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Polymorphism of *SLC14A1* encoding human erythrocytic Kidd antigens in the Chinese population

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ABSTRACT

The *SLC14A1* gene, which encodes important Kidd blood group antigens, has not been systematically analyzed at the molecular level in Chinese individuals. In this study, *SLC14A1* genetic polymorphism was examined in Chinese individuals with Jk(a+b−), Jk(a+b+), and Jk(a−b+) expression. The Kidd phenotype was determined for 146 specimens using monoclonal anti-Jk^a and -Jk^b antibodies. From these, 87 specimens were Jk(a−b+), 21 were Jk(a+b−), and 38 were Jk(a+b+). According to the Kidd phenotype results, 20 specimens were randomly selected from each group, i.e., Jk(a−b+), Jk(a+b−), and Jk(a+b+), for the molecular analyses of exons 3 to 11 of the *SLC14A1* gene. Novel alleles were detected in the *SLC14A1* gene, including IVS3−106A, IVS3−99A, exon3 130G, IVS4−299G, IVS4−293G, IVS4+211C, IVS4 +230C, exon6 499A, exon6 588A, IVS7−68T, IVS9+244G, and IVS10−153T, indicating that the locus harbored significant polymorphism. We also showed that IVS4−299, IVS7−68, and IVS10−153 were novel SNPs absolutely associated with exon 8 nt.838. The minor allele frequencies were all greater than 10% and all SNPs in the Chinese population showed Vel antigen expression on RBC membranes. We identified 12 SNPs in the *SLC14A1* gene in the Chinese population, IVS3−106A, IVS3−99A, exon3 130G, IVS4−299G, IVS4−293G, IVS4+211C, IVS4+230C, exon6 499A, exon6 588A, IVS7−68T, IVS9+244G, and IVS10−153T. Our results also indicated that three novel SNPs produced Jk^a and Jk^b antigens in Chinese individuals.

Keywords: polymorphism, *SLC14A1*, human erythrocytic antigens, Kidd

INTRODUCTION

The Kidd glycoprotein is a transmembrane protein that functions as a urea transporter. Jk(a−b−) is a rare null phenotype lacking the high-incidence Jk3 antigen (Jk_{null}). The *SLC14A1* locus is located on chromosome 18 at 18q11−q12, spans approximately 30 kb containing 11 exons, and constitutes the Kidd (Jk) blood group system (ISBT009)[1].

Jk(a−b−) red blood cells are easily identified by their failure to lyse in 2 mol/L urea, distinguishing them from other Kidd phenotypes with normal urea transporter function[2]. Natural or transfusion-related immune anti-Jk3 has been identified in people with Jk(a−b−) and anti-Jk3 is responsible for severe immediate and delayed hemolytic transfusion reactions and newborn hemolytic disease[3]. A rare blood bank from Shanghai and Taipei reported that Jk_{null} had the highest incidence in the Chinese population[4].

The most common molecular variant causing Jk_{null} is the IVS5−1 mutation, which is an RNA splice site

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error. However, genetic polymorphisms in *SLC14A1* have not been investigated in the Chinese population. Homozygous IVS3−78 A>G mutations have been reported in Jk_{null} individuals and individuals expressing normal Jk(a−b+)[5−11]. We previously performed a pedigree analysis of anti−Jk3−induced newborn hemolytic disease and found that genetic polymorphisms in *SLC14A* may explain the controversial results in the Chinese population, while discussing molecular variation in Jk_{null} individuals[12]. Therefore, we examined *SLC14A1* polymorphism in the Chinese population and expected the results to offer insight into the molecular basis of Jk_{null} individuals.

The aim of our study was to characterize the sequences of normal serological Kidd antigens in Chinese individuals. Notably, this is the first study to investigate *SLC14A1* gene polymorphism in an Asian population.

MATERIAL AND METHODS

Data collection and DNA extraction

EDTA peripheral venous blood specimens were examined by the direct and indirect Coombs tests, and serological Kidd phenotyping was performed by 2 mol/L urea hemolytic tests and monoclonal antibody against Jk^a and Jk^b (Pelikloon anti−Jk^a [IgM] monoclonal and Pelikloon anti−Jk^b[IgM] monoclonal, Sanquin GmbH, Netherlands). The exclusion criteria were positive direct or indirect Coombs tests or positive in the 2 mol/L urea hemolytic test.

Peripheral EDTA−treated anti−coagulated blood was stored at 4℃. Serological tests and DNA extraction were performed within 12 hours of blood acquisition. DNA was purified from the buffy coat samples using micromagnetic technology with a commercial kit (Magnetic Bead; Texas Biotechnology Co., Ltd., Xiamen, China) and an automatic instrument (EZ Bead−32, Texas Biotechnology Co., Ltd., Xiamen, China. All DNA samples were stored at −80℃ until required for molecular analysis.

Genotyping and molecular analysis

Kidd blood group genotyping was performed by polymerase chain reaction (PCR) with sequence−specific primers using a commercial kit (Human Rare Erythrocytic Antigen Genotype; Jiangsu ZhongJi WanTai Biological Pharmaceutical Co., Ltd., Jiangsu, China). Sanger sequencing of Kidd exons 3 to 11 was performed using a commercial Kidd gene sequencing kit (Kidd E3−11 Sequence; Jiangsu ZhongJi WanTai Biological Pharmaceutical Co., Ltd, Jiangsu, China). Sequencing of PCR−purified products was performed by Sangon Biotech (Beijing, China) and the results were analyzed using sequence analysis software (Geneious R9; Auckland, New Zealand). The *SLC14A1* allele sequence (GenBank No. NM 015865.1) template was used as a reference for analyses and to mark mutations.

Genetic linkage in the *SLC14A1* allele could not be identified. The data were grouped according to Jk(a+b−), Jk(a−b+), Jk(a+b+), and Jk(a−b−) phenotypes based on serological results and genetic linkage with nt.838 in exon 8 from *SLC14A1*, which is a single nucleotide polymorphism (SNP) for the Jk^a antigen (nt.838G) and Jk^b antigen (nt.838A) and was examined.

RESULTS

Baseline

All 146 random specimens obtained from employee health examinations were negative in both 2 mol/L urea hemolytic testing and antibody screening. Of the 146 specimens were examined to determine the Kidd phenotype using monoclonal anti−Jk^a and −Jk^b antibodies; 87 specimens were Jk(a−b+), 21 were Jk(a+b−), and 38 were Jk(a+b+). According to the Kidd phenotype results, we random selected 20 specimens from each group, i.e., Jk(a−b+), Jk(a+b−), and Jk(a+b−) for molecular analyses of exons 3 to 11 of the *SLC14A1* gene.

Molecular analysis of exons 3 and 4 of *SLC14A1* gene

Nine single nucleotide variants were found at exons 3 and 4 of the *SLC14A1* gene, including IVS3−106A, IVS3−99A, exon3 130G, IVS4−299G, IVS4−293G, exon4 191G, IVS4+177T, IVS4+211C, and IVS4+230C***(Table 1)***. The seven single nucleotide variants IVS3−106A, IVS3−99A, exon3 130G, IVS4−299G, IVS4−293G, IVS4+211C, and IVS4+230C were polymorphic and did not influence Jk^a or Jk^b antigen expression because all specimens in our study exhibited normal expression of Kidd antigens. The IVS4−299G SNP was absolutely related to nt.838; homozygous IVS4−299CC was associated with homozygous nt.838GG, which expresses the Jk(a+b−) antigen; heterozygous IVS4−299GC was associated with heterozygous nt.838GA, which expresses Jk(a+b+) antigen; and homozygous IVS4−299GG was associated with homozygous nt.838AA, which expresses Jk(a−b+).

Molecular analysis of exons 5 and 6 of *SLC14A1* gene

Five single nucleotide variants were found at exons 5 and 6 of the *SLC14A1* gene, including IVS5−1G,

Table 1 **The single nucleotide variants between exon 3 and 4 of *SLC14A1* gene**

[*n*(%)]

Alleles	Jk(a+b−)	Jk(a+b+)	Jk(a−b+)	Total
Exon8 838G				
GG	20(100)	0(0)	0(0)	20(33)
GA	0(0)	20(100)	0(0)	20(33)
AA	0(0)	0(0)	20(100)	20(33)
IVS3−106A				
AA	20(100)	0(0)	11(55)	31(52)
AG	0(0)	9(45)	9(45)	18(30)
GG	0(0)	11(55)	0(0)	11(18)
IVS3−99A				
AA	12(60)	0(0.0)	5(25)	17(28)
AG	2(10)	9(45)	0(0)	11(18)
GG	6(30)	11(55)	15(75)	32(56)
Exon3 130G				
GG	20(100)	13(65)	0(0)	33(56)
AG	0(0)	7(35)	10(50)	17(28)
AA	0(0)	0(0)	10(50)	10(16)
IVS4−299G				
GG	0(0)	0(0)	20(100)	20(33)
CG	0(0)	20(100)	0(0)	20(33)
CC	20(100)	0(0)	0(0)	20(33)
IVS4−293G				
GG	20(100)	17(85)	9(45)	46(77)
AG	0(0)	3(15)	10(50)	13(21)
AA	0(0)	0(0)	1(5)	1(2)
Exon4 191G				
GG	19(95)	20(100)	20(100)	19(98)
GA	1(5)	0(0)	0(0)	1(2)
AA	0(0)	0(0)	0(0)	0(0)
IVS4+177T				
TT	18(90)	20(100)	20(100)	58(97)
CT	2(10)	0(0)	0(0)	2(3)
CC	0(0)	0(0)	0(0)	0(0)
IVS4+211C				
CC	1(5)	19(95)	18(90)	38(63)
CG	2(10)	1(5)	2(10)	5(8)
GG	17(85)	0(0)	0(0)	17(28)
IVS4 +230C				
CC	10(50)	20(100)	20(100)	50(83)
CT	1(5)	0(0)	0(0)	1(2)
TT	9(45)	0(0)	0(0)	9(15)

IVS5+36 insA, IVS6−27 C, exon6 499A, and exon6 588A***(Table 2)***. Two single nucleotide variants, i.e. exon6 499A and exon6 588A were polymorphic, and we confirmed that these SNPs did not influence Jk^a or Jk^b antigen expression because all specimens in our study showed normal expression of Kidd antigens.

Molecular analysis of exons 7 to 11 of *SLC14A1* gene

Six single nucleotide variants were found at exons 7 to 11 of the *SLC14A1* gene, including IVS7−68T, IVS7+84C, IVS9−46G, IVS9+244G, IVS10−153T, and IVS10−24G***(Table 3)***. The three single nucleotide variants IVS7−68T, IVS9+244G, and IVS10−153T were polymorphic, and we confirmed that these SNPs do not influence the Jk^a or Jk^b antigen expression because all specimens in our study showed normal expression of Kidd antigens. The SNPs IVS7−68T and IVS10−153T were absolutely related to nt.838; homozygous IVS7−68TT and IVS10−153GG were associated with homozygous nt.838GG, which expresses the Jk(a+b−) antigen, heterozygous IVS7−68TC and IVS10−153TG were associated with heterozygous nt.838GA, which expresses the Jk(a+b+) antigen, and homozygous IVS7−68CC and IVS10−153TT were associated with homozygous nt.838AA, which expresses Jk(a−b+).

Table 2 **The single nucleotide variants between exon 5 and 6 of *SLC14A1* gene**

[*n*(%)]

Alleles	Jk(a+b−)	Jk(a+b+)	Jk(a−b+)	Total
Exon8 838G				
GG	20(100)	0(0)	0(0)	20(33)
GA	0(0)	20(100)	0(0)	20(33)
AA	0(0)	0(0)	20(100)	20(33)
IVS5−1G				
GG	18(90)	20(100)	20(100)	58(97)
GA	2(10)	0(0)	0(0)	2(3)
AA	0(0)	0(0)	0(0)	0(0)
IVS5+36				
insA heterozygous	9(45)	2(10)	0(0)	11(18)
IVS6−27C				
CC	20(100)	19(95)	20(100)	59(98)
CT	0(0)	1(5)	0(0)	1(2)
TT	0(0)	0(0)	0(0)	0(0)
Exon6 499A				
AA	10(50)	12(60)	20(100)	42(70)
AG	6(30)	6(30)	0(0)	12(20)
GG	4(20)	2(10)	0(0)	6(10)
Exon6 588A				
AA	6(30)	3(15)	4(20)	13(21)
AG	4(20)	3(15)	6(30)	13(21)
GG	10(50)	14(70)	10(50)	34(58)

DISCUSSION

In this study, we investigated the frequencies of mutations in the *SLC14A1* gene in Chinese individuals with normal expression of Jk^a and Jk^b antigens. Our analysis identified 12 novel alleles in the *SLC14A1* gene, including IVS3−106A, IVS3−99A, exon3 130G, IVS4−299G, IVS4−293G, IVS4+211C, IVS4+230C, exon6 499A, exon6 588A, IVS7−68T, IVS9+244G, and IVS10−153T, indicating that the gene harbors significant polymorphism. We also showed that IVS4−299, IVS7−68, and IVS10−153 were novel SNPs perfectly associated with exon 8 nt.838.

To the best of our knowledge, the sequence of the *SLC14A1* gene in Chinese individuals has not been reported previously. Few studies of Chinese individu−

Table 3 **The single nucleotide variants between exon 7 to 11 of SLC14A1 gene**

[*n*(%)]

Alleles	Jk(a+b−)	Jk(a+b+)	Jk(a−b+)	Total
Exon 8 838G				
GG	20(100)	0(0)	0(0)	20(33)
GA	0(0)	20(100)	0(0)	20(33)
AA	0(0)	0(0)	20(100)	20(33)
IVS7−68T				
TT	20(100)	0(0)	0(0)	20(33)
TC	0(0)	20(100)	0(0)	20(33)
CC	0(0)	0(0)	20(100)	20(33)
IVS7+84C				
CC	1(5)	1(5)	0(0)	2(3)
CT	1(5)	2(10)	1(5)	4(7)
TT	18(90)	17(85)	19(95)	54(90)
IVS9−46G				
GG	20(100)	15(75)	12(60)	47(78)
GA	0(0)	5(25)	8(40)	13(22)
AA	0(0)	0(0)	0(0)	0(0)
IVS9+244 G				
GG	0(0)	1(5)	2(10)	3(5)
GA	2(10)	5(25)	8(40)	15(25)
AA	18(90)	14(70)	10(50)	42(70)
IVS10−153T				
TT	0(0)	0(0)	20(100)	20(33)
TG	0(0)	20(100)	0(0)	20(33)
GG	20(100)	0(0)	0(0)	20(33)
IVS10−24G				
GG	11(55)	11(55)	1(5)	23(38)
GA	2(10)	5(25)	9(45)	16(26)
AA	7(35)	4(20)	11(55)	22(36)

als have reported molecular analyses of subjects with the Jk_{null} phenotype. Because normal controls have not been investigated, it is very easy to infer incorrect associations between nucleotide variants in individuals with the Jk_{null} phenotype[13]. Our study provided data that can serve as a normal control reference and identified 12 novel alleles expressing normal Kidd antigens in the Chinese population. IVS4−299, IVS7−68, and IVS10−153 were novel SNPs expressing Jk^a and Jk^b antigens. In our study, we limited the sequencing analysis to exons 1 and 2 in the *SLC14A1* gene because these two exons encode the urea transporter protein. Whole−gene sequencing may provide additional information on polymorphisms in Chinese individuals.

In summary, our results identified 12 SNPs in the *SLC14A1* gene in the Chinese population, including SNPs at positions IVS3−106A, IVS3−99A, exon3 130G, IVS4−299G, IVS4−293G, IVS4+211C, IVS4+230C, exon6 499A, exon6 588A, IVS7−68T, IVS9+244G, and IVS10−153T. Our study also identified three SNPs that make a genotype expressing Jk^a and Jk^b antigens in Chinese individuals.

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