MY (AAAB)	—	—	T	C	—	—	—	—	—	G	—	C	—	C	—	—	—	—	—
C. family (AABA) *cis-AB*^{Taipei}	—	—	T	C	—	—	—	—	—	G	—	A	—	G	—	—	—	—	—
cis-AB01(AAAB)	—	—	T	C	—	—	—	—	—	G	—	C	—	C	—	—	—	—	—
cis-AB02(BBAB)	—	G	—	G	—	—	T	—	—	A	—	C	—	C	—	—	A	—	—
cis-AB03 (BBBB) *cisABIse*^{1}*01*	—	G	—	G	—	—	T	—	T	A	—	A	—	C	—	—	A	—	—

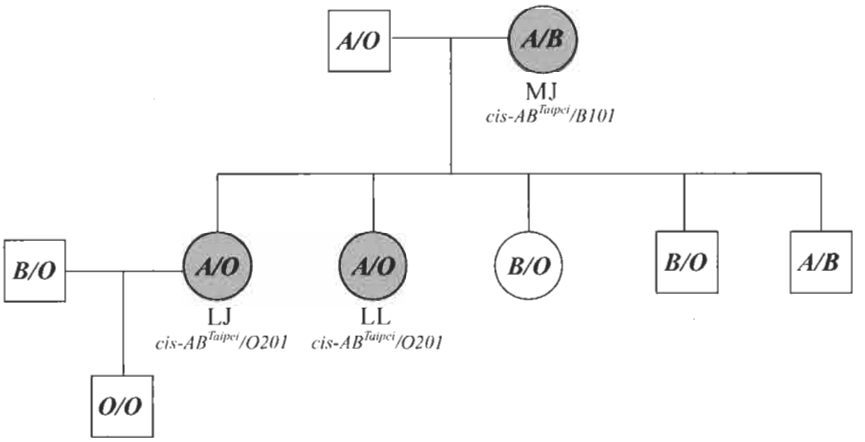

Fig. 2. Pedigree of the C. family. Genotypes after PCR-RFLP (inside shaded circles) and cloning and sequencing of both alleles from exon 6 to exon 7 of ABO gene were shown here for the propositus (LJ), her sister (LL), and mother (MJ). PCR-RFLP only was conducted for the rest of the C. family members. *O201* allele is also known as *O*^{1v} or *O02*.

the third position of the four aa substitutions that discriminate A^{1} and B transferases and, therefore, demonstrates an AABA composition as predicted by Yamamoto and Hakomori[17] in their HeLa cells transfection experiments. In contrast to both *cis-AB02* and *cis-AB03* which have nt substitutions derived form *B101*, *cis-AB*^{Taipei} and *cis-AB01* have substitutions at nt 796 and 803 on *A102*, respectively. A complete pedigree of the C. family demonstrating genotypes after PCR-RFLP and cloning and sequencing of both alleles from exon 6 to exon 7 of the *ABO* gene was shown in Fig. 2 for the propositus, her sister, and her mother, whereas the rest of the family were genotyped by PCR-RFLP only. A Mendelian monofactorial mode of inheritance was demonstrated.

As for the 300 AB-phenotype controls, serologically all had strong agglutination reaction with anti-A, anti-B, and anti-A,B, except for one case that had slightly weak reaction (3+) with anti-A, whereas no reaction was noticed for the entire group on reverse-grouping. Surprisingly, the PCR/single-strand conformation polymorphism study for this group disclosed that a total of six individuals had unusual genotypes as compared with that of the normal AB blood group. Molecular cloning and sequencing were then performed and confirmed a finding of five new variants with one silent and four missense mutations that have not been reported yet in literature (data not shown).

DISCUSSION

We report here a novel *cis-AB* allele with familial transmission. It was first suspected when the propositus, a 38-year-old Taiwanese woman phenotyped A_2B, gave birth to a baby with O phenotype on a routine blood grouping. A parentage testing was carried out and a kinship could not be excluded by a PCR-amplified short-tandem repeat analysis. The pedigree study showed that in addition to the propositus, her mother and one of her three siblings also had this A_2B phenotype. Molecular cloning and sequencing demonstrated a unique 796C>A substitution (AABA) predicting an aa change at residue 266 of leucine to methionine on the *A102* background, which to our knowledge has so far not been reported in the literature. It has been well documented that *cis-AB01* and *cis-AB02*, phenotyped as A_2B_3, and A_2B_{weak}, respectively, are characterized by the presence of A, weakened B, and elevated H antigens on the RBCs, whereas an anti-B is usually detectable in the serum nonreactive against autologous red blood cells (RBCs).[26] In contrast, it is serologically noteworthy for this particular Taiwanese C. family that no anti-B was detected in their sera but varying quantities of irregular anti-A_1 were. Furthermore, in addition to a strong agglutination of RBCs by murine monoclonal anti-A and anti-A,B reagents, murine monoclonal anti-B was also noted to have a parallel strength of agglutination to the trio's RBCs despite a geno-

typing of *cis-AB*^{Taipei}*/O201* for both the propositus and her sister, while a *cis-AB*^{Taipei}*/B* for their mother suggesting a complete B transferase activity for *cis-AB*^{Taipei}. A weak anti-H was found for the mother, probably due to her coexisting B transferase activity. It is then interesting to notice that this novel *cis-AB*^{Taipei} is serologically more like the lately defined *cis-AB03*, which also has an A_2B phenotype but a unique nonsynonymous substitution in exon 7 at position 700 of codon 234, which leads to replacement of a proline by a serine on the background of *B101*(BBBB), and therefore is not exactly the same as the one that was observed by Yamamoto and Hakomori[17] to express only B activity in the HeLa cells transfection experiments. The hydroxyl group-containing polar serine seems to be sufficient to modify the specificity of the enzyme leading to the expression of A substance comparable to that observed in A_2B individuals,[14] despite the fact that the nt substitution does not involve any of the four (nt 526, 703, 796, 803) main positions responsible for specificity differences between A and B transferases. It is therefore claimed that codon 234 of human ABO glycosyltransferase also plays a crucial role. Another allele, *B(A)*^{700} defined by Yu et al.[27] in Taiwan, also has a single substitution in exon 7 for *B101* at codon 234, but the proline was replaced by an alanine. Unlike the hydroxyl group-containing polar serine, the nonpolar alanine results in decreased B transferase activity and very low A transferase activity. On the contrary, the novel *cis-AB*^{Taipei} allele in our case that appears to derive from a single nt substitution for *A102* at position 796, the third position of the four crucial aa substitutions (AABA), does express AB transferase activity as was observed by Yamamoto and Hakomori.[17] As for the three other artificial constructions of A-B transferase cDNA chimeras having the same BA type on the third and fourth aa substitutions, a similar expression of AB transferase activity as *cis-AB*^{Taipei} is awaiting further clinical exploitation.

In summary, we have defined a novel *cis-AB*^{Taipei} allele that has a unique 796C>A mutation in exon 7 on *A102* background. The nt substitution results in the aa substitution Leu266Met, which changes the sugar donor specificity at that specific site to a B-transferase and gives rise to a bifunctional AB transferase activity, which is serologically more like *cis-AB03* rather than *cis-AB01*or *cis-AB02*. Because of the lack of any blood group information about the parents of the mother, a mutation that was demonstrated after both serologic and molecular studies might have happened to the mother herself or even her ancestors then passed to her offspring. In the present study, two unrelated individuals with A_2B_3 phenotype were proved to be of the *cis-AB01/O201* genotype. For the 300 AB-phenotype controls, however, only one case that had slightly weak reaction (3+) with anti-A, whereas no reaction was noticed for the entire group on reverse-grouping. The real incidence of *cis*-AB is unknown for 23 million people in Taiwan, and this is the first time a *cis*-AB has ever presented. Our molecular studies for the first time confirm that *cis-AB01* does exist in the Chinese population in Taiwan and have uncovered a novel *cis-AB* ^{Taipei} allele that arises from the genetic aberration of *A102* allele.

ACKNOWLEDGMENTS

This work was supported by research grants NSC 91-2314-B-075-026 from the National Science Council of Taiwan, the Republic of China and VGH-92-C-252 from the Taipei Veterans General Hospital (Taipei, Taiwan). The authors also thank the Taipei Blood Center for their kind providing of 300 AB-phenotype blood samples.

REFERENCES

1. Landsteiner K. Agglutination phenomena of normal human blood. Wien Klin Wochenschr 1901;113:768-9.
2. Bernstein F. Results of a biostatistics analysis of human blood group heredity. Klin Wochenschr 1924;3:1495-7.
3. Seyfried H, Walewska I, Werblinska B. Unusual inheritance of ABO group in a family with weak B antigens. Vox Sang 1964;9:268-77.
4. Yamaguchi H, Okubo Y, Hazama F. An A_2B_3 phenotype blood showing atypical mode of inheritance. Proc Jpn Acad 1965; 41:316-20.
5. Yamaguchi H, Okubo Y, Hazama F. Another Japanese A_2B_3 blood group family with the propositus having O-group father. Proc Jpn Acad 1966;42:517-20.
6. Madsen G, Heisto HA. Korean family showing inheritance of A and B on the same chromosome. Vox Sang 1968;14:211-7.
7. Yamaguchi H, Okubo Y, Tanaka M. 'Cis AB' bloods found in Japanese families. Jap J Hum Genet 1970;15:198-215.
8. Hummel K, Badet J, Bauermeister W, et al. Inheritance of cis AB in three generations. Vox Sang 1977;33:290-8.
9. Valdes MD, Zoes C, Froker A. Unusual inheritance in the ABO blood group system: a group O child from a group A_2B mother. Vox Sang 1978;35:176-80.
10. Yoshida A, Yamaguchi H, Okubo Y. Genetic mechanism of cis AB inheritance. I. A case associated with unequal chromosomal crossing over. Am J Hum Genet 1980;32:332-8.
11. Yoshida A, Yamaguchi H, Okubo Y. Genetic mechanism of cis AB inheritance. II. Cases associated with structural mutation of blood group glycosyltransferase. Am J Hum Genet 1980;32:645-50.
12. Mifsud NA, Watt JM, Condon JA, et al. A novel cis-AB variant allele arising from a nucleotide substitution A796C in the B transferase gene. Transfusion 2000;40:1276-7.
13. Bennet M, Levene C, Greenwell P. An Israeli family with six cisAB members: serologic and enzymatic studies. Transfusion 1998;38:441-8.
14. Roubinet F, Janvier D, Blancher A. A novel cis AB allele

derived form a B allele through a single point mutation. Transfusion 2002;42:239-46.
15. Yamamoto F, Marken J, Tsuji T, et al. Cloning and characterization of DNA complementary to human UDP-GalNAc: Fucα1->2Galα1->3Gal NAc transferase (histo-blood group A transferase) mRNA. J Biol Chem 1990;265:1146-51.
16. Yamamoto F, Clausen H, White T, et al. Molecular genetic basis of the histo-blood group ABO system. Nature 1990; 345:229-33.
17. Yamamoto F, Hakamori S. Sugar-nucleotide donor specificity of histo-blood group A and B transferases is based on amino acid substitutions. J Biol Chem 1990;265:19257-62.
18. Yamamoto F, McNeill PD, Kominato Y, et al. Molecular genetic analysis of the ABO blood group system: 2. cis-AB alleles. Vox Sang 1993;64:120-3.
19. Hosoi E, Yoshikawa K. Genetic analysis of the genotype ABO and cis-AB blood groups. Rinsyo Byori 1993;1133-40.
20. Fukumori Y, Ohnoki S, Yoshimura K, et al. Rapid detection of the cis-AB allele consisting of a chimera of normal A and B alleles by PC-RFLPs. Transfus Med 1996;6:337-44.
21. Ogasawara K, Yabe R, Uchikawa M, et al. Molecular genetic analysis of variant phenotypes of the ABO blood group system. Blood 1996;88:2732-7.
22. Tzeng CH, Lyou JY, Chen YR, et al. Polymorphisms of twelve short tandem repeat loci in a Taiwanese population and their application in parentage testing. J Formos Med Assoc 1998;97:738-44.
23. Lee JCI, Chang JG. ABO genotyping by polymerase chain reaction. J Forensic Sci 1992;37:1269-75.
24. Yip SP. Single-tube multiplex PCR-SSCP analysis distinguishes 7 common ABO alleles and readily identifies new alleles. Blood 2000;95:1487-92.
25. Sun CF, Chen DP, Lin KT, et al. Molecular genetic analysis of the B_{el} phenotype. Vox Sang 2003;85:216-20.
26. Daniels G. Human blood groups. Oxford: Blackwell, 1995: 50-3.
27. 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 α-(1,3)-galactosyltransferase increases its intrinsic α-(1,3)-N-acetylgalactosamyltransferase activity. Biochem Biophys Res Commun 1999;262:487-93.