A Novel Variant B Allele of the ABO Blood Group Gene Associated with Lack of B Antigen Expression

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ABO genetics · ABO genotyping · B weak · Galactosyltransferase

Summary
Background: The gene locus for the ABO blood group system encodes a glycosyltransferase. Alterations in the DNA sequence are associated with the blood groups and the expression levels of antigens on red blood cells. A number of ABO alleles have been described as the molecular basis of weak A or B antigens. Patients and Methods: Here, we describe a novel variant B allele in a blood donor with discrepant results in routine forward (group A) and reverse (very weak anti-B isoagglutinins) ABO blood grouping. Results: Determination of the ABO genotype using polymerase chain reaction-sequence-specific primers (PCR-SSP) indicated a blood group A$_2$B. Sequencing of the ABO gene exons 6 and 7 showed for 1 allele a G insertion into the GGGGGG sequence at position 811–816 of exon 7. The 816insG mutation (designated ABO*Bw20) led to a frame shift of the coding sequence and subsequent alteration of the protein sequence. The location of the mutation on a B allele was proven by PCR-SSP. Screening for the novel mutation in 211 blood donors with regular ABO phenotypes indicated that *Bw20 is a rare variant. Conclusions: The low levels of anti-B isoagglutinins associated with this novel variant indicate that residual undetectable amounts of B antigen may be present on red blood cells. The serological and molecular analysis of members of the blood donor’s family further proved the phenotype-genotype correlation of the *Bw20 allele with antigen O and individually variable levels of anti-B isoagglutinins. The characterization of novel alleles associated with ABO subgroups may ensure the correct determination of blood groups in which serological methods are combined with molecular genetic approaches.
Introduction

The ABO blood group gene locus is located on the long arm of human chromosome 9, and encodes a glycosyltransferase that mediates the expression of A and B antigens predominantly on erythrocytes. The gene structure consists of 7 exons, and the last 2 exons encompass the major proportion of the coding sequence including the catalytic domain of the glycosyltransferase [1, 2]. A number of studies have shown a clear correlation between DNA sequence variations in the coding region of the ABO gene and the quality (A or B antigens) and quantity (A2, A3, Ael, Ael, B3, B2) etc. phenotypes) of blood group antigens detectable on the erythrocyte surface [reviews in 3, 4]. Phylogenetic analysis predominantly of intron 5 sequences enabled the identification of 5 main lineages of alleles: ABO*O, ABO*B, ABO*O01, ABO*O02, and ABO*O03 [5]. The ABO alleles are listed in the open access database dbRBC on the National Center for Biotechnology Information (NCBI) website [6]. The allele listing currently includes more than 40 different alleles associated with the expression of B antigens. 28 of these alleles were found to be related to weak B phenotypes such as B2, B3a, or Bweak, and are characterized by different single nucleotide exchanges predominantly in exon 7 [7–9].

Correct typing of ABO blood groups of blood donors and recipients is the most important test for compatible transfusion of red blood cells (RBCs). Determination of antigens and the isoglutaminins are routinely performed by serological methods. Depending on the method and the type of reagents, the sensitivity of antigen detection may differ [10]. Especially in cases of substantially reduced levels of antigen expression, serological antigen typing and reverse typing may reveal unclear or discrepant results. In the case of a weak B phenotype, antigen typing may indicate blood group O because of the absence of anti-B isoagglutinins [11]. Genotyping of specific features of the ABO gene sequence may help to interpret such serological findings. In the present study, we report on a novel variant B allele as the molecular basis of a missing B antigen expression in a German blood donor and 2 family members. We established a polymerase chain reaction-sequence-specific primer (PCR-SSP) system for the detection of this variant B allele and used it to screen blood donors with regular ABO phenotypes.

Materials and Methods

Serological ABO Typing

The geographical origin of blood donors of our transfusion service is the southwestern part of Germany. Annually, more than 500,000 blood donations are typed for ABO Routine ABO antigen and reverse typing of blood donors were performed on Olympus PK 7200 automated system (Olympus Optical Co., Tokyo, Japan). For the antigen typing of the first and second time donors, two different sets of monoclonal anti-A (clone A-11H5, SIFIN Institut für Immunpräparate und Nährmedien GmbH, Berlin, Germany; and clone A003 of Biotest AG, Dreieich, Germany) and anti-B (clone B6F9, SIFIN Institut für Immunpräparate und Nährmedien GmbH; and clone B005, Biotest AG) reagents were used. Reverse typing was performed using A1, A2, B, and O red cells (Medion Diagnostics, Düdingen, Switzerland). Known donors with at least 2 previous blood donations were typed for ABO with 1 set of monoclonal anti-A (clone A003) and anti-B (clone B005) reagents. No reverse typing was performed. Samples with unclear antigen typing or discrepant reverse typing results were retested in the tube technique (reference method) using monoclonal anti-A (clones MH04, 3D3, Ortho-Clinical Diagnostics, Rochester, NY, USA) and anti-B (clones NB1.19, NB10.5A5, NB10.3B4, Ortho-Clinical Diagnostics) reagents and A1, A2, B and O red cells (Medion Diagnostics) for the reverse typing. If still discrepant or unclear the molecular tests were performed. For further antigen determination of the samples with the novel B allele an adsorption (using polyclonal anti-B) and elution (DiaMed, Cressier sur Morat, Switzerland) was performed. In the present study, blood samples of family members of the blood donor with the variant B allele were obtained on request. Antigen typing and reverse typing was performed as described above.

DNA Isolation and Standard ABO Genotyping by PCR-SSP

DNA was isolated from an EDTA-anticoagulated blood sample of the blood donor and family members using a commercial system (QIAamp Blood Kit, Qiagen, Hilden, Germany). Typing for the major ABO gene variants *A2, *B, *O01, and *O03 determining the blood groups A2, B, and O was performed as described previously [12].

DNA Sequencing of ABO Exons 6 and 7

Part of the genomic ABO gene region including exon 6, intron 6, and exon 7 was amplified with the use of a forward primer in intron 5 and a reverse primer in the 3’ untranslated region of exon 7. The coding sequences of the 2 exons was analyzed in the blood donor sample using fluorescently labeled sequencing primers and an automated DNA sequencer (LI-COR 4200L, Licor, Germany).

PCR-SSP System for the Variant B Allele

In order to establish the location of the new mutation on a B allele, we established a PCR-SSP system with forward primers specific for nucleotide position 703 (G in non-B alleles; A in B alleles) and reverse primers specific for the new frame shift mutation at position 811–816 (table 1). Part of the β-globulin locus was co-amplified as an internal control PCR fragment (536 bp) using primers β-glob-F (5’-GCTCACTCAGTGTGGCAAAG-3’). Genotyping of specific features of the ABO gene sequence may help to interpret such serological findings. In the present study, we report on a novel variant B allele as the molecular basis of a missing B antigen expression in a German blood donor and 2 family members. We established a polymerase chain reaction-sequence-specific primer (PCR-SSP) system for the detection of this variant B allele and used it to screen blood donors with regular ABO phenotypes.

Results

As a result of the daily routine typing, a first time blood donor with the following discrepancy between the ABO antigen and reverse typing was found: antigen typing group A, no anti-B
isoagglutinins on Olympus PK 7200. In the reference method (tube test), antigen typing confirmed group A and reverse typing showed a very weak detection (+/–) of anti-B isoagglutinins. In the absorption and elution analysis, no B antigen could be detected. Standard ABO genotyping of the donor by PCR-SSP showed an A2/B genotype (data not shown). Direct sequencing of exons 6 and 7 of the ABO gene revealed heterozygous sequences at position 297 (A>G) in exon 6 and at positions 467 (C>T), 526 (C>G), 570 (G>A), 796 (C>A), 803 (G>C), 930 (G>A), and 1059 (C>del) in exon 7 as expected for a *A201/B101 genotype. In addition, we identified for 1 allele the insertion of a G into the GGGGGG (G6) sequence at position 811 to 816 within or very close to the catalytic domain of the encoded glycosyltransferase. The resulting G7 sequence caused a reading frame shift of the coding sequence with an altered protein sequence downstream from amino acid position 272 of the ABO protein. This frame shift led to an extended reading frame with a TAA stop codon at nucleotide position 1174–1176 and a prolonged protein of 391 amino acids in length. According to the nomenclature of dbRBC, the novel variant B allele is designated ABO*Bw20.

To prove the location of the heterozygous 816insG mutation on the B allele, we set up a PCR-SSP system consisting of 4 reactions with primer sets I, II, III, and IV (table 1). The forward primer was either specific for G at position 703 (non-B alleles) in primer sets I and III, or specific for A at position 703 (B alleles) in primer sets II and IV. In primer sets I and II, the forward primers were combined with a reverse primer specific for the wild type sequence (G6 at position 811–816), whereas in primer sets III and IV, the forward primers were combined with the mutation-specific reverse primer (G7 at position 811–817). The blood donor sample with the missing B antigen was analyzed using the PCR-SSP system, and revealed positive PCR bands with primer sets I and IV as expected for the *A201/*Bw20 genotype (fig. 1). A total number of 211 blood donor samples with different ABO phenotypes and genotypes were further analyzed with the PCR-SSP system. All samples showed the pattern of positive and negative am-

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer direction</th>
<th>Sequence (5′-3′)</th>
<th>Sequence specificitya</th>
<th>Allele specificity</th>
<th>In primer setb</th>
</tr>
</thead>
<tbody>
<tr>
<td>703G-F</td>
<td>forward</td>
<td>TCGGCACCCCTGCACCCCG</td>
<td>703G</td>
<td>non-B</td>
<td>I, III</td>
</tr>
<tr>
<td>703A-F</td>
<td>forward</td>
<td>TCGGCACCCCTGCACCCCG</td>
<td>703A</td>
<td>B</td>
<td>II, IV</td>
</tr>
<tr>
<td>810G-R</td>
<td>reverse</td>
<td>TCTTGACGACACCCCCC</td>
<td>811–816G</td>
<td>non-Bw20</td>
<td>I, II</td>
</tr>
<tr>
<td>810C-R</td>
<td>reverse</td>
<td>TCTTGACGACACCCCCC</td>
<td>811–817G</td>
<td>Bw20</td>
<td>III, IV</td>
</tr>
</tbody>
</table>

aSequence position and nucleotide in the ABO gene sequence.
bExpected size of the specific PCR product in each primer set was 151 bp.

Fig. 1. Representative result of PCR-SSP analysis of the sample with the new variant B allele (A) and of samples with different ABO phenotypes (B–D). A A specific PCR-product of 151 bp in size was observed with primer set I (for non-B alleles) and primer set IV which proved the location of the 816insG mutation on the B allele. B Sample with B phenotype (*B101/*B101 genotype); positive result only with primer set II. C Sample with B phenotype (*O03/*B101 genotype); positive result only with primer set I. The internal control fragment of 536 bp in size (arrow head) was detectable in all reactions irrespective of the ABO genotype.

Table 1. Primer sets for PCR-SSP to prove location of the novel G-insertion on a B allele and to screen different ABO blood group phenotypes

Table 2. Genotyping results of 211 blood donors with normal phenotypes and genotypes using the PCR-SSP system specific for nucleotide positions 703 and the novel frame shift mutation of the *Bw20 allele

<table>
<thead>
<tr>
<th>ABO phenotype</th>
<th>ABO genotype</th>
<th>PCR result using sequence-specific primersa</th>
<th>Tested samples, n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Set I</td>
<td>Set II</td>
</tr>
<tr>
<td>A</td>
<td>*A101/*A101</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>*O01/*A101</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>O</td>
<td>*O01/*O01</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>*O01/*O03</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>B</td>
<td>*B101/*B101</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>*O01/*B101</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>*A101/*B101</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

aPrimer sets II and IV were specific for B alleles (bold symbols) and primer sets III and IV were also specific for the novel frame shift mutation (gray columns); primer set III (specific for non-B/Bw20 alleles) was designed to exclude the occurrence of the novel mutation on other ABO alleles; primer set I (specific for non-B/non-Bw20 alleles) was important to obtain the ABO zygosity.
plification as expected from *Bw20-negative genotypes (fig. 1; table 2). These results indicate that the *Bw20 mutation is rare and that it is unlikely to occur on other ABO alleles.

In order to confirm the genotype-phenotype correlation of the novel ABO allele, we included members (mother, father, 2 sisters) of the blood donor’s family and performed serological and molecular ABO analyses. The father (F.H.) and 1 sister (B.H.) revealed phenotype A with normal levels of anti-B isoagglutinins. PCR-SSP genotyping indicated *A101/*A201 (father) and *O01/*A101 genotypes (fig. 2). The other sister (D.H.) also showed phenotype A but with weakened anti-B isoagglutinins (2+). DNA-typing indicated a *A101/*Bw20 genotype. The ABO phenotype of the mother (U.H.) revealed O antigen with normal levels of anti-A1 (4+) and anti-A2 (4+) isoagglutinins. However, anti-B isoagglutinins could be detected only at very low levels (+/–). As expected, the mother also carried the *Bw20 allele in addition to a *O01 allele (fig. 2).

Discussion

DNA sequencing of the ABO gene exons 6 and 7 in a blood donor with missing B antigen expression and very weak anti-B isoagglutinins revealed a heterozygous single nucleotide (G) insertion in exon 7. We could prove the location of the mutation on a B allele using a PCR-SSP system with sequence-specific forward and reverse primers. This novel variant allele (ABO*Bw20) is characterized by a frame shift mutation in exon 7 of a B allele. The insertion of one additional G nucleotide into the GGGGGG sequence at position 811–816 has not been described in other ABO alleles. Other mutations of B alleles associated with weak B antigen expression have been described in similar regions of the ABO gene (*Bw02: 873C>G [7]; Bw08: 863T>G [7]; Bx01: 871G>A [8]; Bel04: 829G>A [13]). Due to the frame shift, the encoded protein sequence is altered downstream from amino acid position 272 and prolonged to a 391 amino acid protein. A similar mechanism has been described for the ABO*Ael01 allele that is characterized by a single G nucleotide insertion into the GGGGGG sequence at position 798–804 [9]. It is assumed that these substantial alterations close to the catalytic domain lead to a significant reduction or loss of the glycosyltransferase activity, and cause a weak or missing expression of ABO antigens. Frame shift mutations in downstream regions such as the C deletion at position 1059 in alleles causing the A2 phenotype [1] have lesser effects on the enzymatic activity. Depending on serological reagents and methods, significantly reduced antigen expression or a lack of the expected isoagglutinins may lead to difficulties or misinterpretation of the ABO group. A discrepancy between routine antigen typing and reverse typing often leads to the identification of weak antigen expression [7]. An example of such difficulties was a discrepancy between routine ABO typing and the result of the bedside test that led to the identification of the ABO*Aw11 allele in a blood donor with suspected 0 blood group [10]. These cases emphasize the importance of both forward and reverse ABO typing. In our case, a blood donor with normal A antigen expression showed almost undetectable anti-B isoagglutinins suggesting a missing B antigen. It is remarkable, that one of the donor’s sisters (phenotype A1) carrying the *Bw20 allele had only a moderate reduction of anti-B (2+), whereas the other sister (also phenotype A) with a slightly stronger, almost normal anti-B (3+) did not have the *Bw20 allele. The reason for the different levels of anti-B isoagglutinins in the 3 *Bw20 carriers (low in O and A2; high in A1 phenotype) is probably the catalytic activity of an additional ABO glycosyltransferase. In all 3 individuals from this family carrying the *Bw20 allele, no B antigen expression could be demonstrated, even though the mother of the donor had the phenotype O. This case, where no other functional ABO glycosyltransferase competes for the H substance, provided further evidence that the *Bw20 allele does not produce serologically detectable levels of B antigen. The correct nomenclature of such alleles is controversial. The *Aw08 allele differs from the non-deletional ABO allele *O03 by a single nucleotide change in exon 7. The phenotype caused by both alleles is very similar: serologically undetectable levels

![Fig. 2. Family pedigree including the blood donor (F.H.; index) with the *Bw20 allele. Carriers of the *Bw20 allele (gray circles) revealed lack of B antigen and individually different levels of anti-B isoagglutinins: mother (U.H.), phenotype O with very weak (+/–) anti-B isoagglutinins; sister 1 (D.H.), phenotype A with moderate reduction (2+) of anti-B isoagglutinins. The father (F.H.) and sister 2 (B.H.) showed normal phenotypes and no *Bw20 allele (plain symbols).](image-url)
of A antigen and weakened anti-A isoagglutinins [14]. Based on our findings of undetectable B antigen expression in *Bw20 allele carriers, the allele may be classified as *O allele. However, from the significantly lower levels of anti-B in allele carriers, we conclude that RBCs express a very low number of B antigens that could not be detected even with sensitive serological methods. In addition, the *Bw20 allele shared all sequence characteristics known for B alleles. The current version of the ABO database (dbRBC) includes 3 O-alleles (*O24, *O41, *O42) with sequence characteristics of B alleles in exon 7, that cause a regular O phenotype. However, these alleles have the 261delG mutation in common which is characteristic for deletional *O alleles. Presumably, these alleles originated from recombination between exon 6 of a deletional *O allele and exon 7 of a *B allele. This is not the case for the *Bw20 allele which acquired a frame shift mutation in a region critical for the enzymatic activity of the glycosyltransferase. We conclude that the glycosyltransferase encoded by *Bw20 is characterized by residual but not absent enzymatic activity.

Based on our results on the screening for *Bw20 alleles in 211 blood donors and based on the study of more than 2,000 German blood donors with missing or weakened isoagglutinins [14] in which the *Bw20 allele was not detected, we suggest that the variant allele is rather rare. It cannot be ruled out that *Bw20 is a private mutation of the ABO gene in this family. Nevertheless, the characterization of novel alleles associated with ABO subgroups may help in the correct determination of blood groups in which serological methods are combined with molecular genetic approaches [15].

References