

Original article

Prediction of Duffy blood group phenotypes through genotypic analysis among Southern Thai blood donors

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Abstract:

Background and Objectives: The Duffy (FY) blood group system is important in transfusion medicine due to the risk of alloimmunization from Fy^a and Fy^b antigens after transfusion or pregnancy. Serological testing can misinterpret Fy^a, Fy^b, and Fy^x antigens; therefore, genotyping of FY*A and FY*B alleles is applied to accurately predict FY phenotypes. This study aimed to determine FY*A and FY*B allele frequencies, predict FY phenotypes, and assess the presence of the FY*X allele in Southern Thai blood donors. **Materials and Methods:** Altogether, 427 DNA samples of blood donors in Southern Thailand were genotyped using in-house PCR with sequence-specific primers (PCR-SSP) to detect FY*A and FY*B alleles. Samples with FY*A/B and FY*B/B genotypes were confirmed and used to determine the FY*X allele by DNA sequencing. **Results:** The FY*A, FY*B and FY*B^{ES} allele frequencies were 0.854, 0.145 and 0.001, respectively. Although the Fy(a-b-) phenotype was not observed, one donor was identified as possessing the FY*A/B^{ES} genotype. Genotype frequencies were FY*A/A (72.83%), FY*A/B (24.82%), FY*B/B (2.11%) and FY*A/B^{ES} (0.23%). Those 115 samples with FY*A/B and FY*B/B genotypes, FY*X allele screening revealed variation only at c.298G>A, which did not affect Fy^b antigen expression. Fy^a and Fy^b alloimmunization risks were significantly higher in Southern Thais compared to Central and Northern Thais and other Asian populations ($p < 0.05$). **Conclusion:** The high frequency of the FY*A and FY*B alleles among Southern Thais contributes to transfusion-induced alloimmunization risks. Consequently, FY genotyping is suggested to minimize transfusion reactions and improve blood transfusion safety.

Keywords : ● Duffy blood group system ● Genotypes ● Alleles ● Phenotypes ● Thais

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นิพนธ์ต้นฉบับ

การทำนายฟีโนไทป์หมู่เลือดระบบ Duffy จากการตรวจจีโนไทป์ในผู้บริจาคเลือดคนไทยภาคใต้

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บทคัดย่อ

บทนำ หมู่เลือดระบบ Duffy (FY) เป็นหมู่เลือดที่มีความสำคัญในด้านเวชศาสตร์การบริการโลหิต เนื่องจากความเสี่ยงของการเกิด Fy^a และ Fy^b alloimmunization ภายหลังจากการได้รับเลือดหรือการตั้งครรภ์ การตรวจแอนติเจนทั้ง Fy^a , Fy^b และ Fy^x พบความผิดพลาดเมื่อใช้การตรวจด้วยวิธีซีโรโลยี ดังนั้นจึงมีการประยุกต์ใช้การตรวจจีโนไทป์เพื่อระบุอัลลีล FY^*A และ FY^*B เพื่อทำนายเป็นฟีโนไทป์ **วัตถุประสงค์** การศึกษานี้มีวัตถุประสงค์เพื่อตรวจอัลลีล FY^*A และ FY^*B เพื่อทำนายเป็นฟีโนไทป์ของ FY และตรวจหาอัลลีล FY^*X ในผู้บริจาคเลือดคนไทยภาคใต้ **วัสดุและวิธีการ** ใช้ตัวอย่างดีเอ็นเอของผู้บริจาคเลือดคนไทยภาคใต้ จำนวน 427 ราย มาทำการตรวจจีโนไทป์เพื่อระบุอัลลีล FY^*A และ FY^*B ด้วยวิธีพีซีอาร์ เอสเอสพี ตัวอย่างที่เป็นจีโนไทป์แบบ FY^*A/B และ FY^*B/B จะตรวจยืนยันเพิ่มเติมด้วยวิธี DNA sequencing เพื่อตรวจหาอัลลีล FY^*X **ผลการศึกษา** ความถี่ของอัลลีล FY^*A , FY^*B และ FY^*B^{ES} เท่ากับ 0.854, 0.145 and 0.001 ตามลำดับ ถึงแม้ว่าฟีโนไทป์แบบ $Fy(a-b-)$ ไม่พบในการศึกษาครั้งนี้ แต่มีผู้บริจาคเลือด 1 ราย ที่พบว่ามีจีโนไทป์แบบ FY^*A/B^{ES} ความถี่ของจีโนไทป์แต่ละชนิดคือ FY^*A/A (72.83%), FY^*A/B (24.82%), FY^*B/B (2.11%) และ FY^*A/B^{ES} (0.23%) ตามลำดับ ตัวอย่างจำนวน 114 ราย ที่มีจีโนไทป์เป็น FY^*A/B และ FY^*B/B ได้นำมาตรวจหาลำดับเบสเพื่อหาอัลลีล FY^*X แต่พบว่าไม่มีตำแหน่งของนิวคลีโอไทด์ที่พบความเปลี่ยนแปลงเฉพาะ c.298G>A ซึ่งไม่ส่งผลต่อการแสดงออกของแอนติเจน Fy^b ความเสี่ยงของการเกิด alloimmunization ต่อ Fy^a และ Fy^b ในคนไทยภาคใต้สูงกว่าคนไทยภาคกลาง ภาคตะวันออก และกลุ่มประชากรเอเชียอื่นอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) **สรุป** ความถี่ของอัลลีล FY^*A และ FY^*B ที่สูงในคนไทยภาคใต้ส่งผลต่อความเสี่ยงของการเกิด alloimmunization จากการได้รับเลือด ดังนั้นจึงแนะนำให้ตรวจจีโนไทป์ของ FY เพื่อลดปัญหาปฏิกิริยาอันไม่พึงประสงค์จากการได้รับเลือด และเพิ่มความปลอดภัยในการให้เลือดต่อไป

คำสำคัญ : ● หมู่เลือดระบบ Duffy ● จีโนไทป์ ● อัลลีล ● ฟีโนไทป์ ● คนไทย

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต. 2568;35:257-69.

Introduction

The Duffy (FY) blood group system consists of five antigens: Fy^a, Fy^b, Fy3, Fy5 and Fy6.¹ Two antithetical antigens, Fy^a and Fy^b, play a vital role in alloimmunization following blood transfusions or pregnancies. Both anti-Fy^a and anti-Fy^b IgG are primarily implicated in hemolytic transfusion reactions (HTRs) and hemolytic disease of the fetus and newborn (HDFN).^{2,3} In general, four FY phenotypes are defined by testing red blood cells (RBC) with anti-Fy^a and anti-Fy^b: Fy(a+b-), Fy(a+b+), Fy(a-b+) and Fy(a-b-). The Fy^a-positive antigen is the most common among Caucasians and Asians, with African Americans being an exception. However, the Fy(a-b-) phenotype is rare across all populations; its frequency is frequently found among African Americans.²⁻⁴ In addition, the Fy^x antigen is produced by reducing the expression of the Fy^b antigen through three mutation points of single-nucleotide polymorphisms (SNPs) of the *FY*B* alleles, which were subsequently defined as the *FY*X* allele. The frequency of the *FY*X* allele is approximately 2% to 3.5% among Caucasians, while not observed among African Americans.^{5,6} Misinterpretation of the Fy^b antigen typing may result in Fy(a-b^w) or Fy(a-b-), leading to difficulties in blood management. Therefore, molecular testing is strongly recommended to identify gene variations and evaluate Fy^b antigen expression.⁵⁻⁷

In Thailand, alloantibodies were found among 1,766 multi-transfused patients reported by the National Blood Centre, Thai Red Cross Society. The most common alloantibodies were in the Rh system (42.2%), followed by MNS (31.9%), Kidd (10.5%), Lewis (5.9%), P1PK (3.0%) and Duffy (2.7%) systems, respectively.⁸ Moreover, the prevalence of alloantibodies among 360 Southern Thai patients was as follows: MNS (37.0%), Rh (30.2%), Lewis (21.5%), Kidd (3.9%), P1PK (3.7%) and Duffy (2.3%) systems, respectively.⁹ Although the frequencies of alloantibodies found among Central Thai patients were similar to those among Southern Thai patients, the prevalence of predicted phenotypes of MNS, Rh, Kidd,

Diego and Dombrock systems in Central and Southern Thai donor populations showed statistically significant differences.¹⁰⁻¹⁵ These findings could be explained by the fact that most Southern Thai Muslims living in three provinces: Pattani, Yala, and Narathiwat near the Malaysian border have distinct ethnic backgrounds from people in other parts of Thailand.

Misinterpretation of blood group antigen typing using serological techniques could occur among patients with positive direct antiglobulin tests, particularly those receiving transfusions, and among patients carrying the Fy^x antigen. Hence, molecular techniques are applied to determine *FY*A* and *FY*B* alleles to predict FY phenotypes. The FY gene has two major alleles; *FY*A* and *FY*B*, which are located on chromosome 1q21-22. In addition, there are another alleles; *FY*A^{ES}* or *FY*B^{ES}* (ES, erythrocyte silent), that are located on the same chromosome as well.

The Fy^a and Fy^b antigens are associated with a bi-allelic polymorphism caused by SNP c.125G>A, p.Gly42Asp (rs12075). *FY*A* encodes Gly42 and *FY*B* encodes Asp42 in exon 2 of the *ACKR1* gene. Additionally, a single mutation in a GATA motif in the *FY*A^{ES}* or *FY*B^{ES}* promoter at c.-67T>C causes a non-expressed antigen among FY-negative individuals.¹⁻⁴ For *FY*X* alleles, the most common was *FY*02W.01* caused by c.265C>T, p.Arg89Cys (rs34599082) and c.298G>A, p.Ala100Thr (rs13962). The other *FY*02W.02* caused by three SNPs, including c.145G>T, p.Ala49Ser (rs1307925062); c.265C>T, p.Arg89Cys (rs34599082), and c.298G>A, p.Ala100Thr (rs13962), are involved in weak Fy^b antigen expression.¹ Related studies revealed only the frequency of *FY*A* and *FY*B* alleles in Central, Northern, and Northeastern areas in Thai blood donor populations.¹⁶⁻¹⁸ Information regarding the frequency of such alleles among blood donors from Southern Thailand remain unknown. Therefore, this study aimed to determine *FY*A* and *FY*B* allele frequencies and investigate the *FY*X* allele to eventually estimate the risk of alloimmunization in the Southern Thai population.

Materials and Methods

Study population and ethics considerations

Altogether, 427 DNA samples obtained from unrelated Thai Muslim blood donors residing in the three southern border regions (Yala, Patani and Narathiwat) between 2018 and 2019 were included. Genomic DNA was isolated using the Genomic DNA extraction kit (REAL Genomics, RBC Bioscience, Taipei, Taiwan) according to the manufacturer instructions and stored at -20°C until used. The Human Research Ethics Committee of Thammasat University (Science), Pathumtani, Thailand (HREC-TUSc), provided approval with the research protocol (COE No. 005/2568).

DNA standards

Eleven DNA samples with known phenotypes confirmed by DNA sequencing, including three Fy(a+b-), three Fy(a+b+), three Fy(a-b+) and two Fy(a-b-) phenotypes of *FY*B^{ES}/B^{ES}* (c.-67CC), were used as controls.¹⁶

Duffy blood group genotyping by PCR-SSP

Genotyping of FY alleles was performed using the PCR-SSP technique, as previously described¹⁶ with some modifications. A set of FY genotyping included four reactions of PCR mixtures to identify *FY*A^{ES}*, *FY*B^{ES}*, *FY*A* and *FY*B* alleles. Each PCR reaction (10 µL) consisted of genomic DNA 50 ng/µL, forward primer

for the FY promoter region polymorphism (GATA-AB-F/*FY*-AB-F) 0.5 µmol/L and reverse primer for the *FY*A* and *FY*B* polymorphism (*FY*-A-R/*FY*-B-R) 0.5 µmol/L. The sequences of primers used in this study are shown in Table 1. In addition, co-amplification of the human growth hormone gene (HGH) using 0.3 µmol/L of each HGH-F primer and HGH-R primer was run as an internal control. The PCR reaction was performed with 5 µL of the 2X PCR reaction mixture (Green HotStart PCR Master Mix Direct-Load, biotechrabbit, Berlin, Germany).

We performed PCR under the following conditions. Initial denaturation was conducted at 94°C for 2 min. The PCR program began with 10 cycles of 94°C for 10 sec and 69°C for 1 min, then 20 cycles of 94°C for 30 sec, 62°C for 1 min and 72°C for 30 sec. The last step was a final extension at 72°C for 5 min. The PCR products were electrophoresed at 100 volts on a 1.5% agarose gel containing 10,000X fluorescent DNA gel stain (StainIN Green Nucleic Acid Stain, highQu GmbH, Kraichtal, Germany) using 1x Tris borate ethylenediamine tetraacetate (TBE) buffer. Products were visualized under a blue-light transilluminator. The PCR product sizes of the *FY*A^{ES}*, *FY*B^{ES}*, *FY*A* and *FY*B* alleles were each 711 bp, while the internal control product size was 434 bp. Known DNA samples for the Fy(a+b-), Fy(a+b+), Fy(a-b+)

Table 1 Primer sequences for FY blood group genotyping

Allele	Primer	Primer sequence (5'→3')	Product size (bp)	Final concentration (µmol/L)
<i>FY*A^{ES}</i>	GATA-AB-F	CTCATTAGTCCTTGGCTCTTAC	711	0.5
	FY-A-R	AGCTGCTTCCAGGTTGGCAC		
<i>FY*B^{ES}</i>	GATA-AB-F	CTCATTAGTCCTTGGCTCTTAC	711	0.5
	FY-B-R	AGCTGCTTCCAGGTTGGCAT		
<i>FY*A</i>	FY-AB-F	CTCATTAGTCCTTGGCTCTTAT	711	0.5
	FY-A-R	AGCTGCTTCCAGGTTGGCAC		
<i>FY*B</i>	FY-AB-F	CTCATTAGTCCTTGGCTCTTAT	711	0.5
	FY-B-R	AGCTGCTTCCAGGTTGGCAT		
<i>HGH</i>	HGH-F	TGCCTTCCCAACCATTCCTTA	434	0.3
	HGH-R	CCACTCACGATTTCTGTTGTGTTTC		
SEQ	SEQ-FY-F	TGGGGTAAGGCTTCCTGATG	996	1.0
	SEQ-FY-R	AGAGCTGCGAGTGCTACCTA		

and Fy(a-b-) phenotypes were included in each run. In addition, 427 DNA samples obtained from Southern Thai blood donors were tested to determine the FY alleles.

Identifying the *FY*X* allele by DNA sequencing

DNA samples from Southern Thai blood donors identified as *FY*A/B* and *FY*B/B* genotypes using PCR-SSP were subjected to Sanger DNA sequencing to identify the *FY*X* allele. A fragment of 996 bp containing polymorphisms of SNPs c.-67T>C, c.125G>A, c.145G>T, c.265C>T, and c.298G>A was obtained using PCR amplification. The PCR reaction mixture comprised genomic DNA 100 ng/ μ L, 2 μ mol/L forward primer (SEQ-FY-F: 5' TGGGGTA-AGGCTTCCTGATG 3'), and 2 μ mol/L reverse primer (SEQ-FY-R: 5'AGAGCTGCGAGTGCTACCTA 3'), 2X PCR reaction mixture (2X PCR Master mix Solution, i-StarMAX II, iNtRON Biotechnology, Gyeonggi-do, Korea) 10 μ L and distilled water 4 μ L, for a total volume of 20 μ L. The PCR program was performed as described: initial denaturation at 95°C for 5 min, followed by 95°C for 30 sec, 62 °C for 40 sec and 72 °C for 30 sec for 35 cycles and a final extension at 72°C for 5 min. Thereafter, PCR products were separated on a 1.5% agarose gel containing DNA Gel Stain, electrophoresed in 1x TBE buffer at 100 volts and visualized under a blue-light transilluminator. The PCR bands were purified using a gel extraction kit (MEGAquick-spin plus, iNtRON Biotechnology, Gyeonggi-do, Korea), and the eluted fragments were sequenced using sequencing-based technology on the Illumina Platform (U2Bio Sequencing Service, Bangkok, Thailand) with the abovementioned PCR primers.

Computerized analysis of the three-dimensional (3D) structure model of c.298G>A variant

The deduced amino acid sequences of the wild type and mutant were uploaded to a ColabFold notebook for Deepmind's AlphaFold2-based 3D structure building, illustrating the impact of a single-point mutation on protein structure.¹⁹ The Discovery Studio Visualizer 2021 was then used to visualize and superimpose both models.

The DynaMut2 web server was used to estimate the impact of a single mutation on the protein's stability based on the calculation of ddG ($\Delta\Delta G$).²⁰ In addition, PolyPhen2 (Polymorphism Phenotyping, Version 2.2.2) was also used to envisage how the mutation will affect the protein structure and functionality.²¹

Statistical analysis

The statistical analysis was conducted using Microsoft Excel and SPSS Version 25.0 (SPSS Inc., Chicago, IL, USA). The genotype and allele frequencies were determined using direct counting. The chi-square (χ^2) test of homogeneity was employed to determine the difference between Thai¹⁶⁻¹⁸ and other populations.²²⁻²⁸ A *p* value was considered statistically significant when less than 0.05. In addition, the probability of successfully obtaining antigen-negative RBCs for alloimmunized patients was calculated by dividing the number of antigen-negative units desired by the incidence of antigen-negative individuals in the donor population.

In addition, the risk estimation of Fy^a/Fy^b alloimmunization was obtained by multiplying the probability of being predicted by the Fy^a/Fy^b negative phenotype frequencies by the likelihood of having a predicted Fy^a/Fy^b phenotype frequency, as described below.

1. Risk of anti-Fy^a production

Fy(a+b+) donor to Fy(a-b+) patient

$$= FY^*A/B \times FY^*B/B$$

Fy(a+b-) donor to Fy(a-b+) patient

$$= FY^*A/A \times FY^*B/B$$

2. Risk of anti-Fy^b production

Fy(a+b+) donor to Fy(a+b-) patient

$$= FY^*A/B \times FY^*A/A$$

Fy(a-b+) donor to Fy(a+b-) patient

$$= FY^*B/B \times FY^*A/A$$

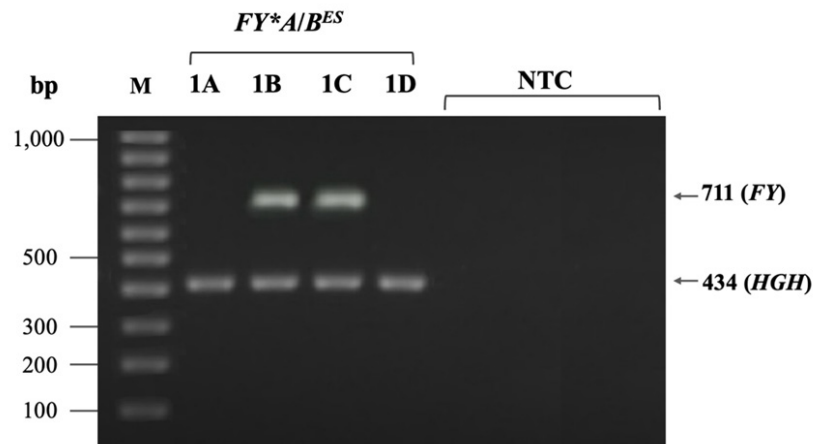
Results

FY genotyping determined using PCR-SSP to predict FY phenotypes

The validated in-house PCR-SSP¹⁶ was applied to genotype 427 DNA samples from Southern Thai blood

Table 2 Frequencies of *FY* genotypes and predicted phenotypes among Southern Thai blood donors

Genotype	Alleles				Predicted phenotype	Number	%
	<i>FY</i> *A ^{ES}	<i>FY</i> *B ^{ES}	<i>FY</i> *A	<i>FY</i> *B			
<i>FY</i> *A/A	Negative	Negative	Positive	Negative	Fy(a+b-)	311	72.83
<i>FY</i> *A/B ^{ES}	Negative	Positive	Positive	Negative		1	0.23
<i>FY</i> *A/B	Negative	Negative	Positive	Positive	Fy(a+b+)	106	24.83
<i>FY</i> *B/B	Negative	Negative	Negative	Positive	Fy(a-b+)	9	2.11
Total						427	100.00



One sample with *FY**A/B^{ES} genotype was found in this study. The PCR amplicon of *FY* is 711 bp, while the *HGH* internal control is 434 bp. Lane M indicates a 100 bp ladder marker. Lane 1A-1D indicates *FY**A^{ES}, *FY**B^{ES}, *FY**A, *FY**B alleles, respectively. NTC indicates no template control.

Figure 1 The PCR-SSP results of *FY**A/B^{ES} genotype.

donors. The frequencies of *FY* genotypes and predicted phenotypes among 427 Southern Thai blood donors are shown in Table 2. The most common genotype was *FY**A/A (311, 72.83%), followed by *FY**A/B (106, 24.83%), *FY**B/B (9, 2.11%) and *FY**A/B^{ES} (1, 0.23%), respectively. The predicted phenotypes observed in this study were Fy(a+b-) 73.06%; Fy(a+b+) 24.83% and Fy(a-b+) 2.11%, while the predicted Fy(a-b-) phenotype was not found.

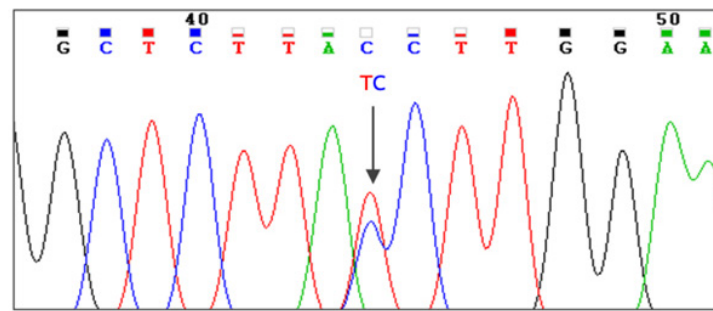
Determining *FYB alleles and identifying the *FY**X allele using DNA sequencing**

To validate the genotyping results of PCR-SSP, one sample of the *FY**A/B^{ES} genotype, 106 samples of *FY**A/B and nine samples of *FY**B/B were selected and analyzed using Sanger DNA sequencing for the GATA-1 promoter and exon 2 of the *ACKR1* gene. For one donor with *FY**A/B^{ES} genotype, the PCR-SSP result (Figure 1) agreed with DNA sequencing (Figure 2A: c.-67TC and 2B: c.125GA) and predicted the Fy(a+b-) phenotype.

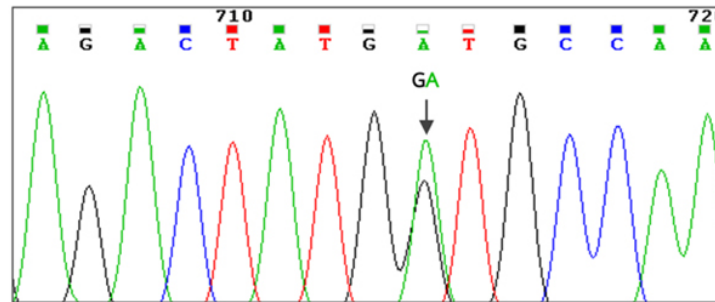
Regarding DNA sequencing results in exon 2 of the *ACKR1* gene at SNP c.125G>A, among 115 samples with *FY**A/B and *FY**B/B genotypes, we found that 106 samples of *FY**A/B genotypes were c.125GA and nine samples of *FY**B/B genotypes were c.125AA (Table 3). Hence, genotyping using PCR-SSP agreed with the DNA sequencing.

For *FY**X allele determination, two alleles including *FY**02W.01 and *FY**02W.02 were identified using DNA sequencing. Regarding the *FY**02W.01 allele, c.265C>T and c.298G>A were analyzed. Among 106 *FY**A/B samples, all were c.265CC; however, 97 samples were c.298GG (Figure 3A), 3 samples were c.298GA (Figure 3B), and 6 samples were c.298AA (Figure 3C). For nine of the *FY**B/B samples, all were c.265CC; however, 6 samples were c.298GG (Figure 4A), and 3 samples were c.298AA (Figure 4B).

(A) SNP. c.-67TC



(B) SNP. c.125GA

**Figure 2** Electropherogram results of the sample with FY^*A/B^{ES} genotype.(A) SNP. c.-67TC in the promoter region and (B) SNP. c.125GA at exon 2 of the *ACKR1* gene.**Table 3** DNA sequencing results of FY^*A , FY^*B , and FY^*X alleles in 115 samples

Pattern	The <i>ACKR1</i> gene at exon 2				Genotype	Predicted phenotype	Frequency
	<i>FY*A/FY*B</i>	<i>FY*X</i>					
	c.125G>A	c.145G>T	c.265C>T	c.298G>A			
1	GA	GG	CC	GG	<i>FY*A/B</i>	Fy(a+b+)	97
2	GA	GG	CC	GA	<i>FY*A/B</i>	Fy(a+b+)	3
3	GA	GG	CC	AA	<i>FY*A/B</i>	Fy(a+b+)	6
4	AA	GG	CC	GG	<i>FY*B/B</i>	Fy(a-b+)	6
5	AA	GG	CC	AA	<i>FY*B/B</i>	Fy(a-b+)	3

 FY^*A and FY^*B allele frequencies in Asian populations

The FY^*A and FY^*B allele frequencies observed in the Southern Thai population were compared with those reported in different populations²²⁻²⁸ and regions in Thailand¹⁶⁻¹⁸ (Table 4). The FY^*A allele was predominant in Thailand and in Asian countries. Its frequency was similar to Northeastern Thai¹⁸ and Malaysian²⁶ populations ($p > 0.05$); however, the difference was observed in Central¹⁶ and Northern Thai¹⁷, Han Chinese²², Taiwanese²³, Korean²⁴, Brazilian Japanese²⁵, Filipino²³, Indian²⁷, and Northern Pakistani²⁸ populations ($p < 0.05$).

Risk of Fy^a/Fy^b alloimmunization in Thai populations

The risk of Fy^a and Fy^b alloimmunization among Thai populations is demonstrated in Table 5. The risk of anti- Fy^a and anti- Fy^b productions in the Southern Thai population was similar to those of Northeastern Thais ($p > 0.05$)¹⁸, but was significantly higher than those of the Central and Northern Thai populations ($p < 0.05$).^{16,17}

3D computational architectures of c.298G>A variant

As we found some donors possessing the variant only at the c.298G>A variant (p.Ala100Thr), we then sought to determine the structural and biochemical changes

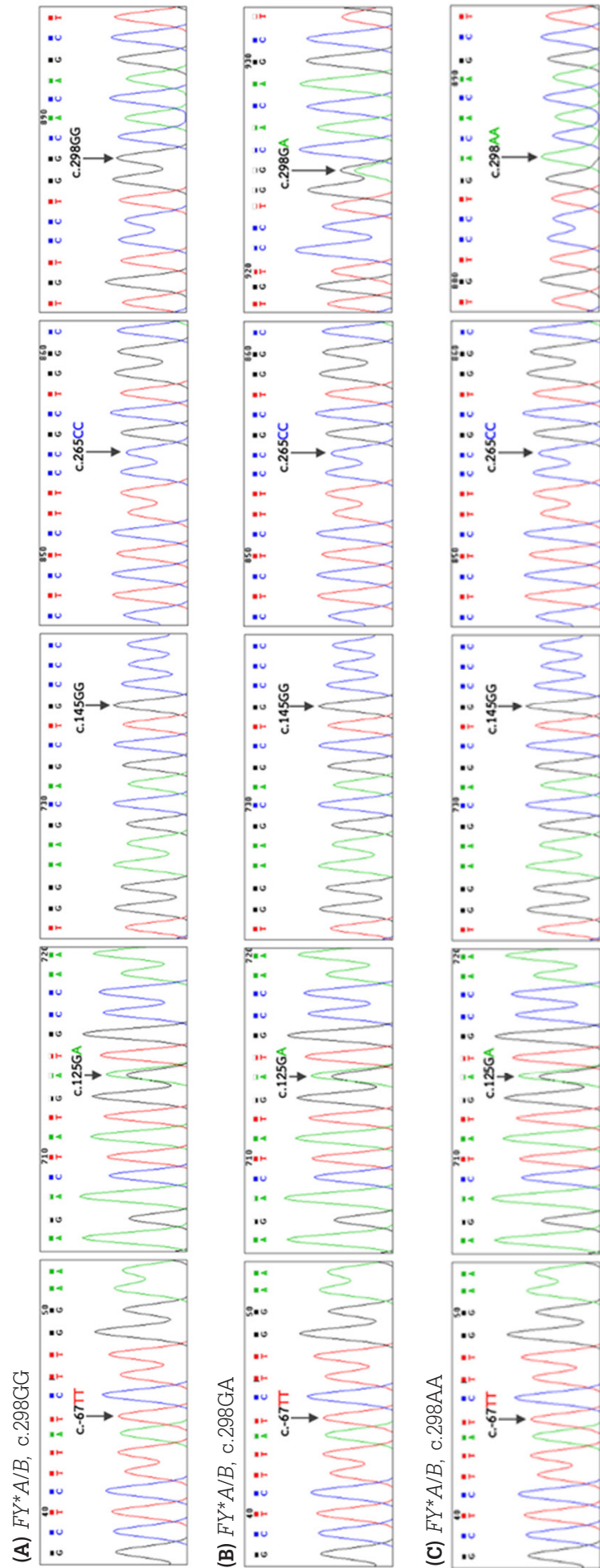


Figure 3 Electropherograms of the *FY* gene at the *FY*^{*}A and *FY*^{*}B polymorphism regions.

DNA sequencing results for different SNP variations at position c.298 illustrate (A) *FY*^{*}A/B with a homozygous c.298GG, (B) *FY*^{*}A/B with a heterozygous c.298GA and (C) *FY*^{*}A/B with a homozygous c.298AA.

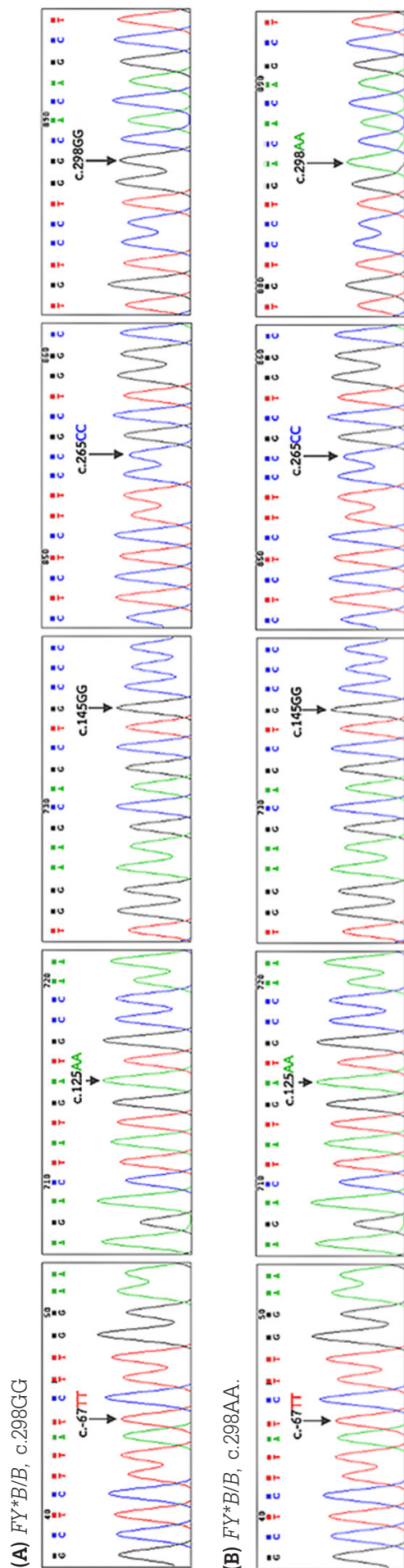


Figure 4 Electropherograms of the *FY* gene at the *FY**B polymorphism region.

DNA sequencing results for different SNP variations at position c.298 illustrate **(A)** *FY**B/B with a homozygous c.298GG and **(B)** *FY**B/B with a homozygous c.298AA.

induced by this SNP. The computerized structure of the wild-type and variant of c.298G>A variant (p.Ala-100Thr) was shown in Figure 5A and 5B, respectively. It has been demonstrated that the anticipated overall structure of the variant shares traits with the wild-type protein model, based on the 3D model and superimposed imaging. The 3D structural model revealed an amino acid modification of the variant in the α -helix region due to a single point mutation (c.298G>A) compared with the wild-type model (Figure 5C).

The structure of the variant was compared with that of the wild-type by the root mean square deviation (RMSD). We found that the main-chain RMSD of the c.298G>A variant structure was 2.418 Å, and the all-protein RMSD was 2.599 Å. These RMSD values suggested a slight but not significant difference between the conformational change of the protein backbone and the overall structure of the wild-type and variant protein.

In addition, an alteration in the protein stability of the variant was predicted. When single amino acid modifications were employed, the change in protein stability yielded a $\Delta\Delta G$ of -0.74 kcal/mol, indicating that the protein variant decreased its stabilization. Likewise, in the protein prediction of stability using PolyPhen-2 analysis, we calculated the thermodynamic stability differences between the wild-type and mutant proteins. This variant was more likely to be detrimental, with a Humdiv score of 0.968 and HumVar score of 0.763. These results suggest that the mutation may affect stability. In summary, the mutant at position 298 alone did not disturb the protein structure and expression but may result in uncertainty of the biochemistry of the protein.

Discussion

In this study, 427 blood donor samples from Southern Thailand were investigated for the *FY* genotypes to predict phenotypes. As a result, the most common genotype was *FY**A/A, followed by *FY**A/B, *FY**B/B, and *FY**A/B^{ES}, consecutively. Hence, the Fy(a+b-) phenotype indicated the highest prevalence, followed by

Table 4 Comparison of *FY**A and *FY**B allele frequencies in different populations

Populations	Number	Allele frequency (N)		χ^2	p-value
		<i>FY</i> *A	<i>FY</i> *B		
Southern Thai	427	0.854 (729)	0.145 (124)	-	-
Central Thai ¹⁶	500	0.962 (962)	0.038 (38)	65.177	< 0.001*
Northern Thai ¹⁷	300	0.938 (563)	0.062 (37)	24.205	< 0.001*
Northeastern Thai ¹⁸	345	0.883 (609)	0.117 (81)	2.355	0.112
Han Chinese ²²	1,412	0.915 (2585)	0.077 (217)	34.867	< 0.001*
Taiwanese ²³	400	0.945 (756)	0.055 (44)	35.942	< 0.001*
Korean ²⁴	306	0.905 (554)	0.095 (58)	7.927	< 0.001*
Brazilian Japanese ²⁵	209	0.785 (328)	0.215 (90)	9.308	< 0.001*
Filipino ²³	230	0.922 (424)	0.078 (36)	11.958	< 0.001*
Malaysian ²⁶	200	0.855 (342)	0.140 (56)	0.018	0.895
Indian ²⁷	3,073	0.648 (3,982)	0.349 (2,146)	142.227	< 0.001*
Northern Pakistani ²⁸	1,000	0.504 (1,008)	0.454 (908)	271.087	< 0.001*

*Statistical significance at $p < 0.05$

Table 5 Estimations of Fy^a and Fy^b alloimmunization risks in Thai populations

Populations	Number	Allele frequencies		Risk of alloimmunization	
		<i>FY</i> *A	<i>FY</i> *B	Anti- Fy^a	Anti- Fy^b
Southern Thai (this study)	427	0.854	0.145	0.021	0.196
Central Thai ¹⁶	500	0.962	0.038	0.004*	0.067*
Northern Thai ¹⁷	300	0.938	0.062	0.000*	0.108*
Northeastern Thai ¹⁸	345	0.883	0.117	0.011	0.173

* Statistical significance at $p < 0.05$

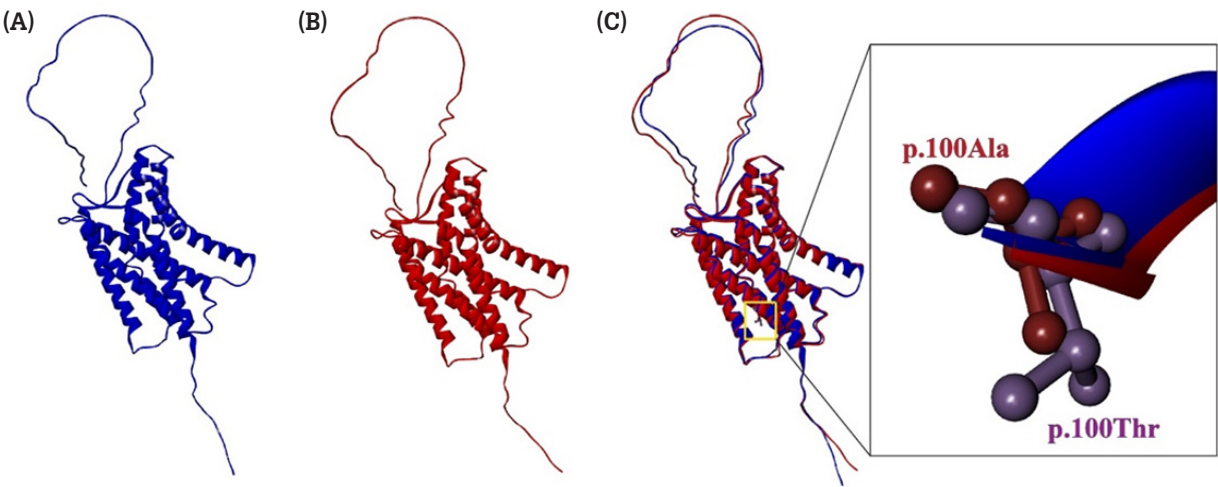


Figure 5 Molecular docking analysis of the wild-type Fy^b antigen and the c.298G>A variant **(A)** The overall structure of the wild-type Fy^b antigen **(B)** The overall structure of the c.298G>A variant **(C)** The comparative structure of wild-type and the c.298G>A variant. The wild-type is shown in blue and the mutant is indicated in red. The specific region of the inset indicates the superimposed structure between Ala100 and Thr100.

Fy(a+b+), and Fy(a-b+), consecutively. Not surprisingly, the Fy(a-b-) phenotype, which derived from FY^*A^{ES}/A^{ES} or FY^*B^{ES}/B^{ES} genotypes and is uncommon in all populations except African and African-American populations⁴, was not observed in this study. However, we found a heterozygous FY^*A/B^{ES} genotype in one donor from the Southern part of Thailand. This finding inevitably suggests that the FY^*B^{ES}/B^{ES} genotype might be discovered in a larger sample size.

Although a serological test is helpful and frequently employed, it nevertheless shows certain drawbacks. Molecular techniques, particularly PCR-SSP, can be used to overcome these limitations. In this study, PCR-SSP testing was used to identify FY^*A , FY^*B , FY^*A^{ES} , and FY^*B^{ES} alleles. Samples with FY^*A/B , FY^*B/B genotypes, and likewise one sample of the FY^*A/B^{ES} genotype were additionally confirmed by DNA sequencing. The SNP at the c.125G>A position¹⁻⁵ indicated the presence of the Fy^a and Fy^b antigens. We found that the result from PCR-SSP was consistent with that of the DNA sequencing results, suggesting the accuracy and reliability of the PCR-SSP approach.

One donor with the FY^*A/B^{ES} genotype confirmed by DNA sequencing also exhibited a heterozygous mutation (c.-67TC) at the GATA-1 region. This individual was predicted to have the Fy(a+b-) phenotype as the FY^*B^{ES} allele results in the absence of Fy^b antigen expression on RBCs, although it may still be expressed on other cell types. A patient with the FY^*A/B^{ES} genotype can safely receive blood from donors with Fy(a+b-), Fy(a+b+), or Fy(a-b+) phenotypes. Related reports showed that patients with sickle cell disease, typically having the FY^*B^{ES}/B^{ES} genotype and the Fy(a-b-) phenotype, can receive Fy(a-b+) blood without developing anti-Fy^b alloantibodies. However, no reports are available of Thai donors or patients with the FY^*B^{ES}/B^{ES} genotypes, indicating a need for further studies in larger populations.

To identify the FY^*X allele, three SNPs including c.145G>T, c.265C>T, and c.298G>A¹⁻⁶, were analyzed using DNA sequencing. Among 115 samples of FY^*A/B

and FY^*B/B genotypes, all were c.145GG and c.265CC, suggesting that both $FY^*02W.02$ and $FY^*02W.01$ alleles were not observed in this study, as these alleles were primarily found in Caucasians and are rare in other populations.^{4,6} These results suggested that no Fy^x antigen was observed in this study. However, we found only a mutation at c.298G>A of both c.298GA and c.298AA; this SNP did not affect the conformational change of the 3D-computerized structure of Fy^b antigen compared to that of wild-type. These data support the finding that only the c.298G>A mutation does not affect the Fy^b antigen expression.^{5,29}

The allele frequencies of the FY blood group system in the Southern Thai population were compared to those of other studied groups. The allele frequencies significantly differed from those in the Central and Northern Thai, Han Chinese, Taiwanese, Korean, Brazilian Japanese, Filipino, Indian and Northern Pakistani populations ($p < 0.05$)^{16,17,22-25,27,28}, but their frequencies were similar to those of the Northeastern Thai and Malaysian populations ($p > 0.05$).^{18,26} Additionally, related studies revealed that the allele frequencies of the Kidd and Diego blood groups from the Southern Thai donors differed considerably from those in other regions ($p < 0.05$) but were comparable with those of donors from the Northeastern region.^{13,14} Given that most people in Southern Thailand are originally from Malaysia, this could be explained by the possibility that their allele frequencies are similar to Malaysians.

In this study, we also calculated the risk of alloimmunization using FY^*A and FY^*B allele frequencies to predict phenotypes among Southern Thai blood donors. The possibility of anti-Fy^a and anti-Fy^b production following alloimmunization was 0.021 and 0.196, respectively. The risk of alloantibody production against both Fy^a and Fy^b antigens was relatively similar to those reported in Northeastern Thai populations ($p > 0.05$).¹⁸ Surprisingly, this risk of alloimmunization among Southern Thais significantly differed from the Central and Northern Thai populations ($p < 0.05$).^{16,17} Therefore, genotyping the

Duffy blood group alleles is a valuable alternative method for anticipating phenotypes, which later contributes to blood safety management for patients.

Conclusion

In summary, this study examined the *FY**A, *FY**B, *FY**A^{ES}, and *FY**B^{ES} alleles of the Duffy blood group system among 427 Southern Thai blood donors using the PCR-SSP technique. The findings demonstrated that all alleles were correctly identified and that all samples' phenotypes could be predicted. The PCR-SSP results were consistent with the DNA sequencing results, suggesting the accuracy and dependability of the in-house procedure. DNA sequencing was used to further examine samples with the *FY**A/B and *FY**B/B genotypes to determine the existence of the *FY**X allele. After considering SNPs linked to decreased expression of the Fy^b antigen, the *FY**X allele was not found in any of the 115 samples that were analyzed. Furthermore, compared with groups in Central and Northern Thailand, the Southern Thai population was shown to have a higher risk of alloimmunization. Accordingly, the results of this study can be applied and used to support the selection of compatible blood donors for transfusion in clinical settings.

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