Comparative Analysis of Serological and Molecular Techniques for Blood Group Typing in Thai Blood Donors and Thalassemic Patients

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Abstract

Background: Blood group antigens are polymorphic, and their immunogenicity has the potential for alloimmunization. Traditionally, serologic red blood cell (RBC) phenotyping is performed in the transfusion service to identify antigen-matched RBCs between donors and patients for safe transfusion. An inaccuracy of RBC phenotyping has been observed due to the limitations of the classical hemagglutination method. Genotyping has recently been introduced to tackle the difficulty of serological phenotyping.

Objective: To observe the discrepancy between phenotype and genotype in both donors and thalassemic patients.

Materials and Methods: A cross-sectional study was conducted between December 2018 and 2019, targeting Thai blood donors who donated blood at least twice a year at the Blood Bank, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, and Thai transfusion-dependent thalassemic patients who had received blood from the Blood Bank. Forty-two blood donors and 66 multiple-transfused thalassemic patients were included in this study. Two blood samples of each donor and patient were used, one for serological RBC phenotype and the other for the BLOODchip® molecular analysis. A comparison between phenotype and genotype was carried out for Rh (C, c, E, e), Kidd (Jk(a), Jk(b)), Duffy (Fy(a), Fy(b)), and MNS (M, N, S, s, Mi(a)) systems.

Results: Twenty-four out of 66 (36.36%) patients showed discordance between phenotyping and genotyping results. A phenotype+/genotype– pattern was more common among thalassemic patients, with the discrepancies occurring most frequently in the Duffy and MNS systems. Among the 42 donors, only one individual (2.38%) demonstrated a discordant result, also in the Duffy system.

Conclusion: The discrepancies between phenotype and genotype were seen in both donors and patients. The patients with the phenotype+/genotype– had a potential for alloimmunization. Blood group genotyping has the benefit over serologic phenotyping, particularly in the thalassemic patients who require lifelong regular transfusion.

Keywords: Genotype; Blood donor; Thalassemia; Red blood cell; Blood group; Thailand

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Polymorphism in blood group antigens is a central challenge in transfusion medicine. When incompatible blood is transfused, patients may develop alloantibodies, which complicate or, in the most severe cases, preclude subsequent transfusions. For patients who already have alloantibodies and require transfusion, it is essential to identify donor units that lack the corresponding antigens to ensure safety. Even in the absence of alloantibodies, providing phenotype-matched RBC units effectively diminishes the risk of

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What is already known about this topic?

Serological phenotyping is often unreliable in chronically or recently transfused patients because donor-derived red cells can produce mixed-field or misleading reactions. Previous studies have shown that molecular genotyping can overcome many of these limitations by providing more accurate antigen prediction in transfusion-dependent individuals.

What does this study add?

This study identifies specific limitations of the BLOODchip® platform, including its inability to detect certain clinically relevant variant alleles found in the Thai population (*Fy*A* null and *JK*01W.01* variants). It also shows that discrepancies between negative serological phenotypes and positive genotyping results require confirmatory DNA sequencing. These findings highlight the need for more comprehensive genotyping panels and emphasize the importance of sequencing in resolving phenotype-genotype conflicts.

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alloimmunization and helps prevent both acute and delayed hemolytic transfusion reactions in chronically transfused patients[1]. Thus, extended RBC phenotyping plays a critical role in supplying antigen-negative blood. Beyond ABO and RhD, blood group antigens are typically determined by classical hemagglutination techniques, performed on platforms such as standard tube tests, microplates, or microcolumn agglutination systems. Although hemagglutination remains the gold standard for antigen typing[2,3], accurate phenotyping is not always achievable. Its limitations include limited sensitivity, susceptibility to technical variability related to reagent quality, and potential interference from patient factors. These challenges have driven increasing adoption of molecular methods for predicting RBC antigen expression. Genotyping is particularly valuable for patients who have been recently or repeatedly transfused, such as those with hemoglobinopathies, congenital or acquired hemolytic anemias[4]. In Thailand, where thalassemia is highly prevalent[5], affected individuals often require lifelong regular transfusions, and more than 17% develop alloantibodies[6]. In such patients, the presence of transfused donor RBCs can cause mixed-field agglutination in the serological assays, complicating interpretation. Therefore, alternative molecular approaches are needed to ensure accurate blood group antigen identification.

In the present study, we aimed to compare serological methods and molecular-based methods for blood group antigen determination in both donors and patients. These data will provide essential comparative evidence to inform their implementation in routine transfusion practice.

Materials and Methods

Setting and Study Design

A cross-sectional study was conducted between December 19, 2018, and December 18, 2019, involving Thai blood donors who donated at least twice per year at the Blood Bank, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, and multiple-transfused thalassemic patients who received blood products from this Blood Bank. This work was approved by the Institutional Review Boards, Faculty of Medicine Ramathibodi Hospital, Mahidol University (COA. No. MURA2018/970).

Inclusion and Exclusion Criteria

Donor inclusion criteria were: (i) voluntary blood donation; (ii) presentation to the Ramathibodi Hospital Blood Bank during the study period; (iii) a history of at least two donations within a year, including the current visit; (iv) prior phenotyping of RBCs for Rh (C, c, E, e), Kidd (Jk(a), Jk(b)), Duffy (Fy(a), Fy(b)), and MNS (M, N, S, s, Mi(a)) at least once; and (v) availability of residual blood samples for testing. Patient inclusion criteria were: (i) Thai thalassemic patients requiring multiple blood transfusions; (ii) active follow-up at the Hematology Clinic, Ramathibodi Hospital, Mahidol University during the study period; (iii) receipt of regular transfusions within the preceding 12 weeks; and (iv) availability of leftover specimens for testing. Exclusion criteria for both donors and patients were: (i) incomplete data, such as missing antigen results from previous phenotyping in donors or incomplete clinical information in patients referred from other hospitals; (ii) insufficient sample volume for phenotyping and genotyping; and (iii) inadequate DNA yield or purity.

Serological Phenotype Determination

Two ethylenediaminetetraacetic acid (EDTA) samples of each donor and patient were used, one for serological RBC phenotype and the other for molecular analysis. The phenotype of the donors' and patients' RBCs was serologically determined using 2 methods. For Rh (C, c, E, e), Kidd (Jk(a), Jk(b)), Duffy (Fy(a), Fy(b)), and MNS system, an automated column agglutination technology (IH-500, BIO-RAD, Switzerland) was used with ID-card phenotype (DiaMed GmbH, Switzerland) containing commercial antisera to determine the presence of the antigens. The antisera for identifying 5 antigens were a mixture of monoclonal and polyclonal antibodies as follows: anti-E (clone MS260), anti-Jk(a) (MS15), anti-Jk(b) (MS8), anti-M (9211 9D5 1G10), and anti-N (1422 C7+MN879), and the rest were polyclonal antibodies produced from human serum. Specific antisera for blood grouping were used against 0.8 and 5% of the RBCs in ID-Diluent (DiaMed GmbH, Switzerland). This was performed according to standard manufacturer's protocols (DiaMed GmbH, Switzerland). The image of the reaction was captured, and hemagglutination was graded using the data management software (IH-500, BIO-RAD, Switzerland). For the Mi(a) phenotype, a standard tube test method was used with an in-house human antiserum. Visible hemagglutination was graded manually, and a mixed-field reaction was recorded as indeterminate.

Genomic DNA Extraction

Genomic DNA was obtained according to the manufacturer's instructions for the QIASymphony™ automated nucleic acid purification system (Qiagen,

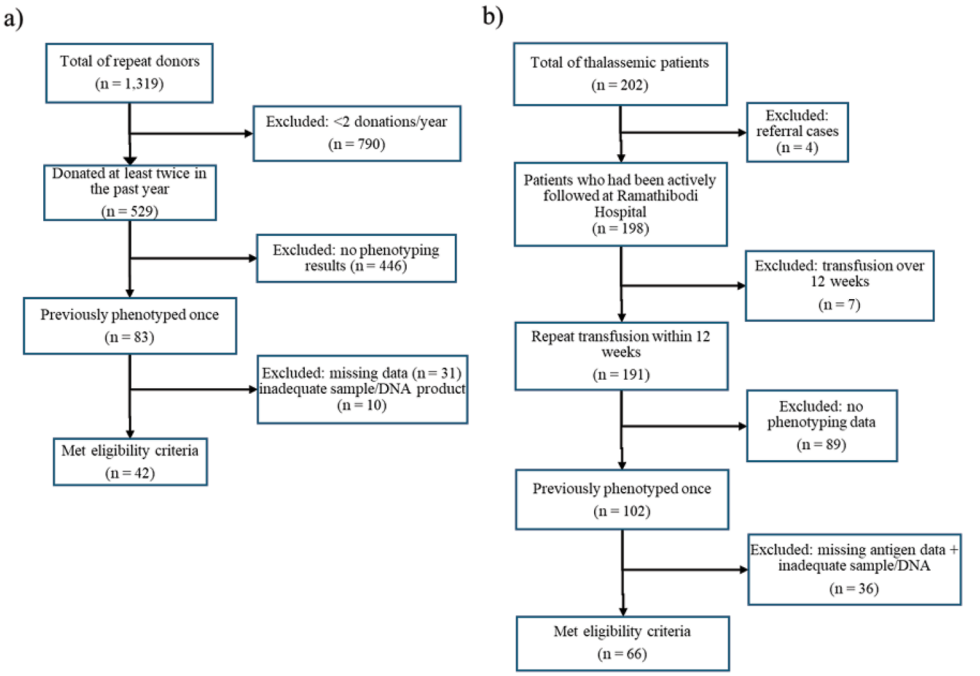


Figure 1. Flowchart of inclusion and exclusion of (a) donors and (b) patients.

Hilden, Germany). The genomic DNA was quantified by measuring the absorbance at 260/280 nm using a NanoDrop™ Lite spectrophotometer (Thermo Fisher Scientific, Delaware, USA). The purity of DNA was also determined by calculating the ratio of corrected absorbance at 260 nm to corrected absorbance at 280 nm before storage at –40 °C.

BLOODchip® analysis

Predicted RBC phenotype by molecular-based method was carried out using the ID CORE XT (Progenika Biopharma, SA, Spain), which can simultaneously determine 37 RBC antigens. This was run on BLOODchip® ID, which is a genotyping platform based on Luminex® xMAP technology. The analysis of genotype and predicted phenotype was performed according to the manufacturer's instructions using analysis software (ID CORE XT™, Progenika-Grifols). A 'No Call' (NC) was identified if the BLOODchip® Reference software is unable to assign a result.

Result Interpretation

Blood group antigen profiles obtained by serological testing and genotyping were recorded for each subject. Each result was analyzed by a well-trained medical technologist and then approved by another well-trained medical technologist. Results from serology were compared with those generated by the BLOODchip®. Any discrepancies were categorized either phenotype–/genotype+ or phenotype+/genotype–. Categorical variables were described using frequencies and proportions.

Sequencing of the Polymerase Chain Reaction (PCR) Products

The genomic DNA of 9 discrepancy samples was selected to be sequenced, covering all 4 blood group systems, in order to determine the accuracy of the BLOODchip® molecular analysis. Standard Sanger sequencing was performed by Grifols IH Center, San Marcos, Texas, using specific primers located in flanking intron regions of the blood group gene. The sequence analysis was carried out using Seqscape software.

Results

A total of 1,319 repeat blood donors and 202 thalassemic patients at Ramathibodi Hospital were identified during the study period. Of these, 1,295 donors and 136 patients were excluded for not meeting the study criteria, as shown in Figure 1. This left 42 donors and 66 patients who met the inclusion criteria and were included in the study. The demographic characteristics of 42 donors and 66 patients are shown in Table 1.

Table 1. Demographic characteristics in donors and patients

	Donors (n=42)	Patients (n=66)
Age (years); range (mean)	25-57 (43.2)	2-41 (11.8)
Sex; n (%)		
Male	27 (64.29)	32 (48.48)
Female	15 (35.71)	34 (51.52)
Blood group		
O	22 (52.38)	26 (39.39)
A	8 (19.05)	16 (24.24)
B	10 (23.81)	21 (31.82)
AB	2 (4.76)	3 (4.55)
Last transfusion; n (%)		
<1 month	-	63 (95.45)
1-3 months	-	3 (4.55)

The serological phenotypes of all donor samples were identical to the previous historical record. For the patients, 16 out of 66 (24.24%) demonstrated a mixed-field agglutination resulting in an indeterminate phenotype, and all of them had their last transfusion within a month. For genotyping, no NC results were observed in any subject. Regarding the correlation between phenotype and genotype, all donors showed concordant results across the four RBC systems, with the exception of one donor (2.38%) who demonstrated a discordant result in the Duffy system (Table 2). Among the 66 patients, 26 (39.39%) demonstrated concordant phenotype-genotype results. Sixteen patients (24.24%) had inconclusive results due to indeterminate mixed-field reactions in RBC phenotyping. The remaining 24 patients (36.36%) showed discordance between phenotype and genotype. The most frequent antigen with discordant results was Fy(b) (14, 21.21%), whereas discordance in Fy(a) and s typing was least common (1, 1.52%). The frequencies of phenotype-genotype correlation of other RBC antigens are presented in Table 2.

Regarding the discordant results between serological phenotyping and molecular genotyping, phenotype+/genotype– discrepancies were more common among thalassemic patients (Figure 2). The three most frequent antigens in this category were Fy(b) and N, each occurring in 11 of 66 (16.67%), followed by S in 7 of 66 patients (10.61%). In contrast, for phenotype–/genotype+ discrepancies, the most common antigens were Jk(a), Mi(a), and Fy(b), observed in 6 (9.09%), 4 (6.06%), and 3 patients (4.55%), respectively.

Serological phenotyping showed that 16 patients (24.24%) had mixed field reactions, resulting in an indeterminate RBC phenotype. The frequencies of indeterminate phenotypes and their corresponding genotype results are presented in Table 3. The Rh system was the most commonly affected, with 10 patients (15.15%) showing an indeterminate C or c phenotype; genotyping subsequently predicted 6 out of these patients to be Rh C+c– and 4 to be Rh C+c+. All indeterminate serologic phenotypes were resolved through genotyping. Notably, serological phenotype for Mi(a) and for antigens in the Duffy system produced clear and unambiguous results. Regarding the phenotype-genotype discrepancies, genomic DNA from 9 samples was selected for Sanger sequencing to determine the consistency between the two molecular assays. Among these, only one donor sample with

Table 2. Phenotype-genotype correlation in donors and patients' RBC antigens

Red blood cell system	Antigen	Donors (n=42)		Patients (n=66)		
		Concordance	Discordance	Concordance	Discordance	Inconclusive[1]
Rh	C (RH2)	42	0	62	3	1
	E (RH3)	42	0	51	3	12
	c (RH4)	42	0	51	5	10
	e (RH5)	42	0	62	2	2
Kidd	Jk(a) (JK1)	42	0	51	7	8
	Jk(b) (JK2)	42	0	53	7	6
Duffy	Fy(a) (FY1)	41	1	65	1	0
	Fy(b) (FY2)	42	0	52	14	0
MNS	M (MNS1)	42	0	63	1	2
	N (MNS2)	42	0	54	11	1
	S (MNS3)	42	0	57	8	1
	s (MNS4)	42	0	65	1	0
	Mi(a) (MNS7)	42	0	61	5	0

[1] Inconclusive result of phenotype-genotype correlation due to serological phenotype showing mixed-field or indeterminate result.

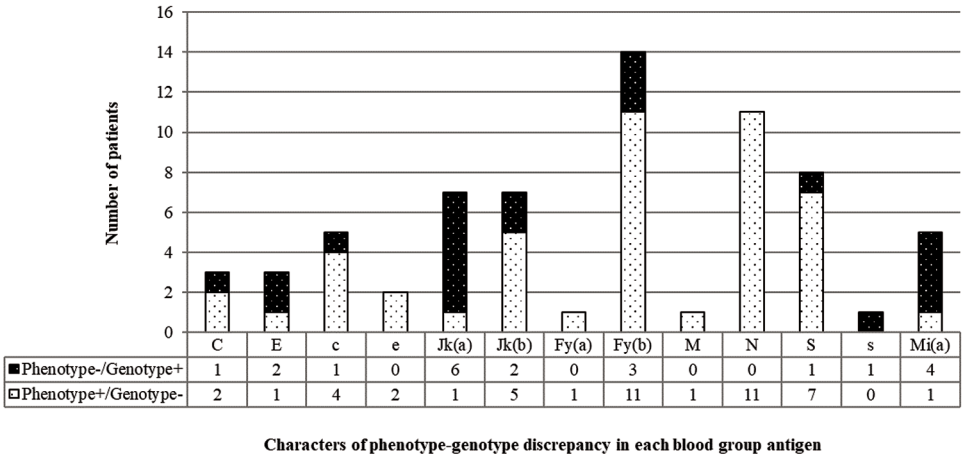


Figure 2. Discrepancies of RBC antigen of patients compared between serological phenotype and molecular genotype.

Table 3. Frequency of mixed-field or indeterminate RBC phenotypes and the corresponding genotypic interpretations in the 16 patients

Mixed field phenotype		Genotype results	
RBC antigens	Occurrence (%)	Predicted phenotypes	Occurrence
C or c	10/66 (15.15)	Rh C+c–	6
		Rh C+c+	4
		Rh C–c+	0
E or e	13/66 (19.70)	Rh E+e-	1
		Rh E+e+	4
		Rh E–e+	8
Jk(a) or Jk(b)	12/66 (18.18)	Jk(a+b–)	3
		Jk(a+b+)	7
		Jk(a–b+)	2
Fy(a) or Fy(b)	0 (0.00)	Fy(a+b–)	0
		Fy(a+b+)	0
		Fy(a–b+)	0
M or N	2/66 (3.03)	M+N–	1
		M+N+	0
		M–N+	1
S or s	1/66 (1.52%)	S+s–	0
		S+s+	0
		S–s+	1
Mi(a)	0 (0.00)	Mi(a+)	0

a discrepant result was included. For cases with a phenotype–/genotype+ pattern, sequencing confirmed the presence of the corresponding blood group alleles. Conversely, in phenotype+/genotype– cases, Sanger sequencing verified the absence of the relevant alleles, consistent with the results obtained using the BLOODchip® technology. Details of the genotypes for 9 samples are shown in Table 4.

Discussion

This study highlights important discrepancies between serological phenotyping and molecular genotyping, demonstrating the clear advantage of molecular methods—particularly in situations where serological testing is unreliable. Although molecular testing is generally considered highly reliable, one notable discrepancy was identified among the 42 donor samples (2.38%). This discrepancy between serology and the BLOODchip® assay in donor samples has not been reported in a previous study[7]. In our study, serology indicated an Fy(a–) phenotype, whereas genotyping showed Fy(a+). Sequencing confirmed the presence of the *FY*01N06* (c.395G>A, exon 2) variant, a mutation known to silence Fy(a) expression[8]. This finding indicates that the BLOODchip® assay failed to detect this particular *Fy*A* null allele (*FY*01N.06*). Importantly, all donors were serologically phenotyped twice by a different clone of antisera, and the results were consistently identical. Therefore, classical hemagglutination for phenotyping the four blood group systems—when performed at least twice—appears to be more accurate and preferable for donor testing, unless molecular methods are expanded to include all known blood group antigen variants.

In transfusion-dependent patients, serological phenotyping is prone to misinterpretation due to the presence of donor-derived RBCs in the circulation[9-12]. In this study, all thalassemia patients who exhibited undetermined RBC antigens due to mixed-field agglutination (24.24%) had received their most recent transfusion within the previous month, confirming the limitation of serological phenotyping in recently transfused individuals. Among the affected cases, Rh

Table 4. The features of phenotype and confirmed genotypes of 9 samples

Sample ID	Serological phenotype	BLOODchip®[1]		Sanger sequencing		Antibody specificity
		Genotype	Predicted phenotype	Genotype	Predicted phenotype	
D36	Fy(a–b+)	*FY*A, FY*B*	Fy(a+b+)	*FY*01N06* (c.395G>A, exon 2), *FY*B*	Fy(a–b+)	Not detected
PT05	Rh C–c+	*RHCE*CE, RHCE*cE*	C+c+[2]	*RHCE*CE, RHCE*cE*	C+c+	Anti-C, -e, -Fy(b), -P_1, -Mi(a)
PT08	S–s–	*GYPB*s*	S–s+	*GYPB*s, GYPB*04.06* (c.251G>C, exon 5)	S-s+	Not detected
PT09	Mi(a+)	*GYPB*s*	Mi(a–)	*GYPB*s*	Mi(a–)	Not detected
PT15	S+s+	*GYPB*s*	S–s+	*GYPB*s*	S–s+	Not detected
PT25	M+N+	*GYPA*M*	M+N–	*GYPA*M*	M+N–	Not detected
PT29	Jk(a+b+)	*JK*A*	Jk(a+b–)	*JK*A*	Jk(a+b–)	Not detected
PT30	Fy(a+b+)	*FY*A*	Fy(a+b–)	*FY*A*	Fy(a+b–)	Not detected
PT44	Jk(a–b+)	*JK*A, JK*B*	Jk(a+b+)	*JK*01W.01* (c.130G>A, exon 3), *JK*B*	Jk(a+b+)[3]	Anti-Mi(a), -E, -c, -S, -Le(b)

[1] Repeated testing was performed by the Grifols IH Center, San Marcos, Texas, [2] Weak C antigen expression, [3] Weak Jk(a) antigen expression

and Kidd systems were the most commonly associated with inconclusive serological results. In contrast, genotyping with BLOODchip® successfully resolved these uncertainties by clearly identifying the patients' predicted blood group phenotypes. This reinforces the value of molecular methods in the management of chronically transfused populations.

When considering concordance across the four blood group systems, nearly 40% of patients showed complete agreement between phenotype and genotype. In contrast, more than 36% showed discrepancies—a finding consistent with previous reports in which 15% to 51% of multiply transfused patients demonstrated genotype-phenotype inconsistencies[9-14]. The MNS system was the most frequently involved, likely due to the large number of antigens within this system. Phenotype+/genotype– discrepancies were common among thalassemic patients. Such discrepancies may reflect a risk for alloimmunization if transfusions are guided solely by serology. Although the underlying causes of these discrepancies remain unclear, possible explanations for falsely serological phenotypes include technical variability, RBCs coated with IgG[15], or interference from autoantibodies. Indeed, nearly 30% of multiply transfused thalassemic patients have been reported to develop autoantibodies[16], which can complicate hemagglutination assays. Molecular testing is not without limitations—such as preferential allele amplification during PCR[15]—yet in this study, five phenotype+/genotype- cases were subjected to confirmatory sequencing and were correctly genotyped by both molecular platforms (Table 4). This strongly suggests that the observed discrepancies were due to serological mistyping. Although these 5 patients had no detectable alloantibodies at the time of testing, they remain at risk of alloimmunization.

For instance, in PT09, serological typing indicated Mi(a+), whereas genotyping by both BLOODchip® and sequencing showed Mi(a–). This discrepancy may be attributed to limitations in either method. The BLOODchip®, at the time of the study, primarily targeted *GYP*Mur*, potentially missing some other variants. Although Sanger sequencing can detect most common hybrid alleles, rare variants may be overlooked if primers are not specifically designed. In the Thai population, common hybrid alleles are GP(B-A-B) hybrids, including GP.Mur and GP.Bun or GP.Bun-like variants (e.g., *GYP*Thai* and *GYP*Thai II*)[17,18]. In contrast, rarer variants such as GP.Vw and GP. Hut are GP(A-B-A) hybrids, which require different PCR starting sites, and their crossover points have not yet been clearly defined[19]. As a result, these variants may not be detected without targeted primers. Alternatively, serological phenotyping with polyspecific human serum may yield false-positive results due to additional antibodies reacting with non-Mi(a) antigens. This issue could be addressed by using currently available monoclonal anti-Mi(a) antibodies. In this case, the results of phenotyping and genotyping remain inconclusive. However, Mi(a–) blood units should be selected for this patient to ensure transfusion safety.

In contrast, phenotype–/genotype+ discrepancies were observed in this study but were less common, with the Kidd system being the most frequently affected. Several factors could account for this type of discrepancy. One possibility is the reduced expression of certain antigens on the RBC surface, potentially associated with variant alleles—many of which may still be uncharacterized[15]. Technical factors may also play a role, including low-quality antisera, differences in IgM versus IgG reagents, misinterpretation, or

other serological typing errors. In this study, three patient samples showing phenotype–/genotype+ were selected for confirmatory sequencing. One sample that typed C-negative by serology was genotyped as *RHCE*CE*, *RHCE*cE* by BLOODchip®. This genotype was confirmed by Sanger sequencing. The C antigen encoded by the *RHCE*CE* allele has been reported to exhibit weaker expression than that encoded by the more common allele *RHCE*Ce*[20]. This haplotype includes the Pro226Ala (E/e) polymorphism, which induces conformational changes in the protein and results in reduced C antigen expression[20]. A previous study demonstrated that individuals with the *RHCE*CE*, *RHCE*cE* genotype express only 8500-9800 C antigen sites per RBC[21], which likely explains the C-negative serologic result. Notably, the presence of anti-C in this patient further supports the interpretation of a weak C variant. In another case, serology indicated an s-negative phenotype, whereas BLOODchip® genotyping showed *GYPB*s*. Sanger sequencing confirmed the presence of *GYPB*s* and *GYPB*04.06* (c.251G>C, exon 5), consistent with a predicted phenotype of S–, s+. This discrepancy is most plausibly attributed to a serological technical error. Previous reports have shown that variability among anti-s reagents can lead to inconsistent s typing results[22], supporting the need for repeat serological testing in such cases. The third discrepancy involved a Jk(a) negative serologic phenotype, while BLOODchip® genotyping revealed heterozygosity for *JK*A* and *JK*B*. Sequencing confirmed *JK*01W.01* (c.130G>A, exon 3) and *JK*B*. The *JK*A* allele carrying the 130G>A substitution in exon 3 encodes a Glu44Lys change in the cytoplasmic N-terminal domain of the Kidd glycoprotein[23], which has been associated with reduced Jk(a) expression. Thus, the patient's predicted phenotype was consistent with weak Jk(a) expression.

This study highlights both the advantages and limitations of genotyping platforms in detecting single nucleotide polymorphisms associated with blood group antigen variation. Genotyping can address key limitations of serological phenotyping, particularly in patients who have recently received transfusions, where mixed red cell populations may confound results. Ongoing advances in genotyping technologies and the identification of additional variants will further enhance their utility, especially in cases where specific antisera are unavailable or serological testing is inconclusive. However, genotyping also has limitations. Incomplete knowledge of DNA sequence variation in less common alleles and the lack of specific primers for their detection may lead to missed or ambiguous results. Consequently, genotyping platforms may not reliably identify all rare variants. Overall, our findings support the integration of molecular genotyping into routine transfusion practice, particularly for multi-transfused patients, where it provides more reliable antigen determination and can help prevent alloimmunization by guiding the selection of truly compatible blood units.

The present study has several notable strengths, including the comparison of advanced molecular platforms with classical serological methods, and the evaluation of both blood donors and multiply transfused thalassemic patients—the latter being the group that stands to benefit most from red cell genotyping. However, this study also has important limitations. First, its cross-sectional design and relatively small sample size introduce the potential for sampling bias, which may limit the representativeness of the cohort and reduce the ability to detect rare allele variants or uncommon blood group phenotypes. Second, because the study population consisted exclusively of Thai individuals, the findings may not be generalizable to other ethnic groups with different allele frequencies or variant distributions. Finally, Sanger sequencing was performed only for selected cases with phenotype–genotype discrepancies due to financial and technical constraints, which may have left additional variants undetected and some discrepancies unresolved.

Future studies should address these limitations by enrolling larger and more ethnically diverse populations, applying comprehensive sequencing to all discrepant or ambiguous cases, and investigating additional or novel variants not captured by the BLOODchip® platform to enhance the accuracy and applicability of red cell genotyping.

Conclusion

This study demonstrates that conventional serological phenotyping may yield inaccurate results in chronically transfused patients, whereas molecular genotyping provides a more reliable method for determining blood group antigens in this population. Molecular testing successfully resolved all serological ambiguities and corrected several mistyped cases, underscoring its value in ensuring appropriate antigen matching and reducing the risk of alloimmunization. These findings support the integration of red cell genotyping—particularly for transfusion-dependent patients such as those with thalassemia—into routine transfusion practice to enhance transfusion safety and compatibility.

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Authors Contributions

SC conducted the literature review; identified, collected, and prepared samples; performed and interpreted the laboratory analyses; prepared figures and tables; and drafted the initial manuscript. SO and PS contributed to the laboratory work, assisted with result interpretation, and reviewed the manuscript. PK provided supervision and critical revision of the manuscript. PP performed the initial literature search, compiled references, collected clinical data, critically evaluated the results, and contributed substantially to drafting and revising the manuscript.

Clinical Trial Registration

As this study did not involve a clinical trial, these documents were not applicable.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Available from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate

This work was approved by the Institutional Review Board, Faculty of Medicine Ramathibodi Hospital Mahidol University (COA. No. MURA2018/970). Given that the study used leftover specimens and anonymized, de-identified datasets, the requirement for informed consent was waived by the Institutional Review Board. No personal identifiers (e.g., names, hospital number, exact dates) were collected or maintained during the research process.

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Use of Artificial Intelligence

The authors used ChatGPT (OpenAI, GPT-5.3) for language editing. All outputs were critically reviewed, and the authors take full responsibility for the final content.

References

1. Flegel WA, Gottschall JL, Denomme GA. Integration of red cell genotyping into the blood supply chain: A population-based study. Lancet Haematol 2015;2:e282-9.
2. Reid ME. Overview of molecular methods in immunohematology. Transfusion 2007;47 Suppl 1:10S-6S.
3. Lomas-Francis C. Testing for rare blood donors by DNA analysis. ISBT Science Series 2006;1:213-9.
4. Fasano RM, Chou ST. Red blood cell antigen genotyping for sickle cell disease, thalassemia, and other transfusion complications. Transfus Med Rev 2016;30:197-201.
5. Jansuwan S, Tangvarasittichai O, Tangvarasittichai S. Alloimmunization to red cells and the association of alloantibodies formation with splenectomy among transfusion-dependent β-thalassemia major/HbE patients. Indian J Clin Biochem 2015;30:198-203.
6. Panich V, Pornpatkul M, Sriroongrueng W. The problem of thalassemia in Thailand. Southeast Asian J Trop Med Public Health 1992;23 Suppl 2:1-6.
7. Manfroi S, Pagliaro P. Genotyping patients' and donors' blood groups for efficient blood therapy. Blood Transfus 2014;12 Suppl 1:s305-7.
8. Höher G, Fiegenbaum M, Almeida S. Molecular basis of the Duffy blood group system. Blood Transfus 2018;16:93-100.
9. Bakanay SM, Ozturk A, Ileri T, Ince E, Yavasoglu S, Akar N, et al. Blood group genotyping in multi-transfused patients. Transfus Apher Sci 2013;48:257-61.
10. Castilho L, Rios M, Bianco C, Pellegrino J Jr, Alberto FL, Saad ST, et al. DNA-based typing of blood groups for the management of multiply-transfused sickle cell disease patients. Transfusion 2002;42:232-8.
11. Guelsin GA, Sell AM, Castilho L, Masaki VL, Melo FC, Hashimoto MN, et al. Benefits of blood group genotyping in multi-transfused patients from the south of Brazil. J Clin Lab Anal 2010;24:311-6.
12. Ribeiro KR, Guarnieri MH, da Costa DC, Costa FF, Pellegrino J Jr, Castilho L. DNA array analysis for red blood cell antigens facilitates the transfusion support with antigen-matched blood in patients with sickle cell disease. Vox Sang 2009;97:147-52.
13. Legler TJ, Eber SW, Lakomek M, Lynen R, Maas JH, Pekrun A, et al. Application of RHD and RHCE genotyping for correct blood group determination in chronically transfused patients. Transfusion 1999;39:852-5.
14. Sarihi R, Oodi A, Dadkhah Tehrani R, Jalali SF, Mardani F, et al. Blood group genotyping in alloimmunized multi-transfused thalassemia patients from Iran. Mol Genet Genomic Med 2021;9:e1701.
15. Reid ME. Applications and experience with PCR-based assays to predict blood group antigens. Transfus Med

Hemother 2009;36:168-78.
16. Dhawan HK, Kumawat V, Marwaha N, Sharma RR, Sachdev S, Bansal D, et al. Alloimmunization and autoimmunization in transfusion dependent thalassemia major patients: Study on 319 patients. Asian J Transfus Sci 2014;8:84-8.
17. Palacajornsuk P, Nathalang O, Tantimavanich S, Bejrachandra S, Reid ME. Detection of MNS hybrid molecules in the Thai population using PCR-SSP technique. Transfus Med 2007;17:169-74.
18. Nathalang O, Khumsuk P, Chaibangyang W, Intharanut K. Characterization of GYP(B-A-B) hybrid glycophorins among Thai blood donors with Mia-positive phenotypes. Blood Transfus 2024;22:198-205.
19. International Society of Blood Transfusion. MNS blood group alleles (ISBT 002), version 5.7 [Internet]. 2023 [cited 2026 Apr 21]. Available from: https://www.isbtweb.org/resource/002mnsalleles.html.
20. Smythe JS, Anstee DJ. Expression of C antigen in transduced K562 cells. Transfusion 2001;41:24-30.
21. Skov F, Hughes-Jones NC. Observations on the number of available C antigen sites on red cells. Vox Sang 1977;33:170-4.
22. Wei L, Zhu S, Wen J, Liao Z, Luo G, Ji Y. The study of variant s antigen expression revealing a novel c.160C>T (p.Arg54Cys) variant on GYPB*s allele associated with partial s phenotype. Transfusion 2024;64:380-7.
23. Wester ES, Storry JR, Olsson ML. Characterization of Jk(a+(weak)): A new blood group phenotype associated with an altered JK*01 allele. Transfusion 2011;51:380-92.