Two Thai Burmese descendants with A4GALT*01N.21, p phenotype, and anti-PP1P\textsuperscript{k}

K. Intharanut, W. Sasikarn, W. Chusri, and O. Nathalang

Anti-PP1P\textsuperscript{k} is produced by p individuals without prior red blood cell alloimmunization. This antibody can react over a wide thermal amplitude, has the potential to bind complement, and has caused hemolytic transfusion reaction, hemolytic disease of the fetus and newborn, and a high rate of spontaneous abortions. This report of two cases describes the genetic basis of p phenotype underlying anti-PP1P\textsuperscript{k} production and the development of a semi-nested polymerase chain reaction (PCR) assay for screening this observed mutation among Thai blood donors. Antibody detection and confirmation were examined by serologic testing. Genomic DNA was extracted from two Thai Burmese descendants with the p phenotype and a history of spontaneous abortions caused by anti-PP1P\textsuperscript{k}; the entire coding region of the A4GALT gene of each was sequenced and analyzed. Additionally, a semi-nested PCR assay of the observed mutation was developed and used for screening the genomic DNA of 1502 Thai blood donors. Anti-PP1P\textsuperscript{k} was identified and the p phenotype was confirmed in the two Thai individuals of Burmese descent. A single-base duplication (c.201dupC in exon 3) in the A4GALT gene was detected in both p patients. The duplication is consistent with the A4GALT*01N.21 allele associated with the p phenotype and anti-PP1P\textsuperscript{k} production. A semi-nested PCR assay was developed and subsequently used for mass screening for this mutation. The mutation was not found among the 1502 Thai blood donors tested with this newly developed assay. *Immunohematology* 2020;36:64–68.

**Key Words:** duplication, p phenotype, anti-PP1P\textsuperscript{k}, Thai Burmese descendant

The P1PK blood group system (International Society of Blood Transfusion [ISBT] number 003) comprises three antigens, P\textsubscript{1}, P\textsubscript{K}, and NOR, and the GLOB blood group system (ISBT number 028) includes the P and PX2 antigens.\textsuperscript{1} Five phenotypes, P\textsubscript{1}, P\textsubscript{2}, P\textsubscript{1k}, P\textsubscript{2k}, and p, are deduced from the presence or absence of the three antigens, P\textsubscript{1}, P\textsubscript{K}, and P on the human red blood cell (RBC) glycoproteins.\textsuperscript{2} Each phenotype can give rise to the naturally occurring antibodies against the lacking antigen(s) without prior RBC or pregnancy sensitization. The absence of all three antigens leads to the rare p phenotype and the production of the mixture component antibody, anti-PP1P\textsuperscript{k}.\textsuperscript{2,3} This antibody is often associated with hemolytic transfusion reaction (HTR), hemolytic disease of the fetus and newborn (HDFN), and spontaneous abortion in early pregnancy.\textsuperscript{4} Anti-PP1P\textsuperscript{k} has been found to be a mixture of IgM and IgG isotypes and able to react over a wide range of temperatures and potentially bind and activate complement.\textsuperscript{3–5} ABO discrepancies caused by unexpected reactions, either hemolysis or agglutination in the reverse grouping, usually occurs. Moreover, panagglutination can be observed in antibody detection and identification testing, requiring further antigen typing to exclude the high-prevalence antigens and to confirm the antibody specificity. Currently, commercial anti-PP1P\textsuperscript{k} is unavailable for routine testing, so finding a way to solve serologic problems caused by this suspected antibody or a compatible RBC transfusion remains challenging and limited.

The P1PK (A4GALT) gene encodes 4-α-galactosyltransferase (α4GalT, A4GALT, EC 2.4.1.228), which was found to give rise to the P1 and P\textsuperscript{K} antigen biosynthesis. This gene is located on chromosome 22 (22q13.2) and encompasses four exons spanning approximately 26.6 kbp of genomic DNA with the whole coding region at exon 3.\textsuperscript{2,6} The molecular basis for the different mutations resulting in the p phenotype includes nonsense mutations, combined deletion and insertion resulting in a premature stop codon, missense mutation, and large deletion in the regulatory upstream region of the A4GALT gene.\textsuperscript{7–11} The rare p phenotype is a recessive inherited trait caused by mutation in both alleles of the A4GALT gene, which was estimated at 5.8 per million individuals in all populations. The highest prevalence of this rare phenotype is found in Swedish and Amish populations (141 per million).\textsuperscript{3} In Thailand, three individuals of the p phenotype with anti-PP1P\textsuperscript{k}, all residing in provinces near the Thailand–Burma (now Myanmar) border, have been reported.\textsuperscript{12} Apart from the ancient relationship between Thailand and Myanmar, the development of the border trade and culture has a long history; consequently, Burmese populations living in Thailand can be categorized into three types: professional migrants working in business, laborers working in the low-skilled professions, and refugees fleeing conflicts. Hence, the Thai health care service system faces managing a number of uncommon or rare blood types, especially the rare p
phenotype. Here, we describe two Thai Burmese women with a history of spontaneous abortion who presented with the rare p phenotype and production of anti-PP1P\(^{k}\). We identified the A4GALT*01N.21 allele in both individuals and developed a semi-nested polymerase chain reaction (PCR) assay to screen Thai blood donors.

**Case Report**

The first case was a 32-year-old female Thai Burmese patient (PX01) who had an unremarkable history of a normal first pregnancy, and her child exhibited healthy growth and development. One year later, she had a second pregnancy and suffered a spontaneous abortion at 8 weeks’ gestation without curettage procedure. With the third pregnancy, she had a late-term spontaneous abortion at 36 weeks’ gestation; a sample was submitted for investigation.

The second case involved a 29-year-old female Thai Burmese patient (PX02) who had preterm labor with her first pregnancy. At 10 weeks’ gestation of her subsequent pregnancy, the patient attended the antenatal clinic of the hospital. Thereafter, the patient was admitted at 24 weeks’ gestation because of spontaneous abortion. Although she did not require a blood transfusion, a sample was sent for investigation.

Initial serologic results indicated both patients to be group B, D+ with positive antibody detection tests (partial hemolysis at 37°C). The antibody detection test was positive with all screening cells (O1 and O2, National Blood Centre, Thai Red Cross Society, NBC-TRC, Bangkok, Thailand) by conventional tube test (CTT) using antihuman globulin (AHG) reagent (Anti-IgG, -C3d; CE-Immundiagnostika GmbH, Eschelbronn, Germany). The initial ABO grouping, D typing, and antibody detection test were performed by the Blood Bank Unit, Bangkok Metropolitan Administration General Hospital (Bangkok, Thailand). Samples from the two patients were referred to the Faculty of Allied Health Sciences, Thammasat University (Pathumtani, Thailand), for further serologic evaluation and molecular testing.

**Results**

For ABO grouping of both patients (PX01 and PX02), the initial result of the forward grouping was B, and the reverse grouping was O (weak agglutination with group B and group O RBCs). The antibody detection test results were positive with screening cells at room temperature, 37°C, and AHG phases, as shown in Table 1. To resolve the ABO discrepancy, antibody identification was performed by CTT using 11 panel RBCs (NBC-TRC, Bangkok, Thailand) along with an autocontrol; panagglutination/partial hemolysis was observed with all panel RBCs, and the autocontrol was negative. The results are summarized in Table 2. The panagglutination/partial hemolysis showed specific features of an antibody to a high-prevalence antigen such as anti-H, anti-Vel, and anti-PP1P\(^{k}\); therefore, screening the patient’s RBCs for possible high-prevalence antigens and testing the patient’s serum with selected rare phenotype and null RBCs were essential in further investigation. To confirm the antibody specificity, the patient’s serum was tested with an extra group O RBC of the p phenotype. RBC phenotyping using anti-H, anti-P1,

**Table 1. Results of ABO grouping, D typing, and antibody detection testing of the two Thai Burmese descendants**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Forward grouping</th>
<th>Reverse grouping</th>
<th>Antibody detection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anti-A</td>
<td>Anti-B</td>
<td>Anti-D</td>
</tr>
<tr>
<td>PX01</td>
<td>0</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td>PX02</td>
<td>0</td>
<td>4+</td>
<td>4+</td>
</tr>
</tbody>
</table>

RT = room temperature; AHG = antihuman globulin; PH = partial hemolysis.

**Table 2. Results of additional testing of p phenotype individuals (PX01 and PX02) with extra RBCs and antisera**

<table>
<thead>
<tr>
<th>Extra RBCs</th>
<th>PX01</th>
<th>PX02</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autocontrol</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group O, p phenotype</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Antigen typing**

| Anti-H | 3+ | NT  | NT  | 4+ | NT  | NT  |
| Anti-P1 | 0  | NT  | NT  | 0  | NT  | NT  |
| Anti-PP1P\(^{k}\) | 0  | 0   | 0   | 0  | 0   | 0   |

RBCs = red blood cells; RT = room temperature; AHG = antihuman globulin; NT = not tested.
and anti-PP1P\textsuperscript{k} was performed, and alloantibodies to other common antigens were ruled out. Anti-PP1P\textsuperscript{k} showing \textit{in vitro} hemolysis was demonstrated in both patients.

Molecular testing was performed using genomic DNA from PX01 and PX02 and DNA (control samples) from one P\textsubscript{1} phenotype (PS01) and one P\textsubscript{2} phenotype (PS02) donor for genetic analysis using DNA sequencing. The whole \textit{A4GALT} gene reading frame, located in exon 3, was sequenced in all samples. The partial sequencing results of the whole coding region at the \textit{A4GALT} gene target are shown in Figure 1. A single-base duplication (c.201dupC) was detected in both p phenotype samples when compared with the sequence deposited data in GenBank (Genomic reference, NG_007495.1) and the two controls.

In addition, we developed a semi-nested PCR assay to detect c.201dupC in the \textit{A4GALT} gene. Two primer pairs were used; the first pair was used as an internal control specific for the \textit{A4GALT} exon 3 region with the product size of 882 bp, and the second pair identified c.201dupC with the product size of 468 bp (Fig. 2). This semi-nested PCR assay was validated using genomic DNA from 10 P\textsubscript{1} and 10 P\textsubscript{2} individuals, and the results showed 100 percent concordance. Additionally, 1502 DNA samples from unrelated Thai blood donors were tested using the semi-nested PCR assay; c.201dupC was not observed in any of these samples. To test for reproducibility, the semi-nested PCR assay was performed repeatedly on 30 randomly selected DNA samples. The results from all samples were the same as those from the first round of testing.

**Discussion**

Anti-PP1P\textsuperscript{k}, formerly known as anti-Tj\textsuperscript{a}, is composed of anti-P, anti-P\textsubscript{1}, and anti-P\textsubscript{2}, and its various components are separable. Anti-PP1P\textsuperscript{k} produced by individuals with the rare p phenotype is invariable and usually associated with HTR and HDFN. Identifying suspected laboratory results including ABO discrepancy, a positive antibody detection test, and incompatible crossmatches before transfusion may decrease the occurrence of severe cases. Regarding the properties of the anti-PP1P\textsuperscript{k}, both cytotoxic IgM and IgG3 forms of anti-P and anti-P\textsubscript{P} have been reported to react against both corresponding antigens on the placental tissues resulting in destruction of the placenta and early pregnancy loss or multiple abortions.\textsuperscript{3-5} Several studies have reported cases of multiple early miscarriages in African, Japanese, and Iranian populations.\textsuperscript{13-15}
Anti-PP1P\textsuperscript{k} is rarely found among Thai patients. In other populations, double filtration plasmapheresis (DFPP) has been used for rapid reduction of highly sustained anti-PP1P\textsuperscript{k} titers less than or equal to 32.\textsuperscript{15−18} Nevertheless, two of seven pregnant Japanese women with anti-PP1P\textsuperscript{k} who underwent plasma exchange and DFPP had abortions in the first 12 and 13 weeks of pregnancy, respectively, due to the phenomenon of rebound antibody production.\textsuperscript{16} Such a treatment regimen to prevent recurrent abortions for pregnant women with the p phenotype and with anti-PP1P\textsuperscript{k} has not been used in Thailand. The growing number of ethnic intermarriages and expansion of immigrants or descendants, particularly among the Burmese population, may lead to an increase in genetic variations of the p phenotype in Thailand. The main obstacles are finding compatible frozen donated blood units as well as autologous blood donations or the patients’ family member blood donations for these cases.

P1 and P\textsuperscript{a} antigens are catalyzed by A4GALT biosynthesis pathways from lactosylceramide by P1/P\textsuperscript{a} synthase, therefore originating the globoseries pathway leading to the P antigen.\textsuperscript{6} In this study, the whole coding region in exon 3 of the A4GALT gene and some parts of the 5′- and 3′-untranslated regions were sequenced and investigated for a mutation. We identified a single-base duplication in two Thai individuals of Burmese descent. This A4GALT\textsuperscript{*01N.21} allele is responsible for a reading frame shift resulting in a gene product with an additional 215 amino acids (p.Thr68Hisfs*215).\textsuperscript{4} Other genetic mechanisms of the p phenotype include a three-base deletion (c.241_243delTTC) and a one-base duplication (c.1029_1030insC) reported in Japanese individuals and two missense (c.783G>A and c.752C>T) and one nonsense mutation (c.201dupC) to predict the p phenotype among Thai blood donors. Interestingly, the one-cytosine base insertion is adjacent to the exonic six-cytosine tandem repeat in the wild-type DNA. The mutation was not found in the 1502 Thai blood donors tested. The semi-nested PCR assay developed in this study could provide accurately targeted amplicons specific to this mutation.\textsuperscript{22} Further studies with larger sample sizes would be beneficial to determine the prevalence of this rare blood type in the Thai population.

Conclusions

We describe two Thai Burmese women with a history of spontaneous abortion who show p phenotype and anti-PP1P\textsuperscript{k} production who were both found to carry the A4GALT\textsuperscript{*01N.21} allele that has a single-base duplication (c.201dupC) in the A4GALT gene. The semi-nested PCR assay described here could be subsequently used for mass screening to identify blood donors with this rare null allele.

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KI conceived and designed the research and wrote for grants, all authors performed experiments, KI and ON analyzed and interpreted the data, KI contributed reagents/analytical tools, and KI and ON wrote the manuscript.

References


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