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Consistency of ABO blood group phenotypes and genotypes in renal transplant patients

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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 A1A1in 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 successfor 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[2–4]. 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[5,6]. Although successful ABO–incompatible renal transplantations have been reported, incompatible live donor transplantation almost excluded due to immunosuppression. It is necessary to create studies aimed at reducing titers of ABO antibodyduring organ transplantation.

ABH antigens as carbohydrate structures are generally conjugatedwith polypeptides to form glycoproteins and antigens(as arehistocompatibility antigensexpressing throughout the body), which are very pertinent to transplantation. The ABO antigen–antibody complex cancause serious hyper rejections 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 ofdeceased or living donors and recipients mustbe recorded in the Organ Procurement and Transplantation Network in U.S.

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In China, column agglutination testing replacing slide and tube methodshas been used to define ABO typesince 2008. The present study was conducted to confirmthe concordance between the ABO serological bloodtypes (as grouped in the historical records)andthe genotypesof 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 transplantationat at the Sir Run Run Shaw Hospital before January 2010. Recipients accepting ABO–major incompatible kidneys were ex–cluded 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 commer–cial 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 tothe Sanger sequence method by a commercial ABO exon 6,7 kit (Jiangsu LiBioMedi–cine Biotechnology, China). Sequencing of exon 6,7 PCR purified products was done by Sangon Biotech (Beijing, China) and the results were analyzed us–ing sequence analysis software (Geneious R9, New Zealand). The A101 allele sequence (GenBank No. AF134412) template was used as a reference to ana–lyze and mark the mutations.

ABO phenotyping and genotyping

Routine ABO blood typing was performed by col–umn 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 re–verse typing on an automated system (Jiangsu LiBio–Medicine Biotechnology, China).

Polymerase chain reaction with sequence–specific primers was performed for theABO blood group gen–otyping by using the commercial kit (ABO basic gen–otype, Jiangsu LiBioMedicine Biotechnology, China).

RESULTS

The genotyping and phenotyping results of the ABO blood groupsof 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, re–spectively; the serological AB patient (*n*=1) was A1B; the serological O patients (*n*=12) were O1O2 and O1O1 in 1 and 11 patients, respectively; the serologi–cal B patients (*n*=12) were BB and BO1 in 6 and 6 patients, respectively. There was a significant con–sistency 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 for–ward typing, and A cell: 3+, B cell: negative in reverse typing; the indirect Coombs test was negative ***(Table 2)***. The result of tube method demonstrated that it was anti–A1: negative and anti–H: 4+.

Table 1 **Historical record, gel agglutinin test results from 2016, and genotyping results of patients under–going renal transplantation prior to 2010 (*n*=34)**

Historical record	Gel agglutinin testing at 2016	Genotyping
A(8, 23.5%)	A (8, 23.5%)	A1A1(3, 8.8%)
		A1O1(5, 14.7%)
O(12, 35.3%)	O (12, 35.3%)	O1O1(11, 32.4%)
		O1O2(1, 2.9%)
AB (1, 2.9%)	AB (1, 2.9%)	A1B (1, 2.9%)
B(13, 38.2%)	B (12, 35.3%)	BB (6, 17.6%)
		BO(6, 17.6%)
	A subgroup B(1, 2.9%)	BO1(1, 2.9%)

The patient showingasuspectedABO discrepancy was a 40–year–old Chinese male with a history of endstagechronic 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 fa–ther and mothers were B(A)04/O101 and B101/O101, andthose of the recipient and his brother were B(A)04/O101 and B101/O101, respectively. The serological

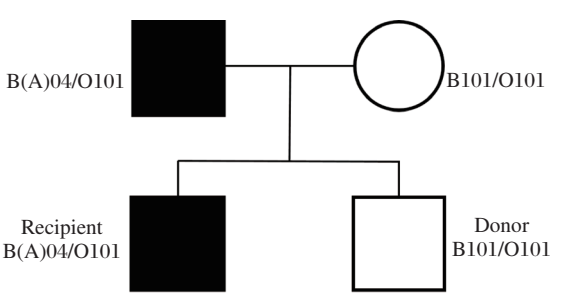

Fig. 1. **Family pedigree of the patient with suspected ABO discrepancy according to the probable ABO exon 6 and 7 sequence results.**

Table 2 **Genotyping and serological ABO blood grouping by gel agglutinin testing of the patient with inconsistent ABO subgroup results and his family**

	Forward			Reverse		
	Anti–A	Anti–B	Anti–D	A cell	B cell	O cell
Recipient	1+	4+	4+	3+	0	0
Donor(Recipient´s brother)	0	4+	4+	4+	0	0
Mother	0	4+	4+	4+	0	0
Father	1+	4+	4+	3+	0	0

results of all family members are shown in ***Table 2.***

The recipient´s sequencingresults of ABO exon 6,7 demonstrated heterozygous 261delG and 297 A/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 causingablood 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.

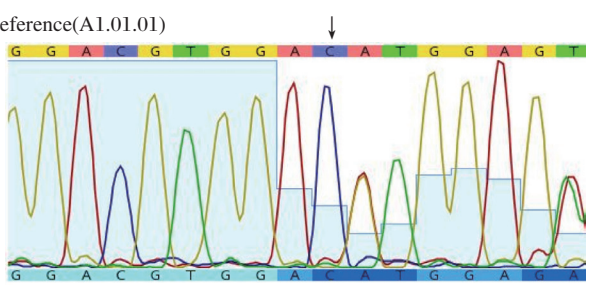

Fig. 2. **Partial ABO exon 7 sequence of recipient.** Arrow indicates 640A>G heterozygous as the key SNP in B(A)04 subgroup.

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, lessstudy 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.

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