What Molecular Has Taught Us About Blood Groups (Old And New)

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Jr Blood Group

• Jr\textsuperscript{a} was first described in 1970 as a high frequency antigen
• Anti-Jr\textsuperscript{a} has caused transfusion reactions but rarely HDFN
• The Jr(a−) phenotype is found Asians (Japanese)
  — also in Northern Europe, Mexico, and Middle East
• Two independent groups identified the Jr\textsuperscript{a} gene
Finding the Jr Gene

- Monoclonal anti-Jr\(^a\) immunoprecipitated a 70 kD protein from cat RBCs
- Mass-Spec identified Abcg2, encoded by the cat ortholog of human transporter gene \(ABCG2\)
- Using homozygosity by descent, 6 Jr(a–) donors were homozygous in a region of chromosome 4q22.1
- This 3.97 kB are contained 4 validated genes: \(MEPE, SPP1, PKD2\) and \(ABCG2\)
  - Only ABCG2 is found on the red cells
What Is ABCG2?

- ATP-binding cassette (ABC) transporter proteins
- A multipass membrane glycoprotein with
  - One nucleotide binding domain
  - One membrane spanning domain
- Also known as breast cancer resistance protein (BCRP)

With permission from Gary E. Kaiser, Ph.D.
The Community College of Baltimore County
Why is Jr\textsuperscript{a} Important?

- The prevalence of Jr(a–) in healthy blood donors, suggests that this phenotype is not detrimental nor results in a medical condition.
- ABCG2 has a wide tissue distribution and substrate specificity and is important role in transporting molecules across membranes.
Lan Blood Group

- Lan was described in 1962 as a high frequency antigen
- Anti-Lan has caused transfusion reactions (some severe) and HDFN (usually mild)
- The rare Lan– phenotype is found in Blacks, Caucasians and Japanese
- Using a similar approach as for JR, the gene responsible for expression of Lan was identified
Finding the Lan Gene

• Using a patient with anti-Lan human monoclonal antibody was produced (OSK43)

• OSK43 used to screen 713,384 blood donors
  – 14 Lan negatives were found (0.002%)

• OSK43 immunoprecipitated a 80 kD protein
  – Identified as ABCB6 by mass spectrometry
  – Confirmed with expression analysis

• Located at chromosome 2q36
  – 10 null mutations identified
What Does ABCB6 Do?

• Family of proteins that transport a wide variety of endogenous or xenobiotic substrates across cell membranes
  – B family = drug resistance

• ABCB6 is a porphyrin transporter
  – May also be involved in drug resistance
  – Increased expression in cancer cells = increased drug resistance
Why is Lan Important?

- Binds heme and porphyrins and functions in their ATP-dependent uptake into the mitochondria
- Plays a role in heme synthesis
- Lan– people appear to be healthy; thus, ABCB6 does not appear to be required for normal erythropoiesis, ie. no anemia
- The Lan– phenotype in apparently healthy blood donors (0.002%), suggests that this phenotype does not lead to an overt medical condition
Vel Blood Group

• First anti-Vel reported by Sussman in 1952
  – Also referred to as anti-Ve\textsuperscript{a}, and anti-Vel\textsuperscript{1}

• Heterogeneity first noted by Levine in 1961
  – 4 generation family study
  – Tested family with proposita’s antibody and 2 other examples of anti-Vel
Finding the Vel Gene

- IT WAS A LONG TIME COMING

- Only one anti-Vel was successful for immunoprecipitation
  - Protein characterized by mass spectrometry, 18 kD
  - De novo peptide sequencing

- Genetic analysis of Vel neg individuals
  - Whole genome sequencing
  - Genome wide SNP profiles
**SMIM1 Gene**

Located on chromosome 1p36.32

4 exons encoding 78 amino acids

- Exons 1 & 2 untranslated, no signal peptide
- 17 bp deletion in exon 3 = Vel neg

With permission from Lionel Arnaud PhD
INTS, National Institute of Blood Transfusion, Paris, France
Small Integral Membrane Protein-1 (SMIM1)

- ~17 kDa on reduced gel, 32 kDa on nonreduced gel
- Forms dimers thru cysteine binding
- Trypsin resistant
- Type II membrane protein
- Unknown function
Genotyping Vel- Donors

- 20 Caucasians studied
  - 15 donors, 5 patients who produced anti-Vel
- All typed as Vel neg with 2 potent anti-Vel
- Genotyped for SMIM1 using StyI PCR-RFLP
  - DNA sequencing performed on heterozygotes

With permission from Lionel Arnaud PhD
INTS, National Institute of Blood Transfusion, Paris, France
Why is Vel Important?
So Now Let’s Investigate Some Typing Discrepancies
Comparing Genotype vs. Phenotype
LifeShare Protocol

• Donor microarray results compared to serological history

• Discordant results investigated
  – Check paperwork
  – Repeat serology
  – Test with other molecular methods

• True discordances
  – Serology negative multiple times
  – DNA positive by multiple assays
Are You Kidding Me?

• Four samples from Black donors typed as Jk(a+b-) serology Jk(a+b+) DNA

• DNA sequencing found a common JK*B null mutation 191G>A (R64Q)
  – Previously observed in Asians
  – Occurs in ~ 1:400 Blacks
  – Allele named JK*02N.09

• 3rd known Jk null mutation among individuals of African descent
Jk Gene

• 10 JK*A and 14 JK*B nulls (7 additional alleles associated with weak or variable reactivity)

• Nonsense mutations, nucleotide deletions, or splice site mutations

• Found in numerous ethnic groups: Polynesians, Finns, Asians, Caucasians, Indians, Hispanics, and Blacks
Does “N” = N?

- A blood center is using microarray to screen for rare blood donors and notes that several Black donors type as N negative by DNA but positive by serology.
- A blood center in San Diego has the same problem.
- A third center, using the same assay, has no problem.
• **N** is carried on GYP*A while “**N**” is on GYP*B

• Many hybrids:
  - Dantu (GP B-A)
  - Mi III (GP B-A-B)
  - Sta (GP A-A)

• Enhanced expression of ‘**N**’ may give false positive reactions with some anti-**N** reagents (monoclonal)
Additional MN 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>
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<tbody>
<tr>
<td>MAb #1</td>
<td>4+</td>
</tr>
<tr>
<td>MAb #2</td>
<td>2+</td>
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<tr>
<td>Rabbit</td>
<td>1+</td>
</tr>
<tr>
<td><em>V.G.</em> lectin</td>
<td>4+</td>
</tr>
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</table>
So Who is Right?

- Serological: Wrong*
- Molecular: Right
When Are You Not You?

- 71 y.o. Black woman with pancreatic cancer
- Anemia from chemotherapy requiring monthly transfusions
- Patient is O neg with anti-D and anti-U
- Phenotypes as S-s-U-
  - Genotype ordered to determine her U status
When Are YoU Not YoU?

- John Moulds quote: “Remember the history”
- Race and Sanger 1975:
  “…all examples of the antiserum (anti-U) agree that S+s-, S+s+ and S-s+ samples are positive, but when it comes to the one new and useful distinction they can make, that between S-s-U- and S-s-U+, their performance is disappointingly varied.”
- Early studies using strong anti-U found that 16% of S-s- were U+
  - Thus the term Uvar was born
U neg at the Molecular Level

• S-s- has 2 major backgrounds
  – deleted GYPB genes, “true” Uneg
  – abnormal GYPB genes = Uvar

• Molecular basis of Uvar
  – 42% of S-s- Uvar have a He specific sequence linked to an intron 5 splice site mutation
  – Exon 5 mutation at bp 230 results in partial exon skipping
  – Produces very low levels of GPB
What Can be Transfused?

Diagram shows different samples with AHG values and transfusion status. Sample 1 has AHG = neg, Sample 2 has AHG = w+, Sample 3 has AHG = 3+, and Sample 4 has AHG = 3+. The graph indicates transfusion status with arrows pointing to 'Tx'd Uvar'.
What Type Are You?

- 37 y.o Caucasian woman, types O+
- Auto control positive
- Anti-D present in serum and eluate
- Is it allo or auto-antibody
  - Requested a RhD genotype
What to Transfuse?

- Genotype = \textit{RHD*DIlla/RHD*DIVa.2}
- Should she receive Rh positive or Rh negative blood?
# SNP Analysis

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<tr>
<th>Variant</th>
<th>186</th>
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<th>455</th>
<th>602</th>
<th>667</th>
<th>1048</th>
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<tr>
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<td>T</td>
<td>C</td>
<td>G</td>
<td>G</td>
<td>G</td>
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<tr>
<td>DIVa.2</td>
<td>T</td>
<td>T</td>
<td>C</td>
<td>C</td>
<td>T</td>
<td>C</td>
</tr>
</tbody>
</table>

Patient can produce allo-anti-D  
Should receive Rh negative blood
What a Knockout ($K_o$)

- Female, 33 y.o.
- Metastatic breast cancer in the bone marrow; Hgb = 5.6 g/dl
- Typed as $K_o$, produced anti-Ku
- 2 $K_o$ units transfused; Hgb = 7.7 g/dl
- No other units in North America

Received 3 units from Finland & Japan
In the Meantime........

• LBC had a patient with anti-Kp^b

• Three O, Kp(b-) donors called to donate

  New policy is to confirm rare donors by both DNA & serology

• One donor genotyped homozygous KP^B

• Needed further investigation of typing discrepancy
Rare Blood Donor

- Caucasian male blood donor, 35 y.o.
  - Mother= Irish
  - Father= Cherokee

- Donated 3x as Kp(b-)
  - O R₁r, Jk(a+b+), Fy(a+b+), Ms, Le(a-b+), P₁, K-

- Donation #4 sent for confirmation by DNA=
  - Kk, Js(a-b+), Kp(a-b+)
A Really Rare Donor

- New DNA sample gave same results on repeat
- RBCs tested and found negative with anti-K, -k, Js\textsuperscript{b}, Kp\textsuperscript{b} and Ku
- Absorption/elution with anti-k was negative
- Concluded donor was K\textsubscript{o} (K\textsubscript{null}), VERY RARE
- Has donated double units 2 more times
Causes For *KEL* Silencing

- **Of 29 known null alleles**
  - 14 nonsense changes = stop codon
  - 6 alternative splice site
  - 4 deletions = frameshift
  - 5 missense = amino acid changes

- **Our donor had a stop codon and a missense mutation**
## KEL Haplotypes

<table>
<thead>
<tr>
<th>K Allele- BP</th>
<th>K Allele- AA</th>
<th>k Allele- BP</th>
<th>k Allele- AA</th>
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<tbody>
<tr>
<td>244C</td>
<td>Arg 82</td>
<td>244T</td>
<td>Cys 82</td>
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<tr>
<td>382C</td>
<td>Arg 128</td>
<td>382T</td>
<td>Stop 128</td>
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<tr>
<td>578T</td>
<td>Met 193</td>
<td>578C</td>
<td>Thr 193</td>
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</tbody>
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Possible Explanations

• *KEL* *244C* harbors another change silencing the gene

• Amino acid change Cys 82 to Arg adversely affects the protein stability
  – Additional glycosylation of arginine?
  – Loss of a S-S bond (due to loss of cysteine) adversely affects protein folding
Possible Explanation

- Charge changes may affect protein structure
  - Cys is small, polar, uncharged side groups
  - Arg is large with positively charged side groups
What is the cost of knowing you have an available blood donor?
Priceless

Patient

Donor

Photos used with individuals’ consent
So when you can’t solve a serological problem

Try peeking into the genes
References for New Blood Groups

JR (ISBT 32)

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