Kidd Blood Group System:

Outwardly Simple with Hidden Complexity

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The need is constant.
The gratification is instant.
Give blood.™

35th International ISBT Congress
Academy Day
June 3, 2018
Disclosures

Honoraria

- Grifols, SA
- Bio-Rad Laboratories, Inc
- Immucor, Inc
Objectives

- Review basic information on JK antigens and antibodies.
- Discuss current information on variant and null alleles and basis of ln(Jk) Jk null phenotype.
- Introduce topology model that locates codons from variant and null SNP alleles in the JK protein.
- Discuss the impact of variant alleles on JK serological studies.
- Examine the role of JK antibodies in renal transplant.
Outwardly Simple
JK System (ISBT 009)

- **Gene SLC14A1**
  - Chromosome 18, 11 exons
  - Exons 4-11 coding

- **Antigens**
  - \( \text{Jk}^a \) (JK1), \( \text{Jk}^b \) (JK2)
  - \( \text{Jk}^3 \) (JK3)
  - Well developed at birth

- **Antibodies implicated in HTR and HDFN**
JK alleles

- \( Jk^a/Jk^b \) defined by 3 single nucleotide substitutions
  - 2 silent
  - 1 missense \( 838G>A \)

Diagram: Wester, et al. Used with permission
## Common JK phenotypes

<table>
<thead>
<tr>
<th></th>
<th>Whites</th>
<th>Blacks</th>
<th>Asians</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jk(a+b-)</td>
<td>26</td>
<td>52</td>
<td>23</td>
</tr>
<tr>
<td>Jk(a+b+)</td>
<td>50</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>Jk(a-b+)</td>
<td>24</td>
<td>8</td>
<td>27</td>
</tr>
</tbody>
</table>

JK System (ISBT 009) Review

- Antigens on multipass membrane protein
  - 45-69 kDa
  - 389 amino acids
  - 10 membrane spanning domains; 5 extracellular loops

- Functions as urea transport
  - Homologous to UT-B
  - Found on red blood cells, kidney, other tissues
  - Allows concentration of urine in kidney
  - Stability of red cell as pass through renal medulla
Anti-Jk<sup>a</sup>/anti-Jk<sup>b</sup>

- Difficult to detect: Dosage. Rapid decrease in titer. Solid phase techniques-more sensitive

- Usually IgG or mixture of IgG/IgM
  - Mostly IgG3 > IgG1; few - IgG2
  - One anti-Jk<sup>b</sup> studied contained anti-IgG4

- 50% of Ab bind complement
  - Only IgM fraction.

- Few literature reports of naturally occurring JK ab
Anti-Jk\textsuperscript{a}/anti-Jk\textsuperscript{b}

- Historically -- relatively low immunogenic potential of Ag
- Neither antibody common
- Anti-Jk\textsuperscript{b} rarer than anti-Jk\textsuperscript{a}

- Tormey and Stack (Blood 2009;114:4279-4282)
  
  Jk\textsuperscript{a}: ranked as 4\textsuperscript{th} most potent immunogen when persistence of antibody considered. Previously 10\textsuperscript{th}
  
  (too few anti-Jk\textsuperscript{b} to evaluate)
JK autoantibodies

Auto anti-Jk\textsuperscript{a}

- AIHA: apparent suppression of Jk\textsuperscript{a}
  - Drug independent: Aldomet
  - Drug dependent: Chlorpropamide

- Benign: several depended on the presence of parabens used as preservative in LISS reagents
JK autoantibodies

- Autoanti-Jk\(^b\): rare
- Autoanti-Jk3: two examples in pregnancy
  - Eckley et al. Transfusion 2013, S78-030L
- Transient suppression of JK antigens: production of anti-Jk3 and anti-Jk\(^a\)
  - Combs et al. Transfusion 2005, S8-30C.
  - Issitt PD Transfusion 1990;30:46-50
Jk null phenotype  Jk(a-b-) JK:-3

- Recessive Jk(a-b-)
  Generally recognized by anti-Jk3 production
  Most frequently Polynesians (0.1-1.4%), Finns
  extremely rare: multiple Asian populations, Caucasian, African (very few)

24 genetic backgrounds recognized by ISBT: JK*01 and JK*02
  exon deletion
  nucleotide substitutions – premature stop codon
  amino acid substitution
  intron changes – splice site mutations

<table>
<thead>
<tr>
<th>JK*02N.01</th>
<th>c.342-1G&gt;A</th>
<th>Polynesian</th>
</tr>
</thead>
<tbody>
<tr>
<td>JK*02N.06</td>
<td>c.871T&gt;C</td>
<td>Finnish</td>
</tr>
</tbody>
</table>

Others reported in abstract form
### 2M urea lysis

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Urea transport</th>
<th>Hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jk(^a)/Jk(^b)</td>
<td>Passive/Active</td>
<td>1 min</td>
</tr>
<tr>
<td>Jk(_{null})</td>
<td>Passive</td>
<td>30 min</td>
</tr>
</tbody>
</table>

Read after 2 min

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**Controls**

*Slide: Wester, et al. Used with permission*
JK null phenotype

- Autosomal dominant Jk(a-b-) In(Jk)
  Garcia-Sanchez F et al. Vox Sang 2017;112(S1):53 // Transfusion 2017; 57(S3):29A
  - Not associated with mutations in SCL14A1
  - Spanish kindreds: all carried 84 bp deletion in ZNF850 gene (19q13)
  - Loss of C2H2 zinc finger-encoding domain

- Normal SLC14A1 mRNA but reduced UT-B1 on RBC membrane
- Similar, but not identical, ZNF850del84 mutation in Japanese In(Jk)

- Inability to concentrate urine
- Mood and/or anxiety disorders in Spanish families
JK variant alleles

- **JK*01 alleles / Jk(a+w )**
  - 5 variants assigned allele number (ISBT)

- **JK*02 alleles / Jk(b+w )**
  - 2 variants assigned allele number (ISBT)

?? weakened expression of antigen OR partial antigen with possible alloantibody production OR both ??
<table>
<thead>
<tr>
<th>JK variant alleles: weak phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>JK*01W.01</strong> c.130G&gt;A p.Glu44Lys most frequent</td>
</tr>
<tr>
<td><strong>JK*01W.02</strong> c.511T&gt;C p.Trp171Arg</td>
</tr>
<tr>
<td><strong>JK*01W.03</strong> c.28G&gt;A p.Val10Met</td>
</tr>
<tr>
<td><strong>JK*01W.04</strong> c.226G&gt;A p.Val76Ile</td>
</tr>
<tr>
<td><strong>JK*01W.05</strong> c.742G&gt;A p.Ala248Thr</td>
</tr>
<tr>
<td><strong>JK*02W.01</strong> c.548C&gt;T p.Ala183Val</td>
</tr>
<tr>
<td><strong>JK*02W.02</strong> c.718T&gt;A p.Trp240Arg</td>
</tr>
</tbody>
</table>

Topology summary – SNP variants

Jk-weak variants
- N-terminal half and cytoplasmic tail
- Top and bottom membrane layers
- Less structurally disruptive

Jk-negative variants
- C-terminal helices near Jk<sup>a/b</sup>
- Mid-membrane layer
- More structurally disruptive
- Some near urea transport pore

from Ramsey G. Transfusion 2017;57(S3):29A
JK variant alleles: discrepancies

- Apparent JK antibody in antigen positive individual
- Discrepancies between prior serological or molecular testing and current results; variation with different reagents
- Apparent Jk(a-b-)
  homzygous for a weak JK allele or an allele with a weakened Ag paired with a silenced allele

\[ \text{JK}^{*01W.01}/\text{JK}^{*01W.01} \quad \text{c.}130\text{G}\rightarrow\text{A} \]

\[ \text{altered JK}^{*01}/\text{JK}^{*02N.08} \quad \text{c.}134\text{T}\rightarrow\text{C} \text{ novel in JK}^{*01} \]

#Vege S et al. Transfusion 2015;55S:35A
### JK antigen typing challenges

<table>
<thead>
<tr>
<th>Sample</th>
<th>Anti-Jk(^a)</th>
<th>Anti-Jk(^b)</th>
<th>Anti-Jk(^a) MS15</th>
<th>Anti-Jk(^b) MS8</th>
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<tbody>
<tr>
<td>1</td>
<td>2+</td>
<td>0</td>
<td>3+</td>
<td>3+</td>
</tr>
<tr>
<td>2</td>
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<tr>
<td>3</td>
<td>1+ to 3+</td>
<td>2+</td>
<td>0</td>
<td>NT</td>
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</table>

From: Whorley T, et al. Transfusion 2009; 49S: 48A
Abstract (S16-040E)
## JK antigen typing challenges

<table>
<thead>
<tr>
<th>Sample</th>
<th>Anti-Jk\textsuperscript{a}</th>
<th>Anti-Jk\textsuperscript{b}</th>
<th>Anti-Jk\textsuperscript{a} MS15</th>
<th>Anti-Jk\textsuperscript{b} MS8</th>
<th>DNA nt change</th>
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<td>2+</td>
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<td>3+</td>
<td>JK\textsuperscript{*A/B} 548C&gt;T (Ala183Val)</td>
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<td>2</td>
<td>3+</td>
<td>2+</td>
<td>0 to +\textsuperscript{w}</td>
<td>3+</td>
<td>JK\textsuperscript{*A/B} 511T&gt;C (Trp171Arg)</td>
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<tr>
<td>3</td>
<td>1+ to 3+</td>
<td>2+</td>
<td>0</td>
<td>NT</td>
<td>JK\textsuperscript{*A/B} 130G&gt;A (Glu44Lys)</td>
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</tbody>
</table>
Case 1: Is it autoantibody?

- 13 year old female with sickle cell anemia
- multiple transfusions
- 2006: phenotyped for Ag-matched transfusions

<table>
<thead>
<tr>
<th></th>
<th>anti-Jk(^a) (polyclonal)</th>
<th>Anti-Jk(^b) (polyclonal)</th>
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</thead>
<tbody>
<tr>
<td>Auto RBC by hypotonic wash</td>
<td>2+</td>
<td>2+</td>
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<tr>
<td>Positive control</td>
<td>2+</td>
<td>2+</td>
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</table>

- 2008: Hospital reports positive antibody screen possible anti-Jk\(^a\) in gel; Positive DAT
### Case 1: plasma/eluate

<table>
<thead>
<tr>
<th>CELL</th>
<th>Rh</th>
<th>Kell</th>
<th>Duffy</th>
<th>Kidd</th>
<th>Lewis</th>
<th>P</th>
<th>MN</th>
<th>LISS</th>
<th>PEG</th>
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<td>+</td>
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<tr>
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<td>+</td>
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<td>0⁺⁺⁺⁺</td>
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<td>+</td>
<td>+</td>
<td>0⁺⁺</td>
<td>+</td>
<td>0⁺⁺⁺⁺</td>
</tr>
</tbody>
</table>

**Note:** Anti-IgG, Anti-IgG, PEG-IgG
Case 1: JK allo or auto?

<table>
<thead>
<tr>
<th></th>
<th>DAT</th>
<th>Anti-Jka Monclonal</th>
<th>Anti-Jka Polyclonal</th>
<th>Plasma PEG-IgG</th>
<th>Eluate PEG-IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008 WB</td>
<td>+ micro</td>
<td></td>
<td></td>
<td>2+</td>
<td>NT</td>
</tr>
<tr>
<td>2008 auto RBC by hypo wash</td>
<td>Neg</td>
<td>3+</td>
<td>2+</td>
<td>1+w</td>
<td>NT</td>
</tr>
</tbody>
</table>

2008: Possible warm autoantibody with anti-Jk<sup>a</sup> specificity
No adsorption studies performed.

Transfused with Jk(a-) RBC from this date.
Case 1: follow-up 2012

- Anti-Jk\(^a\) no longer detectable PEG or gel
- Hypotonic wash autologous RBC:
  - Anti-Jk\(^a\) score 4 \((1^+w)\) monoclonal reagents
  - Anti-Jk\(^b\) score 8 \((2^+)\)
- Sequence JK exons
  - Exon 4: 130G/A (Glu44Lys) weakened Jk\(^a\)
  - Exon 7: 588G/G (silent)
  - Exon 9: 838G/A (Asp280Asn) confirm Jk(a+b+)
- Anti-Jk\(^a\): alloantibody
Autoanti-Jk\textsuperscript{a} reports

- *Auto-anti-Jka* in Evans' syndrome with negative direct antiglobulin test.
- *Autoimmune hemolytic anemia with anti-Jka* specificity detected by means of the gel technic
- Mixed autoimmune haemolysis in a SLE patient due to aspecific and *anti-Jka autoantibodies*
- *Anti-Jka autoimmune* hemolytic anemia in an infant
- Delayed type transfusion reaction due to anti-S antibody in patient with *anti-Jka autoantibody* and multiple alloantibodies

Could variant *JK*\textsuperscript{*01} alleles be involved?
Case 2

Velliquette R, et al. Transfusion 2015;55S:34A

- Filipino male. Not recently transfused
  - Jk(a+b-)
  - Anti-Jk\(^b\)
  - DAT positive: warm autoantibody
  - multiple Jk(a+b-) RBC transfused
Case 2: Subsequent sample

<table>
<thead>
<tr>
<th></th>
<th>Plasma</th>
<th>Eluate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jk(a+)</td>
<td>+micro</td>
<td>1+</td>
</tr>
<tr>
<td>Jk(b+)</td>
<td>2+</td>
<td>+w</td>
</tr>
<tr>
<td>Jk(a-b-)</td>
<td>negative</td>
<td>negative</td>
</tr>
</tbody>
</table>

Adsorption/elution studies with plasma:
Separable anti-Jk\(^a\), anti-Jk\(^b\).
No anti-Jk3
## Case 2: Family Study

<table>
<thead>
<tr>
<th></th>
<th>Phenotype</th>
<th>Genotype</th>
<th>XM with Pt plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>Jk(a+b-)</td>
<td>JK<em>01W.01/JK</em>02N.01</td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td>Jk(a+b-)</td>
<td>JK<em>01W.01/JK</em>02N.01</td>
<td>Neg</td>
</tr>
<tr>
<td>Father</td>
<td>Jk(a+b+)</td>
<td>JK<em>01W.01/JK</em>02</td>
<td>Pos</td>
</tr>
<tr>
<td>Brother</td>
<td>Jk(a+b-)</td>
<td>JK*01W.01/JK01W.01</td>
<td>Neg</td>
</tr>
</tbody>
</table>
JK variant alleles in antibody ID

- Genotyping may be helpful in
  - alloantibody vs. autoantibody determination
  - identifying suitable donors not apparent by serological testing

- Are JK antibodies produced by individuals with partial alleles always clinically significant?
JK and Renal transplant

- UT-1 (JK protein) found on endothelial cells of renal medulla, vasa recta, and renal tubular epithelial cells

- Five reports suggesting JK antibodies may be involved in rejection of incompatible kidney

JK as histocompatibility Ag

- Allograft rejection 2-10 years after transplant
- Simultaneous appearance of JK antibody
  - 2 anti-Jk\(^a\); 2 anti-Jk\(^b\)
- 3 patients reported non-compliance with immunosuppressive regime. (1- no information)
- Transplanted kidney thought to stimulate primary or anamnestic response
JK as histocompatibility Ag

- 5th patient received RBC transfusion 18 years prior to transplant and immediately post renal transplant.
- 2 hours post transfusion: Acute TR due to anti-Jkα
- Rejection of graft
Summary

- Jk\textsuperscript{a}, Jk\textsuperscript{b}, Jk\textsuperscript{3}. Protein involved in urea transport
- JK Ab clinically significant in transfusion reactions and HDFN.
- Allo and autoantibody. May be hard to detect.
- 31 recognized alleles causing weak or silenced JK expression. More reported in abstracts
- Jk(a-b-) ln(Jk) : ZNF850del84 mutation; chromosome 19
Summary

- Weak/silenced SNP alleles cause amino acid changes having patterns in their location within the membrane.
- Variant alleles can cause discrepancies in JK serological/molecular antigen typing, misidentification of Jk(a-b-), apparent alloAb production in JK-positive patient.
- JK antibodies may impact renal graft survival
Thank You!