Who Is Right - DNA or Serology?

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Genotype vs. Phenotype

- Repeat serological typing on DNA sample
- Check donor records, typed more than once?
- Repeat microarray genotype
- Test with other examples of antibodies, lectins, etc.
- Perform additional molecular testing

Duffy Discrepancy

- A donor unit is on the shelf labeled as Fy(a-b-)
- Genotype shows the donor to be homozygous FY*B
- Who is correct?
Duffy (DARC) Protein

- MW 35-43 kD
- 336 aa, 7 membrane spanning domains
- Fy3 on 3rd loop
- Fy6 = aa 19-25
  - Used by P. vivax for RBC invasion
- HIV secondary receptor
- Chemokine receptor

FY Gene (1q22-23)

- Cap site: binds the ribosome
- Promoter: signals polymerase to begin synthesis
- Enhancer: up-regulates translation into protein

FY*X (Weak Duffy b)

mRNA

<table>
<thead>
<tr>
<th>Exon 1</th>
<th>Exon 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>G=A</td>
<td>C&gt;T</td>
</tr>
<tr>
<td>125</td>
<td>285</td>
</tr>
<tr>
<td>298</td>
<td></td>
</tr>
</tbody>
</table>

FY GP NH2

<table>
<thead>
<tr>
<th>42</th>
<th>89</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gly&gt;Asp</td>
<td>Arg&gt;Cys</td>
<td>Ala&gt;Thr</td>
</tr>
</tbody>
</table>
Duffy Genes & Terminology

<table>
<thead>
<tr>
<th>Nucleotide Position</th>
<th>Amino Acid #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene</td>
<td>-67 125 265 42 89</td>
</tr>
<tr>
<td>FY*A</td>
<td>T G T Gly Arg Fy^a</td>
</tr>
<tr>
<td>FY*B</td>
<td>T A T Asp Arg Fy^b</td>
</tr>
<tr>
<td>FY*X</td>
<td>T A C Asp Cys Fy^bw</td>
</tr>
<tr>
<td>FY*Fy</td>
<td>C G/A T Gly/Arg Arg None</td>
</tr>
</tbody>
</table>

Clinical Relevance

- Patients with silenced FY*B due to mutant GATA are genetically Fy(b+)
- Fy(b+) can be safely transfused to these patients
- Castilho reported 28 patients with silenced FY*B who had not produced anti-Fy or anti-Fy3 following multiple transfusions
  
  (Transfusion 2007;47(supp 1):28S-31S)

So Who is Right?

- Serological: **Right**
- Molecular: **Right**
Kidd Discrepancy

- Caucasian patient has a history of multiple antibodies
- Due to recent transfusions a genotype was ordered
  - Genotype is JK*A/JK*B
  - Predicted phenotype is Jk(a+b+)
- Jk^b is discrepant from hospital records

Kidd Protein

- MW = 43,000; 389 aa
- Jk^null has delayed lysis in 2 M urea
- Functions as a urea transporter
- JK gene is at 18q11-12
  - 30 kb; 11 exons
  - Jk^{ab} snp = G838A
  - Asp 280 changed to Asn

JK^A Null Alleles

(ISBT proposed nomenclature)

<table>
<thead>
<tr>
<th>Allele</th>
<th>BP Change</th>
<th>Location</th>
<th>AA Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>JK*01N.01</td>
<td>Δ Exon 4 &amp; 5</td>
<td>Exon 4 &amp; 5</td>
<td>Initiation Absent</td>
</tr>
<tr>
<td>JK*01N.02</td>
<td>202C&gt;T</td>
<td>5</td>
<td>Gln68Stop</td>
</tr>
<tr>
<td>JK*01N.03</td>
<td>582C&gt;G</td>
<td>7</td>
<td>Tyr194Stop</td>
</tr>
<tr>
<td>JK*01N.04</td>
<td>956C&gt;T</td>
<td>10</td>
<td>Thr319Met</td>
</tr>
<tr>
<td>JK*01N.05</td>
<td>561C&gt;A</td>
<td>7</td>
<td>Tyr187Stop</td>
</tr>
</tbody>
</table>
JK* B Null Alleles

<table>
<thead>
<tr>
<th>Allele</th>
<th>BP Change</th>
<th>Location</th>
<th>AA Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>JK*2N.01</td>
<td>IVS5-1g&gt;a</td>
<td>Intron 5</td>
<td>Skip ex. 6</td>
</tr>
<tr>
<td>JK*2N.02</td>
<td>IVS5-1g&gt;c</td>
<td>Intron 5</td>
<td>Skip ex. 6</td>
</tr>
<tr>
<td>JK*2N.03</td>
<td>222C&gt;A</td>
<td>5</td>
<td>Asn74Lys</td>
</tr>
<tr>
<td>JK*2N.04</td>
<td>IVS7+1g&gt;c</td>
<td>Intron 7</td>
<td>Skip ex. 7</td>
</tr>
<tr>
<td>JK*2N.05</td>
<td>723delA</td>
<td>8</td>
<td>Ile262Stop</td>
</tr>
<tr>
<td>JK*2N.06</td>
<td>871C&gt;T</td>
<td>9</td>
<td>Ser291Pro</td>
</tr>
<tr>
<td>JK*2N.07</td>
<td>896G&gt;A</td>
<td>9</td>
<td>Gly299Glu</td>
</tr>
<tr>
<td>JK*2N.08</td>
<td>956C&gt;T</td>
<td>10</td>
<td>Thr319Met</td>
</tr>
</tbody>
</table>

Additional Typing

• Genotyping repeated using a different molecular platform
  - Predicted phenotype now is Jk(a+b-)
• DNA assay #1 does not detect silenced JK
• DNA assay #2 detects common Finnish mutation (JK*2N.06)

So Who is Right?

• Serological: Right
• Molecular #1: Wrong
• Molecular #2: Right
MN Discrepancy

- A blood center is using microarray to screen for rare donors
- As part of their review process, the genotypes are checked against existing donor records
- It is noted that several African-American donors type as N negative by DNA but positive by serology

MNS (GYPA/GYPB) Proteins

- GPA MW = 43,000; 131 aa
- GPB MW = 25,000; 72 aa
- Genes at 4q28.2-31.1
  - GYPA = 7 exons
  - GYPB = 5 exons, plus 1Ψ
- Many hybrids:
  - Dantu (GP B-A)
  - Mi III (GP B-A-B)
  - Mi (GP A-B-A)
  - St (GP A-A)
- Enhanced expression of 'N’ may give false positive reactions with anti-N

Additional Typing

- Phenotype repeated using rabbit, lectin and other examples of monoclonal anti-N
- Red cells were treated with ficin and re-tested with Vicia graminea

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td>1+</td>
</tr>
<tr>
<td>MAb #1</td>
<td>2+</td>
</tr>
<tr>
<td>MAb #2</td>
<td>4+</td>
</tr>
<tr>
<td>N lectin</td>
<td>4+</td>
</tr>
<tr>
<td>N lectin- ficin</td>
<td>4+</td>
</tr>
<tr>
<td>Glycine soja</td>
<td>Neg</td>
</tr>
</tbody>
</table>
So Who is Right?

- Serological: **Wrong**
- Molecular: **Right**

Lutheran Discrepancy

- A request is received from the ARDP for a Lu(a-b-) unit
- You type your donor with one set of antisera as Lu(a-b-)
- Because you don’t have additional ABO compatible sera for typing you have a microarray genotype performed
- The donor is homozygous LU*B
  - Predicted phenotype Lu(a-b+)

Lutheran (B-CAM) Protein

- Lu protein is a member of the IgSF
- 78 & 85 kD isoforms
- Gene at 19q13.2-13.3; 15 exons
  - Lu^B SNP is 229A>G
  - aa His77Arg
Lutheran Null Phenotype

<table>
<thead>
<tr>
<th>Gene</th>
<th>Dominant (InLu)</th>
<th>Recessive</th>
<th>X-linked (XS2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EKLF</td>
<td>Lu</td>
<td>None</td>
<td>GATA1</td>
</tr>
<tr>
<td>Lu antigens</td>
<td>Very weak</td>
<td>None</td>
<td>Very weak</td>
</tr>
<tr>
<td>AnWj</td>
<td>Neg/Weak</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>P1, i</td>
<td>Reduced</td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>

EKLF Affect on Blood Groups

- At day 6, In(Lu) samples had decreased expression of 354 genes:
  - GPA (MN)
  - Band 3 (Di)
  - Aquaporin (Co)
  - ADP-RT4 (Do)
  - Basigin (Ok)
  - DARC (Fy)

- Greater reduction at day 11 (normoblast):
  - B-CAM (Lu)
  - CD44 (In)

- Heterozygous for the mutant EKLF
  - 12 different sporadic mutations

So Who is Right?

- Serological: Wrong*
- Molecular: Wrong*
RH Typing Discrepancy

- Your lab follows NIH guidelines for transfusion of sickle cell patients:
  - C/c, E/e, K1 matched units
- Patient serologically typed as C+ so received random C+/ units
- Patient develops allo anti-C

r's Haplotype

- Contains two linked but altered genes
- Hybrid RHD gene:
  - Type 1 = D(1,2,3)-CE("3", 4,5,6,7)-D(8,9,10)
  - Type 2 = D(1,2,3)-CE(4,5,6,7)-D(8,9,10)
- RHCE gene: exon 5 mutation 733C>G (Leu245Val) and exon 7 mutation 1006G>T (Gly336Cys)

So Who is Right?

- Serological: **Wrong**
- Molecular: **Right**
Kell System Discrepancy

- Rare donor screening finds Kp(b-)
- IRL confirms with second anti-Kp\(b\)
- Sent for genotype
  - Genotypes as Kp(a+b+), kk, Js(a-b+)
- Possible explanations
  - Silenced KP*B gene
  - Kmod or McCleod
  - Ko (Kell null)

Additional Testing

- □ Type for allele (Kp\(a\), or Kp\(c\))
  - Kp(a-)
- □ Type for other Kell antigens
  - Js(b+), k+, Ku +
- □ Perform adsorption/elution
  - Did not adsorb/elute anti-k
- □ Confirm genotype with another assay &/or DNA sequencing
  - KEL*02N.02/Kel*01N.New
  - T244C changes Cys 82 to Arg

So Who is Right?

- Serological: Incomplete
- Molecular: Wrong*
Case Study – HA

• Patient HA is a 40 y/o Hispanic male with diagnosis of hemolytic anemia
• Sample submitted to regional IRL for serological investigation

Case Study – cont.

• Initial panel: all cells positive at RT-AHG 2-3+ by tube LISS
• Auto control: 3+
• DAT: Poly= 3+, IgG= 3+, C3= 2+
• Eluate: all cells react 3+

Case Study – cont.

• Antigen typing with monoclonal reagents
  - R\(_2\)R\(_2\), K+, M+N-
• Antigen typing post EGA treated cells
  - Fy(a+b+) Jk(a+b+) S+s+
• IRL suggested DNA typing be performed with next admission to determine k type since chloroquine treatment failed to remove antibody from patient's RBCs
HEA Genotyping

So Who is Right?

• Serological: **Wrong**
• Molecular: **Right**

What caused discrepant results?

- Was there a sample identification error on either of the two samples?
- Was there incomplete removal of bound antibody despite negative DAT?
- Was polyspecific AHG used instead of anti-IgG?
  - Complement would remain on cells post EGA treatment
In the future........

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