PRESENCE OF DEL261G (O1) AND 803C (B) ON ONE HYBRID ALLELE - A POTENTIAL CAUSE FOR MISINTERPRETATION OF PCR-SSP RESULTS

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Background

In blood group determination, discrepancies between pheno- and genotypes are generally an indicator for the presence of unexpected or unknown, and usually very rare blood group alleles. In the course of routine screening RhD negative donors for the presence of *RHD* genes, a crosslink-ID-control PCR, specific for the *B*-allele of the *ABO* gene, tested positive in one case, although this donor had a record of blood group A at three independent blood donations.

Methode

Standard serology was used. *ABO* genotyping was performed using a commercially available test kit “ABO” (Innotrain, Germany) and in house PCR-SSP technique. Allele-sequencing was accomplished by long range PCRs with generic amplifications of exons 1-3 and allele discriminative, nonO1- and O1-specific amplifications for exons 4 to 7, respectively.

Number of reaction	1	2	3	4	5	6	7	8
PCR-Product (bp)	134	133	194	193	195	194	172	173
Specificity	01	Non 01	02	Non 02	B	Non B	A2	Non A2
Interpretation	Pos	Pos	Neg	Pos	Pos	Pos	Neg	Pos

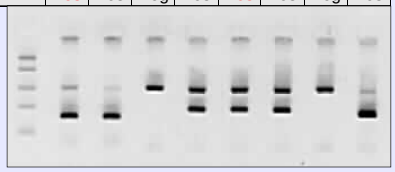

Figure 1: *ABO*-genotyping of the investigated donor revealed genotype *BO1* (“ABO” test kit)

Results

PCR-SSP *ABO*-genotyping at coding nucleotides 261, 802, 803 and 1’061, specific for alleles *O1, O2, B, A2* and *A*, respectively, revealed a *BO1* genotype of the investigated donor (**Fig. 1**). However, allele specific sequencing of exons 4-7 resulted in one “regular” *A*-allele as expected from the serological analysis (blood group A), and a second allele, carrying a G261del deletion, indicative of an *O1* allele, and on the same allele the B-specific 803C (Gassner *et al.*, 1996). The obtained sequences displayed identity to published ABO hybrid alleles ***O24*** (O1v-B; www.ncbi.nlm.nih.gov) and ***O41*** (O1v-B, tlse13), respectively (**Fig. 2**). These alleles have been identified repeatedly in samples from Brazilian blacks and Akans from Ivory Coast (Olson *et al*., 1997; Roubinet *et al.*, 2004).

	Exon 3			Exon 5			Intron 6										Exon 7					
		Exon 4			Exon 6																	
	106	188	189	220	261delG	297	163	179	271	280	446	628	786	891	901	950	526	657	703	796	803	930
***A*[1]**	G	G	C	C	G	A	T	C	A	C	A	A	A	A	G	A	C	C	G	C	G	G
B	G	G	C	C	G	G	C	T	G	T	G	G	G	G	A	G	G	T	A	A	C	A
***O*[1]**	G	G	C	C	261delG	A	T	C	A	C	A	A	A	A	G	A	C	C	G	C	G	G
***O*[24 (O1v-B)]**	T	A	T	T	261delG	G	/	/	/	/	/	/	/	/	/	/	G	T	A	A	C	A
***O*[41 (O1v-B, tlse13)]**	/	/	/	/	261delG	G	C	T	G	T	G	G	G	G	A	G	G	T	A	A	C	A
observed allele	T	A	T	T	261delG	G	C	T	G	T	G	G	G	G	A	G	G	T	A	A	C	A

Figure 2: Multiple alignment of an human ABO blood group gene *O*-allele sequence found in this study, with *O1*, *B*, *O24* and *O41* alleles. The numbering of nucleotide positions in exons 3 to 7 and intron 6 is given by reference to cDNA (position 1 being the A of the start codon) and to intron 6 of allele *A101* (position 1 being the first base of the intron), respectively.

Conclusion

The definitive name of our observed allele is uncertain, since the most similar *O24* lacks sequence information of intron 6, while for *O41*, sequence of exons 1 to 5 is missing in the respective databases. Alternatively, a new allele, having originated from an independent crossing over event, may not be excluded at this time. However, the ethnic origin of our donor points to Brazilian ancestry and makes presence of one of the yet described alleles, *O24* or *O41* more likely. Although PCR-SSP technique is a well-accepted method for allele genotyping, the regular *A* allele in the investigated sample escaped correct identification, due to the pretended independent presence of *O1* and *B*, but which were in fact encoded on one unexpressed allele simultaneously. Direct positive detection of *A* alleles would circumvent such errors, but is handicapped by the need for a 1’050 bp intron 6 crossing amplification, resulting in amplifications lengths, unusual for PCR-SSP.

References

Olsson ML, Guerreiro JF, Zago MA, Chester MA. (1997) Biochem. Biophys. Res. Commun. 234:779-82.
Roubinet F, Despiau S, Calafell F, Jin F, Bertranpetit J, Saitou N, Blancher A. (2004) Transfusion. 44:707-15.