ABO genotyping for diagnosis of unusual ABO blood groups: A Comparative Study in German Blood Donor Centers

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Background:

Unusual ABO typing results are a common problem in blood grouping. Rare *ABO* alleles have been shown to encode for the expression of aberrant ABO phenotypes, resulting in weak antigen activity as well as unexpected findings in reverse typing. Little is known about the frequency and the specificity of phenotypically relevant rare *ABO* alleles in a certain population.

Methods:

Among 142.000 red blood cell donations collected from first- and second-time donors in three German blood donor centers within a six-month period, we analyzed the *ABO* alleles of those donations in which the ABO phenotypes couldn't be defined by automated systems and/or manual serological methods. The presence of rare *ABO* alleles was determined by sequence-specific priming (BAG Health Care, Lich, Germany; Figure 1) and, if necessary, further investigated by sequencing.

Results:

198 (0,14 %) of the red cell donations revealed unusual ABO phenotypes. The majority of these red cell donations (n=107) exhibited an O phenotype with reduced iso-agglutinin activity: the non-deletional *ABO*O* allele *ABO*O03 (O^2)* was implicated in 97, and the genotype *ABO*O01/O01 (O^1)* in 5 of these cases, while known A subgroup alleles *(ABO*Ael, ABO*Aw, ABO*Ax)* were detected in only 5 cases (Figures 1 and 2). The other 91 red cell donations had A and/or B phenotypes with reduced antigen expression and/or inconsistent reverse typing results; in 14 of these cases variant *ABO*A* and *ABO*B* alleles and in one case a chimerism was found. Among the variant *ABO* alleles, twelve known *ABO*A* and *ABO*B* alleles and two new *ABO*B* alleles could be identified. The two new alleles had novel single mutations in exon 6 and in the exon1/intron1 boundary, respectively (Figure 4).

Conclusion:

Rare *ABO* alleles are the major cause of abnormal ABO blood groups in Germany. The use of a PCR-SSP technique which is designed according to population-specific allele frequencies and occasionally supplemented with DNA sequencing is an effective and economic strategy to clarify unusual ABO phenotyping results (Figure 3).

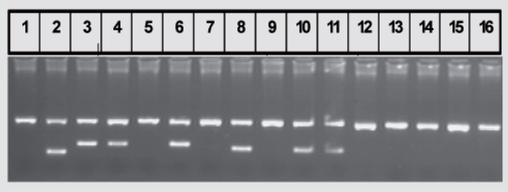
Fig. 1

Evaluation diagram, BAGene ABO-TYPE variant

Reaction no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PCR product (bp)	134	133	194	193	195	194	172	173	240	170	170	238 148	234	235	173	181
Genotype	O^1	non O^1	O^2	non O^2	B^1	non B^1	A^2	non A^2	O^{1v}	A^x B^3	A^x B^3	A^{el} A^w	A^w	A^w	A^3 B^x	B^w
Reaction pattern																
O^2A^x (sample 1)	-	+	+	+	-	+	-	+	-	+	+	-	-	-	-	-
$O^{1v}A^w$ (sample 2)	+	+	-	+	-	+	-	+	+	+	+	-	-	+	-	-

Gel Pictures

Sample 1 O^2A^x



Sample 2 $O^{1v}A^w$

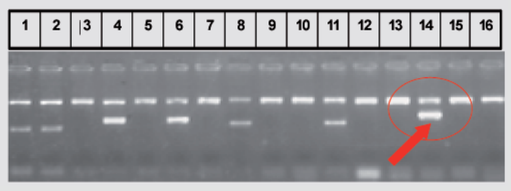


Fig. 2

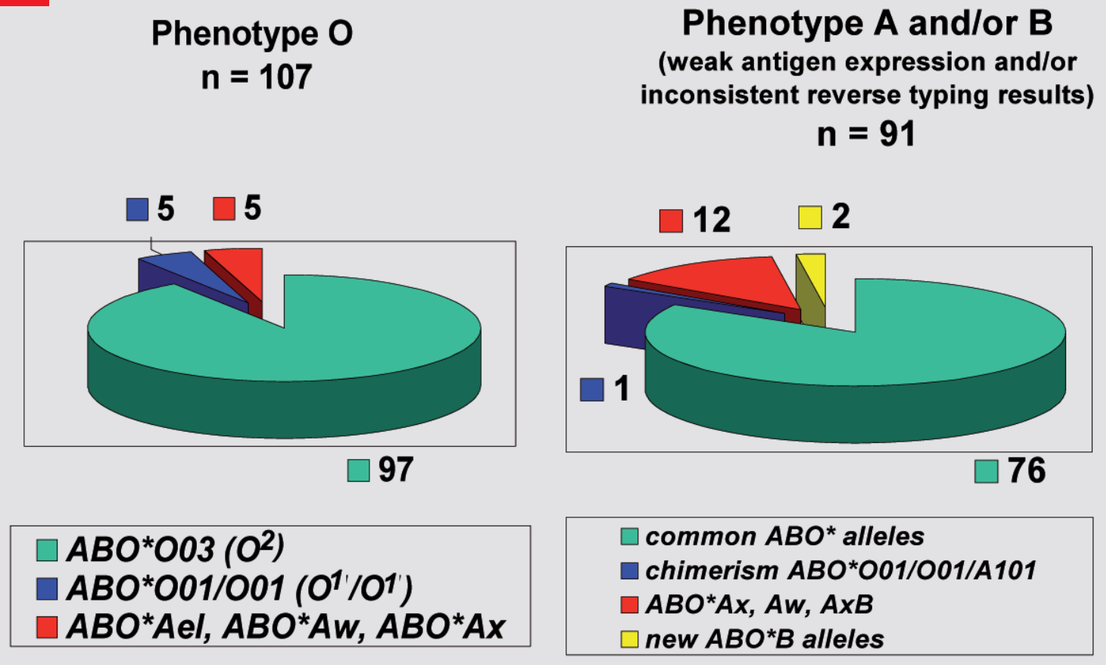


Fig. 3

ABO typing strategy

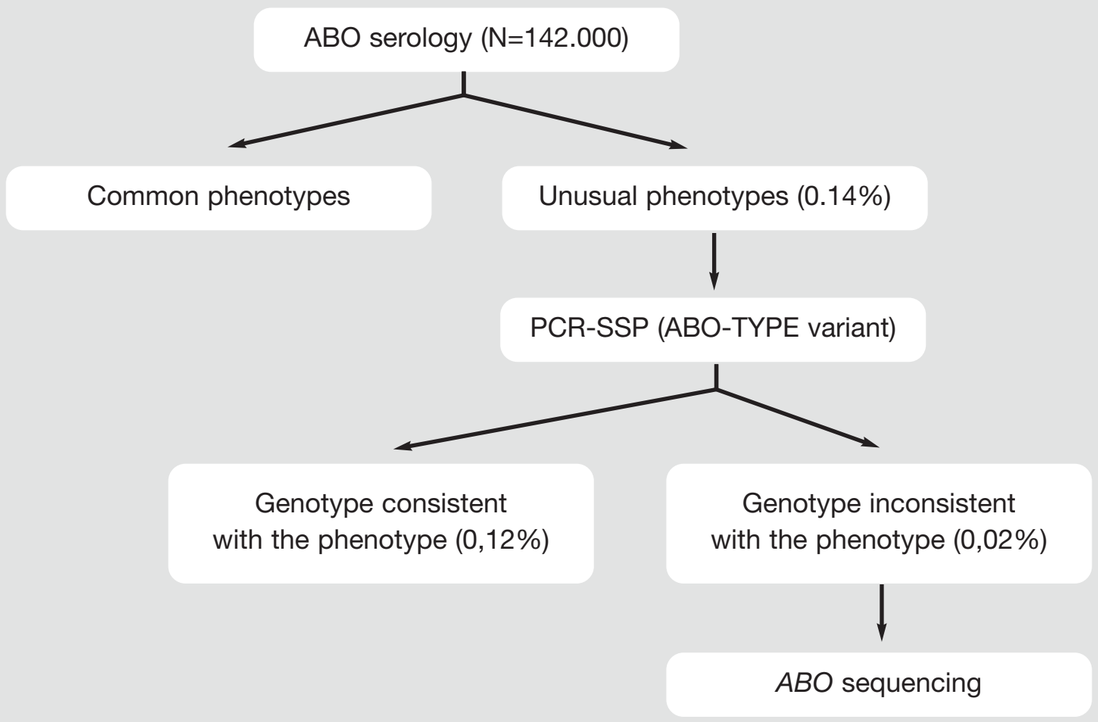


Fig. 4

Splice-site mutation detected in a novel *ABO*B* allele