Objectives

1) Examine the differences between phenotype and genotype testing.
2) Discuss the advantages of genotype testing in certain disease states.
3) Compare the red cell molecular assays that are currently approved by the FDA.

Molecular Testing in the Blood Bank Laboratory

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CLPC, Spring 2019

Molecular diagnostic testing

- From the research lab to the clinical lab
- Sample-to-answer diagnostics
- Accurate, actionable information
- Genetic differences, mutations, disease, cell growth, cancer onset, blood disorders...

Blood groups are biodeterminants

- Protein products = biomarkers in plasma and cell surface
  - Antigens on red cells
  - Antibodies formed in response to antigens
Chromosomes and blood groups

- ABO genes on chromosome 9
- Rh genes on 1
- Kell genes on 7
- Duffy genes on 1
- MNS genes on 4
- Kidd genes on 18
- Lewis genes on 19

- Most blood groups are autosomal
- Only 1 blood group is X-linked

Molecular basics

- RNA splicing
- Translation

Molecular testing in the Blood Bank

- RBC genotyping
  - Determined by single nucleotide polymorphisms (SNPs)
  - Predict the red cell phenotype
  - Using donor or patient DNA
What are SNPs?

- Most common type of genetic variation
- Single base-pair change in DNA sequence
- Occur normally throughout a person’s DNA
- Most occur in non-coding region
- Act as biomarkers
  - Predict response to drugs, toxins
  - Predict risk of disease development
  - Track inheritance of disease genes within families

Human blood groups

- 33 blood group systems
- > 300 antigens
- Population specific
- Markers for ethnicity
  - Diego antigen among people of Mongolian origin
  - Kell Js* antigen exclusive to those of African descent
  - Kidd Jk(a+b+) frequent in Asians and Caucasians
  - Duffy Fy(a-b-) frequent in those of African descent
Things are not always as they seem...

- Traditional BB testing reveals PHENOTYPE (observed characteristics)
  - RBC reaction with antisera (antigens)
  - Plasma reaction with reagent RBC (antibodies)

- Antigens are a collection of epitopes based on the GENOTYPE (actual genes inherited)

## ABO Phenotypes and Genotypes

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>AA or AO</td>
</tr>
<tr>
<td>B</td>
<td>BB or BO</td>
</tr>
<tr>
<td>AB</td>
<td>AB</td>
</tr>
<tr>
<td>O</td>
<td>OO</td>
</tr>
</tbody>
</table>

### Predicting ABO genotypes

- **Punnett square**
  - Gives visual portrayal of offspring potential genotypes
  - Calculate the frequencies of different genotypes and phenotypes of offspring
  - Predict probable genotypes of parents

### Practice

1) Predict possible genotype for the parents, given the following children.

2) Predict possible genotypes of the offspring, given the following parents.

Mendelian law of inheritance, co-dominant expression
## It’s not always that simple...

### Relationship between blood groups and disease/disability

- Many blood groups discovered after some adverse event(s)
- Transfusion reactions
- Hemolytic disease of the fetus and newborn
- Malignancy, cellular adhesion, infectious disease (i.e. West Nile Virus, Zika Virus)
- Management of transfusion dependent diseases

### Transfusion dependent diseases

- Sickle cell disease
- Cancer
- Myelodysplastic diseases
- Various anemias, thalassemia

### Alloimmunization is a major concern

- Worldwide rates in sickle cell patients range from 4.4% - 76%
  - Standard cases range from 2 – 6%
  - Transfusion dependent cases ~ 36%

- Occurs in 15% of patients with myelodysplastic syndrome or chronic myelomonocytic leukemia

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### Transfusion Chart

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCE/DCE</td>
<td>Normal</td>
</tr>
<tr>
<td>DCE/dCE</td>
<td>Rh Negative</td>
</tr>
<tr>
<td>DCE/DcE</td>
<td></td>
</tr>
<tr>
<td>DcE/DcE</td>
<td></td>
</tr>
<tr>
<td>DcE/dcE</td>
<td></td>
</tr>
<tr>
<td>DcE/Dce</td>
<td></td>
</tr>
<tr>
<td>Dce/Dce</td>
<td></td>
</tr>
<tr>
<td>Dce/dce</td>
<td></td>
</tr>
<tr>
<td>dce/dce</td>
<td></td>
</tr>
</tbody>
</table>

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The usual suspects

- Rh blood group
  - 54 antigens identified to date
  - D, C, c, E, e

- Kell blood group
  - 36 antigens identified to date
  - K, k (cellano)

Alloimmunization risk

- Most alloantibodies are formed towards Rh and Kell systems
  - Followed by MNS, Kidd, and Lewis

- Other risk factors include age, gender, and number of transfusions

- Some studies reduce rates by matching units for Rh and Kell

Alloimmunization in the chronically transfused patient

- Requires extended test time for identification
- May require blood separation techniques
- Delays treatment (transfusion)
- Increases healthcare cost

- Average cost for treating SCD up to age 45 is about $1 million
- 90,000 – 100,000 SCD patients in the US

Alloimmunization prevention:
Phenotypically matching blood

- Crossmatching, selecting Ag-neg products
- Widely used, effective

- Should be performed prior to transfusion and formation of antibodies

- May require additional time and resources for the chronically transfused patient
Alloimmunization prevention: Cost effectiveness

- Reagent availability, tech time
- CMS reimbursement rates
- Phenotypic matching is variable, esp in AA
  - High frequency of variant Rh alleles in SCD

- Genotypic matching may save money
  - Not limited by common interferences
  - Recipient and donor are only genotyped once

Genotyping and Sickle Cell Disease

- Sickled cells occlude blood vessels
- Anemia, pain
- Treatment is dependent on transfusions
- Many patients develop multiple antibodies, esp to Rh and Kell
- Matching based on donor pool

Disparities in blood donations

- Majority of donors are Caucasian
- AA donors are under-represented
- Minority donors are less likely to become regular donors

- ~70% of AA are type O and B
- ~56% of Caucasians are type O and B

Blood types by ethnicity

<table>
<thead>
<tr>
<th>Blood Type</th>
<th>Caucasian (%)</th>
<th>AA (%)</th>
<th>Asian (%)</th>
<th>Hispanic (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A positive</td>
<td>33</td>
<td>24</td>
<td>27</td>
<td>29</td>
</tr>
<tr>
<td>A negative</td>
<td>7</td>
<td>2</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>B positive</td>
<td>9</td>
<td>18</td>
<td>25</td>
<td>9</td>
</tr>
<tr>
<td>B negative</td>
<td>2</td>
<td>1</td>
<td>0.4</td>
<td>1</td>
</tr>
<tr>
<td>AB positive</td>
<td>3</td>
<td>4</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>AB negative</td>
<td>1</td>
<td>0.3</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>O positive</td>
<td>37</td>
<td>47</td>
<td>39</td>
<td>53</td>
</tr>
<tr>
<td>O negative</td>
<td>8</td>
<td>4</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>
The SCD Patient versus the Donor

<table>
<thead>
<tr>
<th>Blood group</th>
<th>AA patient</th>
<th>Caucasian donor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh group</td>
<td>Dce</td>
<td>DCe</td>
</tr>
<tr>
<td>Kell</td>
<td>98% are K neg</td>
<td>10% are K pos</td>
</tr>
<tr>
<td>Duffy</td>
<td>68% are Fy(a-b-)</td>
<td>50% are Fy(a+b+)</td>
</tr>
<tr>
<td>Kidd</td>
<td>57% are Jkb neg</td>
<td>72% are Jkb pos</td>
</tr>
<tr>
<td>MNS</td>
<td>31% are S neg</td>
<td>55% are S pos</td>
</tr>
<tr>
<td></td>
<td>1% are U neg</td>
<td>99% are U pos</td>
</tr>
</tbody>
</table>

Genotyping and Rhogam use

- Rhogam used for Rh-neg individual:
  - After delivery of Rh-pos baby
  - Routine prevention at 28 weeks gestation
  - Fetomaternal bleeding (various conditions)
  - Actual or threatened pregnancy loss
  - Ectopic pregnancy
  - After incompatible transfusion of Rh-pos blood products

- Lack of standardization for Rhogam workups
- Weak D phenotypes

- Genotyping to confirm RhD status
  - Avoid 24,700 unnecessary RhIG injections
  - Save $858,078 - $2,356,133
  - Release 48,000 units of RhD negative blood

Genotyping and Multiple Myeloma

- Blood cancer of malignant plasma cells in the bone marrow
- Darzalex (daratumumab) monoclonal antibody treatment (anti-CD38)
Genotyping and Multiple Myeloma

• RBC membrane has CD38 receptors
• Darzalex (anti-CD38) will bind to RBC
  – Causes positive indirect AHG testing (screens/panels)
  – May persist up to 6 months following last treatment
  – Type & screen recommended prior to treatment

Changing the paradigm

• Two red cell genotyping assays approved by the Food and Drug Administration (FDA)
• Detection of single nucleotide polymorphisms (SNPs) on selected genes
• Allows for the prediction of RBC phenotype

Genotyping and Multiple Myeloma

• What if blood is needed during Darzalex treatment?
  – Challenge to blood bank
  – Must determine if there is new underlying antibody
  – DTT is limited
  – Neutralizing anti-CD38 is limited

Blood group genotyping

• Not limited by alloimmunization
• Not limited by mixed RBC population
• No limited by interference of drugs
• DNA can be obtained anytime to determine genotype of red cell antigens
• Blood centers can initiate genotyping on small sets of reliable donors
Immucor PreciseType HEA Molecular BeadChip Test

- FDA approval on May 21, 2014
- Molecular determination of allelic variants
- Predict 35 erythrocyte antigen phenotypes
- Utilizes polymerase chain reaction (PCR) amplification of DNA coupled with elongation of polymorphisms
- Fluorescently labeled
- Compare emissions to image map

Molecular BeadChip

- Ultra-violet, blue, green fluorescent dyes
- Each bead has > 1 M copies of oligonucleotide probes bound to surface
- ~ 4,000 beads are randomly affixed to a silicon chip
- BeadChips are then bonded to slide or plate

Immucor PreciseType HEA Molecular BeadChip Test

- Throughput:
  - 8-test slide or 96-test slide
  - Generate over 3000 results across 36 RBC antigens from a single test

- Turn Around Time: 6 hours
Immucor PreciseType HEA Molecular BeadChip Test

Rh    Kell    Duffy    Kidd
MNS    Lutheran    Dombrock    Landsteiner Wiener
Diego    Colton    Scianna

Detects the Hgb S mutation in the Beta Globin gene

PreciseType Overview

Extract DNA 1 hour
Multiplex PCR 2 hours
Post PCR processing 2 hours
Image Analysis 1 hour

DNA extraction

Specimen collection and prep

Sample
EDTA whole blood

Storage
-20°C or colder

Problems
PCR inhibitors
Citrate
Heparin

Quantity
≥ 15 ng/µL extracted genomic DNA
Multiplex PCR

- Chain reaction makes millions of copies of DNA
- Mutations of interest within amplified segments
- Enzymes separate helix into single strands
- Single DNA strand is combined with reagent
- Pipette onto each BeadChip

Hybridization

- BeadChips placed in hybridization oven
- DNA sequences hybridize to oligonucleotide probes
- If DNA sequence matches probe, probe is elongated with fluorescently tagged nucleotides
- If no match, no elongation

Analysis and Results

- Reader detects fluorescent signal in assay image
- Determines which probe signal associated with
- Software algorithms detail genotype and phenotype

Progenika Biopharma ID Core XT

- FDA approval on October 11, 2018
- Qualitative PCR and hybridization genotyping
- Luminex flow cytometric system
- Provides results for:
  - 29 genetic polymorphisms
  - 53 alleles
  - 37 antigens (of 10 blood groups)
**Progenika Biopharma ID Core XT**

- **Throughput:**
  - 96-well plate
  - 37 antigens yields > 3550 results

- **Turn Around Time:** 4-5 hours

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**ID Core XT Overview**

- **Extract DNA:** 30 mins
- **PCR amplification:** 2.5 hours
- **Hybridization & Labeling:** 1 hour
- **Result Analysis:** 30-45 mins

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**Multiplex PCR**

- Chain reaction makes millions of copies of DNA
- Mutations of interest within amplified segments
- Enzymes separate helix into single strands
- PCR products get bound to microspheres on plate
Hybridization

- PCR products hybridized to probes on microspheres
- Microspheres have unique ratio of fluorescent dyes
- Hybridized DNA is labeled with fluorescent conjugate (reporter)

Analysis and Results

- Green laser quantifies bound reporter molecules
- Red laser identifies target bead i.e. the location within microspheres
- Software outcome is polymorphism genotype, predicted allele genotype and predicted phenotype

BB Molecular testing Overview

- Patient DNA + primers
- PCR
- Hybridize to probes on microbeads
- Add fluorescent labels
- Analysis of fluorescence emission

Benefits of Molecular Testing

**Donor Testing**

- Resolve typing discrepancies
- Antigen testing when antisera is unavailable
- Identify RBC-matched blood for chronically transfused patients
- Adaptable to high throughput platforms and multiplex assays
Benefits of Molecular Testing

Patient Testing
- Resolve typing discrepancies
- Antigen testing when antisera is unavailable
- Predict correct RBC phenotype of:
  - Multiply transfused patient
  - Patient with autoantibodies
  - Patient with positive DAT

- Perform extended RBC phenotype to provide antigen matched blood products

Evolving Transfusion Medicine
- Mass screening of blood donors
- Antigen testing when antisera is not available
- Improve alloimmunization prevention
- Enhanced reagent cell panels
- Accurately identify variant alleles
- NextGen sequencing becomes affordable and standard
- Transfusion recipients can be matched to selected genotyped donors
- Especially important for transfusion-dependent diseases

Questions and Open Discussion

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