AB0 GENOTYPING WITH PCR

Ulrike Schacker, Peter M. Schneider*, and Manuel Zapata

Lange Str. , 76530 Baden-Baden; *Institute of Legal Medicine, 55131 Mainz, Germany

Since Landsteiner discovered the bloodgroup antigens in 1902 more than 20 independent blood group systems have been identified by serological means. Altogether more than 250 different antigens are known to be present on human red cells. Their characterization has made rapid progress due to the developments in molecular biology. The application of DNA sequence analysis has allowed the identification of coding regions of the gene products. The molecular basis of AB0 polymorphism has been known for about 30 years to be mediated via carbohydrate determinants and variant glycosyl transferases encoded by the AB0 locus on the long arm of chromosome 9. Bloodgroup A individuals express an N-acetyl-D-galactosaminyl transferase and the B-encoded enzyme is a D-galactosyl transferase. AB individuals express both enzymes and 0 individuals exhibit none of the two enzymatic activities.

The molecular cloning of the AB0-specific mRNAs was carried out by Yamamoto et al. (1990). Sequence analysis revealed that the A gene differs from B by seven nucleotides which results in four amino acid substitutions. The 0 gene was found to be identical to the A coding sequence except for a single base deletion at nucleotide position 258 which leads to a frame shift mutation resulting in the synthesis of a functionally inactive transferase. In addition, it has recently been reported by Grunnet et al. (1994) that there is a second mutation in the gene also causing nonexpression and thus a second type of the 0 allele. In this allele, position 258 is not deleted, position 526 is identical to the B allel, and the actual mutation is located at position 802. In this position, the glycin has been replaced by an arginine possibly blocking the enzymatic activity. This allele has a frequency of 3.7 % in the population. The minor subtype of A, the A2 transferase, differs from A1 by a single base substitution at nucleotide 464, which can be distinguished by restriction analysis using Nae I (A_1) or Alu I (A_2).

Based on the sequence data a combination of PCR assays was developed. Single mutations can be detected either via allele-specific PCR (ASP) or restriction enzyme digestions of the PCR product. The differentiation of $A_{(1/2)}$ and B gene products was carried out with two allele-specific PCR assays. For the first PCR amplification a 407 bp fragment was chosen which contained the variable nucleotide positions responsible for A1,A2 and A,B differentiation. The nested PCR was carried out with the respective allele-specific primer for the $A_{(1/2)}$ and B loci.

Table 1: PCR primer sequences for AB0 Genotyping

Primer	Sequence (5′ to 3′)
258-01-I	GAC ACC GTG GAA GGA TGT CCT C
258-01-II	CAA TGT CCA CAG TCA CTC GCC
523-I	TCC TGA AGC TGT TCC TGG AGA
523-II	AGT AGA AAT CGC CCT CGT CCT T
523-A	AGC TGT CAG TGC TGG AGG TGC
523-B	AGC TGT CAG TGC TGG AGG TGG

Table 2: PCR primer combinations for AB0 genotyping

Primer combination	*Restriction analysis / size*	*AB0 allele*
258-01-I + 258-01-II	Kpn I [+]: 171/28 bp	0_1
258-01-I + 258-01-II	Kpn I [-]: 200 bp	A, B
Nested PCR with 523-I + II:	407 bp	(use as template)
523-A + 523-II	266 bp	A_1 or A_2
523-B + 523-II	266 bp	B

Using these primers as well as the restriction enzyme Kpn I (Lee et al., 1992), the major blood groups A, B and 0 can be differentiated using the primers listed in Table 1 and combined in individual reactions as described in Table 2. The resulting PCR fragment lengths and patterns are shown schematically in Fig. 1. The smaller Kpn I fragments are clearly visible after polyacrylamide gel electrophoresis and silver staining. All A or B positive samples which show this additional smaller fragment after PCR and following restriction analysis are heterozygote null allele carriers. The ability of PCR analysis to identify AB0 genotypes should be useful for paternity testing and stain analysis.

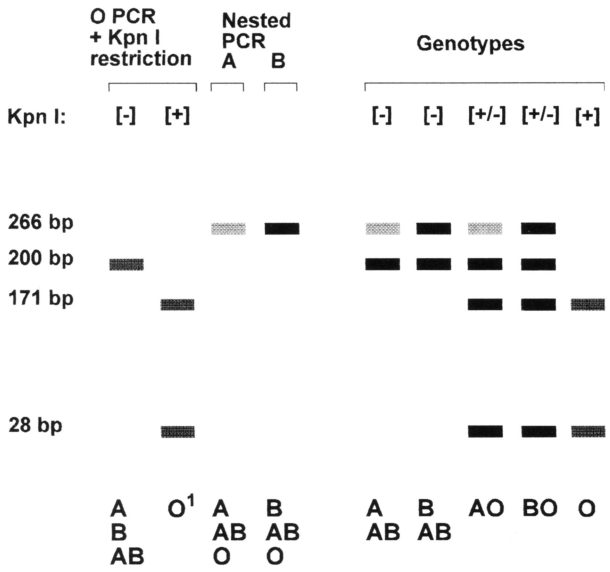

Fig. 1: PCR fragments and restriction fragments for AB0 genotyping. On the left, the individual PCR fragments from each amplification (as well as Kpn I digestion in case of the 200 bp fragment) are shown, and on the right the respective fragment combinations informative for the genotypes.

References

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