RBC Antigen Determination of Thalassemia Patients using Leansequencing process

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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 includind 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

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

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 Biomoleculer 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

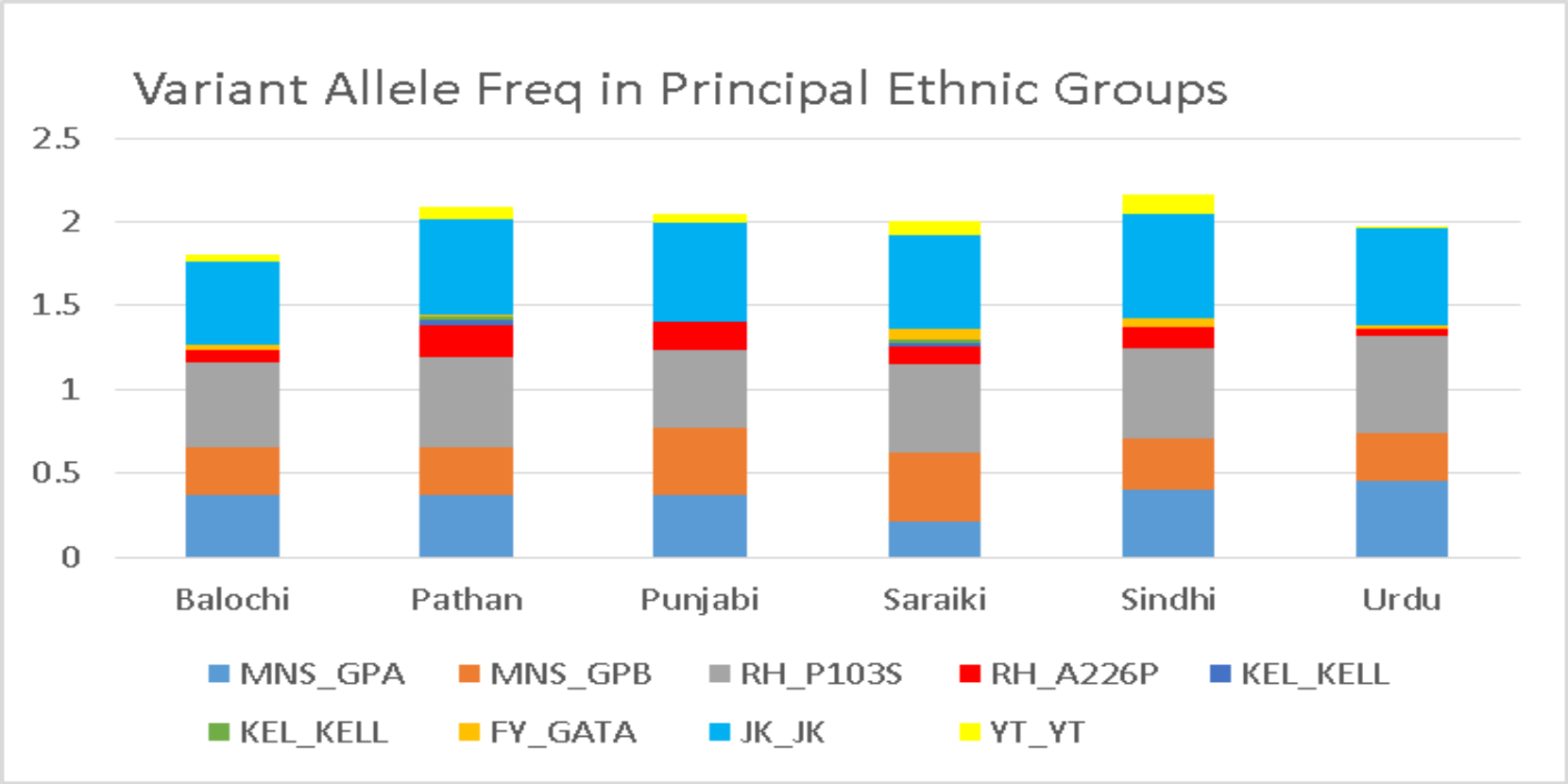
Antigens	Frequency of Negative Phenotype	Antigens	Frequency of Negative Phenotype
K	98%	Dob	19%
Ytb	86.5%	M	14%
E	70%	Fya	13%
S	44%	C	12.5%
N	42%	s	7%
Fyb*	38.6%	Jka	6.4%
Jkb	24%	e	<1%
Doa	21%		
c	20%		

*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.

FREQ > EUR:	N-, Fyb-	> AFA:	
FREQ < EUR:	M-, s-, C-, Fya-, Jka-, Yta-	< AFA:	S-, C-, Fya-, Fyb-, Jkb-

No significant difference in allele frequencies was observed between the 6 principal ethnicities in our cohort.



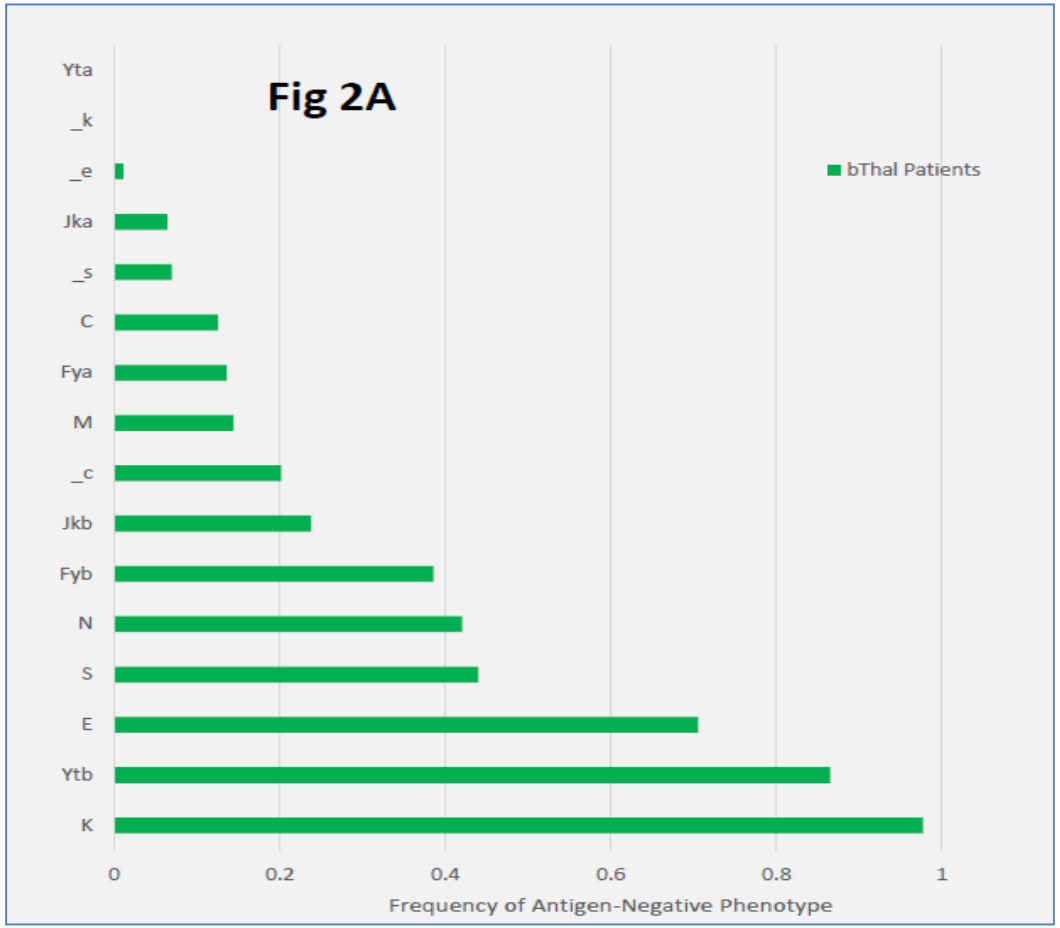


Fig 2A
Yta
_k
_e
Jka
_s
C
Fya
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_c
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N
S
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bThal Patients
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0.8
1
Frequency of Antigen-Negative Phenotype

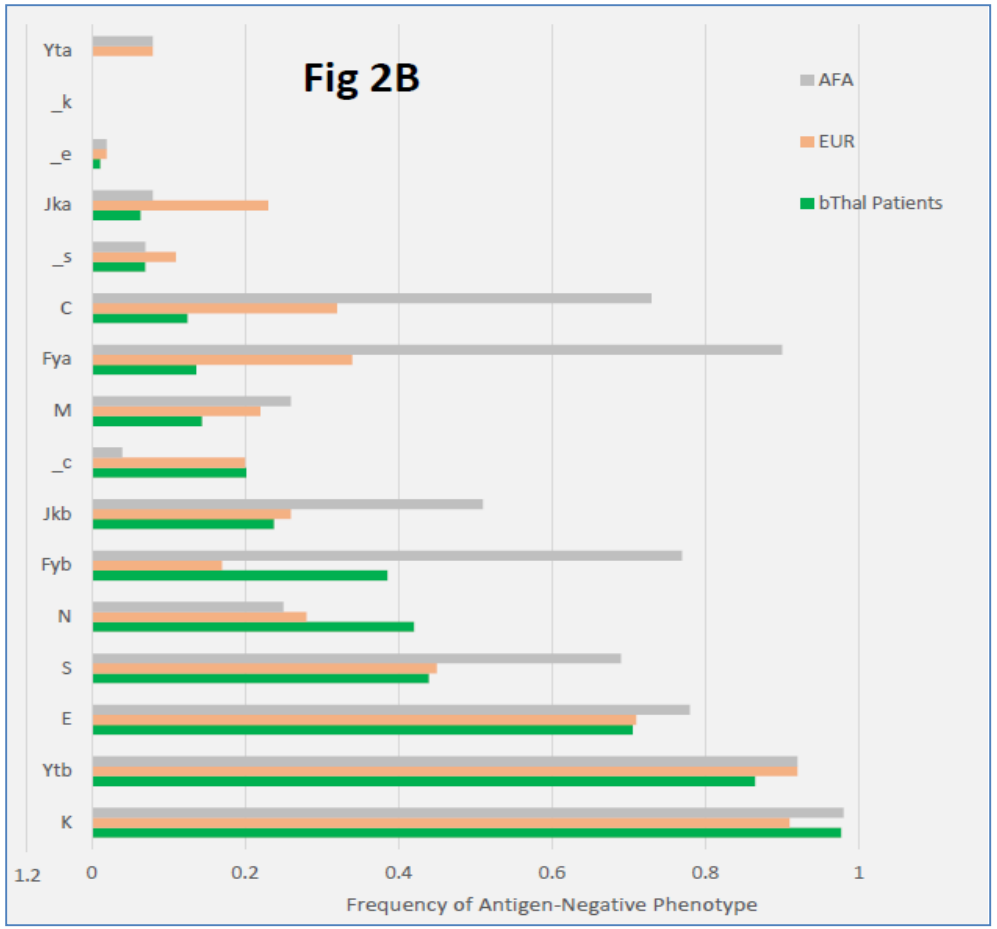
Fig 2B
Yta
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Jka
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bThal Patients
1.2
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0.2
0.4
0.6
0.8
1
Frequency of Antigen-Negative Phenotype

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Review

Emerging strategies of blood group genotyping for patients with hemoglobinopathies

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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 allows 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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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

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Red Cell Genotyping by Multiplex PCR Identifies Antigen-Matched Blood Units for Transfusion-Dependent Thai Patients

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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.

VoxSanguinis
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ISBT International Society of Blood Transfusion

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ORIGINAL PAPER

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

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Vox Sanguinis

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 affective 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 moleculer 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

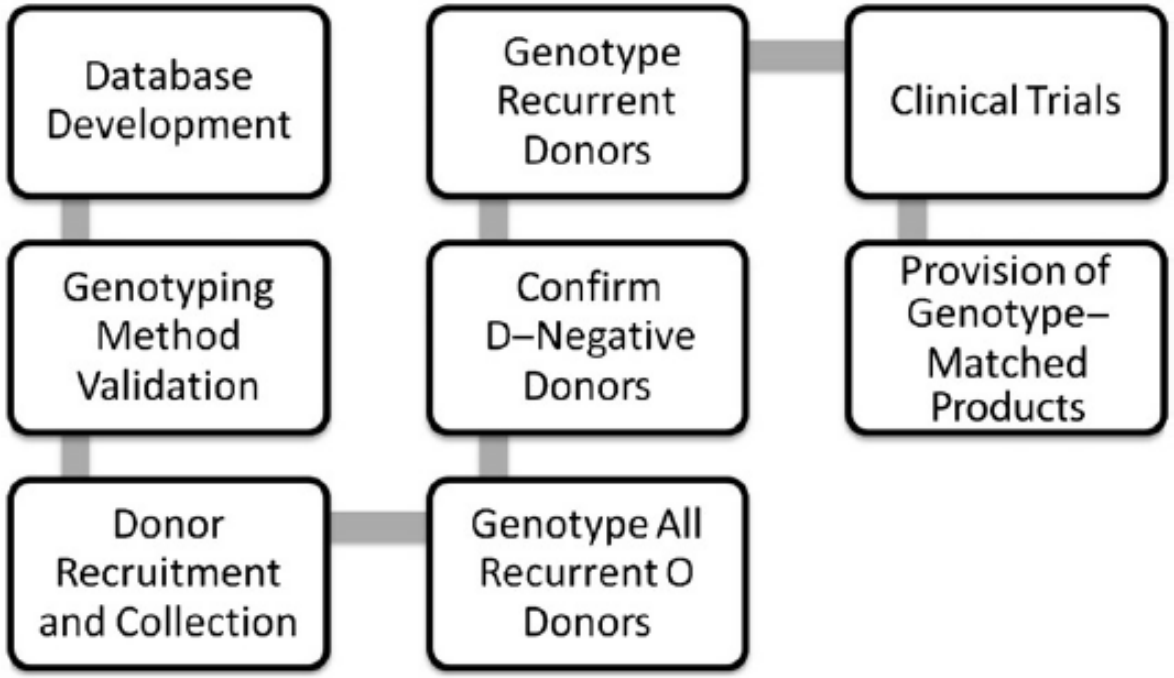

Fig 1. Stepwise process for the implementation of molecular testing in the donor center.

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