

## Original Article

# Rare blood donors screening by multiplex PCR methods in Chinese Zhuang and Dong population and pedigree analysis

Wei Jiao<sup>1\*</sup>, Xiangcheng Liao<sup>1\*</sup>, Hailan Li<sup>1</sup>, Jiao Lan<sup>1</sup>, Zhuning Mo<sup>1</sup>, Ziji Yang<sup>1</sup>, Fei Liu<sup>1</sup>, Ruiping Xiao<sup>1</sup>, Yulei He<sup>2,3</sup>, Luyi Ye<sup>3</sup>, Ziyang Zhu<sup>3</sup>

<sup>1</sup>Department of Blood Transfusion, The People's Hospital of Guangxi Zhuang Autonomous Region, Nanning, China; <sup>2</sup>School of Life Science, East China Normal University, Shanghai, China; <sup>3</sup>Blood Group Reference Laboratory, Shanghai Blood Center, Shanghai, China. \*Equal contributors.

Received November 24, 2014; Accepted February 2, 2015; Epub March 15, 2015; Published March 30, 2015

**Abstract:** There are little data available regarding the frequencies of the blood group antigens other than ABO and RhD in the Chinese Zhuang and Dong population. Knowledge of the antigen frequencies is important to assess risk of antibody formation and to guide the probability of finding antigen-negative donor blood, which is especially useful when blood is required for a patient who has multiple red cell alloantibodies. The aim of this study is sought to massively screen for rare blood donors with antigen-negative (e.g. Fy (a-), s-, k-, Di (b-) and Js (b-)) in an ethnic Zhuang and Dong population of China, for providing precious rare blood type materials which can be used to improve the capability of compatible infusion and reduce the transfusion reactions. Finally, inheritance of the Fy (a-), s-, k-, Di (b-) and Js (b-) phenotypes was investigated by pedigree analysis. We screened for Fy (a-), s-, k-, Di (b-) and Js (b-) phenotypes in blood donors by multiplex polymerase chain reaction. The rare phenotypes Fy (a-), s-, k-, Di (b-) and Js (b-) were verified by flow cytometry and sequencing analysis. The results indicated that there are five Fy (a-), three s (-), two Di (b-) in 4490 Zhuang random samples and three Fy (a-) in 1927 Dong random samples. In conclusion, this study is the first small step to create a rare donor data bank for Chinese Zhuang and Dong population and to prepare antigen negative compatible blood to patients with multiple alloantibodies.

**Keywords:** Screening, rare blood donors, multiplex polymerase chain reaction

## Introduction

There are over 300 antigens within the 33 blood group systems which have been recognized by the International Society of Blood Transfusion (ISBT) [1]. However, some individuals do not express certain the blood group antigens which are usually expressed by others, such as Fy<sup>a</sup>, s, k, Di<sup>b</sup> and Js<sup>b</sup> antigens. If patients with rare blood phenotypes were repeatedly transfused with unmatched blood, they might produce alloimmune responses, which even would be life-threatening. Therefore, to avoid potentially fatal transfusion reactions, it is important to create a donor data bank for providing antigen-negative compatible blood to patients with multiple alloantibodies whenever possible [2].

Hemagglutination-based methods known as the gold standard for blood group typing. These methods are simple and quick. However, they have a critical limitation: lack of rare antisera, typing of subjects with a positive direct anti-globulin test, and of multitransfused patients. Recently, several working groups have demonstrated the feasibility and the advantages of DNA-based assays for high-throughput genotyping of RBC antigens [3-9]. With regard to cost-efficiency, we choose a conventional multiplex PCR in our study. The aim of our work was to use a reliable low-cost, high-throughput multiplex PCR methods for large-scale rare blood groups screening in the Chinese Zhuangs and Dongs, and to provide precious rare blood type materials which can be used to improve the capability of compatible infusion and reduce the transfusion reactions.

## Rare blood pedigree analysis by multiplex PCR

**Table 1.** Primers used for PCR amplification

PCR type	Primer name	Primer sequence (5'-3')	Product size (bp)
Multiplex PCR	HGH-F	CTCCCAACCATTCCTTA	425
	HGH-R	CCACTCAGGATTCTGTGTGTTTC	
	FYA-R716s	GCTGCTTCCAGGTGGGAC	714
	FYA-Fs	GCCCTCATTAGTCCTTGCTC	
	s-R2	ACGATGGACAAGTTGACGCG	600
	Ss-com-F1	TGTGGACTGAGAGGGAAGGTTAGC	
	Dib-F3	TGTGGGTGGTGAAGTCCATTCC	419
	Dib19e-r	CCAAATGACTCGGAAGACG	
	JSb 1910 F	CTAGGCAGCACCAACCCTA	96
	JSb1910R3	GCATGGTTGTACACGCTA	
	JSb2019 F	CTAGGCAGCACCAACCCTA	206
	JSb2019R2	CAACGTCTGCAGCATAGTCT	
	ks	GAGATGGAGATGGAATGGTTATG	281
	k-R4	ACTGACTCATCAGAAGTCTCTTCG	
PCR for sequencing analysis	s-F	GCACAGGTGGAACAGTAAGG	1366
	s-R	GGTTGTCAAGATGGTCCCTAA	
	Dib19e-r	CCAAATGACTCGGAAGACG	595
	Dib19e-f	TCACCCAGATGTGCCCTAC	
	FYA-F	AGAGTCCCTTATCCCTATGCC	1240
	FYA-R	ACCTCACCAGGAAATCCAGTC	

### Materials and methods

#### Subjects and DNA extraction

4490 and 1927 ethylenediaminetetraacetate (EDTA)-anticoagulated blood samples were respectively collected from randomly selected healthy donors of Guangxi Zhuang and Dong ethnic origin. Written consent was taken at the time of subjects screening and the study was performed with the approval of the ethics committee of The People's Hospital of Guanqxi Zhuang Autonomous Region.

Target genomic DNA was extracted from white blood cell fractions using QIAamp DNA blood mini kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions and then was kept at -20°C for long-term storage.

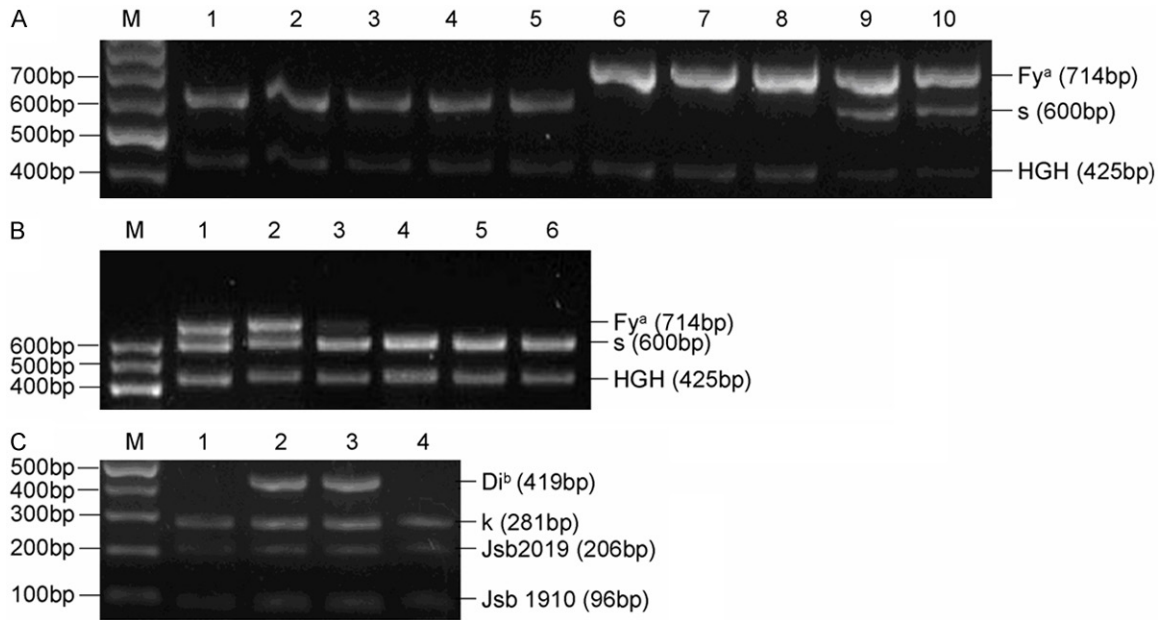
#### Experimental design

Samples were screened for Fy (a-), s-, k-, Di (b-) and Js (b-) phenotypes by the multiplex polymerase chain reaction as previously described [10]. Phenotype identified by the multiplex polymerase chain reaction, were confirmed by flow cytometry and sequencing analysis.

#### Multiplex polymerase chain reaction

The test for 5 rare phenotypes is carried out in two multiplex reaction mixes consisting of different amplification targets per mix. A specific multiplex PCR mix 1 was established to detect alleles Fy<sup>a</sup> and s. Multiplex PCR mix 2 was applied to detect alleles antigens Di<sup>b</sup>, k, Js<sup>b</sup>1910 and Js<sup>b</sup>2019 at the same time. Sequence-specific primers were designed as presented in **Table 1**. The PCR was performed in a final volume of 25 µL, consisting of 50 ng of gDNA, 1.25 mmol/L MgCl<sub>2</sub>, 1× PCR buffer, 200 µmol/L dNTP and 0.625 unit of Taq polymerase (Taq HS, TaKaRa, Kyoto, Japan). The PCR conditions for mix 1 included an initial denaturation at 95°C for 10 min and 35 cycles of 30 s at 94°C, 30 s at 58°C, and 50 s at 72°C, followed by a final elongation step of 10 min at 72°C. Whereas the PCR procedures for mix 2 consisted of an initial denaturation step at 95°C for 2 min and 2 cycles of 30 s at 94°C, 30 s at 65°C and 40 s at 72°C, followed by 15 cycles of 30 s at 94°C, 30 s at 58°C and 40 s at 72°C, and finally 5 cycles of 30 s at 94°C, 30 s at 55°C and 40 s at 72°C. A final elongation step was applied at 72°C for 10 min.

## Rare blood pedigree analysis by multiplex PCR



**Figure 1.** Multiplex PCR screening for the rare blood groups. A. Multiplex PCR screening for the rare blood groups Fy (a-) and s- among Zhuangs. The image indicates that donors 1-5 (lane 1-5) are Fy (a-), whereas donors 6, 7 and 8 (lane 6, 7, 8) are s-. Donors 9 and 10 (lane 9 and 10) are positive controls containing one Fy (a+) sample and one s+ sample. B. Multiplex PCR screening for the rare blood groups Fy (a-) and s- among Dongs. The image indicated that donor 1 and 2 (lane 1 and 2) are positive controls containing one Fy (a+) sample and one s+ sample. The image indicates that donors 3, 4, 5 and 6 (lane 3, 4, 5 and 6) are Fy (a-), whereas donor S4 is Fy (a-b+). The bands of lanes 3 appeared unclearly is Fy (a+b+) sample which confirmed by subsequent Sequence analysis. C. Multiplex PCR detection of the rare blood groups of k-, Di<sup>b</sup> (b-), Js (b-). Donor 1 and 4 (lane 1 and 4) are 2 samples screening by multiplex PCR that are negative for the Di<sup>b</sup> allele. Donor 2 and 3 (lane 2 and 3) are positive DNA controls containing one Di (b+) sample, one Js (b2019+) sample and one Js (b1910+) sample and one k+ sample.

After amplification 10  $\mu$ l of the final PCR products were analyzed on 2% agarose gel.

### Flow cytometry

Whole blood was collected from Chinese Zhuang and Dong volunteer donors, previously identified as Fy (a-), s- and Di (b-), held in tubes containing an anticoagulated with EDTA-2K for a maximum of 24 hours at room temperature, prior to processing. The tubes were centrifuged, plasma was removed and the packed RBCs after washed three times in saline were finally adjusted to a 2% red blood cell saline suspension. 50  $\mu$ l of a 2% red blood cell saline suspension was first incubated with 50  $\mu$ l Human IgG Monoclonal anti-Fya, anti-s and anti-Dib respectively for 30 minutes at 37°C and, subsequently, washed three times in saline, and then incubated again with a secondary antibody (50  $\mu$ l of a 1:60 diluted fluorescein isothiocyanate (FITC)-conjugated anti-human IgG antibody) for 30 minutes at room temperature in the dark. It was then washed three times and resuspended

in saline, and analyzed employing FACS Calibur (BD).

### Sequencing analysis

In addition, to confirm the real genotype, PCR products from these individuals identified as Fy (a-), s- and Di (b-) by multiplex PCR were sequenced on both strands in an ABI PRISM 3730.

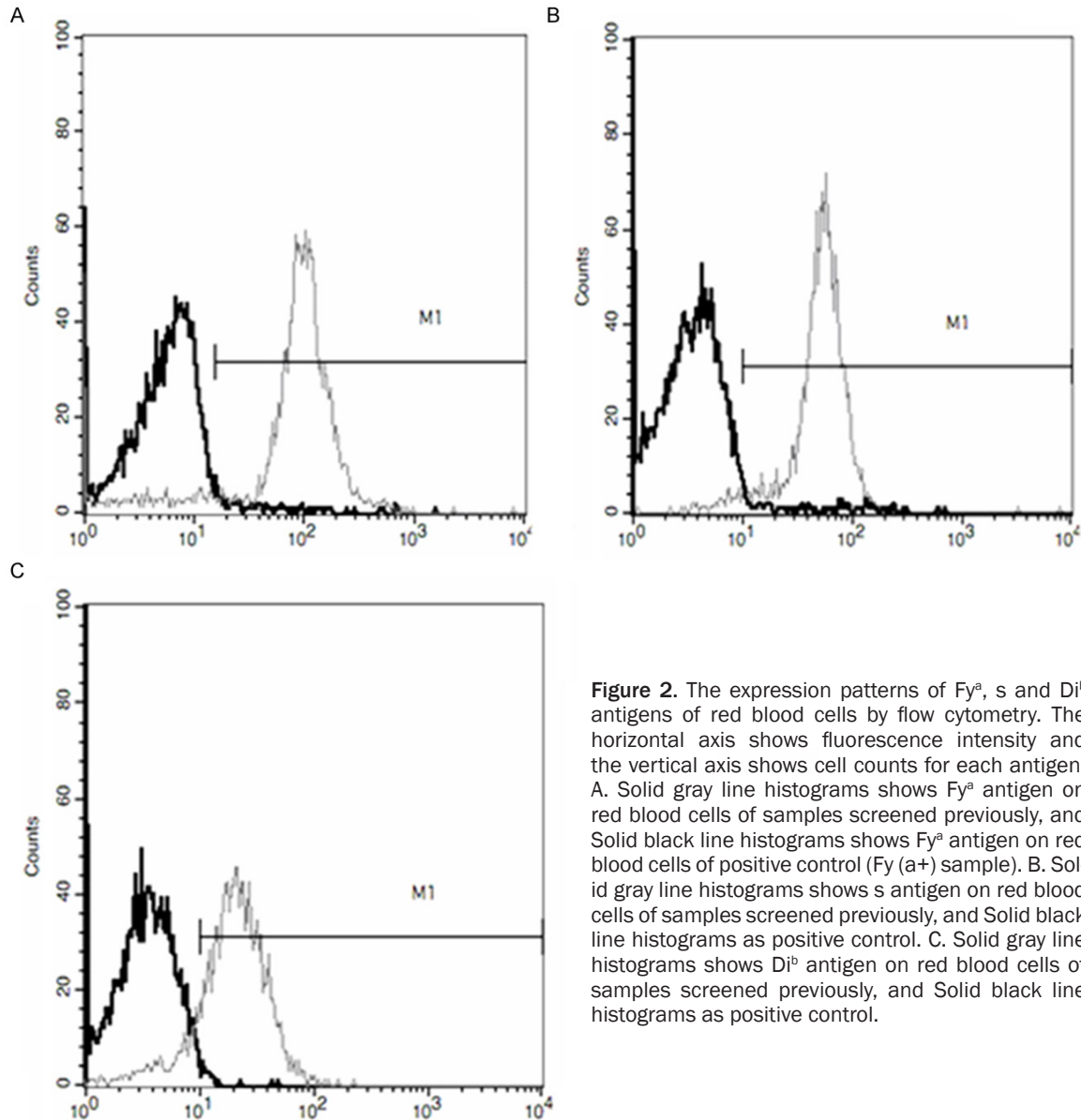
### Pedigree analysis

Family members of those rare blood donors (of phenotype Fy (a-), s- and Di (b-)) we screening were amplified separately and sequenced. By analyzing the phenotypes and genotypes of all the probands and their family members, we draw pedigree charts of those families.

## Results

### Multiplex polymerase chain reaction 1

We determined Fy<sup>a</sup> and s alleles in Zhuangs and Dongs respectively by multiplex PCR mix 1.



**Figure 2.** The expression patterns of  $Fy^a$ , s and  $Di^b$  antigens of red blood cells by flow cytometry. The horizontal axis shows fluorescence intensity and the vertical axis shows cell counts for each antigen. A. Solid gray line histograms shows  $Fy^a$  antigen on red blood cells of samples screened previously, and Solid black line histograms shows  $Fy^a$  antigen on red blood cells of positive control ( $Fy(a+)$  sample). B. Solid gray line histograms shows s antigen on red blood cells of samples screened previously, and Solid black line histograms as positive control. C. Solid gray line histograms shows  $Di^b$  antigen on red blood cells of samples screened previously, and Solid black line histograms as positive control.

Among the 4490 Zhuang donors, 5 donors tested negative for allele  $Fy^a$ , and 3 donors were identified as s-. The PCR results that included all negative ones are presented in **Figure 1A**. In addition, among the 1927 Dong donors, 3 donors tested negative for allele  $Fy^a$ , and 1 donor with unclear result was presumed to  $Fy(a+b+)$ , this was subsequently demonstrated by sequence analysis, whereas there were no s-donors discovered, as shown in **Figure 1B**.

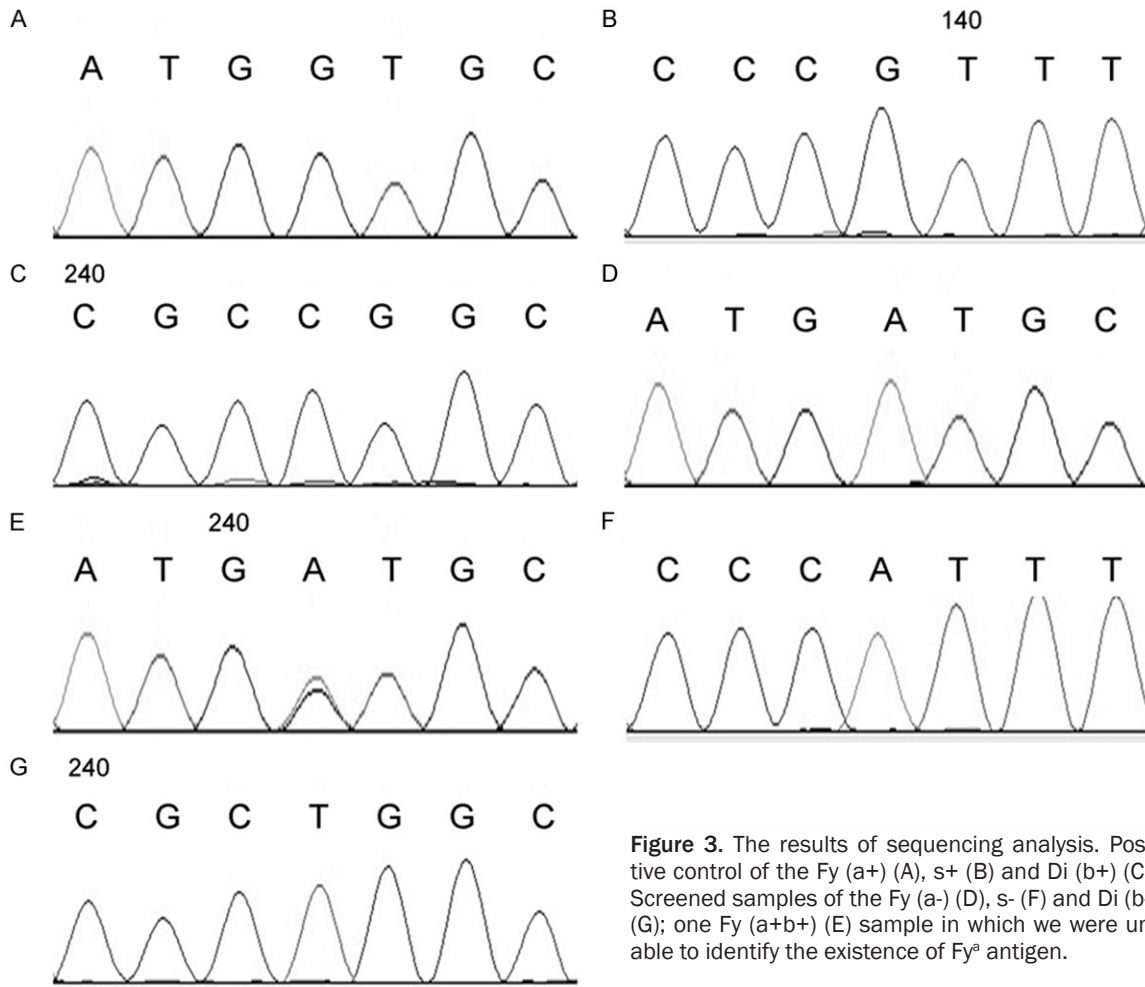
*Multiplex polymerase chain reaction 2*

We determined k,  $Di^b$ ,  $Js^b$  alleles in Zhuang and Dong two ethnic donors respectively by multi-

plex PCR mix 2. Among the 4490 Zhuang and 1927 Dong donors, only 2 Zhuang donors tested negative for allele  $Di^b$ , whereas others were all identified as  $Di(a+)$ . There were no k- and  $Js(b-)$  donors discovered. The result was shown in **Figure 1C**.

*Flow cytometry*

The rare phenotypes [8  $Fy(a-)$  samples, 3s-samples and 2  $Di(b-)$  samples] screened out of the 4490 Zhuang and 1927 Dong donors were confirmed with flow cytometry. The results revealed that all subjects identified as  $Fy(a-)$ , s- and  $Di(b-)$  by multiplex PCR failed to express



**Figure 3.** The results of sequencing analysis. Positive control of the Fy (a+) (A), s+ (B) and Di (b+) (C); Screened samples of the Fy (a-) (D), s- (F) and Di (b-) (G); one Fy (a+b+) (E) sample in which we were unable to identify the existence of Fy<sup>a</sup> antigen.

corresponding antigen of high-frequency allele on red blood cells when quantitatively compared by fluorescence labeling. The results obtained were in complete concordance with those obtained for previous phenotypes by multiplex PCR. One of each of these phenotypes as shown in **Figure 2**.

#### Sequence analysis

Sequence analysis of transcripts obtained from these individuals (of phenotype Fy (a-), s- and Di (b-)), illustrates the presence of a point mutation at nucleotide position 125 (G → A), which results in a glycine to aspartic acid substitution at amino acid position 42, among all Fy (a-) samples, and a single C to T substitution at nucleotide position 1822 for all s- samples. The homozygous 2561C>T mutation was confirmed in both Di (b-) samples. In all cases, there was concordance between sequence analysis and the PCR result (**Figure 3**).

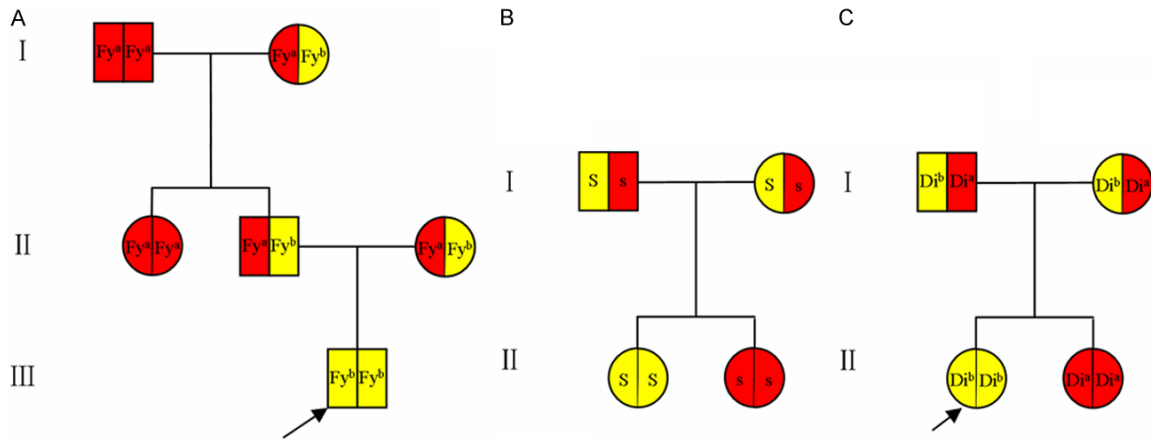
#### Pedigree analysis

A segregation study of those proband's families gave the expected results in which the inheritance of all of the three types rare blood groups accord with Mendel's laws. Representative pedigree charts of those families for each of the three screened rare phenotypes (Fy (a-), s- and Di (b-)) are shown in **Figure 4**.

#### Discussion

Due to limitations of agglutination based assays, the contribution of molecular techniques to immunohematology is continuously growing [11]. However, In China, due to cost-efficiency and feasibility, most previous studies have used PCR-SSP for genotyping blood group antigens [12-16]. However, the assay can only be used in medium-throughput settings, and is not ideal for large-scale applications. In this study, we use an optimal multiplex PCR method for

## Rare blood pedigree analysis by multiplex PCR



**Figure 4.** Pedigree chart of Fy (a-), s- and Di (b-) family. A. A pedigree chart of Fy (a-) family. B. A pedigree chart of s- family. C. A pedigree chart of Di (b-) family.

large-scale rare blood groups screening. The results were always confirmed by flow cytometry and sequence analysis. Using this method, could be a strategy to bridge the gap until higher resolutions techniques, such as microarray technology, are available at an affordable price in developing countries and small-scale laboratories.

Antigens Fy<sup>a</sup>, s, k/Js<sup>b</sup> and Di<sup>b</sup> have high incidence in Chinese people, which belong to 7 common clinically relevant blood group systems (i.e., Duffy, MNS, Kell, and Diego). Kell, Duffy and Diego blood group systems are clinically important in transfusion medicine, Alloantibodies to antigens in these systems may cause hemolytic transfusion reactions or hemolytic disease of the fetus and newborn (HDFN) [17-19]. Anti-S, -s, and anti-U in the MNS system have been implicated in hemolytic transfusion reactions (immediate and delayed) and in HDFN [20]. Therefore, to avoid potentially fatal complications, the urgent task seems to be donor screening the availability of blood units that lack the corresponding antigen(s) and considering appropriate matched transfusions whenever possible.

In addition to the Han population, the Zhuang ethnic group, with a population size of approximately 16.2 million, is the largest ethnic population in China. Most of the Zhuang live in the Guangxi Zhuang Autonomous Region in southern China. In Guangxi, another important indigenous population is Dong. Blood group antigen distributions not only distinguish Asian from

European populations, but also illustrate the varied genetic compositions of different Chinese ethnic populations [12]. These differences in blood group antigen distribution are important due to their influence on the clinical practice of transfusion medicine. The phenotypes frequencies of rare blood groups, such as s, Fy (a-), s-, k-, Di (b-) and Js (b-) in Zhuang and Dong population of Guangxi Zhuang Autonomous Region remain unclear. This is the first study where frequency of clinically significant rare blood groups is studied in Chinese Zhuangs and Dongs using multiplex PCR methods.

In our study, 5 Fy (a-) samples, 3s- and 2 Di (b-) samples have been discovered from 4490 Zhuang samples, whereas 3 Fy (a-) samples have been discovered from 1927 Dong samples. We did not find s- among Dongs; nor did we find k- and Js (b-) sample among Zhuangs and Dongs. Since the null alleles, such as the rare phenotypes Fy (a-b-) and S-s-, are mainly found in individuals of African descent and they are very rare among non-African populations, we can ignore the possibility that donors carrying the rare null alleles. Hence, The Fy (a-) samples from the multiplex PCR previously were regarded as Fy<sup>b</sup>/Fy<sup>b</sup>, and the s- samples were regarded as S/S. After calculated, the rare Fy<sup>b</sup> gene frequencies among Zhuang and Dong population are 3.34% and 3.95%, respectively. Which are similar to the frequencies reported in other studies published from China (3.6%-6%) [12, 15, 16, 21], whereas it is more common among Caucasian, Black and Indian than Chinese identified 83.0, 23.0 and 57.7%,

respectively [22]. The frequency of S obtained in our study among Zhuang population is 2.58%, whereas those among Caucasian, Black and Indian are 55.0, 31.0 and 54.8%, respectively [22]. Di<sup>a</sup> antigen is very rare among Caucasians and Blacks, but is relatively common among South American Indians and Asian population, especially in people of Mongolian origin. It is considered to be a useful anthropologic marker. In contrast, Di<sup>b</sup> is found in almost 100 percent of other ethnic groups [23]. Blood donors with Di (b-) phenotype have been included in the rare blood donor category by the ISBT Working Group on Rare Blood Donors [24]. Since rare phenotypes Di (a-b-) has not yet been found, the Di (b-) samples in our study were regarded as Di<sup>a</sup>/Di<sup>a</sup> and the frequencies of Di<sup>a</sup> allele was calculated as 2.11%, which is consistent with previous findings in Shenzhen, China [25]. In the current study, no donor was typed as k and Js<sup>b</sup> negative. The prevalence of cellano (k) antigen is almost 100% in the Chinese population, whereas 0.2 per cent of the Caucasians are k negative [26]. The occurrence of Js<sup>a</sup> antigen is almost exclusively among African population [27], China has not yet found Js (b-) phenotype.

This study has provided us with rare donor data with antigen negative of a part of minor blood group for Chinese Zhuang and Dong population. Further study should be carried out for other clinical relevant blood group systems which can obtain a more detailed determination of the rare blood types.

### Acknowledgements

The authors are grateful to Shanghai Blood Center for their cooperation and suggestions. This work was supported by the science and technology development plan project of Guangxi Municipality, China (Grant No. Guangxi Branch attack 0993003B-26) and the special foundation of Health Care, China (Grant No. 201002005).

### Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Wei Jiao, Department of Blood Transfusion, The People's Hospital of Guanxi Zhuang Autonomous Region, Nanning 530021, Guangxi, China. E-mail: gxjw2005@yeah.net; Dr. Ziyuan Zhu, Blood Group Reference Labo-

ratory, Shanghai Blood Center, Shanghai, China. E-mail: zhuziyan@sbc.org.cn

### References

- [1] Reid ME. ISBT scientific series-State of the art invited speaker. Protein blood group systems: Berlin and beyond 2012; 7: 41-43.
- [2] Sadeghian MH, Keramati MR, Badiei Z, Ravarian M, Ayatollahi H, Rafatpanah H, Daluei MK. Alloimmunization among transfusion-dependent thalassemia patients. *Asian J Transfus Sci* 2009; 3: 95-98.
- [3] Gao SP, Liang S, Pan M, Sun RL, Chen C, Luan H, Jiang MH. Interleukin-6 genotypes and serum levels in Chinese Hui Population. *Int J Clin Exp Med* 2014; 7: 2851-2857.
- [4] Montpetit A, Phillips MS, Mongrain I, Lemieux R, St-Louis M. High-throughput molecular profiling of blood donors for minor red blood cell and platelet antigens. *Transfusion* 2006; 46: 841-848.
- [5] Karpatidou K, Drago F, Crespiatico L, Paccapelo C, Truglio F, Frison S, Scalomogna M, Poli F. Blood group genotyping for Jk (a)/Jk (b), Fy (a)/Fy (b), S/s, K/k, Kp (a)/Kp (b), Js (a)/Js (b), Co (a)/Co (b), and Lu (a)/Lu (b) with microarray beads. *Transfusion* 2008; 48: 505-512.
- [6] Avent ND. Large-scale blood group genotyping: clinical implications. *Br J Haematol* 2009; 144: 3-13.
- [7] Jungbauer CC, Hobel CM, Schwartz DW, Mayr WR. High-throughput multiplex PCR genotyping for 35 red blood cell antigens in blood donors. *Vox Sang* 2012; 102: 234-242.
- [8] Fu X, Guo L, Jiang ZM, Zhao LS, Xu AG. An miR-143 promoter variant associated with essential hypertension. *Int J Clin Exp Med* 2014; 7: 1813-1817.
- [9] Latini FR, Gazito D, Arnoni CP, Muniz JG, de Medeiros Person R, Carvalho FO, Baleotti W, Castilho L, Barreto JA. A new strategy to identify rare blood donors: single polymerase chain reaction multiplex SNaPshot reaction for detection of 16 blood group alleles. *Blood Transfus* 2014; 12: s256-263.
- [10] Jiao W, Xie L, Li H, Lan J, Mo Z, Yang Z, Liu F, Xiao R, He Y, Ye L, Zhu Z. Application of multiplex PCR for the screening of genotyping system for the rare blood groups Fy (a-), s-, k-, Di (b-) and Js (b-). *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 2014; 31: 242-246.
- [11] Jungbauer C. Routine use of DNA testing for red cell antigens in blood centres. *Transfus Apher Sci* 2011; 45: 61-68.
- [12] Yan L, Zhu F, Fu Q, He J, ABO, Rh, MNS, Duffy, Kidd, Yt, Scianna. Colton blood group systems in indigenous Chinese. *Immunohematology* 2005; 21: 10-14.

## Rare blood pedigree analysis by multiplex PCR

- [13] Song N, Zheng SR, Yao ZQ, Wei TX, Chen JX, Chen Q. Study on genotyping of Kidd blood group system in Sichuan Chinese Han. *Chin J Blood Transfus (Chin)* 2008; 21: 926-927.
- [14] Han Y, Zhao L, Jiang Z, Ma N. Analysis of the expression of KIR and HLA-Cw in a Northeast Han population. *Exp Ther Med* 2013; 5: 300-304.
- [15] Zhao XM, Li ZQ. Research of genetic polymorphism of rare blood group system in Shanghai Han Population. *J Chin Transfus Lab Med (Chin)* 2009; 11: 220-223.
- [16] Fan Y, Xia Y, Zhao H, Zhang J, Li S, Tian Y, Weng X, Qiu G. Complications analysis of posterior vertebral column resection in 40 patients with spinal tumors. *Exp Ther Med* 2014; 8: 1539-1544.
- [17] Daniels G. *Human blood groups*, 2nd edition. UK: Blackwell Science Ltd. 2002. pp. 232-367.
- [18] Tohyama H. *Blood transfusion reaction due to Diego blood group*. Tokyo: Chuugai Medical Publishers; 1989. pp. 379-380.
- [19] Lee SM, Im SJ, Park SE, Lee EY, Kim HH. A case of severe hemolytic disease of the newborn due to anti-Dia antibody. *Korean J Lab Med* 2007; 27: 373-376.
- [20] Reid ME. MNS blood group system: a review. *Immunohematology* 2009; 25: 95-101.
- [21] Wu DL, Lan JC, Ding H, Cao Q. Study on genotyping of Duffy in Guangzhou Chinese Han. *Chin J Blood Transfus (Chin)* 2000; 13: 202-202.
- [22] Makroo RN, Bhatia A, Gupta R, Phillip J. Prevalence of Rh, Duffy, Kell, Kidd & MNSs blood group antigens in the Indian blood donor population. *Indian J Med Res* 2013; 137: 521-526.
- [23] Daniels GL. *Human blood groups*. Oxford: Blackwell Science; 1995. pp. 452-455.
- [24] Anstee D, Levene C, Mallory D, Overbeeke M, Poole J, Reile J, Reid M, Smart E, Tani Y, Wendel S, Woodfield G. Rare blood: An ISBT Working Party report on rare blood donors. *Vox Sang* 1999; 77: 58-62.
- [25] Wu GG, Su YQ, Yu Q, Jin SZ, Zhao TM. Development of a DNA-based genotyping method for the Diego blood group system. *Transfusion* 2002; 42: 1553-1556.
- [26] Reid ME, Lomas-Francis C. *The blood group antigen facts book*. New York: Elsevier Academic Press; 2004.
- [27] Lin-Chu M, Broadberry RE, Chang FJ. The distribution of the blood group antigens and alloantibodies among Chinese in Taiwan. *Transfusion* 1988; 28: 350-352.