Molecular genetic analysis of a B3 allele in a rare subtype A1 B3blood-type individual.

土井, 和子

(Degree)
博士（保健学）

(Date of Degree)
2007-03-25

(Date of Publication)
2009-04-07

(Resource Type)
doctoral thesis

(Report Number)
甲3981

(URL)
https://hdl.handle.net/20.500.14094/D1003981

博 士 論 文

Molecular Genetic Analysis of a B^3 Allele in a Rare Subtype A_1B_3 Blood-type Individual

平成 19 年 1 月 22 日

神戸大学大学院医学系研究科保健学専攻

土 井　和 子

Molecular Genetic Analysis of a B^3 Allele in a Rare Subtype A_1B_3 Blood-type Individual.

（稀な血液型 A_1B_3 に認められた B^3 対立遺伝子の遺伝子解析）

Kazuko Doi and Kaoru Nishiyama

Department of Medical Technology, Faculty of Health Sciences, Kobe University School of Medicine, Kobe, Japan 654-0142

Running Title: B^3 Allele in an A_1B_3 Individual

Correspondence: Kaoru Nishiyama

E-mail: kaoru12@kobe-u.ac.jp, Tel & Fax: +81-078-796-4542

ABSTRACT

Molecular genetic analysis of a rare subtype, A_1B_3, is reported. The targets of nucleotide alterations were within exons 6 and 7. After sequencing analysis, two typical SNPs of 547G>A and 467C>T were observed. Adenine nucleotide at nt 547 was identified as B^{var} (ref. Cho *et al*., 2004) on the *B* allele in an A_1B_3 by ASP-PCR-RFLP without a cloning procedure. This variant could be rapidly identified by the ASP-PCR with a variant-specified primer. The patterns of PCR-SSCP near nt 547 and 467 were altered. Finally this A_1B_3 individual was identified as $A^{1v}B^3$.

Key Words:

ABO gene, A_1B_3 blood group, B^3 allele, Genetic polymorphism, Mini-gel SSCP, ASP-PCR-RFLP

INTRODUCTION

In 1990, the *A*[1] allele of human blood group typing (histo-blood group A transferase) was successfully cloned and the consensus sequence of the *A*[1], *B* and *O*[1] allele was established[1-3]. In 1993, molecular genetic alterations at the ABO locus in individuals in subgroups were first reported[4-7]. Each variant was identified by the sequencing of exons 6 and 7. Usually the B_3 variant is characterized by mixed field hemagglutination of red blood cells with anti-B and with anti-A, B (O sera) antibodies[8]. Yamamoto reported on his molecular genetic analysis of three B_3 cases consisting of one case of A_1B_3 as the *B*[3] allele with a 1054C>T substitution near the 3'-terminal of the *B* allele and two cases of a B_3 with a normal sequence around exons 6 and 7[4]. Ogasawara indicated that *B109* as a B_3 species might be generated by a gene conversion-like event between *B101* and *O201*, and that, around the nt 646 area, a 646T>A substitution and lack of B specific polymorphism at nt 657 might indicate a hot spot for mutation[9]. Recently 14 cases of B_3 were clarified[10]. Of these, 13 had a mutation at the + 5 nucleotide of intron 3 (intervening sequence 3 [IVS3] + 5 G>A mutation) and one had a 247 G>T mutation in exon 6. There have been reports of novel B weak subtypes[11] (eight cases) with missense mutations in exon 7, of a pedigree analysis of B weak subtypes[12] with a substitution at nt 556A>G, and of a novel *B*[variant] *(B*[var]*)* allele with a 547G>A mutation found in A_1B_3 donors in two pedigrees[13]. In this

study, the authors explored a case of B_3 phenotype (A_1B_3) and compared it with known B_3 variants.

MATERIALS AND METHODS

Preparation of DNA specimens and PCR amplification

Blood specimens of the weak subtypes such as A_1B_3 and A_2B classified by their serological testing (data not shown) were offered by the kindness of Hyogo Red Cross Blood Center. Common blood samples were collected from healthy volunteers (A; 22, AB; 6, O; 5 and B; 5 individuals, respectively) after obtaining their consent. Common ABO blood typing was based on a method in Japanese Association of Medical Technologists (JAMT) Library XII with a test tube. The following reagents were used: anti-A and anti-B murine monoclonal antibody (Ortho Clinical Diagnostic, Japan), and anti A_1-lectin from *Dolichos biflorus* (Ortho Clinical Diagnostic, Japan). A_1, B and O red cells were prepared from healthy ABO blood cells after checking A_1, B and O antigens for reverse testing.

The genomic DNAs were prepared using a DNA extraction kit (Dona Quick II, Dainihon Co. Ltd, Japan). 1 μl of DNA (100 ng) was added to 10 μl of PCR buffer containing 0.2 mM of dNTPs, 0.2 μM of each primer and 0.2 U of *AmpliTaq Gold* (Applied Biosystems, USA). The PCR steps were an initial heating at 95 °C for 10 min, followed by 30 cycles of 94 °C for 30 sec, 62 °C for 30 sec and 72 °C for 90 sec, and a final extension at 72 °C for 5 min. All of the primers for the PCR, PCR-SSCP, PCR-RFLP and the Amplification Refractory Mutation System (ARMS) test are listed

in Table 1.

Genotyping by the SSCP analysis

We have previously reported on genotyping using SSCP analysis[14]. PCR products were diluted 9 - 20 times with a denature-reagent, 95 % formamide-25 mM EDTA- 0.05 % xylene cyanol pH 8.0, heated at 98 °C for 10 min and then chilled on ice water immediately. Each treated aliquot was applied to a polyacrylamide gel (PAG) mini-slab without glycerol, 120 (W) × 90 (H) × 0.75 (D) mm, and electrophoresed with a 0.5 × TBE buffer. The buffer temperature was maintained at 15 – 25 °C with a heat exchanger (Tempcon, AE-6370, Atto Co., Japan) equipped with an external thermo-statically controlled circulator (Superstat MINI, AB-1600, Atto Co., Japan). After electrophoresis, SSCP-patterns were visualized by silver staining[15]. SSCP analysis for exon 6 was carried out with an 8 % PAG mini-slab at 7 W, 1 hr, 15 °C. For the analyses of exon 7 in the ABO alleles, the first PCR consisted of nt 385 to 1079 (695 bp) with ex710 and ex720 as the primer pair. For SSCP analyses of 3'-terminal area, nested secondary PCR products between nt 430 and 701 (272 bp) with ex711 and ex712 as the primer pair, between nt 445 and 701 (257 bp) with ex716 and ex712 and between nt 847 and 1079 (233 bp) with ex715 and ex720 as the primer pair were amplified. Electrophoresis was performed with a 9 % or an 11 % PAG mini-slab at 5 W or 7 W, 2-2.5 hrs, 25 °C.

The ARMS test for products of exons 6 and 7

The first PCR products of exons 6 or 7 were amplified in two tubes with a variant or wild allele specific primer (v-ASP or w-ASP) and a pair of consensus primers, respectively. V-ASP-PCR products, W-ASP-PCR products and the control PCR products were obtained at the same time. The PCR products were applied to 2 % agarose gel with ethidium bromide and electrophoresed with a 1 × TAE buffer at 100 V for 20 min using an electrophoresis system (Mupid-2 plus, TaKaRa, Japan) with an M4 molecular size marker (M4, *Φ*X174/*Hae* III digest, Nippon gene) or a 100 bp ladder marker.

PCR-RFLP analysis

547A or 547G was used as the variant/consensus allele specific primer (0.2 μM) and ex713 (0.2 μM) was used as the antisense primer for the ASP-PCR. Three μl of these products (264 bp) and 2 μl of *Alu* I solution (2 μl of *Alu* I; 12 U, 5 μl of 10 × buffer and 13 μl of sterilized water) were incubated at 37 °C for 1 hr. Then the reaction mixture was applied to 2 % agarose gel with ethidium bromide and electrophoresed with a 1 × TAE buffer at 100 V for 20 min. PCR-RFLP analysis of the IVS3+5G>A mutation was performed with the IVS2 and IVS3 as primers for the PCR, and Bsm I was applied in the same manner as *Alu* I for RFLP analysis (*Bsm* I and *Alu* I were purchased from Nippon Gene Co., Ltd., Japan).

Sequence analysis

Sequence analysis was developed by the dye-termination method with the Sequencer AB-310 (Applied Biosystems, USA). Ex710 and ex714 as the sequencing primers were applied to the forward major part and 3'-terminal part, respectively.

RESULTS

Direct sequencing analysis of exon 7 in the A_1B_3 individual

Compared with ordinal AB sequence, this A_1B_3 had two points of single nucleotide polymorphisms (SNPs). The first SNP, C>T, could be detected at nt 467 (Fig. 1-A). This was assumed to be derived from the A^{1v} allele. The second SNP was detected at nt 547 with a dominant A and a little of G (Fig. 1-B). Hence in the common AB type G at this site was considered as a single sharp spike. The A_1B_3 individual had two SNPs at nt 467 and 547 in exon 7 of the ordinal A and B alleles.

SSCP analyses of exons 6 and 7

Exon 6 exhibited differences at nt 261 and 297 among the common *A, B,* O^1 and O^{1v} alleles. The corresponding nucleotides were 261G and 297A for the *A* allele, 261G and 297G for the *B* allele, 261G-del and 297A for the O^1 allele and 261G-del and 297G for the O^{1v} allele. Thus, 10 patterns *AA,* AO^1, AO^{1v}, O^1O^1, O^1O^{1v}, $O^{1v}O^{1v}$, *AB, BB,* BO^1 and BO^{1v}, arose in these common alleles. Nine SSCP patterns are shown in Fig. 2-A. Specifically, the *A, B,* O^1 and O^{1v} homozygous alleles had their own electrophoretic patterns. Their combinations were observed as heterozygous alleles. The SSCP patterns of the variant blood groups A_1B_3 and A_2B proved to be similar to the ordinary AB blood groups, respectively, as shown in Fig. 2-B.

In exon 7, SSCP analysis was carried out in two parts of the PCR product, a

5’-terminal area, 272 bp (nt 430-701) and 257 bp (nt 445-701), and a 3’-terminal area, 233 bp (nt 847-1079). The A^1 allele and the O^1 allele had the same sequence in the 5’-terminal area (nt 430-701). Compared with the A^1 allele, the *B* allele had two points of substitutions at nt 526C>G and 657C>T, and O^{1v} allele had two points of substitutions at nt 646T>A and 681G>A, respectively, in this area. Significant bands of 272 bp in SSCP analysis migrated into two groups (Fig. 3-A). In the first group, the migrated bands of the A^1 and O^1 alleles were distinguishable from those of the *B* allele, but not from those of the A^{1v} allele under this condition. The pattern of the A_1B_3 genome obviously differed from those of the common A^1B, BB, $A^{1v}A^{1v}$ and O^1O^1 alleles. Another SSCP analysis of a 5’-terminal area PCR product (257 bp) was performed with a 9 % PAG mini-slab at 5 W, 140 min, 25 °C. The *A* allele of A_1B_3 individual was confirmed to be the same as the control A^{1v} allele but distinct from the $A^1(O^1)$ allele (Fig. 3B). The migration pattern of the B^3 allele (Fig. 3B lane 2, duplet arrows) was a little different from that of the ordinal *B* allele (Fig. 3B lane 3, arrows). Thus, this A_1B_3 individual was determined as the heterozygote of an A^{1v} and a variant of B^3 alleles. For SSCP analysis of the 3’-terminal area, another nested PCR between nt 847 and 1079 (233 bp) was performed. Among the ordinal A^1, *B*, O^1 and O^{1v} alleles, only the *B* allele had a substitution at nt 930G<A. The SSCP pattern of A_1B_3 (Fig. 3-C lane 1) with an 11 % PAG mini-slab at 7 W, 2 hrs, 25 °C was identical to that of the ordinal AB blood group (Fig. 3-C lane 4), but differed from that of the A_2B blood group with A^2 allele as

1054C>T (Fig. 3-C lane 2).

Simple confirmation analysis of nucleotide substitutions of G>A at nt 547 and C>T at nt 467 by the ARMS test

The products of two paired V- and W-characteristic bands (174/173 bp with 547A/G primers and 256/255 bp with 467T/C primers, respectively) and a control band (317 bp) from the A_1B_3 individual are shown in Fig. 4. However, 174 bp product was not observed in the left lane with 547A of the AB individual (Fig. 4-Test 2-V). The results of the ARMS tests confirmed the existence of the A^{1v} allele and the B^3 allele in this A_1B_3 genome (Fig. 4-Test 1).

Localization of an nt 547G>A alteration in the A_1B_3 genome

ASP-PCR 264 bp products with 547G and ex713 between nt 529 and 792 in the AB individuals could be digested into 174 and 90 bp fragments by *Alu* I, but fragmentation from the A_1B_3 individual could not be observed (Fig. 5, Analysis-1). Since the targeting site of *Alu* I in the *B* allele was at nt 703G, 547G should not exist on the *B* allele in the A_1B_3 individual. Another ASP-PCR-RFLP analysis with 547A and ex713 showed obvious fragmentation by *Alu* I in the A_1B_3 individual, but not in the AB individual (Fig. 5, Analysis-2). In this A_1B_3 genome, the guanine nucleotide was proven to be present at nt 547 in the A^{1v} allele and the adenine nucleotide was found at nt 547 in the B^3 allele.

Other genetic information about the A_1B_3 individual

There have been several reports regarding genomic analyses of the B^3 allele; the substitution of nt1054 C>T from an A_1B_3 individual, a gene conversion-like event by the *B* and O^{1v} alleles, two-point substitutions at 646T>A and 657C in the *B109* (B^3) allele and IVS3+5G>A or 247T>G mutations. We checked whether these variable points were present in our A_1B_3 individual. The PCR-SSCP patterns of the PCR products from nt 847 to 1079 are shown in Fig. 3-C. The pattern of A_1B_3 was similar to that of AB but clearly differed from that of A_2B with a 1054C>T change in the A^1 allele. This fact was confirmed by direct-sequencing and the ARMS test with 1054T/C as the variant/ wild-ASP. Clearly, the nt 1054 of this A_1B_3 individual was not T but C as in ordinary *A* and *B* alleles. Direct sequencing proved there were no abnormalities at nt 646 and 657. In addition, it was confirmed that there was no IVS3+5G>A mutation in this A_1B_3 individual by the ARMS test (data not shown) with IVS3+5A or IVS3+5G as the specific primer and by RFLP analysis with *Bsm* I (data not shown). Regarding the 247T>G substitution checked by the ARMS test (data not shown) and the SSCP analysis of exon 6 (Fig. 2), it was shown to be a common B sequence at this point in these A_1B_3 individual.

DISCUSSION

A blood group system usually consists of one or more antigens. In addition, weak B subgroups, such as B_3, B_x, B_m and B_{el}, are very rare and are classified into subgroups as A variants. Generally, the B^3 allele is rare and may be heterogeneous. In fact, the major substitution site of the B^3 allele in the A_1B_3 individual reported here was not nt 1054, nt 646 nt 657, nt 247 or IVS3+5, but nt 547. This 547A>G variant found in a Japanese individual is the second case of the B^{var} allele that was originally described by Cho *et al.*[13]. The present study showed that a variation site could be pinpointed without the complicated cloning process. For primary genetic sorting of the *ABO* alleles and their subtype alleles, the PCR and electrophoresis techniques were applied as in other genetic investigations. SSCP analysis was effective in recognizing the SNPs under selective conditions, such as gel concentration, temperature, the length of PCR products and electrophoresis time for suitable conditions. As a result, the fine mini-slab gel SSCP-pattern was obtained. By SSCP analyses of the exon 6 and the 3'-terminal area between nt 847-1079 in exon 7, it was confirmed that the genome in this A_1B_3 individual consisted of an A^{1v} allele and a *B* allele (Fig. 2 and 3-C lane 4). And the corresponded allele to an A^1 allele was neither a *cisAB* allele nor an O^2 allele.

The B^3 allele with nt 547G >A mutation was detected on a 9 % PAG mini-slab and distinguished from other $A^1(O^1)$, A^{1v} and *B* alleles. If the presence of A^{1v} in a

genome could be known before the SSCP analysis, the B^3 allele could be found as variant allele. A variant could also be found with the 11 % PAG mini-slab electrophoresis, but under this condition, it was not clear enough to confirm the variant as the B^3 allele, nor enough to distinguish them into $A^1(O^1)$ and A^{1v} alleles. With the ARMS test or ASP-PCR-RFLP analysis, the presence of known variants in a PCR product could also be verified by simple procedures. All the procedures used in this study were based on simple electrophoresis with PAG mini-slab gel or agarose gel, after the selection of primers for the PCR and a restriction enzyme for cleaving.

The clusters predominantly between nt 536-548, 641-721, 829-873 and 965-1060 are related to the active sites of the A and B glycosyltransferases[12,16]. The amino acid substitutions in these regions can affect protein conformation. The surrounding region between aa 180 and 186, RWQDVSM, is a notable cluster which is highly preserved for its enzyme activity and has been suggested to be involved in the binding site for nucleotide sugars to the enzyme[16] and in product release[17]. Among the other B weak specimens, two SNPs have been observed as nt 539G>A (Arg 180 His) and nt 548A>G (Asp 183 Gly) in the above cluster[18]. In addition, a 556A>G variant observed in the B^w allele has been reported to cause a change in a Met by a Val at position 186[12]. Owing to this amino acid change, it was concluded that its reactivity deriving from the B^w allele was poor. The exact nt 547 position in the B^3 allele found here localized in this RWQDVSM cluster and corresponded to the substitution of G to A.

Therefore, an amino acid change at 183 of an asparagine from an aspatic acid could be predicted. This change did not seem as drastic as the change of a valine from a methione in the B^w allele.

Another substitution of the nt 467C>T substitution was observed in the A^{1v}*(A102)* allele. The ARMS test showed the allelic frequencies of A^1 and A^{1v} to be 4 (11.8 %) and 30 (88.2 %), respectively, in A and AB blood types from healthy volunteers (29 individuals; *AA*:5, *AO*:18, *AB*:6, total 34 *A* alleles, unpublished data). Indeed, the A^{1v} allele is dominant in Japan[19,20] and China[21]. The A_1B_3 individual in this study also had a 467C>T mutation (Fig. 4) and was found to be a heterozygote with the A^{1v} allele with 467C>T mutation and B^3 allele with 547G>A mutation genetically.

Acknowledgements

The authors are grateful to Dr. Teruo Harano, a former associate professor of Kawasaki Medical School, for his continuous support. The authors express thanks to Mrs. Mariko Yamashita, Hyogo Red Cross Blood Center, for her kindness.

REFERENCES

1. Yamamoto F, Marken J, Tsuji T, *et al.* Cloning and characterization of DNA complementary to human UDP-GalNAc: Fuc α1-2Gal α1-3GalNAc transferase (histo-blood group A transferase) mRNA. J Biol Chem 265: 1146-1151, 1990.

2. Yamamoto F, Hakomori S. Sugar-nucleotide donor specificity of histo-blood group A and B transferase is based on amino acid substitution. J Biol Chem 265: 19257-19262, 1990.

3. Yamamoto F, Clausen H, White T, *et al*. Molecular genetic basis of the histo-blood group ABO system. Nature 345: 229-233, 1990.

4.Yamamoto F, McNeill PD, Yamamoto M, *et al*. Molecular genetic analysis of the ABO blood group system: 1. Weak subgroups: A^3 and B^3 alleles. Vox Sanguinis 64: 116-119, 1993.

5. Yamamoto F, McNeill PD, Kominato Y, *et al*. Fujimura Y. Molecular genetic analysis of the ABO blood group system: 2. *cis-AB* alleles. Vox Sanguinis 64: 120-123, 1993.

6. Yamamoto F, McNeill PD, Yamamoto M, *et al*. Molecular genetic analysis of the ABO blood group system: 3. A^x and $B^{(A)}$ alleles. Vox Sanguinis 64: 171-174, 1993.

7. Yamamoto F., McNeill PD, Yamamoto M., *et al*. Molecular genetic analysis of the ABO blood group system: 4. Another type of *O* allele. Vox Sanguinis 64: 175-178, 1993.

8. Daniels G. Human blood groups. London, Blackwell Science Ltd, pp.43-47, 1995.

9. Ogasawara K, Yabe R, Uchikawa M, *et al*. Recombination and gene conversion-like events may contribute to *ABO* gene diversity causing various phenotypes. Immunogenetics 53: 190-199, 2001.

10. Yu LC, Twu YC, Chou ML, *et al*. Molecular genetic analysis for the B^3 allele. Blood 100: 1490-1492, 2002.

11. Olsson ML, Irshaid NM, Hosseini-Maaf B, *et al*. Genomic analysis of clinical samples with serologic ABO blood grouping discrepancies: identification of 15 novel *A* and B subgroup alleles. Blood 98: 1585-1593, 2001.

12. Estalote AC, Palatnik M, Chester MA, *et al*. A novel blood group B subgroup: serological and genetic studies. Transfus Med 14: 173-180, 2004.

13. Cho D, Kim SH, Ki CS, Choi KL, *et al*. A novel B_{var} allele (547G>A) demonstrates differential expression depending on the co-inherited *ABO* allele. Vox Sanguinis 87: 187-189, 2004.

14. Doi K, Harano T. ABO blood group typing with a PCR-SSCP method (*in Japanese*). Jap J Med Technol: 50, 927-932, 2001.

15. Bassam BJ, Caetano-Anolles G, and Gresshoff PM. Fast and sensitive silver staining of DNA in polyacryamide gels. Anal Biochem 196: 80-83, 1991.

16.Gastinel LN, Bignon C, Misra AK, *et al*. Bovine alpha 1, 3- galactosyltransferase catalytic domain structure and its relationship with ABO histo-blood group and

glycosphingolipid glycosyltransferases. The EMBO Journal 20: 638-649, 2001.

17. Unligil UM, Zhou S, Yuwaraj S, *et al*. X-ray crystal structure of rabbit N-acetylglucosaminyl transferase I: catalytic mechanism and a new protein superfamily. The EMBO Journal19: 5269-5280, 2000.

18. Chester MA, Olsson ML. The ABO blood group gene: a locus of considerable genetic diversity. Review. Transfus Med Rev. 15:177-200. 2001.

19. Ogasawara K, Bannai M, Saitou N, *et al*. Extensive polymorphism of ABO blood group gene: three major lineages of the alleles for common ABO phenotypes. Hum Genet 97: 777-783, 1996.

20. Ogasawara K, Yabe R, Uchikawa M, *et al*. Different alleles cause an imbalance in A_2 and A_2B phenotypes of the ABO blood group. Vox Sanguinis 74, 242-247, 1998.

21. Yip SP. Single tube multiplex PCR-SSCP analysis distinguishes 7 common *ABO* alleles and readily identifies new allele. Blood 95: 1487-1492, 2000.

LEGENDS OF THE FIGURES

Fig. 1 Partial sequencing data of exon 7 from the A_1B_3 individual.

The nucleotide sequences between nt 385 and 1079 of the ABO gene are shown. (A) near nt 467, (B) near nt 547.

Fig. 2 Mini-slab gel PCR-SSCP patterns for the PCR products of exon 6 in the ABO genes.

Genomic DNA fragments of exon 6 were amplified, as described in Materials and Methods. The PCR-SSCP was performed with an 8 % PAG mini-slab and 0.5 x TBE buffer at 7 W, 15 ºC for 1 hr. (A) lanes 1-9; *AA,* AO^1, AO^{1v}, $O^{1v}O^{1v}$, O^1O^1, O^1O^{1v}, A^1B, *BB* and BO^1 alleles, respectively. (B) lanes 1-3; rare subtypes, A_1B_3, A_2B and AB.

Fig. 3 The close-up mini-slab gel PCR-SSCP patterns for exon 7 in the ABO gene.

Genomic DNA fragments of exon 7 were amplified as described in Materials and Methods. Electrophoresis for PCR-SSCP was performed on 9 and 11 % PAG mini-slabs and with 0.5 x TBE buffer at 5-7 W, 25 ºC for 2- 2.5 hrs. (A) 11 % PAG at 7 W, 25 ºC for 2.5 hrs. Lane 2; product (nt 430-701) from A_1B_3, as a subtype. Arrow are used to distinguish the variant from other types. Lanes 1 and 3-5; products from four common A^1B, *BB,* $A^{1v}A^{1v}$ and O^1O^1 alleles as the control. (B) 9 % PAG at 5 W, 25 ºC for 140 min. Lane 2; product (nt 445-701) from A_1B_3, as a subtype. Lanes 1 and 3-8;

products from common $A^{1v}B$, BB, $A^{1v}A^{1}$, BO^{1}, $O^{1}O^{1}$, $A^{1v}O^{1}$, $O^{1v}O^{1v}$ alleles. Arrows and doublet arrows showed the B and the B^{3}(547G>T) alleles, respectively. (C) 11 % PAG at 7 W, 25 ºC for 2 hrs. Lanes 1-6; products (nt 847-1079; 3'-terminus) from the $A^{1v}B^{3}$, $A^{2}B$, $A^{1}O^{1}$, $A^{1}B$, $A^{1}A^{1}$ *and* BB alleles. The arrow showed the variant.

Fig. 4 ARMS test for A^{1v} and B^{3} alleles of 467C>T or 547G>A substitution.

The PCR products of 256/ 255 bp with 467T/C (2.0 mM) and 174/173 bp with 547A/G (0.1 mM) as the V- and W-ASPs, and 317 bp with a pair of consensus primers (ex710, 0.2 mM and ex712, 0.2 mM) as the PCR control were separated on 2 % agarose gel, as described in Materials and Methods. The paired ARMS test was composed of the products in the left lanes derived from 467C>T or 547G>A mutation of the A^{1v} or B^{3} allele (arrow). The right lanes were derived from 467C or 547G of the common A^{1}, B and O^{1} alleles. Test 1: A_1B_3, Test 2 :AB individuals, respectively. M4: molecular size marker.

Fig. 5 ASP-PCR-RFLP patterns with *Alu* I.

The ASP-PCR amplification products for A^{1}, A^{1v}, B and B^{3} alleles were obtained with 547G or 547A for a sense primer and ex713 for an anti-sense primer. After cleavage by *Alu* I, the lysates were simultaneously separated by 2 % agarose gel electrophoresis. Analysis-1 with 547G, and Analysis-2 with 547A. M4: molecular size marker.

Table 1. List of Primers for ABO genotyping in this study

primer names	s/as	Sequence (5' > 3')	Position	Experiment
fy57[1)]	s	gaattcatgtgggtggcaccctg	IVS5	
fy46[1)]	as	gaattcactcgccactgcctggg	IVS6	SSCP(exon 6), ARMS test 4
ex710	s	ttcctgaagctgttcctggag	385-405	1st PCR for exon 7, sequencing, ARMS test
ex720	as	ccccggcagccgctcac	1062-1079	SSCP for 3'-term.
ex711	s	ggccaccgtgtccactacta	430-449	SSCP(exon 7),
ex716	s	tactatgtcttcaccgaccag	445-465	SSCP(exon 7),
ex712	as	gggtgcagggtgccgaaca	683-701	SSCP(exon 7), ARMS test
ex713	as	gtagaaatcgccctcgtcctt	772-792	ASP-PCR-RFLP(*Alu* I)
ex714	s	gacgagggcgatttctactac	775-795	sequencing
ex715	s	gcctgccaccaggccatg	847-864	SSCP for 3'-term., ARMS test 2
ex730	as	acaacaggacggacaaaggaa	1147-1169	ARMS test 2
467T	s	actatgtcttcaccgaccagcT	446-467	
467C	s	ctatgtcttcaccgaccagcC	447-467	ARMS test for A^{1v}
547A	s	cgcctacaagcgctggcagA	528-547	ARMS test for B^{3} , ASP-PCR-RFLP(Alu I)
547G	s	gcctacaagcgctggcagG	529-547	

1) Yamamoto F, Hakomori S : J. Biol. Chem. 265: 19257-19262,1990.

Figures

Fig.1

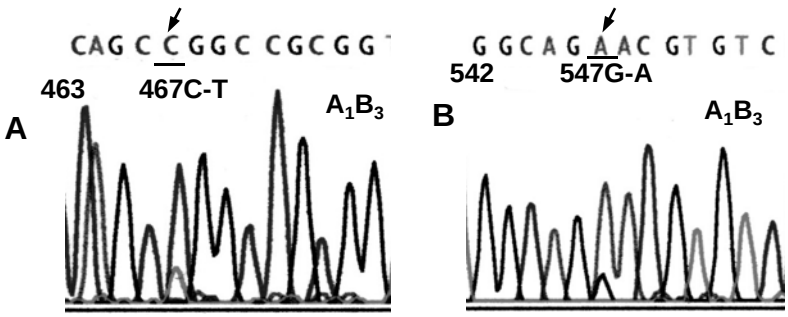
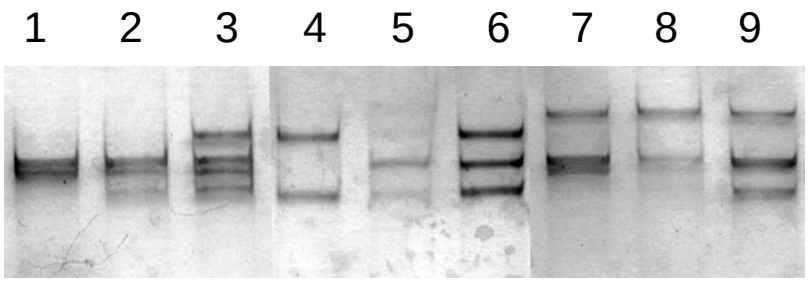


Fig.2

A



B

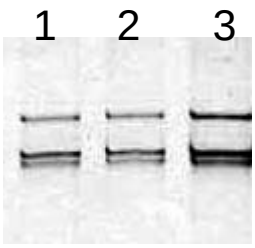


Fig.3

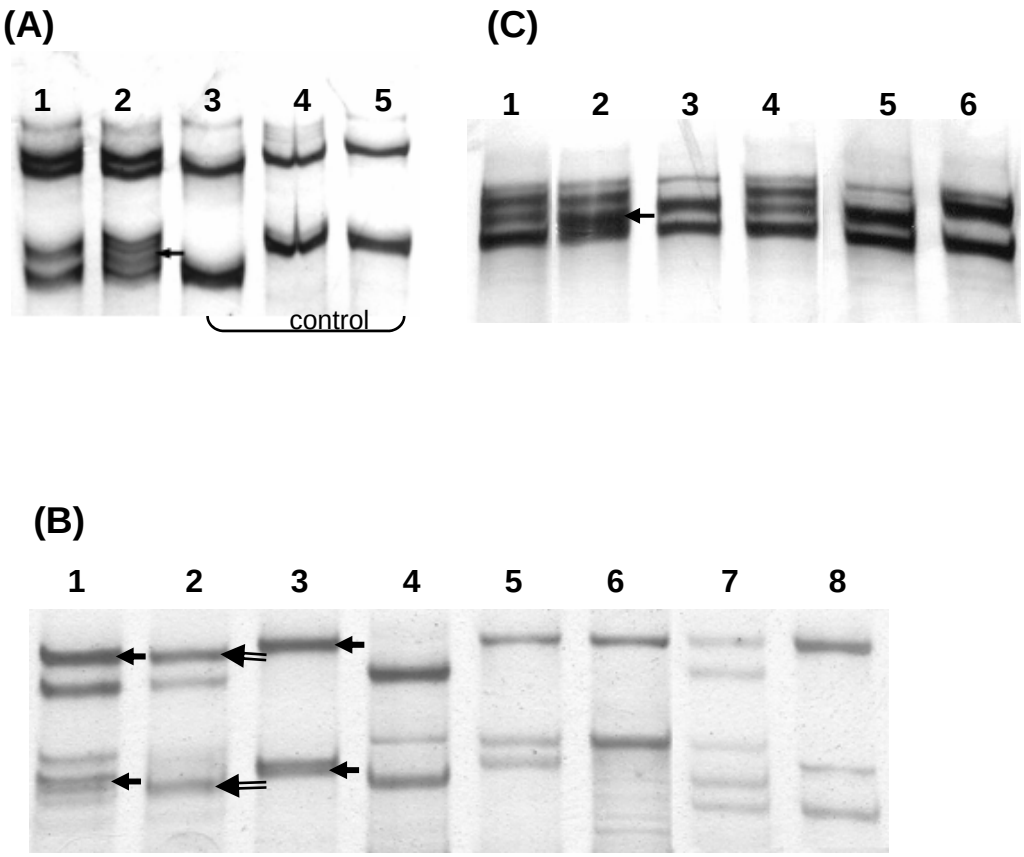


Fig.4

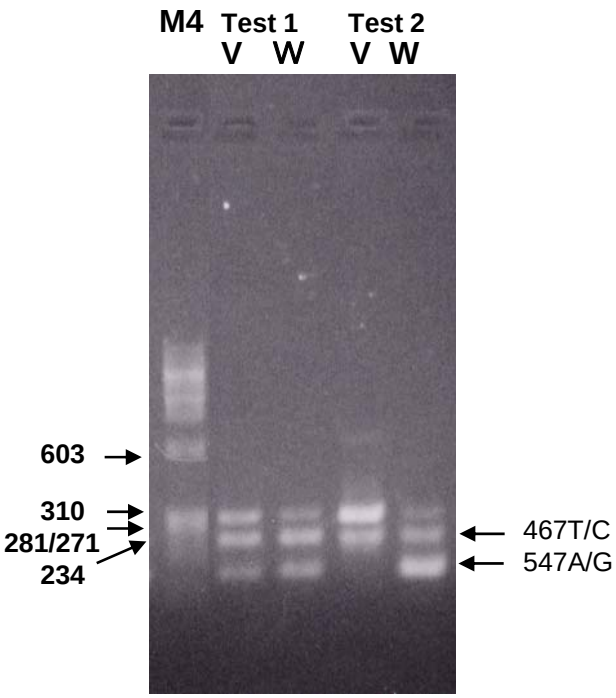
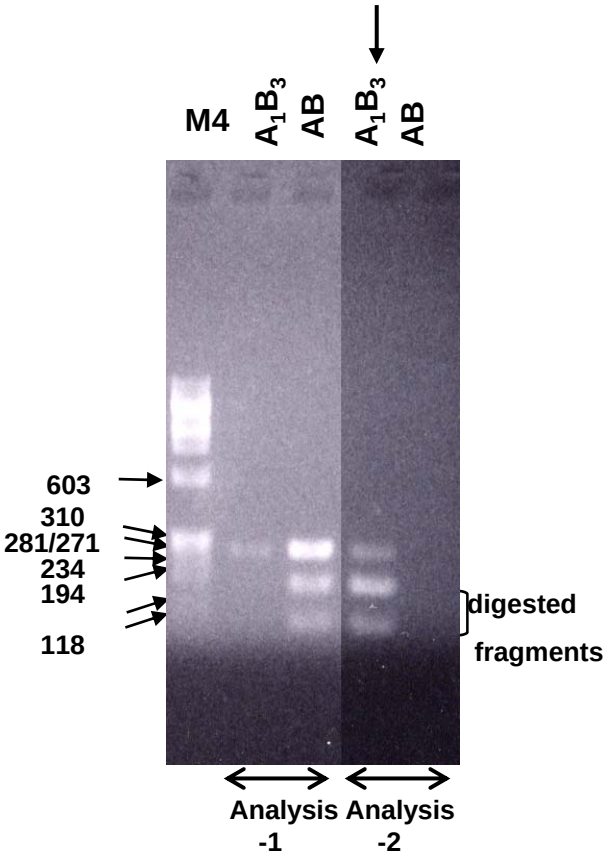


Fig.5


M4
A1B3
AB
A1B3
AB
603
310
281/271
234
194
118
digested
fragments
Analysis -1
Analysis -2