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Serological and molecular analysis of anti-Tja: Case report

Zhiyuan Xu[1], Haochun Chang[2,3,4], Xiaojie Zhu[2,4], Chen Cao[2,4], Tianhong Miao[1], Jiwu Gong[4,5], Yu-Shiang Lin[2,4*]

[1]*Blood Group Lab, Beijing Red Cross Blood Center, Beijing 100088, china*

[2]*Nobel Prize Winner Erwin Neher Research Institute, Jiangsu Zhongji Wantai Biological Pharmaceutical Co., Ltd. Jiangyin, Jiangsu 214400, china*

[3]*Department of Clinical Medicine, School of Basic Medical Sciences, Peking University Health Science Center, Beijing 100191, china*

[4]*The United Reference Laboratory for Blood Group Gene Detection, National Center for Clinical Laboratories, Beijing 100730, china*

[5]*Department of Transfusion, National Center of Gerontology, Beijing Hospital, Beijing 100730, China*

ABSTRACT

P antigen frequency is very low in the Chinese population. However, the presence of anti-P1PP^k (anti-Tja) is a huge risk for patients undergoing clinical transfusions and recurrent abortions. This report aims to describe p antigen and anti-Tja serological test features and suggests ways in which we may better identify the p antigen. Polymerase chain reaction was used to amplify A4GALT and B3GALNT1, which were then analysed for polymorphisms using Sanger sequencing. The A4GALT sequence results revealed c. 547-548delAT (HE818933), which resulted in a frame shift at aa 183 stopping at aa 281 (M183fs, 281X). Compared with the reference sequence, B3GALNT1 did not show any variations in any of the subjects assessed. Eggs from *Columba livia* were used in the neutralised P substance test, but failed to neutralise anti-Tja. The serological test and molecular analysis confirmed that the P blood antigens are caused by A4GALTc. 547–548 AT deletion, and the neutralised P substance test cannot identify anti-PP1Pk from RBC alloantibodies against high frequency antigens.

Keywords: anti-Tja, P blood group, p phenotype, A4GALT

INTRODUCTION

Very few reports have demonstrated the presence of anti-Tja in the Chinese population. According to recent news from the National Rare Blood Bank of China, type P is a very rare blood type, with only several cases found among millions of people[1]. Our report aims to describe features of a serological test for p antigen and anti-Tja, and suggests improved techniques for the identification of p blood antigens.

CASE REPORT

A 61-year-old female requiring exact diagnosis at our centre due to antibody identification demonstrated suspect to alloantibodies against high frequency antigens of red blood cells. The antibody screening and identification tests showed that all cells had an agglutinin level of 3+ when combined with patient plasma. Meanwhile, the auto-control and direct Coombs test were negative. The phenotype was reported as P1negative, Fy(a+b-)and Jk(a-b+).

Samples and serological tests

EDTA-treated, anti-coagulated peripheral blood was stored at 4°C. Serological tests and DNA extraction were performed within 12 hours of blood acquisition. The DNA was purified from buffy coat samples using micromagnetic technology with a commercial kit (Magnetic Bead, Texas Biotechnology Co., Ltd., Xiamen, China) and an automatic instrument (EZ

*Correspondence to: Yu-Shiang Lin, Nobel Prize Winner Erwin Neher Research Institute, Jiangsu Zhongji Wantai Biological Pharmaceutical Co., Ltd. Jiangyin, Jiangsu 214400, China. E-mail:1448639963@qq.om

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Bead-32, Texas Biotechnology Co., Ltd., Xiamen, China.) . All DNA samples were stored at -40°C until molecular analysis.

Routine ABO, RhD blood typing and antibody screening and identification used micro-column tests [ABO RhD Blood Typing Card, ABO cells, screen cells, and Anti-Human Globulin Card (anti-IgG and anti-C3d), Jiangsu Libio Biotech Co.Ltd, Jiangsu, China; Panel cells, Jiangsu ZhongJi Wantai Biological Pharmaceutical Co Ltd, Jiangsu, China]. P1 phenotyping was done using monoclonal anti-P1 (Jiangsu ZhongJi Wantai Biological Pharmaceutical Co Ltd, Jiangsu, China).

The neutralised P1 substance test

Turtle dove eggs were used for the neutralised P1 substance test. The egg was diluted 100× through normal saline and mixed up and down 20 times. This diluted solution (10 μL) was mixed with 200 μL of patient plasma and incubated at room temperature for 15 min. Monoclonal anti-P1 was used as a positive control and normal saline as a negative control. After incubation, the neutralised plasma reacted with the P1+ screen cells. The neutralised P substance test was negative. When the monoclonal anti-P1 and saline controls were incubated with P substance and cross-matched with P1+ cell, the result was negative, in contrast with when plasma from a patient with p phenotype was used, which resulted in a recording of 3+.

Fluo-genotyping

Multi-erythrocytic blood group genotyping was performed by polymerase chain reaction (PCR) with sequence-specific primers using a commercial kit (Human Multi-Erythrocytic Antigen Fluo-Genotype; Jiangsu ZhongJi WanTai Biological Pharmaceutical Co., Ltd., Jiangsu, China). The fluo-genotype was Ccee for RHCE, Lw(a+b-) for Landsteiner-Wiener, Fy(a+b-) for Duffy, Jk(a-b+) for Kidd, MMssMur- for MNSs, Di(a-b+), Wr(a-b+) for Diego kk and Kp(a-b+) for kell, Co(a+b-) for Colton, Do(a-b+) for Domocrock, Au(a-b+) for Augestine, and Yt(a+b-) for Yt (***Fig 1***). These results excluded related common RBC alloantibodies against high frequency antigens, including the Jknull, RH variant, Fya-, Dib-, Lw a-, Sc+, Colton and Domcrock, among others.

Molecular analysis

Sanger sequencing of the A4GALT and B3GALNT1 genes was performed using a commercial Rare Blood Group Sequencing Kit [P blood group (A4GALT and B3GALNT1) sequence kit, Jiangsu ZhongJi WanTai Biological Pharmaceutical Co., Ltd, Jiangsu, China]. Sequencing of PCR-purified products was performed by GENEWIZ (Jiangsu, China) and the results were analysed using sequence analysis software (GeneiousR9; Auckland, New Zealand). The A4GALT (GenBank No. AJ245581) and B3GALNT1 (GenBank No. AB050855) allele sequence templates were used as references for analyses and to mark mutations. JMH, Vel, Lan, Lutheran and p. A4GALT variants were confirmed while B3GALNT1 showed the same sequence as the reference. A4GALT sequencing revealed a c. 547–548delAT (HE818933) variant (***Fig 2***), which results in a frame shift at aa 183 stopping at aa 281 (M183fs, 281X). When compared to the reference, the B3GALNT1 sequence was invariant for all subjects.

DISCUSSION

Few studies have examined the P blood group variant in Chinese people. Two variants of A4GALT have been described, and only one study has reported that anti-Tja induces recurrent abortion[2–5]. However, to our knowledge, at least four cases of RBC alloantibodies against high frequency antigens are strongly suspected to be due to anti-Tja. To date, there is no serological test for the rapid identification of anti-Tja. Our study used the neutralised P substance test and molecular tests to examine p phenotype, and our results have demonstrated that sequence analysis is a superior method to examine P blood groups, especially for p phenotype.

Expansion of the P system was described in 1955 by the Sanger group, following the injection of rabbits with human red cells[6]. In the "Practical Guide to Transfusion Medicine" by Marian Petrides et al., there is a chapter on "P Blood Group and GLOB Collection" by Laura Cooling. This indicated that two glycotransferases synthesised from the common precursor, lactosylceramide, form "P1", "Pk","P" or "LKE" antigens on the red blood cell membrane through the globoside and paragloboside series, including α 1,4- galactosyltransferase (A4GALT), which is located at chromosome 22q13.2, and β1,3-N-acetylgalactosaminyltransferase (B3GALNT1), which is located at chromosome 3q25+[2,6]. In the globoside series pathway, A4GALT catalyses the transfer of galactose to lactosylceramide (Gb2) to produce Gb3 (Pk antigen) and B3GALNT1 catalyses the transfer of acetylgalactosamine to Gb3 to produce Gb4 (P antigen)[7–8]. In contrast, in the paragloboside series pathway, ceramide first links to Gb2 and A4GALT, then transfers galactose to produce P1 antigen. Alloantibodies against the P system are more commonly of the P1 phenotype, including anti-P1, due to weak

ID: Sample 03		RH	Ccee	LW	LW(a+b-)	Duffy	Fy(a+b-)
Kidd	Jk(a-b+)	MNSs	MMss	Mur	Mur-	Sciana	Sc-
Diego	Di(a-b+)	Wright	Wr(a-b+)	Kell	kk	Kp	Kp(a-b+)
Co	Co(a+b-)	Do	Do((a-b+)	Augestine	Au((a-b+)	Yt	Yt(a+b-)

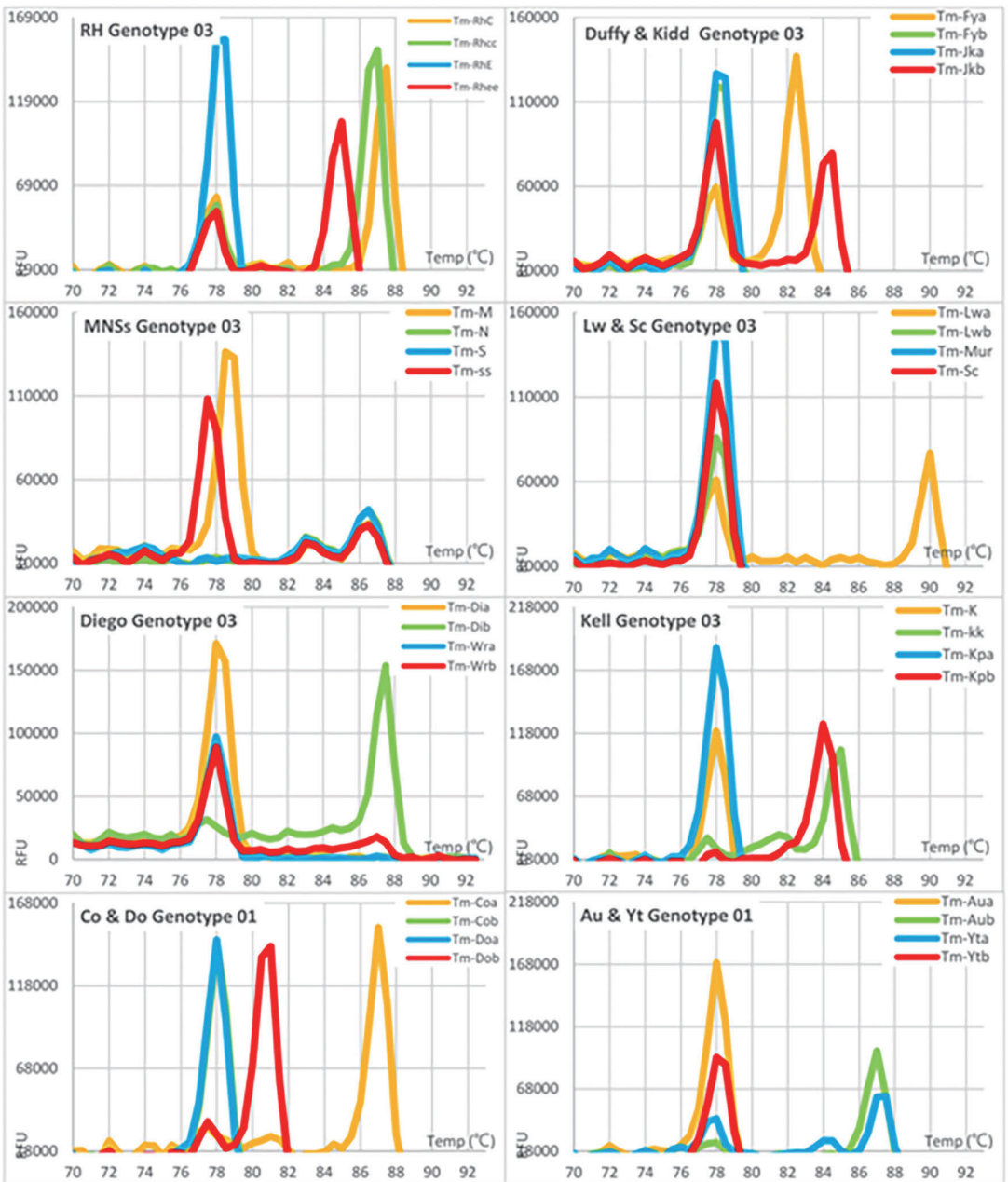

Fig. 1 **The fluo–genotyping of multi–erythrocytic blood groups for the patient.** X axis means temperature and Y axis means log value of Fluorescence. The curve means internal control for PCR at 76°C–78°C. The curve presented shows positive for PCR through sequence specific primers over 80°C. With the exception of PCR for MNSs sequence specific primers, the curve mean internal control for PCR at over 80°C, while the curve presented means positive for PCR through sequence specific primers between 76°C–78°C.

A4GALT-formed P^k and P antigen.The P2 phenotype has a lower frequency and consists of anti-P alloantibodies due to the B3GALNT1 variant resulting in a lack of P antigen[9]. Very rare alloantibodies against the P system include anti-PP1P^k (also called anti-Tja),

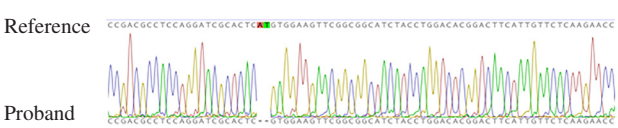

Fig.2 **The A4GALT variat of c. 547–548 del AT (HE818933)**

which results from A4GALT variants[10].

We attempted to use *Columba livia* eggs as a neutralised P substance with anti-P1PP^k from a p phenotype with A4GALT 547–548 homozygous deletions. These neutralization tests were unsuccessful. It has been reported that gastric carcinoma produces 'illegitimate' P antigens, and that these P antigens act as receptors for the Parvo B 19 virus and HIV-1. It is almost certain that anti-PP1P^k in the sera of p women is the cause of the observed abortions and mild haemolytic diseases in new-borns. One reported case known to us involved a woman from the Yunnan province in China, which is abundant in minorities, from where there is an increased incidence of anti-Tja. Thus, the conduction of a localized systemic investigation would likely yield positive results.

In summary, the serological tests combined with molecular analyses was able to successfully identify the p blood antigens caused by A4GALTc. 547–548 deletion AT variant and the neutralised P substance test cannot identify anti-Tja from RBC alloantibodies against high frequency antigens.

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