RESEARCH ARTICLE

HNA-1, -3, -4, and -5 genotyping using multiplex PCR among southern Thais: Developing continual HNA-1 null detection

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Background: Antibodies against human neutrophil antigens (HNAs) are involved in various clinical conditions including transfusion-related acute lung injury and auto/alloimmune neutropenia. We aimed to determine HNA-1, -3, -4, and -5 frequencies among southern Thais using multiplex PCR and to develop HNA-1 null detection.

Methods: Samples obtained from 427 southern Thai blood donors were genotyped HNA-1, -3, -4, and -5 using multiplex PCR and compared their allele frequencies with those previously reported in Thai populations. HNA-1 null samples were tested by newly developed PCR-SSP and PCR-RFLP and confirmed by DNA sequencing.

Results: The frequencies of *FCGR3B**01, *FCGR3B**02, *FCGR3B**03, *SLC44A2**01, *SLC44A2**02, *ITGAL**01, and *ITGAL**02 among southern Thais differed from other Thai populations, except *ITGAM**01 and *ITGAM**02 frequencies. Two samples without specific fragments of *FCGR3B**01, *FCGR3B**02, and *FCGR3B**03 tested by multiplex PCR were confirmed by PCR-RFLP to identify *FCGR3B* deficiency (HNA-1 null). Moreover, to reduce test steps, the newly developed PCR-SSP for *FCGR3B* deficiency was validated and tested in all samples and the results were in agreement with DNA sequencing.

Conclusions: This was the first study to report HNA-1, -3, -4, and -5 frequencies among southern Thais. The indeterminate results of multiplex PCR for HNA-1 genotyping led to establish HNA-1 null detection using PCR-SSP, which is simple, convenient and cost-effective and can be used to identify *FCGR3B* deficiency.

KEYWORDS

allele frequencies, HNA-1 null, human neutrophil antigens, multiplex PCR, southern Thais

1 | INTRODUCTION

Human neutrophil antigens (HNAs) have been categorized in 5 systems: HNA-1, -2, -3, -4, and -5 comprising different glycoproteins encoded by *FCGR3B*, *CD177*, *SLC44A2*, *ITGAM*, and *ITGAL* genes, respectively.^{1,2} The epitopes of HNA-1a, -1b, -1c, -1d, -3a, -3b, -4a, -4b, -5a, and -5b contribute single nucleotide polymorphisms (SNPs) of those genes that give rise to different alleles.² However, the HNA-2 and -2 null samples have not yet been examined for their molecular mechanisms.² HNA antibodies have been involved in causing various clinical conditions of neonatal allo/autoimmune neutropenia,

transfusion-related acute lung injury (TRALI), febrile transfusion reaction, immune neutropenia after bone marrow transplantation, refractoriness to granulocyte transfusion, and drug-induced immune neutropenia.^{2,3}

Several molecular typing methods such as PCR with sequence-specific primers (PCR-SSP) have been implemented for individual HNA-1, -3, -4, and -5 genotyping among different populations in Thailand including central, northern, and northeastern Thais.⁴⁻⁸ According to related studies of HNA genotype frequencies among central and northern Thais, their frequencies were similar to HNA-4 and -5 and differed from HNA-1 and -3.⁵⁻⁷ Moreover, only HNA-4

genotype frequencies among northeastern Thais were similar to central Thais.⁸ To determine more convenient HNA genotyping, multiplex PCR has been applied to reduce costs and time-consuming tasks by performing the simultaneous detection of HNA-1, -3, -4, and -5.⁹ The data of HNA among southern Thais remain unknown.

Regarding the HNA-1 system, only one HNA-1 null was found among northeastern Thais.⁸ The HNA-1 null phenotype occurs among individuals with homozygous chromosomal deletion involving the *FCGR3B* gene deficiency.^{10,11} Several cases of neonatal neutropenia caused by HNA-1 antibodies of *FCGR3B*-deficient mothers were reported in different populations.^{10,12,13} Regarding related studies, genotyping of the HNA-1 null individuals using PCR-SSP and/or multiplex PCR showed no bands of *FCGR3B**01, *FCGR3B**02, and *FCGR3B**03 allele detections.^{8,9} The PCR-restriction fragment length polymorphism (RFLP) is suggested to be used to confirm *FCGR3B*-deficient individuals.^{10,11} This study aimed to determine HNA-1, -3, -4, and -5 frequencies among southern Thais using multiplex PCR and to develop HNA-1 null detection.

2 | MATERIALS AND METHODS

2.1 | Study population

Peripheral venous blood was collected in EDTA-anticoagulated blood from 427 unrelated healthy blood donors from Regional Blood Centre 12th Songkhla, Thai Red Cross Society, Songkhla, Thailand. Informed consent was obtained from each subject. This study was approved by the Committee on Human Rights Related to Research Involving Human Subjects, Thammasat University, Pathumtani, Thailand (COE No. 081/2560).

2.2 | Laboratory testing

2.2.1 | Genomic DNA extraction and HNA-1, -3, -4, and -5 genotyping

Genomic DNA was extracted from all samples using a Genomic DNA extraction kit (REAL Genomics, RBC Bioscience, Taipei, Taiwan) and was then stored at -20°C until used for genotyping. In addition, known DNA samples of HNA-1, -3, -4, and -5 phenotypes, provided by Dr. Núria Nogués, Laboratori d'Immunohematologia, Banc de Sang i Teixits, Passeig Taulat, Barcelona, Spain, confirmed by DNA sequencing were used as controls.

HNA-1, -3, -4, and -5 genotyping, using multiplex PCR, was performed as previously described.⁹ Briefly, the multiplex PCR for HNA-1a, -1b, -1c, -3a, -3b, -4a, -4b, -5a, and -5b genotyping contained four multiple reaction sets (A to D) of each different amplification target per mix. Each multiplex set contained two to three HNA-specific primer pairs, and one human growth hormone (HGH)-specific primer pair served as the internal control. PCR reaction mixtures consisted of 10 µL of the 2× PCR reaction

mixture (OnePCR Plus, GeneDirex, New Taipei City, Taiwan), 1 µL (50-150 ng) of genomic DNA, 1 µL of each HNA-specific primer, 1 µL of HGH primers, and 1 to 3 µL of PCR grade water in a final volume of 20 µL.

PCR amplification of four multiplex sets was performed in a T100 Thermal cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The cycling parameters for the PCR program were as follows: 1 cycle of 5 minutes at 95°C; 30 cycles each of 30 seconds at 95°C, 60 seconds at 57°C, and 30 seconds at 72°C; and final extension for 5 minutes at 72°C. The product was then kept at 4°C until used for analysis. PCR products were electrophoresed at 100 volts with a 1.5% agarose gel using 1× tris-borate-ethylenediaminetetraacetic acid (TBE) buffer. Products were visualized under a blue-light transilluminator. All DNA samples of southern Thai blood donors were tested, and the known DNA controls were run through the entire test along with the unknown samples.

HNA-1, -3, -4, and -5 genotyping results by multiplex PCR were demonstrated as follows: multiplex set A identified HNA-1a and -5a with the PCR product size of 141 and 297 bp, while multiplex set B identified HNA-1b and HNA-5b with the PCR product size of 219 and 297 bp. Additionally, multiplex set C identified HNA-4a, -1c, and -3a with the PCR product size of 124, 191, and 289 bp, whereas multiplex set D identified HNA-4b and HNA-3b with the PCR product size of 124 and 289 bp. In each multiplex set, the HGH internal control gave an expected band of 434 bp, showing that amplification had occurred optimally.

2.2.2 | Determination of HNA-1 null by PCR-RFLP

For HNA-1 null determination, the *FCGR3B* gene deletion was identified using PCR-RFLP, as previously described with some modifications.¹⁰ The amplified a 507-bp fragment contained part of 3' untranslated and transmembrane regions of *FCGR3A* and *FCGR3B* genes. For the PCR reaction, 2 µL of genomic DNA (50 ng/µL) was amplified in a total volume of 50 µL using 1 µL of 10 µmol/L forward primers (5'-TTTCCCCTCTAAACTGGG-3') and 1 µL of 10 µmol/L reverse primer (5'-GTCTCTTCTGCTTGATGG-3'). PCR was performed with 25 µL of 2× PCR reaction mixture (Phusion High-Fidelity PCR Master Mix, New England BioLabs, MA, USA) and 21 µL of sterile distilled water in a T100 Thermal cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

PCR was performed under the following conditions: 95°C for 5 minutes, 30 cycles of 95°C for 30 seconds, 60°C for 40 seconds, and 72°C for 30 seconds, and the last step involved a final extension 5 minutes at 72°C. Afterward, 15 µL of amplified PCR products was digested with 5U TaqI restriction endonuclease (Thermo Fisher Scientific Inc., MD, USA) in a total volume of 25 µL at 65°C for 2 hours. In addition, the digested mixtures were resolved in 14% polyacrylamide gel containing 0.5% glycerol. The electrophoresis was run in 1× TBE by applying 20 mA electric power for each slab gel and then stained using ethidium bromide and visualized with a UV transilluminator.

2.2.3 | PCR-SSP development for HNA-1 null detection

The HNA-1 null was detected using an in-house PCR-SSP technique. Individual tests to identify *FCGR3A* and *FCGR3B* genes included two sets of PCR reaction mixture. Briefly, 1 μ L of genomic DNA (50 ng/ μ L) was amplified in a total volume of 10 μ L using 1 μ L of 10 μ mol/L HNA-1-null forward primer (5'-ATCATGGGCTTTCCCTTCCA-3') and 1 μ L of 10 μ mol/L *FCGR3A*-C reverse primer (5'-CTATATTCTCTGTGAAGACAAACATTC-3') for *FCGR3A* detection (set A) and 1 μ L of 10 μ mol/L HNA-1-null forward primer and 1 μ L of 10 μ mol/L *FCGR3B*-T reverse primer (5'-CTATATTCTCTGTGAA GACAAACATTT-3') for *FCGR3B* detection (Set B). The *HGH* gene was co-amplified using 1 μ L of 10 μ mol/L *HGH*-F forward primer

(5'-TGCCTTCCCAACCATTCCTTA-3') and 1 μ L of 10 μ mol/L *HGH*-R reverse primer (5'-CCACTCACGGATTCTGTTGTGTT TC-3') and then run as the internal control in both sets. PCR was performed with 5 μ L of 2 $\times$ PCR reaction mixture (OnePCR Plus, GeneDirex, Taiwan) in a T100 Thermal cycler.

PCR was performed under the conditions described below, that is, 95°C for 5 minutes. The cycle parameters of the PCR program began with the first step of 10 cycles of 10 seconds at 95°C and 60 seconds at 69°C and then 20 cycles of 10 seconds at 95°C, 50 seconds at 61°C, and 30 seconds at 72°C. The last step was final extension 5 minutes at 72°C. Thereafter, PCR products were electrophoresed at 100 volts with 1.5% agarose gel using 1 $\times$ TBE buffer and visualized under a blue-light transilluminator.

TABLE 1 Comparing HNA genotype and allele frequencies among Thai populations

Frequencies	Epitope	Southern ^a (n = 427)	Central ^a (n = 500)	Northern ^a (n = 300)	Northeastern ^b (n = 400)
Genotype frequencies					
<i>FCGR3B</i> *01/ <i>FCGR3B</i> *01	HNA-1a1a	0.415	0.224	0.467	0.518
<i>FCGR3B</i> *01/ <i>FCGR3B</i> *02	HNA-1a1b	0.403	0.642	0.420	0.358
<i>FCGR3B</i> *02/ <i>FCGR3B</i> *02	HNA-1b1b	0.155	0.126	0.113	0.122
<i>FCGR3B</i> *02/ <i>FCGR3B</i> *03	HNA-1b1c	0.014	0.002	0.000	0.000
<i>FCGR3B</i> *01/ <i>FCGR3B</i> *02/ <i>FCGR3B</i> *03	HNA-1a1b1c	0.009	0.006	0.000	0.000
<i>FCGR3B</i> *null (<i>FCGR3B</i> gene deletion)	HNA-1null	0.005	0.000	0.000	0.002
<i>SLC44A2</i> *01/ <i>SLC44A2</i> *01	HNA-3a3a	0.616	0.528	0.600	0.623
<i>SLC44A2</i> *01/ <i>SLC44A2</i> *02	HNA-3a3b	0.384	0.380	0.349	0.325
<i>SLC44A2</i> *02/ <i>SLC44A2</i> *02	HNA-3b3b	0.000	0.092	0.051	0.052
<i>ITGAM</i> *01/ <i>ITGAM</i> *01	HNA-4a4a	0.946	0.950	0.930	0.945
<i>ITGAM</i> *01/ <i>ITGAM</i> *02	HNA-4a4b	0.054	0.050	0.070	0.055
<i>ITGAM</i> *02/ <i>ITGAM</i> *02	HNA-4b4b	0.000	0.000	0.000	0.000
<i>ITGAL</i> *01/ <i>ITGAL</i> *01	HNA-5a5a	0.431	0.642	0.560	0.470
<i>ITGAL</i> *01/ <i>ITGAL</i> *02	HNA-5a5b	0.450	0.258	0.377	0.412
<i>ITGAL</i> *02/ <i>ITGAL</i> *02	HNA-5b5b	0.119	0.100	0.063	0.118
Allele frequencies					
<i>FCGR3B</i> *01	HNA-1a	0.619	0.548*	0.677	0.696*
<i>FCGR3B</i> *02	HNA-1b	0.365	0.452*	0.323	0.301*
<i>FCGR3B</i> *03	HNA-1c	0.012	0.004	0.000	0.000
<i>FCGR3B</i> *null	HNA-1null	0.005	0.000	0.000	0.002
<i>SLC44A2</i> *01	HNA-3a	0.808	0.718**	0.775	0.785
<i>SLC44A2</i> *02	HNA-3b	0.192	0.282**	0.225	0.215
<i>ITGAM</i> *01	HNA-4a	0.973	0.975	0.965	0.972
<i>ITGAM</i> *02	HNA-4b	0.027	0.025	0.035	0.028
<i>ITGAL</i> *01	HNA-5a	0.656	0.771**	0.748**	0.676
<i>ITGAL</i> *02	HNA-5b	0.344	0.229**	0.252**	0.324

^aDetermined by multiplex PCR.

^bDetermined by PCR-SSP.

*P < 0.01.

**P < 0.001.

2.2.4 | DNA sequencing

Twenty selected DNA samples of HNA-1a, -1b, -1c, and HNA-1 null tested by PCR-SSP were sequenced to confirm the HNA-1 null. A fragment of 507 bp was obtained from PCR amplification of both *FCGR3A* and *FCGR3B* target genes, containing SNP, rs#374752953 (NC_000001.11:g.161624517A>G). The PCR amplification for DNA sequencing was identical to HNA-1 null determination by PCR-RFLP. Thereafter, the PCR products were purified using a gel extraction kit (AccuPrep Gel Purification, Bioneer, Inc., Daejeon, Korea), and eluted fragments were then sequenced using those PCR primers.

2.3 | Statistical analysis

Gene and allele frequencies were calculated using the gene-counting method. The chi-square test of homogeneity was used to determine differences between southern Thais and those reported in other Thai populations.⁵⁻⁸ A *P* value of equal or less than 0.05 was considered significant.

3 | RESULTS

3.1 | HNA-1, -3, -4, and -5 genotyping among southern Thais

A total of 427 samples of southern Thai blood donors were simultaneously typed for *FCGR3B**01, *FCGR3B**02, *FCGR3B**03, *SLC44A2**01, *SLC44A2**02, *ITGAM**01, *ITGAM**02, *ITGAL**01, and *ITGAL**02 using the multiplex PCR technique (Table 1). The HNA-1 system, *FCGR3B**01/*FCGR3B**01, was the most frequent (0.415, 177/427), followed by *FCGR3B**01/*FCGR3B**02 (0.403, 172/427), *FCGR3B**02/*FCGR3B**02 (0.155, 66/427), *FCGR3B**02/*FCGR3B**03

(0.014, 6/427), and *FCGR3B**01/*FCGR3B**02/*FCGR3B**03 (0.009, 4/427). Notably, no amplified products of 141, 219, and 191 bps for *FCGR3B**01, *FCGR3B**02, and *FCGR3B**03 detections were found in two donor samples (SM237 and SM402), as shown in Figure 1. In addition, the genotype frequencies of *SLC44A2*, *ITGAM*, and *ITGAL* of HNA-3, -4, and -5 systems are shown in Table 1.

3.2 | Comparison of HNA genotype and allele frequencies in Thai populations

The distribution of HNA-1, -3, -4, and -5 genotypes based on the allele frequencies was compared with those reported in other Thai populations (Table 1).⁵⁻⁸

Of the three allele frequencies, that is, *FCGR3B**01, *FCGR3B**02, and *FCGR3B**03 among 427 southern Thais, the *FCGR3B**01 was the most frequent, found at 0.619, followed by *FCGR3B**02 at 0.365, with *FCGR3B**03 being the least frequent at 0.012 and *FCGR3B**null was found at 0.005. The frequencies of *FCGR3B**01 and *FCGR3B**02 were similar to northern Thais but significantly differed (*P* < 0.01) from those among central and northeastern Thais.

The allele frequencies of *SLC44A2**01 and *SLC44A2**02 were found at 0.808 and 0.192 in southern Thais, respectively, similar to those of northern and northeastern Thais. On the contrary, their frequencies significantly differed from central Thais (*P* < 0.001). Additionally, *ITGAM**01 and *ITGAM**02 were found at 0.973 and 0.027 among southern Thais, respectively, consistent with other Thai populations.

The allele frequencies of *ITGAL**01 and *ITGAL**02 were found at 0.656 and 0.344 among southern Thais, respectively, and the frequency of *ITGAL**01 was significantly lower than northern and central Thais (*P* < 0.001).

FIGURE 1 A representative agarose gel electrophoresis profile to detect HNA-1, -3, -4, and -5 genotyping using multiplex PCR. The genotype was deduced from the presence or absence of amplification products specific for HNA alleles. Multiplex set A identified HNA-1a and -5a with a PCR product size of 141 and 297 bp, while multiplex set B identified HNA-1b and HNA-5b with a PCR product size of 219 and 297 bp. Additionally, multiplex set C identified HNA-4a, 1c, and -3a with a PCR product size of 124, 191, and 289 bp, whereas multiplex set D identified HNA-4b and HNA-3b with a PCR product size of 124 and 289 bp. In each multiplex set, the HGH internal control gave an expected band of 434 bp. Lane M: 100-bp DNA ladder marker (GeneRuler 100 bp, Fermentas, Carlsbad, CA, USA), lanes 1A to 1D, sample SM025: HNA-1a1b1c, -3a3b, -4a4a, and -5a5a, Lanes 2A to 2D, sample SM237: HNA-1 null, -3a3a, -4a4a, and -5a5b

3.3 | HNA-1 null determination and confirmation

In two samples of the HNA-1 null (SM237 and SM402), the multiplex PCR assay of the *FCGR3B* gene did not detect the specific fragments of *FCGR3B**01, *FCGR3B**02, and *FCGR3B**03. We also performed a PCR-RFLP assay to investigate the presence or absence of the *FCGR3B* gene among these donors. A 507-bp PCR amplification of both *FCGR3A* and *FCGR3B* genes was then subjected to digestion with TaqI. For *FCGR3A* gene products, the digested fragments contained 76, 206, and 225 bps, while digested fragments of *FCGR3B* contained 225 and 282 bps. Hence, the PCR-RFLP of the *FCGR3B* gene in these two samples did not detect the 282-bp fragment after TaqI digestion (Figure 2). This suggested that the *FCGR3B* gene might have been deleted in both donors. Moreover, the validity testing of multiplex PCR and PCR-RFLP was performed using 20 randomly selected DNA samples including 2 HNA-1 null samples, and the results were in agreement with DNA sequencing.

3.4 | Detection of the HNA-1 null by PCR-SSP

In this study, we developed an in-house PCR-SSP for *FCGR3B* gene deletion of the HNA-1 null. Two primer sets were used to determine the *FCGR3A* and *FCGR3B* genes. The first set identified *FCGR3A* while the second set identified *FCGR3B* with the PCR product size of 275 bp. In each set, the HGH internal control gave an expected band of 434 bp (Figure 3). The validity of *FCGR3A* and *FCGR3B* gene detections using the in-house PCR-SSP was determined using known *FCGR3A* and *FCGR3B* genes, and *FCGR3B* gene deletion and the results were in agreement. Additionally, this PCR-SSP technique was applied among 427 southern Thais, and the results were consistent with the results obtained from multiplex PCR and PCR-RFLP.

4 | DISCUSSION

Antibodies specific for HNA-1, -2, and -3 can induce TRALI after blood transfusions. Moreover, the passive transferring antibodies of HNA-1 to -5, caused by maternal alloimmunization, are implicated in neonatal neutropenia.^{1,14} Hence, HNA genotyping is used not only to confirm whether the patients produced the corresponding HNA antibodies with deliberate immunization but also to screen appropriate antigen-negative blood.

This study was the first to report frequencies of HNA-1, -3, -4, and -5 among southern Thai blood donors. They were Thai-Muslims living in the three southern border provinces of Pattani, Yala, and Narathiwat that have their own specific religion, culture, and lifestyle. Other genetic markers including HLA and Diego blood group alleles among southern Thais demonstrated significant differences from central, northeastern, and northern Thais.^{15,16} For the HNA-4 and -5 systems, *ITGAM**01 and *ITGAM**02 frequencies among southern Thais were similar to other Thai populations. Homozygous individuals *ITGAM**02 were not observed in this study. Homozygous mothers *ITGAM**02 could develop anti-HNA-4a, rarely found among Thais, and could cause neonatal alloimmune neutropenia. Additionally, a lower prevalence of *ITGAL**01 frequencies among southern Thais compared with northern and central Thais leads to decreased alloimmunization risk of anti-HNA-5a production.

Alloantibodies against HNA-3a are often implicated in severe and fatal TRALI.^{14,17} A related study reported that a high risk of HNA-3a incompatibility and alloimmunization among central Thais was demonstrated.⁶ In this study, *SLC44A2**01 and *SLC44A2**02 allele frequencies significantly differed from central Thais but were consistent with northern and northeastern Thais. Importantly, *SLC44A2**02/*SLC44A2**02 was not observed and may imply scarcely occurring TRALI cases due to anti-HNA-3a among southern Thais. Further studies should require a large sample size to confirm this indication.

FIGURE 2 A representative acrylamide gel electrophoresis profile for PCR-restricted fragment patterns of *FCGR3A* and *FCGR3B* obtained using the TaqI restriction enzyme. The *FCGR3A* gene was deduced from the presence of 206- and 76-bp fragments, and the *FCGR3B* gene was deduced from the presence of a 282-bp fragment. Lane M: DNA ladder marker (Low Molecular Weight DNA Ladder, New England BioLabs, MA, USA), Lane U: the undigested 507-bp PCR products, lanes 1 to 4, samples SM026, SM064, CTR01, and CTR02: the digested PCR products amplified from samples (HNA-1a1a and HNA-1a1b) and controls (HNA-1a1b1c and HNA-1b1b), lanes 5 to 8, samples SM237, SM402, CTR03, and CTR04: the digested PCR products amplified from *FCGR3B* gene deficiency (HNA-1 null) samples and controls

FIGURE 3 A representative agarose gel electrophoresis profile for the *FCGR3B* gene deletion of HNA-1 null by PCR-SSP. The gene was deduced from the presence or absence of amplification products specific for *FCGR3A* (A) and *FCGR3B* (B) genes. Lane M: 100-bp DNA ladder marker (GeneRuler 100 bp, Fermentas, Carlsbad, CA, USA), lanes 1A-1B, sample SM026: HNA-1a1a, lanes 2A-2B, sample SM064: HNA-1a1b, lanes 3A-3B, control CTR01: HNA-1a1b1c, lanes 4A-4B, sample SM237: HNA-1 null, lanes 5A-5B, sample SM402: HNA-1 null and lanes 6A-6B: no-template controls

HNA-1 allele frequencies have been described in Thai populations.^{5,8} The frequencies of *FCGR3B**01 and *FCGR3B**02 among southern Thais were consistent with those of northern Thais but significantly differed from northeastern and central Thais. A high frequency of *FCGR3B**01 among southern Thais is more prone to idiopathic pulmonary fibrosis compared with two other Thai groups.¹⁴ The *FCGR3B**03 is a rare allele in Asian populations,¹⁸ however, we found a higher *FCGR3B**03 frequency compared with central Thais (0.012 vs 0.004). Notably, we identified two samples with *FCGR3B* deficiency, which multiplex PCR did not detect the *FCGR3B**01 or *FCGR3B**02 and *FCGR3B**03-specific fragments in MIX A, B, and C, respectively. To ensure the results, PCR-RFLP of exon 5 of the *FCGR3B* gene was performed, similar to a related study.¹⁰ The PCR-RFLP results confirmed our findings by multiplex PCR, but involved test limitations due to the difficulty in distinguishing between 206- and 225-bp fragments after *TaqI* digestion. It took a long time from start to complete analysis and was considered unsuitable for mass screening. To overcome the PCR-RFLP limitations, we developed an in-house PCR-SSP technique to identify *FCGR3A* and *FCGR3B* gene fragments from the restriction site of *TaqI* (rs#374752953). After implementing this technique among 427 subjects from southern Thais, the results were in agreement with multiplex PCR and PCR-RFLP. In addition, validity testing among 20 randomly selected subjects including 2 subjects with *FCGR3B* deficiency (HNA-1 null) was consistent with DNA sequencing. Antibodies against HNA-1 can be involved in neutropenia and TRALI. A related study revealed that individuals with *FCGR3B* deficiency were strongly influenced in systemic autoimmunity susceptibility.^{14,19} Therefore, screening for *FCGR3B* deficiency using PCR-SSP may be helpful in identifying patients with either HNA-1 alloimmunization or a risk of systemic sclerosis.

In conclusion, HNA-1, -3, -4, and -5 frequencies were firstly reported among southern Thais. Only HNA-4 frequencies were similar to those of other Thai populations. The indeterminate results

of multiplex PCR for HNA-1 genotyping led to establishing HNA-1 null detection by PCR-SSP, which is simple, convenient and cost-effective and can be used to identify *FCGR3B* deficiency.

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