FREQUENCIES OF PREDICTED MIA ANTIGEN AMONG SOUTHERN THAI BLOOD DONORS

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Abstract

**Background:** The Mi^a^ antigen (MNS7) of the MNS blood group system is clinically important in Asian populations. Anti-Mi^a^ has been implicated in hemolytic transfusion reactions and hemolytic disease of the fetus and newborn in Thai populations. However, data of this antigen frequency among southern Thais remains unknown.

**Objective:** This study aimed to determine and predict Mi^a^ antigen frequencies among southern Thai blood donors and to estimate the risk of alloimmunization among Thais.

**Methods:** A cross-sectional study was conducted. Altogether, 400 southern and 500 central Thai blood samples were genotyped for GYP(B-A-B) and GYP(A-B-A) MNS hybrids using polymerase chain reaction with sequence-specific primer (PCR-SSP).

**Results:** Among them, 19 of 400 (4.45%), and 28 of 500 (9.33%) were positive with the set of GP.Hut, GP.HF, GP.Mur, GP.Hop, and GP.Bun. No GP.Vw phenotype was found among southern and central Thais. The predicted Mi(a+) frequency among southern Thais was significantly lower than among central and northern Thais (p<0.05). Its frequency was similar to Vietnamese, Taiwanese, and Southern Han Chinese populations (p>0.05) but significantly differed from Indonesian, Filipino, and Chinese (Guangzhou) populations (p<0.05). The risk of Mi^a^ alloimmunization among southern Thais was significantly lower than among both Thai groups (p<0.05).

**Conclusion:** This constitutes the first study to report Mi(a+) frequencies among southern Thais, supporting the estimation risk of alloimmunization and providing transfusion safety among Thai populations.

**Keywords:** Genotyping, MNS blood group system, Predicted Mi(a+) frequency, Southern Thais
Introduction

The Miltenberger (Mi) subsystem was formally classified by Cleghorn in 1966. The antigens carried on glycophorin A (GPA), and GPB and encoded by the glycophorin genes consisted of GYP(A), GYP(B), and GYP(E) genes of the MNS blood group system. The formation of GYP(A-B-A) and GYP(B-A-B) hybrid genes encode various GP hybrid molecules expressed on the red cell membranes, and these hybrid GPs display an implicit phenotype profile of Mi antigens currently established as MNS7. Mi is an antigen illustrated in 7 hybrid GPs consisting of GP.Vw, GP.Hut, GP.HF, GP.Mur, GP.Hop, GP.Bun, and GP.Kip. These antigen frequencies are rare in Caucasians (0.012%), African Americans (0%) and Indian (0.2%) populations; however, higher frequencies are reported in Thai (9.7%) and Chinese (7.3%) populations. Anti-Mi has been implicated in hemolytic transfusion reactions (HTRs), hemolytic disease of the fetus and newborn (HDFN), and a case of hydrops fetalis. Hence, including Mi(a+) cells in reagent red cells would noteworthy for antibody detection in Asian populations. Southern Thai Muslim populations are concentrated mainly in three provinces: Pattani, Yala and Narathiwat near the border with Malaysia, and have different ethnic origins compared with other regions of Thailand. Concerning a related study, the frequency of Mi antigen varies among Malaysian blood donors in three ethnic groups consisting of Malay (2.08%), Chinese (4.9%), and Indian (3.0%). The prevalence of this antigen may involve alloantibody formation among patients regularly transfused in different ethnic groups. To date, the data of Mi antigen frequency among southern Thai blood donors remains unknown. This study aimed to determine and predict Mi antigen frequency among southern Thai blood donors to estimate the risk of alloimmunization in Thai populations.

Methods

This study was approved by the Committee on Human Rights Related to Research Involving Human Subjects, Thammasat University, Pathumtani, Thailand COE No. 013/2564). Informed consent was signed by all study participants. The study included EDTA-anticoagulated blood from 900 unrelated healthy Thai blood donors. In all, 400 and 500 samples were obtained from the Regional Blood Centre 12th Songkhla, Thai Red Cross Society, Songkhla, and the National Blood Centre, Thai Red Cross Society (NBC-TRC), Bangkok, Thailand. All 400 samples were from Thai-Muslim donors living in the three southern border provinces of Pattani, Yala and Narathiwat.

Genomic DNA was extracted from peripheral blood samples using the DNeasy Blood & Tissue Kit according to manufacturer instructions (QIAGEN GmbH, Valencia, CA, USA), then stored at -20°C until used for genotyping. A PCR-SSP technique to detect two MNS hybrid GPs; GYP(B-A-B) and GYP(A-B-A) were performed using two sets of primers, according to a related study. Control DNA of known Mi(a+) positive phenotypes was from our in house collections and GP.Vw DNA controls were provided by Dr. Genghis H. Lopez, Research and Development Laboratory, Clinical Services and Research Division, Australian Red Cross Blood Service, Brisbane, Australia. In the PCR, 1 µL of genomic DNA (50 ng/µL) was amplified using 0.5 µL of 10 µM F2 primer (5’-CCCTTCTCAACTTCTCTTATATGC AGATAA-3’) and 0.5 µL of 10 µM Rccgg primer (5’-GAGCAACTATTTTTAAACTAGAACA TACCCG-3’) for the first set of GYP(B-A-B) detection. For the second set of GYP(A-B-A) detection, 0.5 µL of 10 µM F1 primer (5’-CAGCATTTCTCTAAAGGCCTAACCAAGAGATG TA-3’) and 0.5 µL of 10 µM RIN primer (5’-CA TATGTGTCCCCGTGGTGCACA-3’). Moreover, 0.5 µL of 6 µ MHGH-434-F primer (5’-TGCTTCTCAACTTCTCTTATATGC AGATAA-3’) and 0.5 µL, 6 µM of HGH-434-R primer (5’-CACCTCCTCTCTAACCAAGAGATGTA-3’) were included in both sets of GYP(B-A-B) and GYP(A-B-A) detections. PCR of each MNS hybrid GP detection was performed using 5 µL of 2X PCR reaction mixture (Green Hot Start PCR Master Mix, Biotechrabbit GmbH, Hennigsdorf, Germany), and 2 µL of distilled water added to a final volume of 10 µL.

PCR amplification of two sets was performed in a T100 Thermal cycler (Bio-Rad Laboratories, Inc., USA). For the first set of GYP(B-A-B)
detection, the cycling parameters for the PCR program consisted of 1 cycle at 95°C for 5 min, followed by 30 cycles at 95°C for 30 sec, 62°C for 40 sec 72°C for 30 sec, with a final extension at 72°C for 5 min. For the second set of GYP(A-B-A) detection, the cycling parameters for the PCR program consisted of 10 cycles at 95°C for 30 sec, followed by 10 cycles at 95°C for 10 sec, 64°C for 1 min, 30 cycles at 95°C for 10 sec, 61°C for 50 sec, 72°C for 30 sec, with a final extension at 72°C for 5 min. PCR products were electrophoresed at 100 V with a 1.5% agarose gel containing 10,000X fluorescent DNA gel stain (SYBR Safe DNA gel stain) using 1X TBE buffer. Products were visualized under a blue-light transilluminator.

Altogether, 900 samples of Thai blood donors including 400 and 500 from southern and central Thais were employed for GYP (A-B-A) and GYP (B-A-B) hybrid detections using PCR-SSP.

**Statistical analysis**

The frequencies of predicted Mi(a+) were calculated using the gene counting method. The differences in Mi(a+) frequencies between southern Thai and other populations were compared using a chi-square test of homogeneity. In addition, the risk estimation of Mi\(^\text{a}\) alloimmunization was obtained by multiplying the probability of having a predicted Mi(a-) phenotype frequency by the probability of having a predicted Mi(a+) phenotype frequency. All statistical analyses were conducted using SPSS, Version 16.0 (SPSS Inc., Chicago, IL, USA). A p-value less than 0.05 was considered statistically significant.

**Results**

The PCR-SSP results of MNS hybrid GP detections are shown in Figure 1. The GYP(B-A-B) hybrids of GYP*Hut, GYP*Mur, GYP*Hop, GYP*Bun, and GYP*HF were amplified with the first set of primers (the product size of 148 or 151 bp), whereas GYP*Vw of the GYP(A-B-A) hybrids was amplified using the second set of primers (the product size of 296 bp). The DNA controls were also tested using these two sets of primers, and the results agreed.

![Figure 1](image_url)

**Figure 1.** PCR-SSP results of MNS hybrid GP detections, (a) PCR products in Lanes 1-6 were amplified using the first set of primers, amplifying the fragments of the GYP(B-A-B) hybrids of GYP*Hut, GYP*Mur, GYP*Hop, GYP*Bun, and GYP*HF. (b) PCR products in Lanes 1-6 were amplified the second set of primers, amplifying the fragment of the GYP(A-B-A) hybrid of GYP*Vw. Lane NTC was the negative control (nontemplate control). Lane M: molecular weight 100-bp DNA Ladder.
Table 1. Occurrence of $GYP(B-A-B)$ hybrids in Asian populations determined by PCR-based assays

<table>
<thead>
<tr>
<th>Population</th>
<th>Number</th>
<th>$GYP(B-A-B)$ hybrids</th>
<th>$\chi^2$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Southern Thai (This study)</td>
<td>400</td>
<td>Mi(a+) 19, Mi(a-) 381</td>
<td>9.202</td>
<td>0.002</td>
</tr>
<tr>
<td>Central Thai (This study)</td>
<td>500</td>
<td>Mi(a+) 51, Mi(a-) 449</td>
<td>49.183</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Northern Thai (15)</td>
<td>300</td>
<td>Mi(a+) 67, Mi(a-) 233</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vietnamese (16)</td>
<td>160</td>
<td>Mi(a+) 10, Mi(a-) 150</td>
<td>0.524</td>
<td>0.469</td>
</tr>
<tr>
<td>Taiwanese (16)</td>
<td>167</td>
<td>Mi(a+) 7, Mi(a-) 160</td>
<td>0.084</td>
<td>0.772</td>
</tr>
<tr>
<td>Indonesian (16)</td>
<td>285</td>
<td>Mi(a+) 5, Mi(a-) 280</td>
<td>4.417</td>
<td>0.035</td>
</tr>
<tr>
<td>Filipino (16)</td>
<td>262</td>
<td>Mi(a+) 20, Mi(a-) 242</td>
<td>179.851</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Southern Han Chinese (17)</td>
<td>3,104</td>
<td>Mi(a+) 201, Mi(a-) 2,903</td>
<td>1.792</td>
<td>0.181</td>
</tr>
<tr>
<td>Chinese (Guangzhou) (18)</td>
<td>528</td>
<td>Mi(a+) 51, Mi(a-) 477</td>
<td>7.864</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Table 2. Estimations of risk for Mi$^a$ alloimmunization in Thai populations

<table>
<thead>
<tr>
<th>Population</th>
<th>Number</th>
<th>The frequencies of predicted Mi$^a$ phenotype</th>
<th>Risk of Mi$^a$ alloimmunization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Southern Thai</td>
<td>400</td>
<td>Negative 0.953, Positive 0.047</td>
<td>0.045</td>
</tr>
<tr>
<td>Central Thai</td>
<td>500</td>
<td>Negative 0.898, Positive 0.102</td>
<td>0.092*</td>
</tr>
<tr>
<td>Northern Thai</td>
<td>300</td>
<td>Negative 0.777, Positive 0.223</td>
<td>0.173*</td>
</tr>
</tbody>
</table>

* $p<0.05$

Among 400 southern and 500 central Thai blood donors, 19 (4.45%) and 28 (9.33%) were positive only with the set of primers specific for GP.Hut, GP.HF, GP.Mur, GP.Hop, and GP.Bun. No GP.Vw phenotype was identified by PCR-SSP technique among southern and central Thais. Regarding the positive results of the MNS hybrid GPs by PCR-SSP, the predicted Mi(a+) frequency among southern Thais was significantly lower than that among central ($p=0.002$) and northern Thais ($p<0.001$). The Mi(a+) frequency between southern Thais and other populations was compared. The frequencies were similar to Vietnamese, Taiwanese, and Southern Han Chinese populations ($p>0.05$). On the contrary, its frequency significantly differed from Indonesian, Filipino, and Chinese (Guangzhou) populations ($p<0.05$), as shown in Table 1. In addition, the risk of Mi$^a$ alloimmunization among southern Thais was significantly lower than those among central and northern Thais ($p<0.05$), as shown in Table 2.

Discussion

In Thailand, blood group allele frequencies have been studied among central, northern and southern Thais. The $DI^A$ allele (predicted Di$^A$ antigen) was significantly lower among southern Thais, while the $JK^*01$ allele (predicted Jk$^a$ antigen) and $KEL^*01$ allele (predicted K antigen) were significantly higher among southern Thais than among central and northern Thais. However, the Mi$^a$ frequencies have not yet been reported in southern Thailand. This study constitutes the first to identify the predicted Mi$^a$ frequencies in Thai Muslim populations, which have their own religion, culture and sustainable lifestyle.

Regarding the genotyping of MNS hybrid GP results using PCR-SSP among the four types; GP.Mur, GP.Hop, GP.Bun, and GP.HF of $GYP$
hybrids, GP.Mur is commonly found in Asian and Australian populations.\(^{(4, 14, 16-18)}\) Notably, GP.Vw of the \(GYP(A-B-A)\) hybrids was not found among southern and central Thais, similar to related studies in Thai and other Asian populations.\(^{(14, 16-18)}\) The predicted Mi(a+) phenotype among southern Thais (4.8%) is closely related to Chinese-Malaysians (4.9%) but unrelated to Malay and Indian Malaysians (2.08% and 3.0%, respectively).\(^{(13)}\) Even though the probability of finding the Mi(a+) phenotype among southern Thais is lower than among central and northern Thais, anti-E and anti-Mi\(^a\) are frequently found among southern Thai patients requiring repeated transfusions.\(^{(20, 21)}\) Hence, the use of PCR-based assay to predict Mi\(^a\) antigen in blood donors and chronically transfused patients would be helpful to reduce alloimmunization risks and the above-mentioned adverse events. This study confirmed that it would pose no difficulty to find Mi\(^a\) antigen-negative donors for Thai patients with anti-Mi\(^a\) except among chronically transfused patients with multiple antibodies including anti-Mi\(^a\). The possibility of finding compatible donor blood in complex cases caused by multiple alloantibodies may be more difficult concerning potential donors with a compatible blood type. Concerning patients requiring repeated transfusions such as thalassemia, cancer, and chronic renal diseases, performing Mi\(^a\) antigen typing is suggested before transfusion therapy and Mi\(^a\)-compatible donor(s) should be provided to patients. In concordance with the Clinical Practice Guidelines for diagnosis and management of thalassemia, the antigen typing of Rh (C, c, E, e) and MNS7 (Mi\(^a\)) is minimally required before the first transfusion.\(^{(22)}\) This particular blood group antigen frequency could support not only determining genetic variation but also enhancing the characterization of Thai populations, especially in blood transfusion therapy.

**Conclusion**

This study is the first to report Mi (a+) frequencies among southern Thai blood donors. This finding is helpful to estimate the risk of alloimmunization and to provide transfusion safety in Thai populations.

**Disclosures**

The authors declare they have no conflicts of interest.

**Acknowledgement**

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**References**