# Genomic characterisation of the Jk(a–b–) phenotype in Thai blood donors

Pramote Sriwanitchrak[1], Kanchana Sriwanitchrak[2], Jintana Tubrod[3], Pawinee Kupatawintu[3], Chollanot Kaset[1], Oytip Nathalang[1]

*[1]Department of Medical Technology, Faculty of Allied Health Sciences, Thammasat University, Pathumtani; [2]Department of Pathology, Ramathibodi Hospital, Mahidol University; [3]National Blood Centre, Thai Red Cross Society, Bangkok, Thailand*

**Background.** The Kidd (JK) blood group antigens are encoded by the *JK* gene. The rare Jk(a–b–) phenotype can be caused by homozygosity for a silent *JK* allele. Currently, $JK^{null}$ alleles have been identified among different populations; however, information on its presence among Thais is not available.

**Materials and methods.** Screening for the Jk(a–b–) phenotype by the urea lysis test was performed in 25,340 blood samples from Thai blood donors. The Jk(a–b–) phenotypes were confirmed by an indirect antiglobulin test (IAT). Additionally, polymerase chain reaction amplification and sequence analysis of the *JK* gene were performed using previously described methods.

**Results.** Five samples were confirmed as having a Jk(a–b–) phenotype by a urea lysis test and IAT; four of these samples were investigated. Two samples of *JK*02* alleles were homozygous for a g>a mutation at the 3' acceptor splice site of intron 5 of the JK gene, as in previous studies in Asians and Polynesians. Moreover, one sample of *JK*02* alleles was homozygous for an 896G>A mutation at exon 9 (Gly299Glu), as in a previous study in Polynesians. Interestingly, missense dual mutations of *JK*01* alleles from a female blood donor were identified. The first mutation was 956C>T (Thr319Met) in exon 10, as in a recent study in African-Americans. The second mutation was 130G>A (Glu44Lys) at exon 4, as in previous studies among Caucasians.

**Conclusion.** There are various different molecular bases of the Jk(a–b–) phenotype. This is the first report of $JK^{null}$ alleles among Thais. The information presented in this study could be beneficial in planning genotyping strategies for blood donors and patients.

**Keywords:** Jk(a–b–) phenotype, genotype, Thai blood donors.

## Introduction

The Kidd (JK) blood group system (ISBT 009) was discovered in 1951[1]. Three antigens, $Jk^a$, $Jk^b$ and Jk3, have been recognised; however, only three phenotypes, Jk(a+b–), Jk(a–b+), and Jk(a+b+), are common in different populations. Anti-$Jk^a$, and anti-$Jk^b$ are usually detected in Jk(a–b+) and Jk(a+b–) individuals after a transfusion or pregnancy. In contrast, the Jk(a–b–) phenotype is rare and anti-Jk3 can be found after immunisation, causing acute and delayed haemolytic transfusion reactions and creating difficulties in finding compatible blood donors[2-8]. Routinely, the Jk(a–b–) phenotype can be identified by the absence of $Jk^a$ and $Jk^b$ antigens when testing red blood cells with specific antiserum using the indirect antiglobulin test (IAT)[2]. The Jk(a–b–) phenotype has been considered a rare phenotype among Thai people[9,10].

The Kidd glycoprotein is a red blood cell urea transporter and the Jk(a–b–) phenotype is associated with absence of this transporter. The red blood cells of subjects with the Jk(a–b–) phenotype are, therefore, resistant to lysis by 2M urea, as previously described[11,12] whereas red blood cells from subjects with the other phenotypes are not; hence, the urea lysis test can be used as a screening test for the Jk(a–b–) phenotype and is practical for mass screening[13].

In addition, genomic level analysis of the coding sequence and splice sites of the *JK* alleles has been performed in various populations (Polynesians, Asians

and Finns). The typical mutation in Polynesians and Asians is that of the invariant g residue to a in the 3' acceptor splice site of intron 5; another missense mutation, 896G>A (Gly299Glu) in exon 9, is also found in Polynesians. The other missense mutation, 871T>C (Ser291Pro) in exon 9, is commonly found among Finns[14-17]. In a previous study in which Thai blood donors were screened for the Jk(a–b–) phenotype by serological testing, this phenotype was found in only 0.025% of cases[13]; however, the molecular basis of the phenotype is still unknown. The aim of this study was to characterise the genomic organisation of the *JK* gene in Thai blood donors with the Jk(a–b–) phenotype.

## Materials and methods

### Subjects

Blood samples taken from 25,340 Thai blood donors at the National Blood Centre, Thai Red Cross Society, Bangkok, Thailand, were included in this study.

### Blood group serology

All samples were screened for Jk(a–b–) phenotypes using a direct urea lysis test as previously described[13]. $Jk^a$ and $Jk^b$ antigens in Jk(a–b–) phenotypes, identified by the urea lysis test, were confirmed by IAT using anti-$Jk^a$ and anti-$Jk^b$ (DiaMed AG, Switzerland) with a standard tube test. After centrifugation, the reactions were read macroscopically and the agglutination reactions were graded as 4+, 3+, 2+, 1+ and w+. After reading the negative reaction under a microscope, IgG-coated red blood cells were added to check the validity of the antiglobulin test[2].

### Molecular biology

Molecular biology analyses were conducted on various EDTA-blood samples from Thai blood donors with different JK phenotypes, identified by the urea lysis test and standard serological techniques. The samples analysed were: four samples of Jk(a–b–), five samples of known Jk(a+b–), five samples of known Jk(a–b+) and five samples of known Jk(a+b+).

Genomic DNA was extracted from peripheral blood by the Diatom binding method[18] and diluted in sterile distilled water to 100 ng/µL. Primers used in this study for polymerase chain reaction (PCR) amplification and sequencing of *JK* gene fragments exons 4 through 10 and flanking intron regions (±50 nucleotides) at the splicing site were similar to those previously described,[14,16] as shown in Table I.

In the PCR, a total volume of 20 µL, containing approximately 100 ng of genomic DNA per reaction, was amplified using GoTaq® Colorless Master Mix, 2X (Promega Corporation, Madison, WI, USA). The reaction mixture contained 4 mM $MgCl_2$, 2X Colorless GoTaq® Reaction Buffer (pH 8.5), 400 µM dATP, 400 µM dGTP, 400 µM dCTP, 400 µM dTTP and 1µM of each set of JK primers (forward and reverse). Each set of reactions was performed in

**Table I -** Primers used for PCR amplification and sequencing of exons 4 to 10 of the JK gene.

| Primer name | Primer sequence (5'® 3') | Amplified fragments | PCR product size (bp) |
|---|---|---|---|
| E4-1F[16] | GGAAAATGGTGCTCTCTTAG | Exon 4 | 437 |
| E4-2R[16] | TAAAAGTAGAAAAATGGTGAGTAA | | |
| E5-1F[16] | GCCTGTGGTTGAAGAGTATC | Exon 5 | 410 |
| E5-2R[16] | ATTCCCTGACCTCTGACTAA | | |
| Jkin5F3[14] | CAAGTGCAACCAAAGCTCAC | Exon 6 | 216 |
| Jkin6R[14] | CTGCCATATAACAACTCCCATTC | | |
| E7-1F[16] | ATAGCGATTCCGTGTGTCA | Exon 7 | 357 |
| E7-2R[16] | ACACCCGTGGACAGTTGAC | | |
| E8-1F[16] | AGTTGTTTTGGTAGCCTCAT | Exons 8-9 | 606 |
| Jkin9R2[14] | ACTGCTTATCCTTGATTGAG | | |
| E10-1F[16] | GCTCATGCTTGTAATCAGG | Exon 10 | 251 |
| E10-2R[16] | AGTGGACTTCAGGAGCATT | | |

a thermal cycler (Mastercycler® gradient, Eppendorf AG, Hamburg, Germany).

The cycling conditions for amplification of exons 4, 5, 6, 7, 8 to 9 and 10 were modified from those previously described[14,16] as follows: initial denaturation at 95 °C for 5 min; 40 cycles at 94 °C for 30 sec, 60 °C for 40 sec and 72 °C for 40 sec and a final extension at 72 °C for 10 min before quenching at 4 °C. In addition, the annealing temperature was changed from 60 °C to 64 °C for amplification of exon 6. PCR products were sequenced.

The DNA sequence was then determined using a cycle sequencing kit (BigDye, Applied Biosystems, Foster City, CA, USA) and a genetic analyser (ABI 3100, Perkin Elmer, Applied Biosystems), according to manufacturers' instructions. The sequences were analysed by software (BioEdit Sequence Alignment Editore software, Carlsbad CA, California) and compared to the reference sequence of *JK* genes (GenBank Accession Number AY942197.1).

## Results

Altogether, 25,340 blood samples were screened for the Jk(a–b–) phenotype. Five samples were confirmed as having a Jk(a–b–) phenotype by serological testing using the urea lysis test and IAT. The gene expression of *JK*01* and *JK*02* alleles in known blood samples of Jk(a+b–), Jk(a–b+) and Jk(a+b+) phenotypes was investigated, and the gene sequences were found to be similar to those of the reference gene sequence. Unfortunately, one sample with a Jk(a–b–) phenotype was unavailable for the molecular characterisation study. Thus, only four of five Jk(a–b–) phenotypes were investigated. Samples 1 and 2 were A/A at nucleotide 838 in exon 9 predicting the $JK^b$ background. The homozygosity of *JK*02* alleles for a G>A mutation at the 3' acceptor splice site of intron 5 of the *JK* gene was demonstrated in both samples. Moreover, homozygosity of *JK*02* alleles for an 896G>A mutation in exon 9 (Gly299Glu) was found in sample 3. Interestingly, missense dual mutations of *JK*01* alleles from a female blood donor were identified. The first mutation was 956C>T (Thr319Met) in exon 10 and the second mutation was 130G>A (Glu44Lys) in exon 4. Comparisons of polymorphic nucleotide positions in exons 4, 9, 10 and intron 5 of the *JK* gene in this study are shown in Table II and Figure 1.

## Discussion

The frequency of the Jk(a–b–) phenotype is very low in all populations except Polynesians and Finns. It is, therefore, laborious to find compatible blood for patients with anti-Jk3 in their serum. The molecular basis for the absence of the JK glycoprotein from red blood cells in Polynesians, Finns, Europeans and Asians was previously elucidated[14-17,19,20].

This study provides two results. Firstly, the *JK*01* and *JK*02* gene sequences in Thais are similar to the reference gene sequence. Secondly, we have shown that the molecular bases of the Jk(a–b–) phenotype in Thai populations are dissimilar. It was found that the invariant G residue in the 3' acceptor splice site was mutated to A in two Thai Jk(a–b–) samples, as in previous studies in Polynesian, Chinese, Taiwanese, Fujian, Filipino and Indonesian populations[14-17,19]. Additionally, one Jk(a–b–) sample had the missense mutation 896G>A (Gly299Glu) in exon 9, located at the transmembrane region and likely leading to the Jk(a–b–) phenotype, as in a previous study in Polynesians[15]. Surprisingly, the last Jk(a–b–) sample from a female blood donor with homozygosity for the *JK*01* allele had dual missense mutations. The first mutation was 956C>T (Thr319Met) in exon 10, as recently found in an African-American male blood donor[14].

**Table II -** Comparison of polymorphic nucleotide positions in exons 4, 9, 10 and intron 5 of the JK gene in this study.

| Type | Phenotype | Allele | Intron 5 | Exon 4 | Exon 9 | | Exon 10 |
|---|---|---|---|---|---|---|---|
| | | | -1 | 130 | 838 | 896 | 956 |
| Known | Jk(a+b-) | *JKA* | G | G | G | G | C |
| Known | Jk(a-b+) | *JKB* | G | G | A | G | C |
| Sample 1 | Jk(a-b-) | $Jk^{null}$ | A | G | A | G | C |
| Sample 2 | Jk(a-b-) | $Jk^{null}$ | A | G | A | G | C |
| Sample 3 | Jk(a-b-) | $Jk^{null}$ | G | G | A | A | C |
| Sample 4 | Jk(a-b-) | $Jk^{null}$ | G | A | G | G | T |

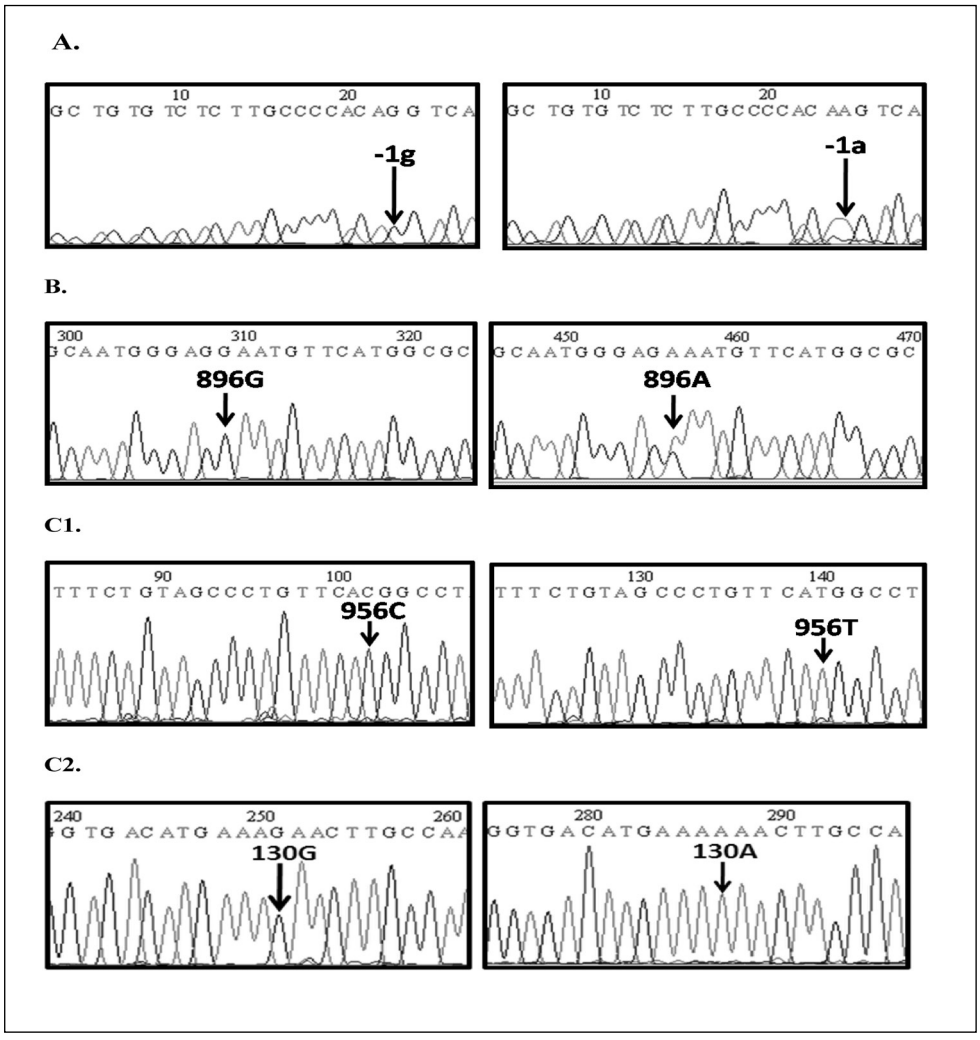


**Figure 1 -** Chromatogram of alleles in samples from Thai homozygous Jknull subjects and normal controls. (A) Thai Jknull allele-related g>a mutation in intron 5. (B) Thai Jknull allele-related-896G>A mutation in exon 9. (C) Dual allelic missense mutations with 956C>T in exon 10 and 130G>A in exon 4. The positions of mutations are indicated with arrows.

The second mutation was 130G>A (GLU44Lys) in exon 4, as in previous studies in Caucasians[20]. Concerning a nucleotide 130G>A in exon 4, from a previous study, it was found that this nucleotide change in a female donor was associated with weak or altered $Jk^a$ antigen expression and production of alloantibodies[21]. A family study of this donor is needed in order to confirm that the two mutations are on separate alleles, but the study has had to be postponed because the woman is pregnant.

In conclusion, this study shows that the underlying molecular bases of the Jk(a–b–) phenotype vary among populations and also clarifies the molecular bases of $Jk^{null}$ in Thais, thus providing useful information for planning genotyping strategies for donors and patients.

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**Correspondence**: Oytip Nathalang
Department of Medical Technology
Faculty of Allied Health Sciences, Thammasat University
Pathumtani, Thailand
e-mail: oytipntl@hotmail.com