RBC Antigen Determination of Thalassemia Patients using Leansequencing process
Afzaal Memorial Thalassemia Foundation (AMTF) Karachi.

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Primary Team of the study

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Primary Investigator - *Dr. Asim Qidwai*, **Co-investigators**: Dr. Neelum Mansoor, Anwari Syeda, (Pak)

Joint Project

Afzaal Memorial Thalassemia Foundation (Pak)
Biomolecular Analytics (U.S.A)
SKH Foundation (U.S.A)
Introduction

- Thalassemia is the most common inherited blood disorder in Pakistan with a carrier rate of 7%
- Red cell alloimmunization is a serious problem in chronically transfused patients
- 8.6% develop antibodies to red cell antigens
- Accurate determination of red blood cell antigens is very important in multitransfused patients including beta thalassemics.
- Need to design transfusion therapy with reduced risk of alloimmunization
• DNA based methods; provide reliable information, not dependent on serologic immunoglobulin reagents
• Results are not affected by presence of circulating transfused RBCs, positive DAT and alloantibodies
  – Identification of variant antigens, weak expression, null phenotype; a major limitation of serological testing
• By developing an extended blood group genotyping system, incidence of acute and delayed hemolytic transfusion reaction can be decreased
## Applications of red cell genotyping

<table>
<thead>
<tr>
<th>Indication</th>
<th>Subject</th>
</tr>
</thead>
<tbody>
<tr>
<td>Determining blood group of recently transfused patient</td>
<td>Patient</td>
</tr>
<tr>
<td>Determining frequency of blood group polymorphisms in a population</td>
<td>Donors</td>
</tr>
<tr>
<td>Screening blood donors to find rare blood group phenotypes</td>
<td>Donors</td>
</tr>
<tr>
<td>Optimize the inventory of multiple antigen negative screened units</td>
<td>Patients/Donors</td>
</tr>
<tr>
<td>Blood group typing of patients with autoimmune hemolytic anemia</td>
<td>Patients</td>
</tr>
<tr>
<td>Extensive blood group typing of donors for alloimmunized patients</td>
<td>Donors/Patients</td>
</tr>
<tr>
<td>Provides blood types when antisera are not available</td>
<td>Donor/Patient</td>
</tr>
<tr>
<td>Fetal DNA-typing</td>
<td>Foetus</td>
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</tbody>
</table>

- Determining blood group of recently transfused patient: Helps in identifying the blood group of patients who have received blood transfusions recently.
- Determining frequency of blood group polymorphisms in a population: Useful for understanding the genetic diversity within a population.
- Screening blood donors to find rare blood group phenotypes: Helps in identifying blood donors with rare phenotypes.
- Optimize the inventory of multiple antigen negative screened units: Assists in managing blood inventory efficiently.
- Blood group typing of patients with autoimmune hemolytic anemia: Essential for patients suffering from autoimmune hemolytic anemia to ensure correct blood matching.
- Extensive blood group typing of donors for alloimmunized patients: Important for patients who are alloimmunized to ensure safe blood transfusions.
- Provides blood types when antisera are not available: Helps in blood typing when specific antisera are not available.
- Fetal DNA-typing: Useful in identifying the blood group of the fetus.
Objective

• To determine red blood cell antigen profile of thalassemia patients by leansequencing process.
• To reduce post-transfusion complications results from subtle antigenic differences between donors and patients.
Materials and Methods

• Total 304 paediatric and adolescent beta thalassemia patients of diverse ethnic background were included in the study.

• Duplicate bar coded buccal swab samples were collected.

• Crude extract was amplified without DNA purification in a multiplexed PCR reaction.

• Red cell antigens of MNS, RHCE, LU, KEL, FY, JK, DI, YT, DO and CO along with 32 RHCE alleles were determined.
Materials and Methods

• The antigen detection was configured optimally per the antigen frequencies in the target population.

• Samples were analyzed by novel Leansequencig process, developed at Biomolecular Analytics.
• It achieves scalability by simultaneously determining multiple variants for multiple samples, using gene specific PCR.
• Genotypes were determined and phenotypes were predicted by proprietary software.
LeanSequencing (BioMolecularAnalytics)

The protocol has 3 analytical steps

- Single multiplex PCR with barcoding of amplicons
- Allele-specific amplification and labeling of pooled amplicons
- Read-out by capillary electrophoresis
- Phenotype prediction by proprietary software
# Results

<table>
<thead>
<tr>
<th>Antigens</th>
<th>Frequency of Negative Phenotype</th>
<th>Antigens</th>
<th>Frequency of Negative Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>98%</td>
<td>Dob</td>
<td>19%</td>
</tr>
<tr>
<td>Ytb</td>
<td>86.5%</td>
<td>M</td>
<td>14%</td>
</tr>
<tr>
<td>E</td>
<td>70%</td>
<td>Fya</td>
<td>13%</td>
</tr>
<tr>
<td>S</td>
<td>44%</td>
<td>C</td>
<td>12.5%</td>
</tr>
<tr>
<td>N</td>
<td>42%</td>
<td>s</td>
<td>7%</td>
</tr>
<tr>
<td>Fyb*</td>
<td>38.6%</td>
<td>Jka</td>
<td>6.4%</td>
</tr>
<tr>
<td>Jkb</td>
<td>24%</td>
<td>e</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Doa</td>
<td>21%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>20%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*including 13 instances of GATA silencing mutation and 3 of 265C>T
None of the patients had variant RHCE alleles encoding partial phenotypes, while interestingly these patients were highly polymorphic for c.48G>C (RHCE*01.01) known to be associated with "weak e" phenotype.
A comparison of the frequencies shows that, for most of the principal antigens, frequencies for our patients are lower than those for Europeans (EUR) and Africans (AFA), with some exception.
No significant difference in allele frequencies was observed between the 6 principal ethnicities in our cohort.
Review

Emerging strategies of blood group genotyping for patients with hemoglobinopathies

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\textbf{ARTICLE INFO}

\textbf{ABSTRACT}

Red cell alloimmunization is a serious problem in chronically transfused patients. A number of high-throughput DNA assays have been developed to extend or replace traditional serologic antigen typing. DNA-based typing methods may be easily automated and multiplexed, and provide reliable information on a patient. Molecular genotyping promises to become cheaper, being not dependent on serologic immunoglobulin reagents. Patients with hemoglobinopathies could benefit from receiving extended genomic typing. This could limit post-transfusional complications depending on subtle antigenic differences between donors and patients. Patient/donor compatibility extended beyond the phenotype Rh/Kell may allow improved survival of transfused units of red blood cells (RBC) and lead to reduced need for blood transfusion and leading to less iron overload and reduced risk of alloimmunization. Here we discuss...
Molecular blood genotyping in patients with Thalassemia major in Tehran Adult Thalassemic Clinic

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²Tehran Adult thalassemia Clinic, Iran
³England Blood Transfusion Organization, Bristol, UK
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Abstract

Background and Objectives
Blood typing by serologic methods after transfusion has limitations due to presence of donor red cells in recipients. Accurate determination of red blood cells (RBCs) antigens is very important in multitransfused patients including beta-thalassemics and sickle cell anemics. So, the aim of this study was to evaluate DNA-based methods as supplement to the hemagglutination technique to determine the red blood cell (RBC) antigen profile of multitransfused patients with beta-thalassemia.
Allele-specific oligonucleotide polymerase chain reaction for the determination of Rh C/c and Rh E/e antigens in thalassaemic patients

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Background. Thalassaemia is a genetic disease in which there is a relative or complete lack of alpha or beta globin chains. Patients with moderate to severe forms of thalassaemia need transfusions from the early years of life. Antibody production against blood group antigens may cause many problems in preparing compatible blood units for transfusion. The identification of definite blood group phenotypes by the haemagglutination method can be difficult because of the mixed population of red blood cells from the donor and recipient.

Materials and methods. Forty multiply transfused thalassaemic patients, and ten healthy
Red Cell Genotyping by Multiplex PCR Identifies Antigen-Matched Blood Units for Transfusion-Dependent Thai Patients

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*b National Blood Centre, Thai Red Cross Society, Bangkok, Thailand;
*c Blood Bank Section, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand
Summary
Background: Antigen-negative red cell transfusion is required for transfusion-dependent patients. We developed multiplex PCR for red cell genotyping and calculated the possibility of finding compatible predicted phenotypes in Thai blood donor populations according to red cell alloantibodies found among Thai patients. Methods: 600 DNA samples obtained from unrelated healthy central and northern Thai blood donors were tested with the newly developed multiplex PCR for FY*A, FY*B, JK*A, JK*B, RHCE*e, RHCE*E, DI*A and GYP*Hut, GYP*Mur, GYP*Hop, GYP*Bun, and GYP*HF allele detections. Additionally, the possibility of finding compatible predicted phenotypes in two Thai blood donor populations was calculated to estimate the minimal number of tests needed to provide compatible blood. Results: The validity of multiplex PCR using known DNA controls and the phenotyping and genotyping results obtained by serological and PCR-SSP techniques were in agreement. The possibility of finding at least one compatible blood unit for patients with multiple antibodies was comparable in Thai populations. Conclusions: The multiplex PCR for red cell genotyping simultaneously interprets 7 alleles and 1 hybrid GP group. Similar strategies can be applied in other populations depending on alloantibody frequencies in transfusion-dependent patients, especially in a country with limited resources.

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High-throughput multiplex PCR genotyping for 35 red blood cell antigens in blood donors

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2Department of Blood Group Serology and Transfusion Medicine, Medical University of Vienna, Vienna, Austria

Background and Objectives One to two per cent of patients in need of red cell transfusion carry irregular antibodies to red blood cell (RBC) antigens and have to be supplied with specially selected blood units. To be able to respond to those requests, blood centres have to screen a significant number of donors for a variety of antigens serologically, which is a costly and through the shortage of reagents, also limited procedure. To make this procedure more efficient, the Austrian Red Cross has developed a genotyping assay as an alternative approach for high throughput RBC typing.
Importance of Extended Blood Group Genotyping in Multiply Transfused Patients

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Overview

The protocol offers a fast and cost effective solution to analyze hundreds of samples in 24 hours by providing:

- Lean workflow
- Pooled sample analysis
- Rapid determination of extended patient and donor RBC antigen profiles for management of chronically transfused patients
Limitations

• High cost
• Turnaround time
• Need for specialized equipment
• Trained technologists
• Expertise for interpretation
• Absence of information systems for handling the results
Conclusion

• Our analysis indicated that, Pakistani thalassemia patients are polymorphic for only a limited set of clinically significant RBC antigens known to be associated with alloimmunization including RHCE (Cc,Ee), K (Kk, Kpa,b), JK, FY, MNS and YT.

• The configurability of LeanSequencing allowed us to customize a set of polymorphic markers

• These markers are polymorphic in all the principal ethnic groups, the selection of candidate donors without taking into account their antigen expression profiles, exposes patients to significant alloimmunization risk
• Our customized LeanSequencing application will facilitate the cost-effective, large-scale screening of blood donors to design individualized transfusion management programs for our patients.

• Under such a program:
  – each newly diagnosed patient will be analyzed for the selected RBC antigens,
  – suitable donors will be chosen, from the screened candidate donors (Hashmi et al, AABB2017) to minimize the risk of alloimmunization.
Future perspective

• This approach of genotype matching of donors and recipients is currently not practical, but it may become standard practice when molecular typing for clinical applications implemented at affordable cost.

• Large scale donor genotyping is necessary to determine the clinical impact that RBC genotyping may have in patients with hemoglobinopathies.
Integrating Molecular Technologies for Red Blood Cell Typing and Compatibility Testing Into Blood Centers and Transfusion Services

Christopher D. Hillyer, Beth H. Shaz, Anne M. Winkler, and Marion Reid

Fig 1. Stepwise process for the implementation of molecular testing in the donor center.
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Thank you