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 con-
error. However, genetic polymorphisms in SLC14A1 have not been investigated in the Chinese population. Homozygous IVS3–78 A>G mutations have been reported in Jknull 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 SLC14A1 may explain the controversial results in the Chinese population, while discussing molecular variation in Jknull individuals\(^12\). Therefore, we examined SLC14A1 polymorphism in the Chinese population and expected the results to offer insight into the molecular basis of Jknull 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 Jka and Jkb (Pelikloon anti–Jka [IgM] monoclonal and Pelikloon anti–Jkb [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 anticoagulated blood was stored at 4°C. 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°C 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 Jka+b−, Jka−b+, Jka+b+, and Jka+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 Jka antigen (nt.838G) and Jkb 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–Jka and –Jkb antibodies; 87 specimens were Jka(a+b−), 21 were Jka(a+b−), and 38 were Jka(a+b+). According to the Kidd phenotype results, we random selected 20 specimens from each group, i.e., Jka(a−b+), Jka(a+b−), and Jka(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 Jka or Jkb antigen expression because all specimens in our study exhibited normal expression of Kidd antigens. The IVS4–299G SNP was associated with homozygous Jka and Jkb antigen expression because all specimens in our study exhibited normal expression of Kidd antigens. The IVS4–299G SNP was associated with homozygous Jka and Jkb antigen expression because all specimens in our study exhibited normal expression of Kidd antigens. The IVS4–299G SNP was associated with homozygous Jka and Jkb antigen expression because all specimens in our study exhibited normal expression of Kidd antigens.

**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,
Six single nucleotide variants were found at exons 7 to 11 of the SLC14A1 gene, including IVS7–68T, IVS7+48C, 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<sup>a</sup> or Jk<sup>b</sup> 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, and homozygous IVS7–68CC and IVS10–153TT were associated with homozygous nt.838AA, which expresses Jk(a−b+).

**DISCUSSION**

In this study, we investigated the frequencies of mutations in the SLC14A1 gene in Chinese individuals with normal expression of Jk<sup>a</sup> and Jk<sup>b</sup> antigens. Our analysis identified 12 novel alleles in the SLC14A1 gene, including IVS3–106A, IVS3–99A, 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<sup>a</sup> or Jk<sup>b</sup> antigen expression because all specimens in our study showed normal expression of Kidd antigens.
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\textsuperscript{[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\(^c\) and Jk\(^d\) antigens in Chinese individuals.

### References


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