Int J Legal Med (1996) 109:90–93

ORIGINAL ARTICLE

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Genotyping of the ABO blood group system: analysis of nucleotide position 802 by PCR-RFLP and the distribution of ABO genotypes in a German population

Received: 19 October 1995 / Received in revised form: 9 May 1996

Abstract Genotypes of the ABO blood group system were studied by PCR-RFLP analysis of the eight polymorphic nucleotide positions (nps) 261, 467, 526, 646, 703, 796, 802 and 803 of the cDNA from A transferase. In 169 unrelated German individuals, 17 genotypes were found and the calculated allele frequencies of A(Pro), A(Leu), B, O(T), O(A) and O^2 were 0.2130, 0.0770, 0.0473, 0.4260, 0.2160 and 0.0207, respectively. These frequency data may provide useful additional information for disputed paternity and stain testing. A variant O allele, O^2, was fout at a polymorphic frequency. As the nucleotide (np 261) of the O^2 allele is the same as that of A and B alleles, the analysis of at least three nucleotide positions, i.e. nps 261, 526 and 802, is necessary to avoid mistyping of the ABO genotype.

Key words ABO blood group · Genotyping · PCR-RFLP · German population

Introduction

The ABO blood group system is one of the major blood group systems in man and is important in transfusion medicine, forensic serology and anthropological genetics. Since the descriptions of the molecular genetic basis of the ABO system by Yamamoto et al. [1, 2], nucleotide substitutions in several ABO subgroup alleles have been disclosed [3–6], and the ABO genotyping methods using PCR (polymerase chain reaction)-based technology have been applied to forensic science. Lee and Chang [7] described the genotyping by PCR-RFLP (restriction fragment length polymorphism) for the analysis of two nucleotide positions (np), np 261 and np 703, of the cDNA from A transferase. Improved and simplified methods, based on the method of Lee and Chang [7], for stain analyses were also reported in subsequent papers [8, 9]. We previously reported a PCR-RFLP method for the analysis of seven positions (nps 261, 467, 526, 646, 703, 796 and 803), and common A and O alleles were divided into suballeles called A(Pro), A(Leu), O(T) and O(A) [10]. Recently, a variant O allele O^2 [11] or $O_{(3)}$ [12] was described. The O^2 allele is characterized by the lack of a nucleotide deletion at np 261, and the nucleotides at nps 526, 703, 796 and 803 of the O^2 allele are the same as those of B, A, A and A alleles, respectively. In addition, a G→A substitution at np 802 was found by DNA sequencing. The existence of the O^2 allele in Danes with the phenotypes O was confirmed [11].

In the course of ABO genotyping by the PCR-RFLP method for the analysis of seven positions (nps 261, 467, 526, 646, 703, 796 and 803) [10], we found a variant allele which was suspected to be the O^2 allele. We designed a new primer and analysed the nucleotide at np 802 of this variant allele using the PCR-RFLP method instead of DNA sequencing. The present paper describes the PCR-RFLP method of np 802 analysis and the results of ABO genotyping in a German population.

Materials and methods

EDTA-blood was collected from 169 healthy unrelated German blood donors from the blood bank of the Bavarian Red Cross Mu-

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nich, Germany. ABO phenotypes were analysed by the routine serological typing method. Genomic DNA was prepared as described elsewhere [13]. Nps 261, 467, 526, 646, 703, 796 and 803 of the ABO gene were analysed by PCR with the primers GA16 (5′-AGAAGCTGAGTGGAGTTCCAGGTG-3′), GA17 (5′-TGATGGCAAACACAGTTAACCC-3′), GA01N (5′-TCCTGGAGACGGCGGAGAAGCA-3′), GA13 (5′-ACCGACCCCCCCGAAGAACG-3′) and GA14 (5′-ACCGACCCCCCGAAGAACC-3′), and RFLP with restriction enzymes BstPI, KpnI, BssHII, BanI, HapII, AluI, MvaI, PvuII and MboI, and ABO genotypes were determined as described previously [10, 14]. The nucleotide at np 802 was also analysed by PCR-RFLP. PCR was carried out with the primers GA14 which was specific to A and O alleles, and GA43 which was newly designed in this study. The GA43(5′-GCCTACATCCCCAAGGACGCG-3′) is a mismatched primer with the same sequence as nps 760–780 of the A allele except for np 779 (A→C). The PCR amplification was carried out as described previously [14]. The amplified 62bp fragments were treated with the restriction enzyme Mnl I (New England Bio Labs, Beverly, Ma), and the digestion patterns were separated on 10% polyacrylamide gels by electrophoresis. When the amplified fragment contained the nucleotide A at np 802, Mnl I cut the 62bp fragment into 35bp and 27bp fragments.

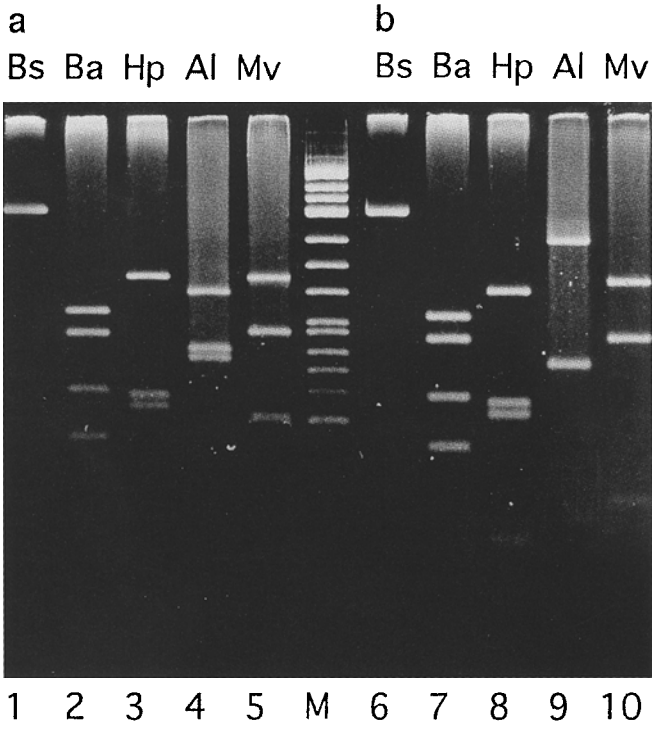

Fig. 1 RFLP patterns of amplified DNA from an individual with phenotype B (genotype B/O). **a** PCR of the B allele amplified with the GA01N/GA13 primer set (C at np 803). From the RFLP patterns, the nucleotides at np 526 (lanes 1 & 2), np 703 (lanes 3 & 4) and np 796 (lane 5) correspond to those of B allele (G, A, and A, respectively). **b** PCR of the O allele amplified with the GA01N/GA14 primer set (G at np 803). The nucleotides at np 526 (lanes 6 & 7), np 703 (lanes 8 & 9) and np 796 (lane 10) were G(=B allele), G(=A allele) and C(=A allele), respectively. Details of the RFLP patterns were given in our previous report [9]. Abbreviations of restriction enzymes used are as follows: Bs, BssHII; Ba, BanI; Hp, HapII; Al, AluI; and Mv, MvaI. M: Size marker (Marker 9, Nippon Gene, Tokyo, Japan)

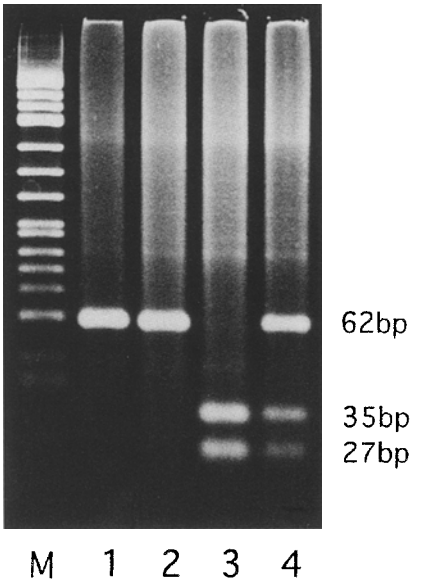

Fig. 2 RFLP patterns of DNAs amplified by PCR with the GA14/GA43 primer set. Lane 1: A allele (62bp), lane 2: O allele (62bp), lane 3: variant allele (= O^2; 35bp + 27bp), lane 4: O and the variant allele (62bp and 35bp + 27bp; genotype of the specimen was O(T)/O^2), M: Size marker (Marker 9)

Results

When the seven nucleotide positions (nps 261, 467, 526, 646, 703, 796 and 803) of the ABO gene were analysed by PCR-RFLP, two suballeles of A [A(Pro) and A(Leu)], two suballeles of O, [O(T) and O(A)], one B allele and one variant allele were detected in 169 unrelated German samples. The variant allele was found in four individuals with O phenotype, one with A phenotype and two with B phenotype. The nucleotide at np 261 of the variant allele was G which is also found in A and B alleles. The nucleotides of the allele at nps 526, 703, 796 and 803 were G(specific to B allele), G, C and G(A allele), respectively. The RFLP patterns are shown in Fig. 1. The nucleotides at nps 467 and 646 of the variant allele were C and T, respectively. The variant allele was suspected to be the O^2 allele, and the nucleotide of np 802 was analysed by PCR-RFLP.

The electrophoretic patterns of PCR products after digestion with Mnl I are shown in Fig. 2. The amplified products of the variant allele were digested by the enzyme Mnl I into 35bp and 27bp fragments, while those of A and O alleles were not digested. From these results, it was confirmed that the variant allele was O^2. The nucleotides at the eight positions (nps 261, 467, 526, 646, 703, 796, 802 and 803) of six alleles are summarized in Table 1.

The distribution of genotypes and allele frequencies are shown in Table 2, and 6 alleles and 17 genotypes were found in the German population. The observed genotype numbers did not differ significantly from those expected under the assumption of Hardy-Weinberg equilibrium.

Table 1 Nucleotides at the eight positions of six alleles

	np261	np467	np526	np646	np703	np796	np802	np803
A(Pro)	G	C	C	T	G	C	G	G
A(Leu)	G	T	C	T	G	C	G	G
B	G	C	G	T	A	A	G	C
O(T)	–	C	C	T	G	C	G	G
O(A)	–	C	C	A	G	C	G	G
O^2	G	C	G	T	G	C	A	G

np: nucleotide position

Table 2 Distribution of ABO genotypes and allele frequencies in the German population

Genotypes	Observed		Expected	
	n	(%)	*n*	(%)
A(Pro)/A(Pro)	8	(4.7)	7.7	(4.5)
A(Pro)/A(Leu)	6	(3.5)	5.5	(3.2)
A(Pro)/O(T)	29	(17.2)	30.7	(18.2)
A(Pro)/O(A)	19	(11.2)	15.6	(9.2)
A(Leu)/O(T)	11	(6.5)	11.1	(6.6)
A(Leu)/O(A)	6	(3.5)	5.6	(3.3)
B/O(T)	4	(2.4)	6.8	(4.0)
B/O(A)	4	(2.4)	3.5	(2.1)
O(T)/O(T)	32	(19.0)	30.7	(18.2)
O(T)/O(A)	32	(19.0)	31.1	(18.4)
O(A)/O(A)	6	(3.5)	7.9	(4.7)
O(T)/O^2	4	(2.4)	3.0	(1.8)
A(Leu)/O^2	1			
B/B	1			
B/O^2	2			
A(Pro)/B	2	8 (4.7)	9.8	(5.8)
A(Leu)/B	2			
Others	0			
Total	169	(100.0)	169.0	(100.0)
Allele frequencies	ABO*A(Pro)= 0.2130 ABO*A(Leu)= 0.0770 ABO*B= 0.0473 ABO*O(T)= 0.4260 ABO*O(A)= 0.2160 ABO*O^2= 0.0207 (χ^2 = 3.348, df.15, $P > 0.99$)			

Discussion

The ABO blood group system is one of the most important genetic markers in paternity testing and forensic stainwork, although many markers including VNTR and STR polymorphisms have been reported so far. In the present study, eight mutation sites of the ABO gene were investigated to construct a database of the allele frequencies for the ABO polymorphism. We performed the PCR-RFLP method for the analysis of np 802 responsible for the O^2 allele, because the method was much simpler than the DNA sequencing technique. For this purpose, we designed a new mismatched primer GA43. Amplification with the primer pair GA43 and GA14 produced a fragment with 62bp as expected. Subsequently, np 802 was successfully analysed by the digestion of the 62bp fragment with the restriction enzyme Mnl I. Since the 20 th nucleotide (np 779) from the 5′-end of the GA43 primer was C instead of A, the primer was not digested with Mnl I. If a non-mismatched primer (A at the 20th nucleotide) is used for PCR, the same size (62bp) of PCR product containing A at np 802 (= O^2 allele) is cut by Mnl I into 12bp, 23bp and 27 bp fragments, and the product containing G at np 802 (= A and O alleles) is cut into 12bp and 50bp fragments. It is very difficult to detect a 12bp fragment, and to distinguish the 23 bp fragment from the 27bp fragment on a conventional 10% polyacrylamide gel.

We found that the O^2 allele was present with a polymorphic frequency (ABO*O^2 = 0.0207) in the German population, and a recent report [11] described that the O^2 allele occurred at a frequency of 3.7% in Danish individuals with phenotype O and is therefore not a rare allele in European populations. The analysis of nps 261 and 526 has been employed for ABO genotyping [7]. The O^2 allele will be mistyped as the B allele from the analysis of nps 261 and 526, and as the A allele from the analysis of nps 261 and 703 (or 796 or 803). The analysis of at least three nucleotide positions, i.e. nps 261, 526 and 802, is therefore necessary for ABO grouping.

The frequency of the A allele (ABO*A = 0.2900) in the German population is very similar to that in a Japanese population (ABO*A = 0.2875) [10]. However, the A(Pro) suballele frequency in the German population is higher and the A(Leu) frequency is lower than those in a Japanaese population [ABO*A(Pro) = 0.0712 and ABO*A(Leu) = 0.2163]. The German population has a higher frequency of ABO*O(T) (= 0.4260) than the Japanese population (= 0.2731). The O^2 allele may be characteristic of Caucasian populations because the allele has not yet been found in a Japanese population [10].

The allele frequencies in the extended ABO polymorphism may provide useful additional information in cases of disputed paternity and stainwork. The isolated exclusion probability would be raised to 48.6% compared with 12.7% in the classical ABO blood group systems of three common alleles. The discriminating power was calculated to be 87.8%.

Acknowledgements This study was partly supported by a grant-in-aid for scientific research (No. 06670460) from the Ministry of Education, Science, Sports and Culture, Japan.

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