Analysis of SERF in Thai blood donors

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The Cromer blood group system consists of nine high-prevalence and three low-prevalence antigens carried on decay-accelerating factor (DAF). We recently described one of these Cromer high-prevalence antigens, SERF, the absence of which was found in a Thai woman. The lack of SERF antigen in this proband was associated with a substitution of nucleotide 647C>T in exon 5 of *DAF,* which is predicted to be a change of proline to leucine at amino acid position 182 in short consensus repeat (SCR) 3 of DAF. This study reports on PCR-RFLP analysis of the *SERF* allele with *Bst*NI restriction endonuclease on more than one thousand Thai blood donor samples. One new donor homozygous (647T) and 21 donors heterozygous (647C/T) for the *SERF* allele were found. Among this cohort of random Thai blood donors, the *SERF* allele frequency was 1.1 percent. Thus, like other alleles in the Cromer blood group system, *SERF* is found in a certain ethnic group. ***Immunohematology* 2005,21:66–69.**

Key Words: Cromer blood group system, decay-accelerating factor, DAF, CD55, SERF, high-prevalence antigen, Thais

The antigens of the Cromer blood group system are carried on decay-accelerating factor (DAF; CD55), which is a member of the regulators of complement activation family of proteins.[1] DAF has four homologous short consensus repeat (SCR) domains followed by an *O*-glycosylated serine- and threonine-rich region attached to a glycosylphosphatidylinositol (GPI) membrane anchor.[2] The Cromer blood group system consists of nine high-prevalence and three low-prevalence antigens.[3,4] Each antigen, with the exception of IFC, is associated with a single amino acid change in DAF. The location of the various Cromer antigens on DAF was accomplished by testing the corresponding antibodies against stable transfectants expressing full-length and deletion mutants of DAF.[5] $Tc^a/Tc^b/Tc^c$, Es^a, and WES^a/WES^b are located in SCR 1; Dr^a and SERF are within SCR 3; and Cr^a, UMC, and GUTI are within SCR 4.[4-8] The Dr(a–) phenotype is characterized by a single nucleotide substitution that is predicted to change serine to leucine at position 165 of DAF. This single nucleotide substitution exposes a cryptic splice site that leads to aberrant RNA splicing, causing a profound decrease in expression both of full-length RNA and of RBC-surface DAF, thus Cromer antigens are very weakly expressed on Dr(a–) RBCs.[9,10] RBCs of the Cromer null phenotype, known as Inab, totally lack DAF expression, with more than one mutation in the *DAF* gene reported.[9,11-13]

Interestingly, the absence of a high-prevalence Cromer blood group antigen is restricted to a certain ethnic group. The Cr(a–) and Tc(a–b+) phenotypes are found predominantly in the Black population.[1] Similarly, the Dr(a–) phenotype is restricted to Jews originating from the Bukharan area of Uzbekistan[9,14,15] and to the Japanese.[16-18] The mutation associated with the lack of the GUTI antigen has been found in 15 percent of Mapuche Indians in Chile.[8] We recently showed that the allele associated with an absence of the SERF antigen was present in 2 percent of Thai donors. The SERF polymorphism is associated with a single nucleotide change of 647C>T in exon 5, which is predicted to encode an amino acid change of proline 182 to leucine.[4]

This report describes a PCR-RFLP analysis for the *SERF* allele in 1041 Thai blood donors.

Materials and Methods

Samples

Three milliliters of peripheral blood samples were collected from each of 1041 healthy volunteer blood donors at the National Blood Centre, Thai Red Cross Society. Institutional Review Board (IRB) approval was obtained.

Genomic DNA extraction from blood

Genomic DNA was extracted from the buffy coat that was collected after centrifugation of the whole blood at 10,000 rpm for 5 minutes. RBC lysis buffer of 155 mM NH_4Cl, 10 mM $KHCO_3$, and 0.1 mM EDTA, pH 7.4, was added; the tube was centrifuged at 10,000 rpm for 10 minutes; and the supernatant was discarded. The white blood cell pellets were resuspended in 200 µL TE

buffer (25 mM Tris and 5 mM EDTA), and lysed by the addition of 10% SDS and proteinase K at 37°C for 1 to 2 hours. DNA was purified by chloroform-isoamyl alcohol extraction and precipitated by isopropanol at –20°C.[19] DNA was collected by centrifugation at 10,000 rpm at 4°C for 10 minutes, washed with cold 70% ethanol, and air-dried. DNA was solubilized in 50 µL of 0.5X TE buffer at 60°C for 10 minutes and stored at –20°C.

The quality and concentration of DNA was determined by comparison with known concentrations of λDNA/*Hin*dIII on a 0.7% agarose gel. Alternatively, the amount of DNA was measured by a spectrophotometer, using the absorbance at 260 nm, and calculated based on the definition that 1 OD_{260} of dsDNA is equivalent to 50 µg/mL DNA.

PCR-RFLP analysis with Bst*NI*

We used the PCR-RFLP assay previously described.[4] In brief, we used the sense primer, SERF 5´ (5´gtgtagtaaatattttaagataataacc 3´) that is located in intron 4, and the antisense primer, SERF 3´ (5´ cttacCTCTGCACTCTGGCACC 3´); the underlined C is a deliberate A>C change) that is located in exon 5 and extends 5 nucleotides into intron 5 (sequence data as per Douglas M. Lublin, MD, verbal communication). The primers were synthesized by Life Technologies, Inc. (Gaithersburg, MD). Two microliters (equivalent to 0.1 µg) of DNA per reaction were amplified using 5 U of *Taq* DNA polymerase (HotStarTaq, QIAGEN Inc., Valencia, CA) in a 50-µL reaction mixture containing 1.5 mM $MgCl_2$, 1X PCR buffer, 0.2 mM dNTPs, and 100 ng sense and antisense primers. PCR amplification was performed in a thermal cycler (9700, Perkin Elmer, Norwalk, CT) as follows: 35 cycles of 94°C for 20 seconds, 55°C for 20 seconds, and 72°C for 20 seconds, followed by a final extension of 10 minutes at 72°C. PCR products were analyzed on a 1.2% agarose gel and then subjected to digestion with *Bst*NI (New England Biolabs, Beverly, MA) overnight and analyzed on 8% polyacrylamide gel.

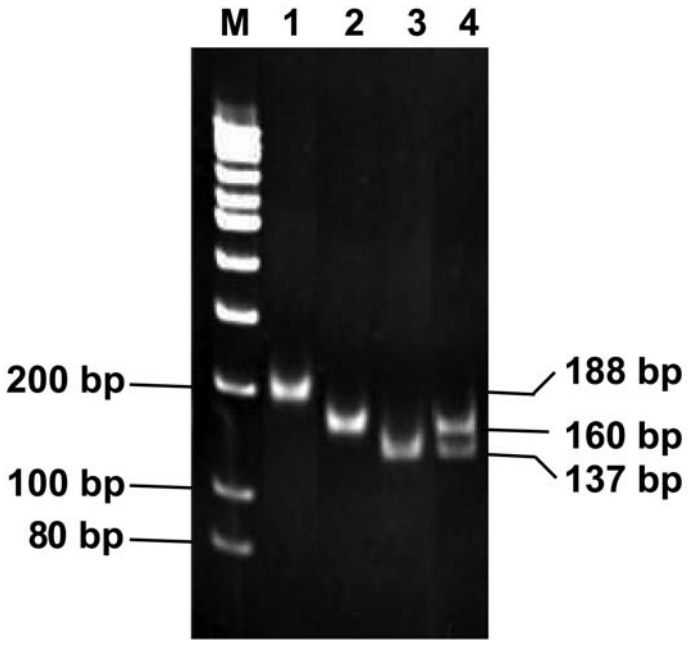

Fig. 1. PCR-RFLP analysis using *Bst*NI of DAF exon 5. **Lane M:** Molecular weight 100 bp DNA ladder marker. **Lane 1:** 188 bp uncut PCR amplicon. **Lane 2:** The digested PCR amplicon from a normal control DNA, which shows the 160 bp band. **Lane 3:** The digested PCR product of a sample, homozygous for the mutation, with a band of 137 bp. **Lane 4:** The digested PCR product from a sample, heterozygous for the mutation and wild-type with bands of 160 bp and 137 bp. The 28 bp band associated with wild-type and mutated alleles, and the 23-bp band associated with the mutated allele ran off the gel and thus are not visible.

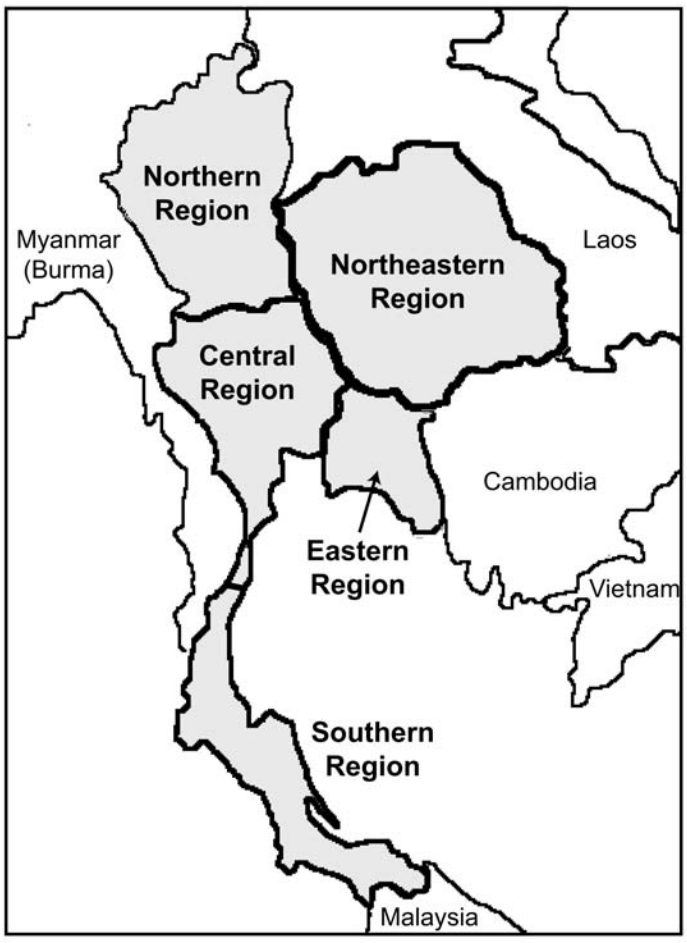

Fig. 2. Map of Thailand showing regions.

Results

The resulting PCR product of 188 bp, when digested with *Bst*NI, demonstrated one of three patterns (Fig. 1). Of the 1041 samples tested, which includes the 100 Thai donor samples previously reported,[4] we found one homozygous sample (in addition to the original proband) and 21 heterozygous samples. This represents an allele frequency of 1.1 percent in the 1041 random Thai donors. The allele was found in northern, eastern, and central regions, but not in the southern region of Thailand (Fig. 2). The region of origin was unknown for 35 of the donors. The *SERF*-negative allele was not found in 50 samples from the southern region. A summary of our findings is given in Table 1.

Table 1. Frequency of the *SERF*-negative allele in random Thai blood donors

Part of Thailand	Donors tested Number	(%)	Donors with *SERF*-negative allele Number	(%)
Northern	65	(6.2)	1	(0.8)
Northeastern	111	(10.7)	3	(1.4)
Eastern	19	(1.8)	1	(2.6)
Central	761	(73.1)	16*	(1.1)
Southern	50	(4.8)	0	(0)
No information	35	(3.4)	1	(1.4)
Total	1041		22	(1.1)

*One homozygous and 15 heterozygous samples

Discussion

SERF is a high-prevalence antigen in the Cromer blood group system and is associated with a proline residue at position 182 in *DAF*. The lack of SERF antigen on RBCs is associated with a single nucleotide mutation (647C>T) in exon 5, predicting an amino acid change (Pro182Leu) in the third SCR of *DAF*. Absence of the SERF antigen was found in the original Thai proband[4] and in one additional donor in this study. In the previous report,[4] two heterozygous samples were found in 100 random Thai donors. In this report, we tested 941 additional blood donors and confirmed that the mutated *SERF* allele has a frequency of 1.1 percent in Thailand. Thus, a total of 23 individuals (21 heterozygous random donors, one homozygous random donor, and the original proband) have been found to have the *SERF*-negative allele. Taken together, this represents an allele frequency of 1.2 percent (25/2084 × 100).

The SERF– phenotype joins other ethnically restricted Cromer phenotypes, the Cr(a–) and Tc(a–b+) phenotypes that are found predominantly in Blacks[1]; the Dr(a–) phenotype that is restricted to Jews originating from the Bukharan area of Uzbekistan[9,14,15] and to the Japanese[17,18]; and the mutation associated with the GUTI antigen, which has only been found in Mapuche Indians in Chile.[8] The Cromer phenotypes associated with certain ethnicities are summarized in Table 2. It should be noted that these ethnic associations have been determined by knowing the ethnicity of antibody producers and not by extensive testing in various ethnic groups.

Table 2. Antigens of the Cromer blood group system

Antigen name	Frequency	Predominant ethnic association(s)
Cr^a	High	Cr(a–) in Blacks 1 Spanish-American
Tc^a	High	Tc(a–) in Blacks Tc(a–) in Whites
Tc^b	Low	Tc(b+) in Blacks
Tc^c	Low	Tc(c+) in Whites
Dr^a	High	Dr(a–) in Uzbekistani Jews and Japanese
Es^a	High	No association
IFC	High	3 Japanese 2 Italian American 1 Jewish American
WES^a	Low	WES(a+) in Finns and Blacks
WES^b	High	WES(b–) in Finns and Blacks
UMC	High	Japanese
GUTI	High	GUTI– in Chileans
SERF	High	SERF– in Thais

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