ABSTRACT

The aim of this study was to confirm the concordance between the ABO phenotype and genotype in 34 patients undergoing renal transplant before 2010 in Sir Run Run Shaw Hospital. The ABO genotyping kit and column agglutination test (CAT) were used to examine the ABO type, and ABO subgroup was checked by sequence analysis of ABO exons 6 and 7. We found that the genotypes of serological A, AB, O, and B patients were A1A1 in 3 patients and A1O1 in 5 patients, A1B, O1O2 in 1 patient and O1O1 in 11 patients, and BB in 6 patients and BO1 in 6 patients, respectively. However, one patient, who was originally reported as serological B in the 2010 medical record and CAT showed Asub B in 2016 and sequence analysis of ABO exons 6 and 7 demonstrated B(A)04/O1. The ABO column agglutination testing combined with genotyping may provide additional value in pre-renal transplantation laboratory examinations, and it may be safe to transplant a B/O1 kidney to a B(A)04/O1 recipient since the transplantation has been successful for 6 years.

Keywords: ABO subtype, genotype, phenotype, sequence analysis

INTRODUCTION

The ABO blood group is a major clinical concern in kidney transplantation, because renal tissues express ABO antigens[1]. Rare cases of ABO discrepancies caused by ABO subgroups, immunosuppression, and it causes risks for organ transplantation[3-6]. There are many methods to identify ABO typing including slide method, tube technique and gel agglutinin test, and the sensitivity of ABO subgroups by the use of different typing methods have been reported[7-10]. Although successful ABO–incompatible renal transplantsations have been reported, incompatible live donor transplantation almost excluded due to immunosuppression. It is necessary to create studies aimed at reducing titers of ABO antibody during organ transplantation.

ABH antigens as carbohydrate structures are generally conjugated with polypeptides to form glycoproteins and antigens (as are histocompatibility antigens expressing throughout the body), which are very pertinent to transplantation. The ABO antigen–antibody complex can cause serious hyper reactions such as haemolytic disease in the new and fatal hemolytic transfusion reactions. Therefore, confirmation of the A or AB subtype must occur prior to proceeding with transplantation, and ABO results of deceased or living donors and recipients must be recorded in the Organ Procurement and Transplantation Network in U.S.
In China, column agglutination testing replacing slide and tube method has been used to define ABO types since 2008. The present study was conducted to confirm the concordance between the ABO serological blood types (as grouped in the historical records) and the genotypes of patients.

**MATERIALS AND METHODS**

**Study design and population**

This retrospective study was conducted in 2016 to observe the concordance between the genotypes and phenotypes of major ABO blood groups of patients, who underwent renal transplantation at the Sir Run Run Shaw Hospital before January 2010. Recipients accepting ABO-major incompatible kidneys were excluded from the study.

**DNA sample collection and molecular genetic analysis of ABO exons 6 and 7**

DNA was extracted from peripheral EDTA-treated anti-coagulated blood using a whole blood commercial kit (TIANamp Blood DNA Kit, Tiangen Biotech CO., LTD, Beijing, China). The sequence primers and amplification protocol for ABO exons 6 and 7 were designed according to the Sanger sequence method by a commercial ABO exon 6, 7 kit (Jiangsu LiBioMedicine Biotechnology, China). Sequencing of exon 6, 7 PCR purified products was done by Sangon Biotech (Beijing, China) and the results were analyzed using sequence analysis software (Geneious R9, New Zealand). The A101 allele sequence (GenBank No. AF134412) template was used as a reference to analyze and mark the mutations.

**ABO phenotyping and genotyping**

Routine ABO blood typing was performed by column agglutination testing using monoclonal reagents for forward typing of the A, B, and D antigens and using commercial A1, B, and O red blood cells for reverse typing on an automated system (Jiangsu LiBioMedicine Biotechnology, China). Sequencing of exon 6, 7 PCR purified products was done by Sangon Biotech (Beijing, China) and the results were analyzed using sequence analysis software (Geneious R9, New Zealand). The A101 allele sequence (GenBank No. AF134412) template was used as a reference to analyze and mark the mutations.

**RESULTS**

The genotyping and phenotyping results of the ABO blood groups of patients who received matching ABO blood type kidney transplantations are presented in **Table 1**. The genotypes of serological A patients \( (n=8) \) were A1A1 and A101 in 3 and 5 patients, respectively; the serological AB patient \( (n=1) \) was A1B; the serological O patients \( (n=12) \) were O102 and O1O1 in 1 and 11 patients, respectively; the serological B patients \( (n=12) \) were BB and B01 in 6 and 6 patients, respectively. There was a significant consistency between the genotype and serotype reported in the historical records, except one patient, who was reported as serological B type in the historical record, but the results showed anti-A: 1+, anti-B: 4+ in forward typing, and A cell: 3+, B cell: negative in reverse typing; the indirect Coombs test was negative (**Table 1**).

**Table 1**

<table>
<thead>
<tr>
<th>Historical record</th>
<th>Gel agglutinin testing at 2016</th>
<th>Genotyping</th>
</tr>
</thead>
<tbody>
<tr>
<td>A(8, 23.5%)</td>
<td>A (8, 23.5%)</td>
<td>A1A1(3, 8.8%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A101(5, 14.7%)</td>
</tr>
<tr>
<td>O(12, 35.3%)</td>
<td>O (12, 35.3%)</td>
<td>O1O1(11, 32.4%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>O1O2(1, 2.9%)</td>
</tr>
<tr>
<td>AB (1, 2.9%)</td>
<td>AB (1, 2.9%)</td>
<td>A1B (1, 2.9%)</td>
</tr>
<tr>
<td>B(13, 38.2%)</td>
<td>B (12, 35.3%)</td>
<td>BB (6, 17.6%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BO (6, 17.6%)</td>
</tr>
<tr>
<td>A subgroup B(1, 2.9%)</td>
<td></td>
<td>BO1(1, 2.9%)</td>
</tr>
</tbody>
</table>

2). The result of tube method demonstrated that it was anti-A1: negative and anti-H: 4+.

The patient showing a suspected ABO discrepancy was a 40-year-old Chinese male with a history of endstage chronic kidney disease, who was placed on the active waiting list for renal transplantation. Both the donor—the recipient’s brother—and the recipient were identified as serological blood type B during the pre-transplantation laboratory examinations in 2006. We investigated the family pedigree of the ABO blood groups (**Fig 1**). The genotypes of the recipient’s father and mothers were B(A)04/O101 and B101/O101, and those of the recipient and his brother were B(A)04/O101 and B101/O101, respectively. The serological
results of all family members are shown in Table 2.

The recipient’s sequencing results of ABO exon 6,7 demonstrated heterozygous 261delG and 297A/G in exon 6, heterozygous 526C/G, 640A/G, 657C/T, 703G/A, 796C/A, 803G/C, 930G/A and 1096G/A in exon 7 (Fig 2). These mutations were in conformity with B(A)04/O101.

**DISCUSSION**

Considering the challenge of donor kidney availability and the frequencies of the different serological blood types, renal transplantation from a close blood relative is preferred in China. Rare cases of ABO subgroups causing blood type mismatch compared to the blood type described in the historical record have been reported since column agglutination testing was firstly certified by the Chinese Food and Drug Administration in 2008 [7]. Furthermore, the first case of ABO major incompatible renal transplantation in China was reported in December 2006 [8]. In the present study, we reviewed the concordance between the genotyping and serological ABO blood group typing results in 34 patients, who received a kidney with the matched ABO blood type, and found a high consistency between the phenotypes and genotypes, except one case of ABO discrepancy and mismatch compared to the historical record. Consequently, family pedigree investigation based on the phenotype and the sequencing analysis of ABO exons 6 and 7 was performed.

This mismatched case indicates the feasibility of renal transplants may walk beyond the B1O1/B(A)04O1 donor–recipient barrier. ABO-incompatible renal transplants are considered routine surgery; however, less study on renal transplantation between ABO subgroup–mismatched individuals have been reported. Fadeyi et al. successfully performed renal transplant across the A1B/A2B donor–recipient barrier without pre–transplantation antibody reduction therapy [9].

Exact ABO blood grouping in both the donor and recipient is a very important issue during the pre–transplant laboratory examination, as renal tissues express ABO antigens, and antibody–mediated rejection can hence occur as the result of the presence of antibodies against the donor endothelium [10]. To our knowledge, this case is the first report of a B(A)04 patient receiving a serological type B kidney and in whom ongoing stable clinical status without allograft rejection at the latest follow-up 9 years after the transplant.

In conclusion, gel agglutinin testing represents a highly sensitive tool for determining ABO groups, and genotyping may be necessary for confirming the serological results. Furthermore, based on the case presented herein, it may be safe to transplant a B/O1 kidney to a B(A)04/O1 recipient, which needs further studies.

**References**


(Received 17 January 2017, Revised 22 February 2017, Accepted 04 March 2017)