DNA Analysis is Our Ally: Tales from the Immunohematology Frontline

Christine Lomas-Francis

Laboratory of Immunohematology and Genomics
New York Blood Center

49th Annual HAABB Spring Meeting; April 2016

Immunohematology frontlines

Immunohematology "frontline" is the facilitation of "safe" blood transfusion

- **Procedures to accomplish this include:**
  - **Antibody identification**
  - **Antigen typing of patients**
  - **Antigen typing of donors**
  - **Discrepancy resolution in both patients and donors**
  - **Screening for antigen-negative donors**
  - **Cross-matching**

……..And much more

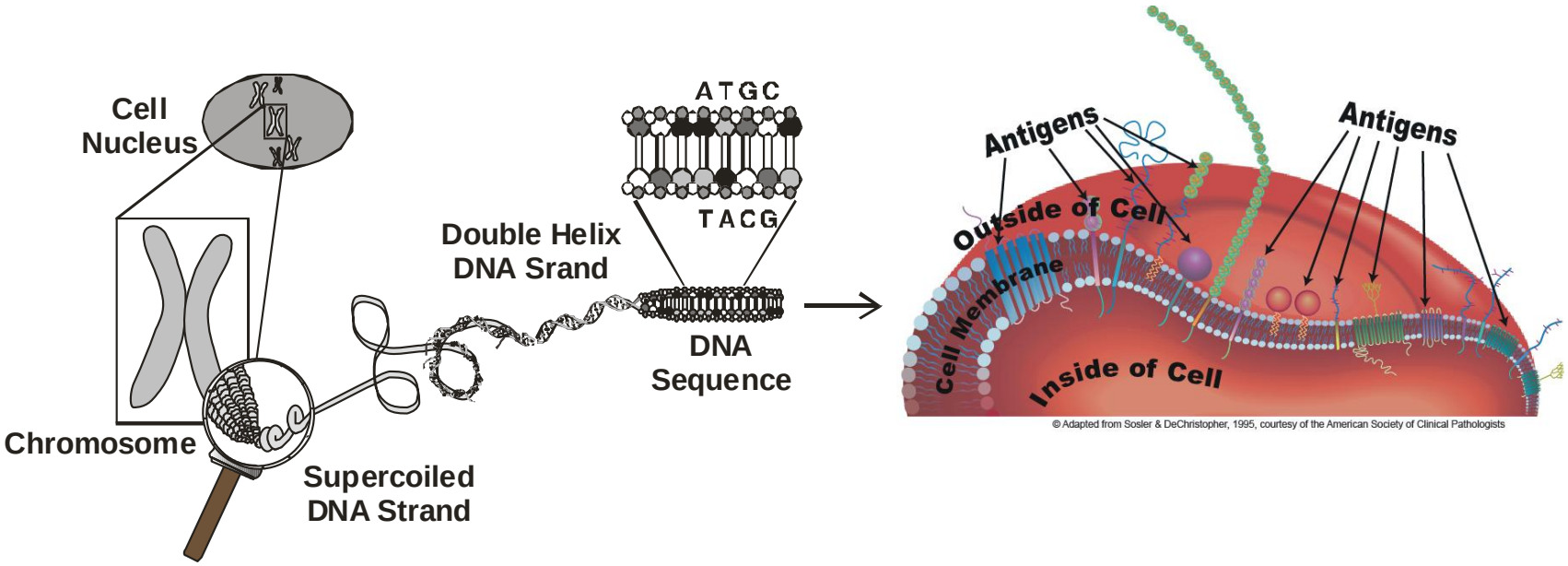
Our traditional arsenal of tools

- Various test media (e.g., LISS, PEG, Alb, Sal) and phases of reactivity (e.g., 4C, RT, 37C, IS, IAT, DAT)
- Absorption and elution
- Treatment of panel RBCs with enzymes and chemicals (DTT, EGA)
- Null phenotype RBCs for testing with patient's plasma
- Inhibition of antibody (natural substances)
- Typing of patient's RBCs for common and high/low prevalence antigens (hemagglutination)
- Availability of extensively typed RBCs (hemagglutination) [Routine and selected panel(s)]

Our traditional arsenal of tools is expanding

- Various test media (e.g., LISS, PEG, Alb, Sal) and phases of reactivity (e.g., 4C, RT, 37C, IS, IAT, DAT)
- Absorption and elution
- Treatment of panel RBCs with enzymes and chemicals (DTT, EGA)
- Null phenotype RBCs for testing with patient's plasma
- Inhibition of antibody (natural substances; recombinant proteins)
- Typing of patient's RBCs for common and high/low prevalence antigens (hemagglutination; DNA typing)
- Availability of extensively typed RBCs (hemagglutination; DNA typing) [Routine and selected panel(s)]
- Molecular technology; an aid for many aspects and levels

DNA and RBC antigens



- **Genes encoding the 36 blood group systems have been cloned and sequenced**
- **The molecular bases of most blood group antigens and phenotypes have been determined, with most determined by single nucleotide polymorphisms (SNPs)**

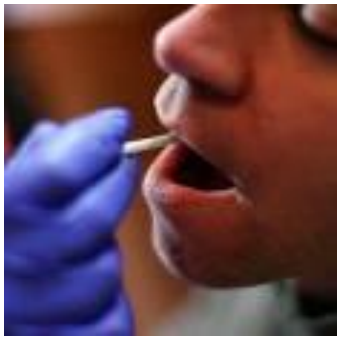
Sample type for DNA

Any nucleated cell

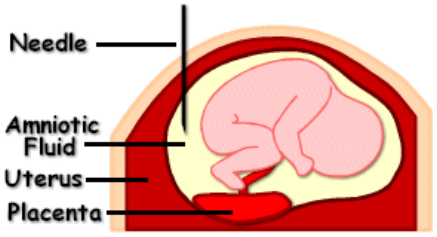
- No sample age requirement
- Patients:
  - Can be post-transfusion
  - Allogeneic stem cell transplant; discrepancy between WBCs and buccal swab
- Donors:
  - <u>Cannot</u> isolate from leukoreduced segments

Blood
(White cells)

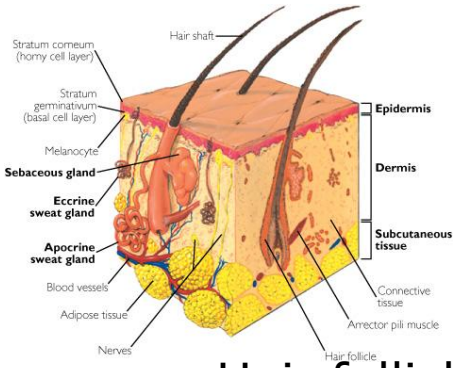
Check cells/
Buccal swabs



Amniocytes



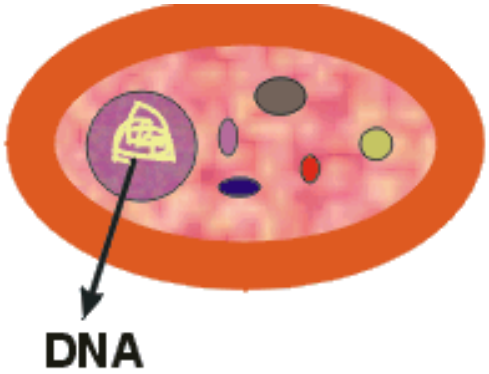
Tissue



Hair follicles

How do you get the DNA?

Sample - cells



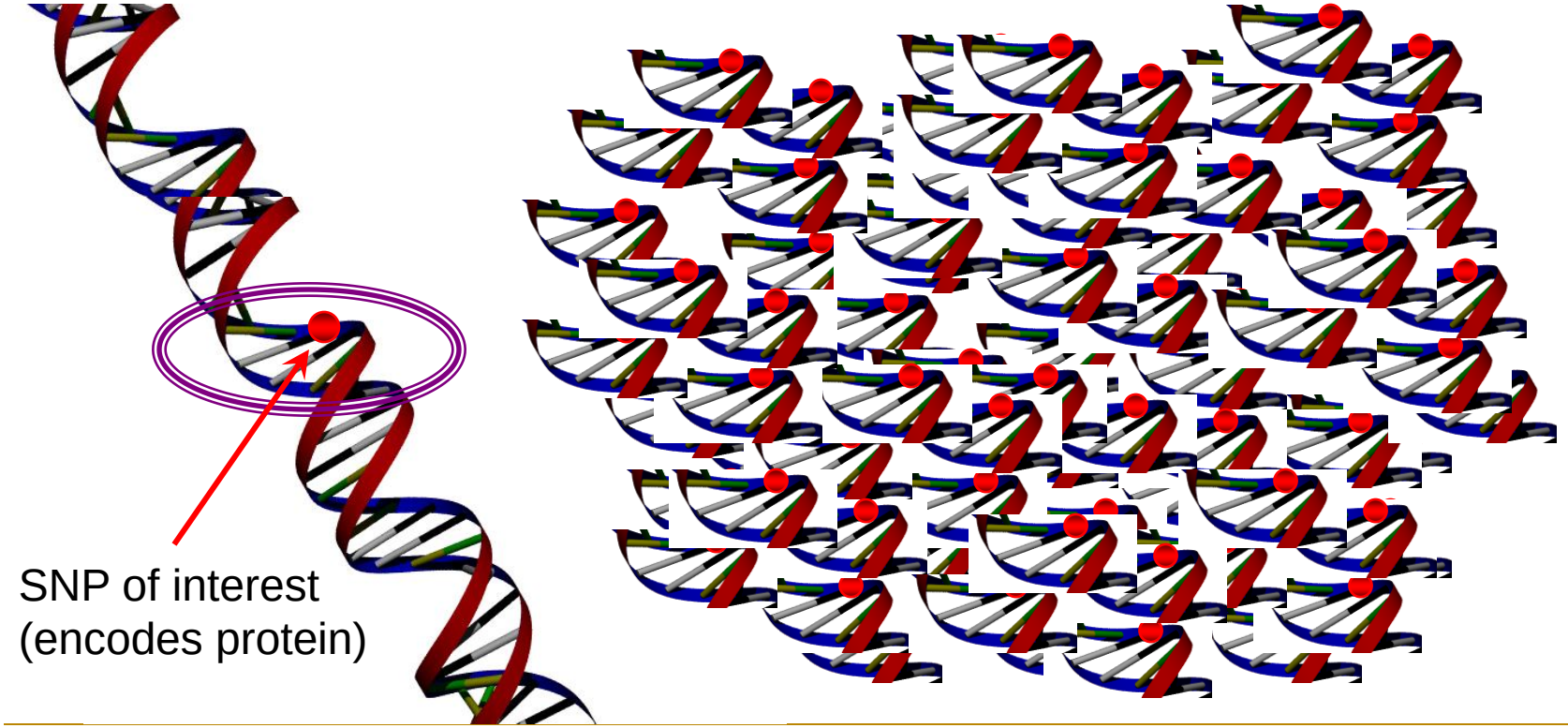
Lyse cells

DNA

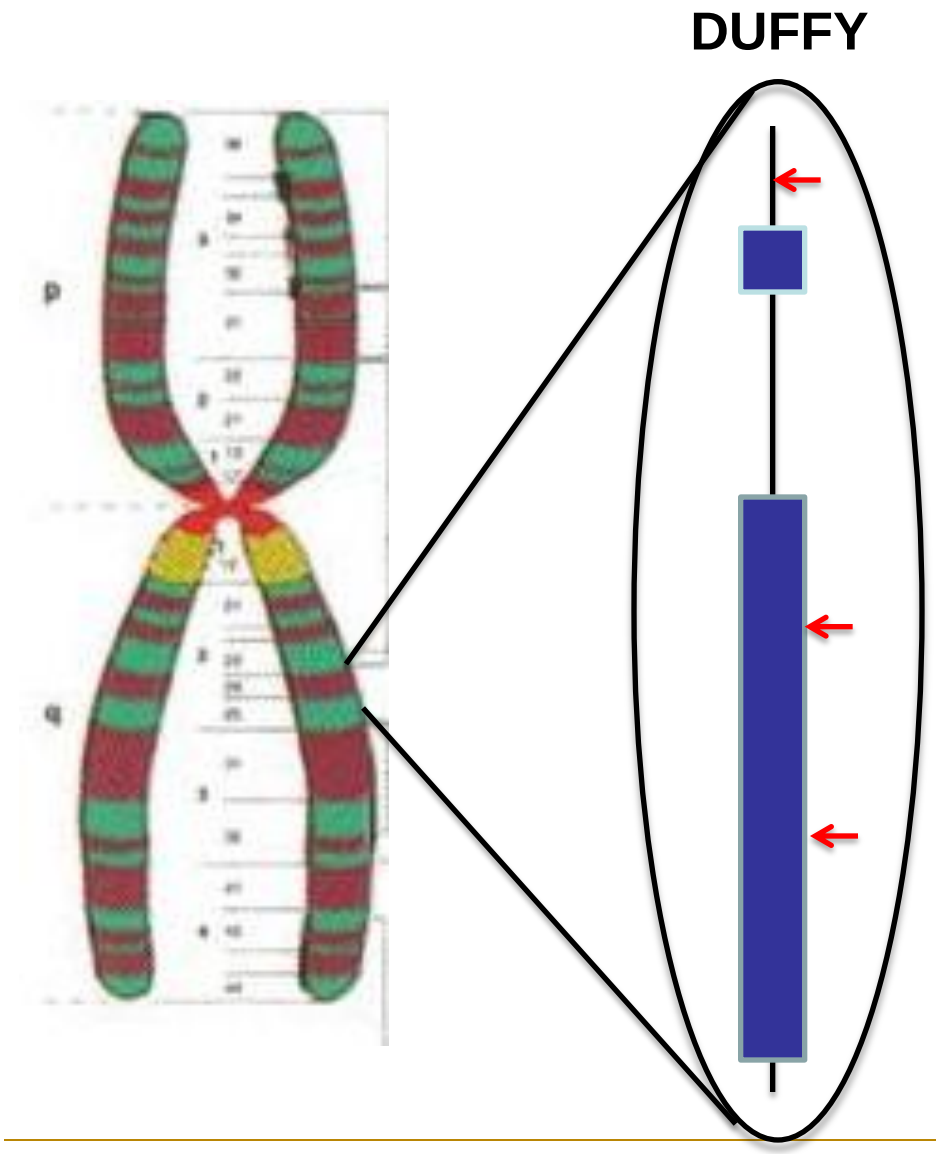
- Commercial kits
- Automation - BioRobots
  - Process 96 samples in 3 hrs

Polymerase Chain Reaction (PCR)

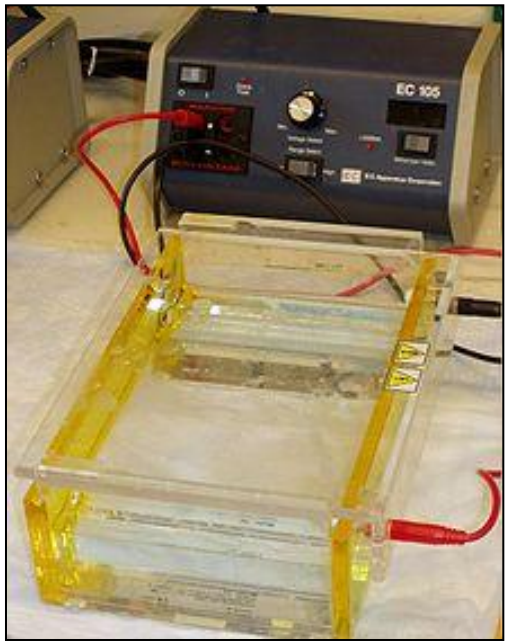
- Amplify a particular segment of DNA that contains SNP or polymorphism(s) of interest
- Generate millions of copies of that segment for further analysis

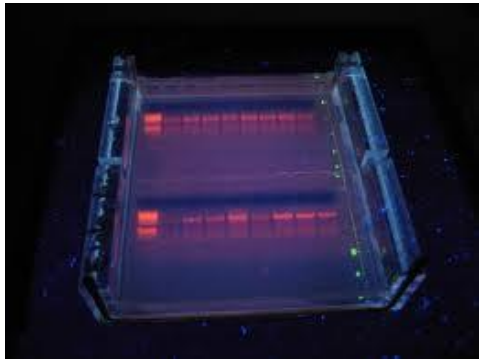


For single or few SNPs



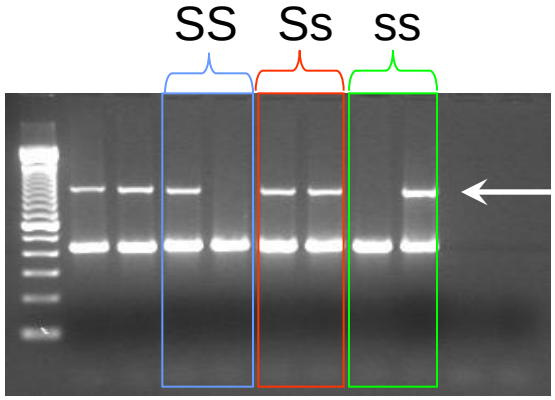
MANUAL ASSAYS

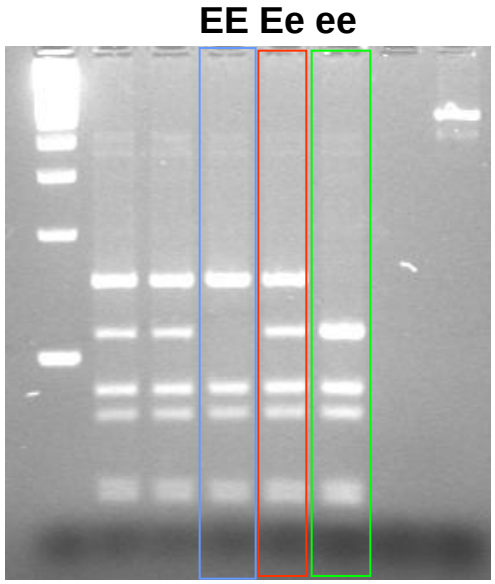




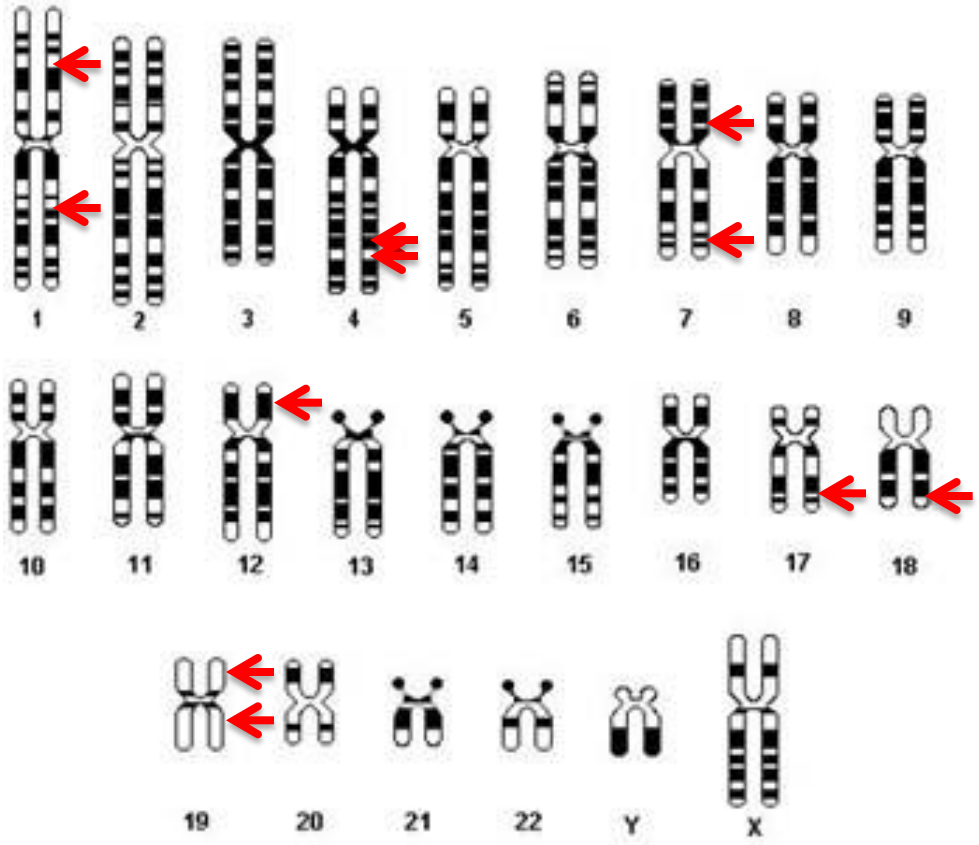
Manual DNA Assays

- Allele-specific PCR (AS-PCR)
  - Primers are specific for SNP/allele
- PCR-Restriction Fragment Length Polymorphism (RFLP)
  - PCR is digested with restriction enzyme and alleles are identified by resulting pattern
- Require electrophoresis for results
  - Gels stained with ethidium bromide
- Time consuming
- Interpretations are done manually

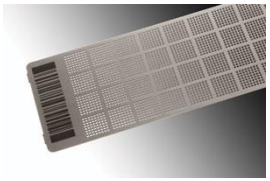


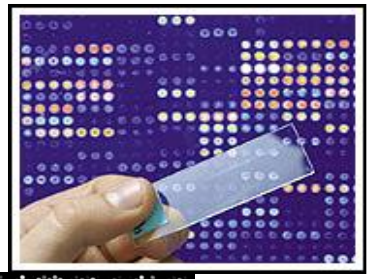


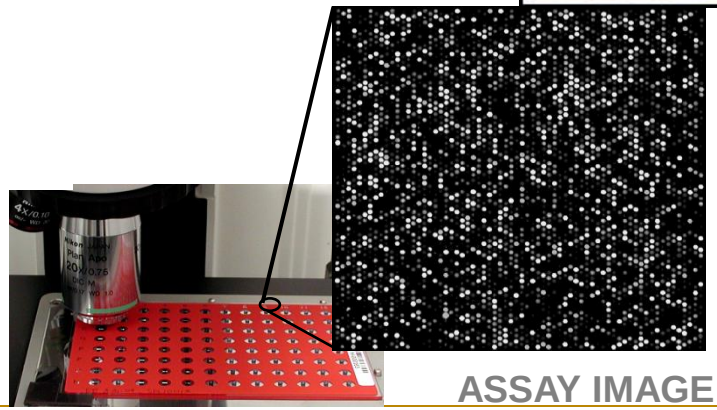
Many SNPs in many genes



- DNA arrays
  - One multiplex PCR
  - Fluorescent read-out for each SNP of interest
  - Interpretation by software
  - Results ~ 5 hours (+ time to extract DNA)







ASSAY IMAGE

Hemagglutination versus DNA-based Assays

Hemagglutination-based assays:

- **Directly determine the presence or absence of an antigen through agglutination, or lack thereof, when antibody and RBCs are combined**

DNA-based assays:

- **Test for the presence or absence of a nucleotide or a sequence of nucleotides within a gene**
- **Indirectly predict the likely presence or absence of an antigen**
- **Provide a "snapshot" of a gene at a single location; as mostly only a few selected nucleotides are tested for**
- **Does not require special reagents**

Case Studies

Case 1

The "Invisible Antibody"

Case 1

- 59 year old female diagnosed with AIHA
- Multiple transfusions; unable to phenotype
- History of anti-E and anti-K
- All units incompatible
- Repeated alloadsorptions with R_1R_1, R_2R_2, rr RBCs
- No new antibodies demonstrated
- However, patient has overt post-transfusion hemolysis

No answer from serology……

DNA typing to the rescue

Case 1: DNA testing predicts the RBC phenotype

- **Patient sample submitted for DNA typing**
- **Probable genotype:**
  - ***RHD, RHCE*C/c, RHCE*e/e, KEL*2/2, JK*A/A, GYPB*S/s, FY*A/B* (with wild type GATA box)**
- **Predicted RBC phenotype:**
  - **D+C+E–c+e+, K–k+, Jk(a+b–), S+s+, Fy(a+b+)**

- **Was the post-transfusion hemolysis caused by (serologically undectable) alloanti-Jk^b?**
- **Transfused with E–K–Jk(b–) blood**
- **No post-transfusion hemolysis**

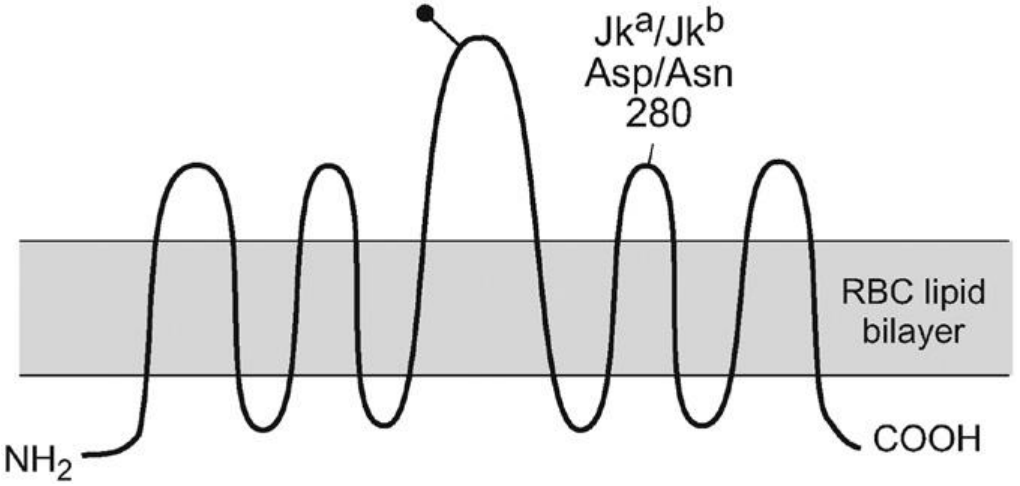
Patients with autoimmune hemolytic anemia

- **Challenge to find appropriate RBC units for transfusion**
- **Due to the presence of a strong autoantibody:**
  - **All RBC samples on the antibody screening and identification panels will be agglutinated**
  - **Difficult to detect and exclude underlying alloantibodies**
  - **Adsorption techniques, either allo or auto, cannot be done in all facilities and are time-consuming**
- **20% to 40% of patients have underlying clinically significant alloantibodies**

Patients with AIHA: Prediction of RBC Phenotype

- **Should determining patient's phenotype and providing prophylactic antigen-matched RBCs become routine?**
- **Provides flexibility in for transfusion management, but maintains safety and avoids or simplifies pre-transfusion adsorption studies**
- **DNA-based assays make prediction of RBC phenotype feasible**
- **Level of antigen-matching to be decided!**

No Kidding around with Kidd



Case 2: history

- 49 yo Hispanic woman; congestive heart failure
- Last transfused in 2005
- Hbg: 9.5g/dL HCT: 28.5%
- Hospital suspects anti-Jk^a

IRL results

- Group O Rh-positive
- DAT: + weak with polyspecific, anti-IgG, anti-C3
- Anti-Jk^a by albumin IAT, PEG IAT and IgG gel test
- Selected panels ruled out other specificities
- Warm autoantibody (no specificity)

Testing for Jk antigens: serology and DNA

- DNA extracted from whole blood
- HEA array performed for common red cell antigens

Array Results:

Detail	c	C	e	E	K	k	Kpa	Kpb	Jsa	Jsb	Jka	Jkb	Fya	Fyb	M	N	S	s	Lua	Lub	Dia	Dib	Coa	Cob	Doa	Dob	Joa	Hy
VS+V+	+	0	+	+	0	+	0	+	0	+	+	+	+	0	+	+	0	+	0	+	0	+	+	0	+	+	+	+

Partial e
Could make allo anti-e

GATA mutation
Not at risk for anti-Fyb

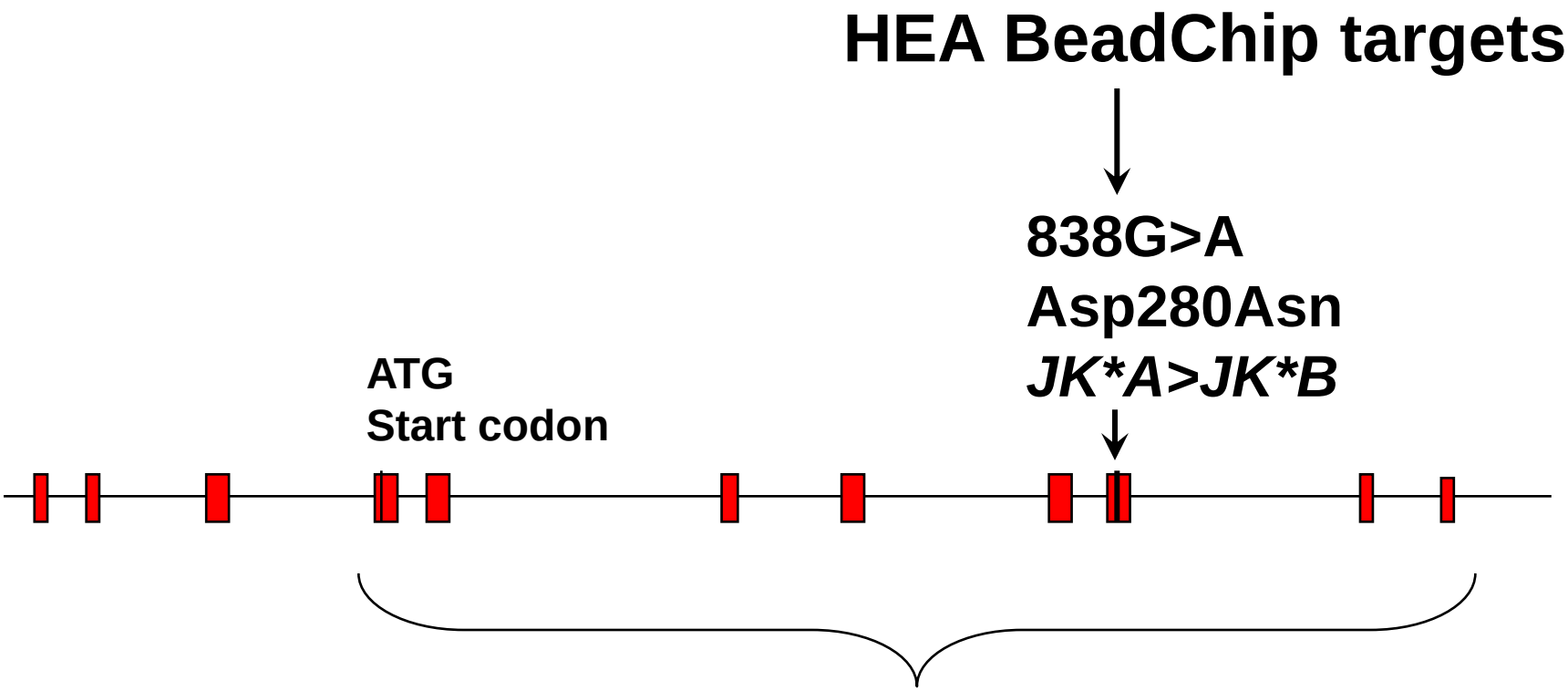
Predicted Jk(a+)

Anti-Jk^a in plasma

RBCs	Jk^a	Jk^b
Patient EGA treated	3+	3+
Jk(a+b+) control	3+	3+

JK gene

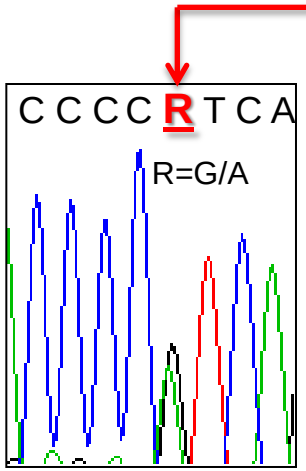
11 exons



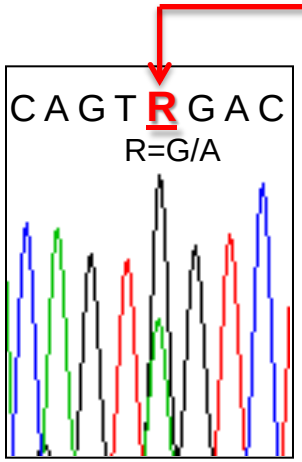
Exons 4 to 11 to be sequenced

Case 2: sequencing of *JK*A* and *JK*B*

- Sequenced exons 4 to 11
- Confirmed *JK*A*/*JK*B* as predicted by HEA
- Exon 5 sequence:
  - Heterozygous for 226G>A (Val76Ile) and 303G>A (silent)



226G>A
Val76Ile



303G>A
No change in amino acid

- Allele was previously reported: *JK*01W.04*
- Predicted phenotype for patient: Jk(a+^{w}b+)
- *JK*A* (*JK*01W.04*) encodes partial Jk^{a} antigen
- Patient's anti-Jk^{a} likely alloantibody; transfuse Jk(a–) units

Case 3: history (first admission)

Patient:

- 33 year old Filipino male with sepsis and cirrhosis
- Transfusion urgently required
- Positive antibody screen; DAT+
- Transfused 4 months ago; negative antibody screen at that time

Plasma contained:

- Anti-E**, anti-Jk^{b}**, warm autoantibody

Patient's RBCs:

- **Jk(a+b–) by serology**

DNA predicts Jk(a+b+) by HEA
Additional testing initiated

Transfusion:

- Patient transfused with Jk(b–) E– RBCs

Case 3: HEA array analysis

USA

Blood Group	Antigen	Result	Notes
Rh	c	+	
	C	+	
	e	+	
	E	0	
Kell	K	0	
	k	+	
	Kpa	0	
	Kpb	+	
	Jsa	0	
	Jsb	+	
Kidd	Jka	+	
	Jkb	+	
Duffy	Fya	+	
	Fyb	+	
MNS	M	+	
	N	+	
	S	0	
	s	+	
Lutheran	Lua	0	
	Lub	+	
Diego	Dia	0	
	Dib	+	
Colton	Coa	+	
	Cob	0	
Dombrock	Doa	0	
	Dob	+	
	Joa	+	
	Hy	+	
Landsteiner-Wiener	LWa	+	
	LWb	0	
Scianna	Sc1	+	
	Sc2	0	

- Predicted to be Jk(a+b+)
- Silenced *JK*B* suspected based on serology
- Additional DNA testing initiated

Case 3: first admission – serological results

RBC testing

- DAT 1+ IgG
- An eluate made from the patient's RBCs :
  - Reacted weakly (1+) in the IAT with all panel cells tested
  - Reacted with the EGA-treated autocontrol indicating probable warm autoantibody
- EGA-treated RBCs typed Jk(a+b–) with Immucor polyclonal reagents
- Untreated RBCs also typed Jk(a+b–) with Ortho BioClone reagents
- The Jk^a typing with both reagents was weaker than with Jk(a+b+) control RBCs

Case 3: the patient returns

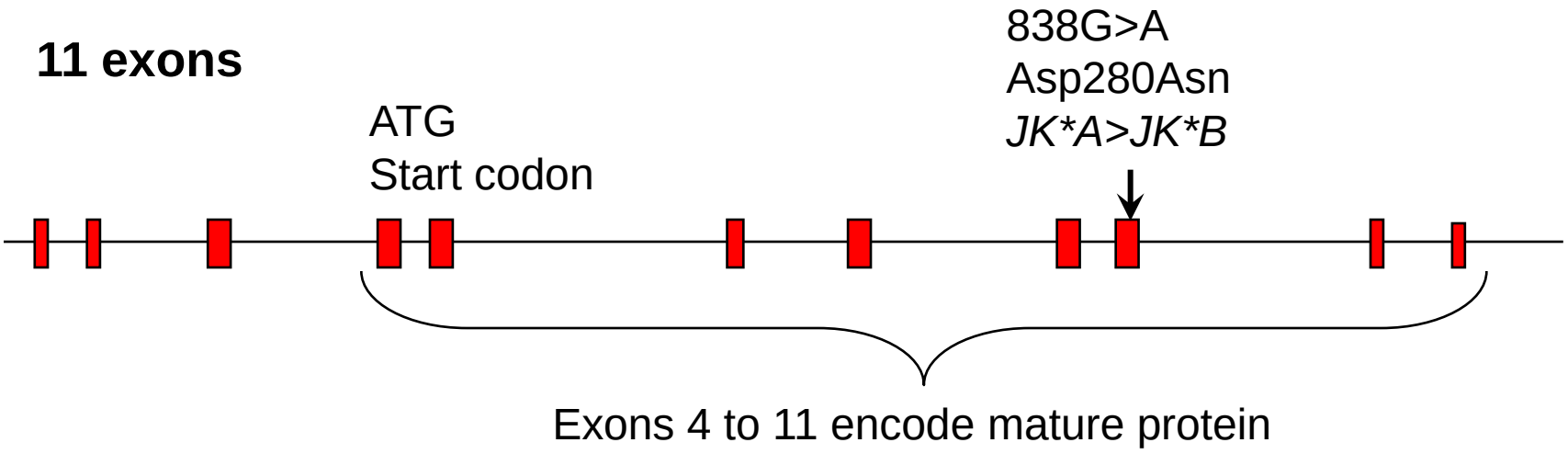
- **Patient subsequently readmitted**
  - **Had been multiply transfused with Jk(a+b−) RBCs**
  - **Plasma reacted weakly micro in the IAT with Jk(a+b–) RBCs**
  - **DAT 1+ IgG**
- **An acid eluate reacted weakly (+/- to 1+) with all panel cells tested**
    - **Jk(a+b–) RBCs reacted 1+**
    - **Jk(a–b+) RBCs reacted +/-**
- **However, the eluate was non-reactive with Jk(a–b–) RBCs**
    - **Does the eluate contain anti-Jk3?**
- **The patient's sample was exhausted and QNS for further testing**

Case 3: two weeks later……..

- Plasma reactivity strength increased
  - $1+^{s}$ to $2+^{w}$ with all panel cells tested
  - Jk(a+b–) and Jk(a–b+) reacted equally
  - Jk(a–b–) RBCs non-reactive (n=2)
  - Is the plasma antibody anti-Jk3?
- Adsorption and elution studies of the patient's plasma undertaken to define specificity/ies
  - The patient's plasma contained separable anti-Jk^{a} and anti-Jk^{b}
  - Ruled out anti-Jk3 in plasma
- Due to lack of sample, adsorption/elution studies could not be performed on eluate to look for anti-Jk3

Case 3: DNA testing results

- **HEA testing predicted Jk(a+b+)**
- **Silenced *JK*B* suspected based on Jk(b−)serology**
  - **HEA does not target silenced *JK or* variant *JK***
- ***JK* gene sequencing initiated to determine molecular basis of the apparent silenced *JKB***



Case 3: gene sequencing of *JK* exons 4 and 6

- **Exon 9: c.838G/A confirmed *JK*A/*B***
- **Exon 4: Heterozygous c.130G/A predicting Glu44Lys**
  - associated with weak/variant Jk^{a}
  - has now been found in several populations
- **Exon 6**
  - **Intron 5, heterozygous IVS5-1 g>a**
    - associated with skipping of exon 6 and a silenced (null) JK*B allele
    - Predicts a Jk(b–) phenotype
    - Not uncommon in Polynesians
- **Patient's *JK* genotype: *JK*01W.01/JK*02N.01***
- **Predicted RBC phenotype: Jk(a+^{w}b–)**

- Wester, E.S., et al., 2011. Characterization of Jk(a+^{weak}): a new blood group phenotype associated with an altered JK*01 allele. Transfusion 51, 380–392
- Whorley T, et al. Transfusion 2009; 49S: 48A Abstract (S1 6-040E)

Case 3: transfusion

- **Patient was initially transfused Jk(a–b–) RBCs**
  - **Exceedingly rare phenotype, mostly found in Polynesians, Filipinos and Finns**
- **Use of Jk(a+b–) RBCs was considered because of the unknown clinical significance of the anti-Jk^a made when *JK*A* 130G>A change is present**
  - **Patient appeared to tolerate Jk(a+b–) blood as reflected by no acute transfusion reaction reported before the anti-Jk^a was identified**
- **Patient's family was tested for possible donors**

Case 3: Family Study

Sample	Jk^a	Jk^b	*JK* 01 (JK*A)*	*JK*02 (JK*B)*	JK genotype
Proband	1+	0	Exon 4: c.130G>A Glu44Lys	Exon 6/intron 5 IVS5-1g>a skipping of exon 6	*JK*01W.01/*02N.01*
Father	3+	3+	Exon 4: c.130G>A Glu44Lys	No changes Consensus *JK*B*	*JK*01W.01/*02*
Mother	1+	0	Exon 4: c.130G>A Glu44Lys	Exon 6/intron 5 IVS5-1g>a skipping of exon 6	*JK*01W.01/*02N.01*
Brother	3+	**0**	Exon 4: c.130G>A Glu44Lys (Homozygous)	No *JK*B*	*JK*01W.01/*01W.01*

- ABO-compatible mother and brother are expected to be suitable donors
- DNA sequencing revealed compatible donors that would have been considered unsuitable based only on RBC testing with anti-Jk^a/Jk^b

When is a positive not a positive?

DNA analysis helps to explain an antigen typing discrepancy

Case 4: what's up with E?

- Caucasian female blood donor, Group O+
- 10 prior donations
- On 2 donations RBCs typed D+C+E−c+e+
- Unit labeled and shipped as E−
- Re-typing by the hospital indicated E+

Testing with anti-E reagents

	Gamma clone (GAMA402)	SeraClone (MS60/12)	ALBA clone (DEM1)	In-house polyclonal
Donor RBCs	4+	4+	2+^{mf}	very weak

E+ with four strongly reactive with two

	Ortho BioClone (C2)	Immucor polyclonal	Immucor Series I (MS12)	In-house polyclonal
Donor RBCs	0	0	0	0

E− with four

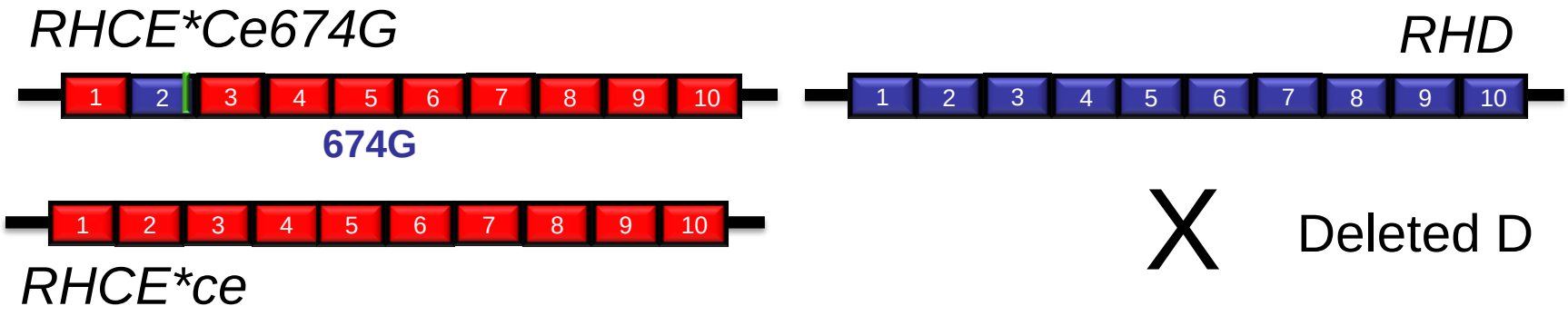
Case 4: DNA results

- HEA Beadchip:
  - **Negative for *RH*E***
  - Genotype: *RHCE*Ce* and *RHCE*ce*
- RHCE Beadchip:
  - **Negative for *RH*E***
  - Genotype: *RHCE*Ce* and *RHCE*ce*
  - Predicted phenotype C+**E-**c+e+
- Manual PCR-RFLP for E/e:
  - 676G/G, predicted **E-**e+
- *RHCE* exon 5 sequencing:
  - 676G/G, predicted **E-**e+
  - Novel nt 674C>G (Ser225Cys)

Is the change present on the *RHCE*Ce* or *RHCE*ce*?

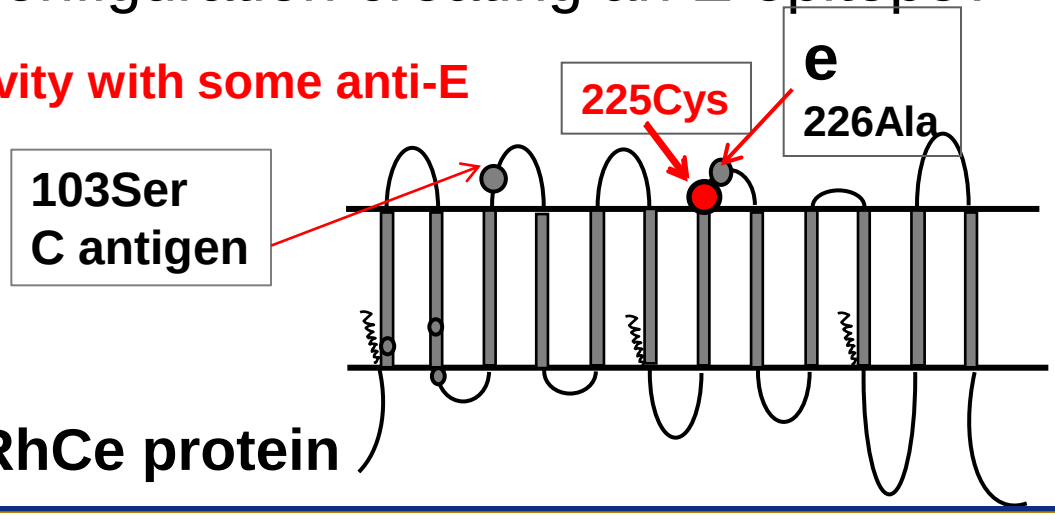
Case 4: Robust (variable) E expression on RhCe

- RNA/cDNA sequence showed 674C>G is on *RHCE*Ce*



- Must alter the protein configuration creating an E epitope?

225Cys in Ce protein = reactivity with some anti-E



Case 4: more questions than answers?

- As donor, unit should be E+ or E−?
- If crossmatched for patient with anti-E, will it be incompatible?
- Possible clue: reactivity with polyclonal anti-E
- Might it stimulate anti-E in a E− patient?
- If patient, should he/she be considered E+ or E−?

	Gamma clone (GAMA402)	SeraClone (MS60/12)	ALBA clone (DEM1)	In-house polyclonal
Donor RBCs	4+	4+	$2+^{mf}$	very weak

	Ortho BioClone (C2)	Immucor polyclonal	Immucor Series I (MS12)	In-house polyclonal
Donor RBCs	0	0	0	0

E antigen typing discrepancy reveals a novel 674C>G change (Ser225Cys) on RhCe responsible for expression of E epitopes. S Vege, C Lomas-Francis, Z Hu, K Hue-Roye, P Patel, C M. Westhoff 2012 Transfusion Abstract

Name that antibody

What is the specificity?

Patient is a White 35 year old woman
Transfused 6 month ago

	Rh-hr					Kell		Kidd		Duffy		MNSs					PEG	Papain
Cell	D	C	E	c	e	K	k	Jk^a	Jk^b	Fy^a	Fy^b	M	N	S	s		IAT	IAT
1	+	+	0	0	+	0	+	+	0	+	0	0	+	+	0		2+	3+
2	+	+	0	0	+	+	+	+	0	+	0	+	+	0	+		2+	3+
3	+	0	+	+	0	0	+	+	0	+	+	0	+	+	+		0^v	0^v
4	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0		0^v	0^v
5	0	0	0	+	+	0	+	0	+	0	+	+	0	+	0		2+	3+
6	0	0	0	+	+	+	+	+	0	+	0	+	+	+	+		2+	3+

Panel indicates presence of anti-e (allo or auto?)
Additional testing has ruled out other underlying antibodies

What is the specificity?

Patient is a White 35 year old woman
Transfused 6 month ago

	Rh-hr					Kell		Kidd		Duffy		MNSs					PEG	Papain
Cell	D	C	E	c	e	K	k	Jk^a	Jk^b	Fy^a	Fy^b	M	N	S	s		IAT	IAT
1	+	+	0	0	+	0	+	+	0	+	0	0	+	+	0		2+	3+
2	+	+	0	0	+	+	+	+	0	+	0	+	+	0	+		2+	3+
3	+	0	+	+	0	0	+	+	0	+	+	0	+	+	+		0^v	0^v
4	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0		0^v	0^v
5	0	0	0	+	+	0	+	0	+	0	+	+	0	+	0		2+	3+
6	0	0	0	+	+	+	+	+	0	+	0	+	+	+	+		2+	3+
Auto	+	0	+	+	0	0	+	+	0	0	0	+	+	0	+		0^v	0^v

Patient's RBCs are e−
Panel indicates presence of alloanti-e

What is the specificity?

Patient is a White 72 year old man
Never transfused

DAT
PS: 2+ IgG: 2+ C3: 0

	Rh-hr					Kell		Kidd		Duffy		MNSs					PEG	Papain
Cell	D	C	E	c	e	K	k	Jk^a	Jk^b	Fy^a	Fy^b	M	N	S	s		IAT	IAT
1	+	+	0	0													2+	3+
2	+	+	0	0													2+	3+
3	+	0	+	+	0	0	+	+	0	+	+	0	+	+	+		0^v	0^v
4	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0		0^v	0^v
5	0	0	0	+	+	0	+	0	+	0	+	+	0	+	0		2+	3+
6	0	0	0	+	+	+	+	+	0	+	0	+	+	+	+		2+	3+
auto	+	+	0	0	+	0	+	+	0	0	0	+	+	0	+		3+**	4+**

Autoanti-e, of course!

** EGA-treated RBCs

Patient's RBCs are E−e+
Panel suggests presence of anti-e
Autoanti-e?

Autoadsorption removed all reactivity

Case 5: what is the specificity?

67 year old African American female
Hgb/HCT: 9.0/27.2
Chest pain
Hospital suspects autoanti-e

Initial panel suggests anti-e
No indication of autoantibody
Additional testing ruled out other underlying antibodies

	Rh-hr					Kell		Kidd		Duffy		MNSs					PEG	Papain
Cell	**D**	**C**	**E**	**c**	**e**	**K**	**k**	**Jk^a**	**Jk^b**	**Fy^a**	**Fy^b**	**M**	**N**	**S**	**s**		**IAT**	**IAT**
1	+	+	0	0	+	0	+	+	0	+	0	0	+	+	0		2+	3+
2	+	+	0	0	+	+	+	+	0	+	0	+	+	0	+		2+	3+
3	+	0	+	+	0	0	+	+	0	+	+	0	+	+	+		0^v	0^v
4	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0		0^v	0^v
5	0	0	0	+	+	0	+	0	+	0	+	+	0	+	0		2+	3+
6	0	0	0	+	+	+	+	+	0	+	0	+	+	+	+		2+	3+
auto	+	0	0	+	+	0	+	+	0	0	0	+	+	0	+		0^v	0^v

How can this e+ patient make an apparent alloanti-e?

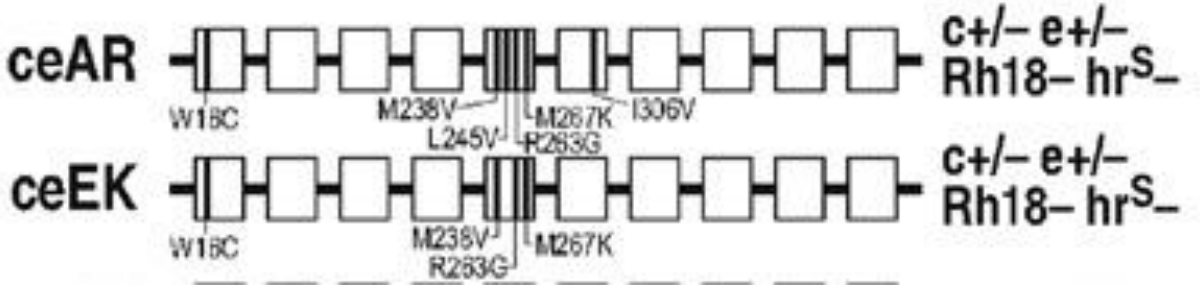
Partial RHCE Antigens

- **Analogous to RhD, altered forms of RHCE proteins express partial antigens**
- **Revealed when:**
  - **Antigen-positive patient makes the corresponding antibody, for example, alloanti-e or alloanti-C or alloanti-c in plasma of patients with e+ or C+ or c+ RBCs, respectively**
  - **Variable results are obtained when antigen typing**
- **Many altered RHCE alleles have been reported**
- **Distinguishing between auto- and alloantibody in a transfused patient or in the presence of warm autoantibodies can be difficult**
- **Analysis of *RHCE* genes can provide valuable insight**

Case 5: result of DNA analysis

***RHCE*: 2 altered *RHCE*ce* alleles**

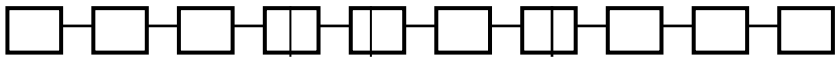
Compound heterozygote: *RHCE*ceAR* with *RHCE*ceEK*



Often partial RHCE phenotypes paired with partial D

***RHD:* Homozygous for an allele that encodes partial D**

RHD*DAR
Partial D



Genotype:
RHD*DAR-RHCE*ceAR/ RHD*DAR-RHCE*ceEK
Potential to make: Anti-D, -C, -E, -e/hr^S (-c, -f), Rh18

Case 5: testing with reagent anti-e

Anti-e reagent (clone/s)	RhceAR	RhceEK
Gamma-Clone (MS16, MS21, MS63)	3+	4+
Ortho Bioclone (MS16)	4+	4+
Biotest/Bio-Rad (Seraclone) (MS16, MS21, MS63)	4+	4+

Some partial e phenotypes give strong reactions with monoclonal anti-e
Difficult to recognize with routine reagents

Case 5: transfusion

- R_2R_2 RBCs suitable until patient makes anti-E, anti-D, etc.
- Donor screening with anti-hr^S or patient plasma
- RH genotype any donors identified as $hr^S–$
  - Very few D– $hr^S–$ donors
- Search for donors with similar *RHD* and *RHCE* genotype
- Often Rh-negative (rr) RBCs can be transfused
- Lack of documented experience with regard to the clinical significance of most anti-e-like antibodies
- Autologous donation if patient's clinical state permits

Patient 6: History

- 54-year-old female orthopedic patient
- Hgb 8.9
- Recently transfused 3 units
- Previous antibody history (Hosp ID):

  Anti-C, anti-E, WAA, unspecified antibody
- Request for antibody identification

Case 6: initial RBC testing

- Reactions suggested the antibody was directed at a high prevalence antigen
- Antibody to an Rh antigen was suspected
- The plasma was non-reactive with −D− RBCs
- The patient's RBCS were hr^{B}+ and hr^{S}–
- 4 of 8 C− E− hr^{S}− units were compatible

Case 6: initial results for RHCE DNA Analysis

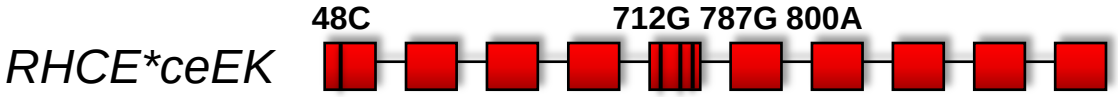
- **Predicted C−E+c+e+**
- **Serology results: C−E−c+e+**
- **Molecular testing confirmed with manual PCR-RFLP for E/e and exon 5 sequencing**

- **Race indicated as White**
- **After investigation, patient is Hispanic**

- **Possible silenced RHCE*cE allele or altered allele with very weak antigen expression**

Case 6: additional results

- **RHCE beadchip:**
  - **Negative for cE variants (EI, EIII, EIV, and EKH)**
- **PCR-RFLP for 907delC: silenced cE found in Hispanics §**
  - **Heterozygous for 907 deletion**
  - **Predicts E−**
- **Other allele: *RHCE*ceEK***
  - **partial c and e, Rh18−, hr^S−**
  - **Associated with production of alloanti-e, -ce(f), -hr^S and/or –Rh18**

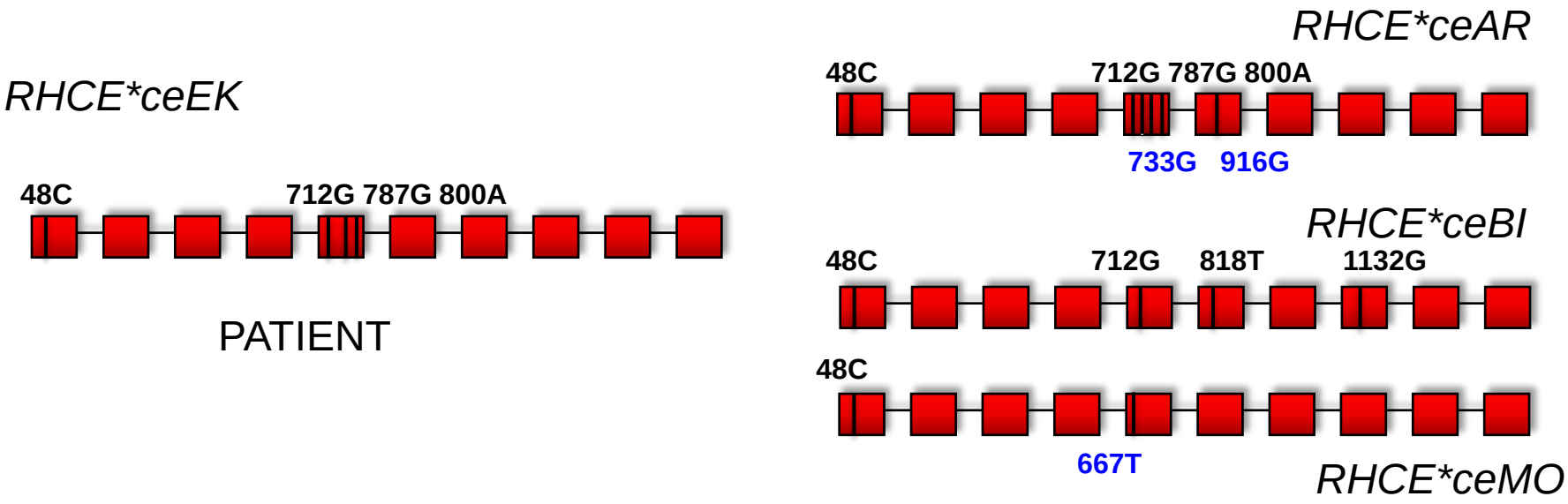


§ Westhoff *et al.* Transfusion 2011 51:2142-7.

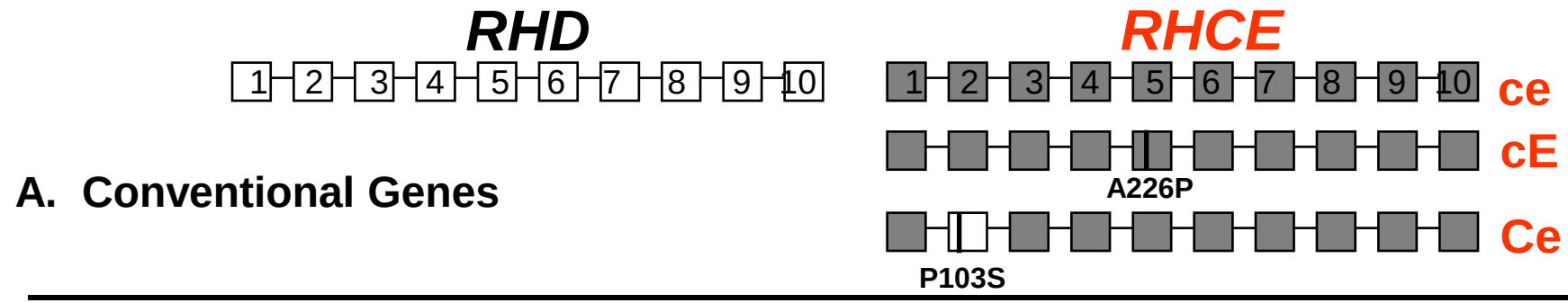
Case 6: compatible hr^{S}– cells

- **Compatible with 4 of 8 hr^{S}– samples**
- **Many *RHCE* backgrounds give the hr^{S}– phenotype**
- **Full RH genotype known on 3 of the samples**
  - **R*HD*DAR – *RHCE*ceAR* homozygous**
  - ***RHD*DAU0 – RHCE*ceMO* homozygous**
  - ***RHD*DAU0 – RHCE*ceMO / RHD*DOL – RHCE*ceBI***

Units transfused



Variant RHD and RHCE genes common in African-Americans (and some Hispanics)



A. Conventional Genes

B. Variant RHD Genes

D/CE/D
No D antigen, altered C antigen

DIIIa
N152T T201R F223V

DIII Type 4
L62F A137V N152T

DAU
T379M

DAR
T201R F223V I342T

DOL
M170T F223V

DIVa
L62F N152T D350H

C. Variant RHce Genes

ce^{S} hr^{B-} — W16C L245V

ce^{S} hr^{B-} — W16C L245V G336C

ce^{MO} hr^{S-} — W16C V223F

ce^{EK} hr^{S-} — W16C M238V R263G M267K

ce^{AR} hr^{S-} — W16C M238V L245V R263G M267K I306V

ce^{BI} hr^{S-} — W16C M238V A273V L378V

Adapted from: Westhoff CM., *Semin.Hematol.* 2007;44:42-50.

DNA typing to predict if a fetus is at risk for anemia of the fetus and newborn

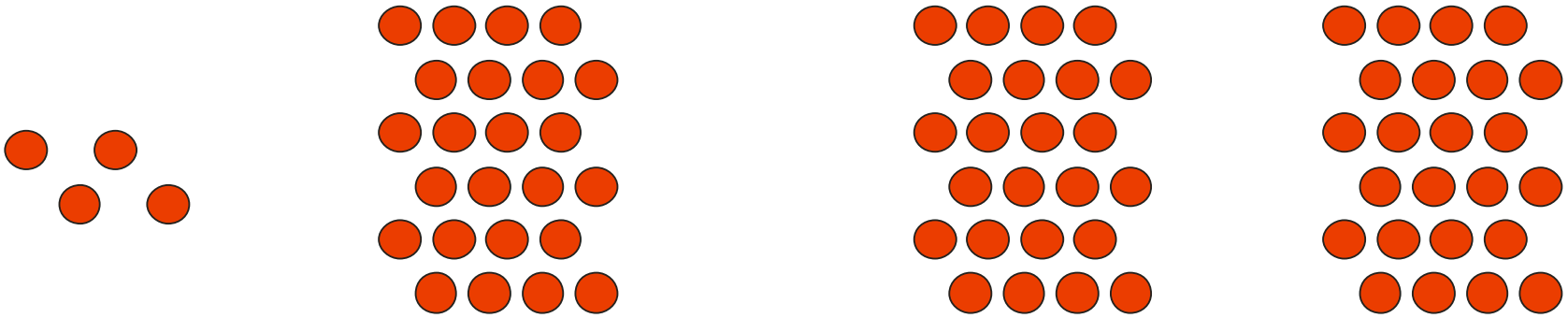
Anti-K in pregnancy

- **Titer of anti-K: not predictive**
  - **Low titer: severely affected fetus**
  - **High titer: K– fetus**
- **Bilirubin level in amniotic fluid: not predictive**
- **Different mechanism from HDFN due to anti-D**

Suppression of erythropoiesis

Erythroid Progenitors (BFU-E & CFU-E) from

K+ D+ cord blood **K– D+ cord blood**



Anti-K **Anti-D** **Anti-K** **Anti-D**

Anti-K, but not anti-D, suppressed their growth
"Anemia of the fetus and newborn" more appropriate designation

Use DNA testing to determine if fetus at risk

Vaughan, *et al*., *N. Engl. J. Med.* 1998;338:798

DNA analysis for Kell antigens in pregnancy

- **A valuable tool to determine if fetus is at risk for anemia of the fetus and newborn**
- **Points to remember:**
  - **Maternal anti-K may have been stimulated through transfusion, not pregnancy**
  - **Test of the baby's father strongly recommended**
  - **Only serological testing of father may be sufficient?**
  - **Only DNA analysis of father may be sufficient?**

Case 7: a cautionary tale

- **Pregnant woman with anti-K**
- **Sample from baby's father submitted for Kell genotyping**
  - ***KEL*01/02*** **(HEA DNA array)**
  - **Predicts the RBCs will be K+k+**
  - **Predicts 50% probability the baby's RBCs will be K+**
- **Sample also submitted for K antigen typing**
  - **K+k−**
  - **Predicts 100% probability the baby's RBCs will be K+**
  - **Father has a silenced *k* allele**
- **An example of the power of combining serological and DNA testing**
- ***Select test method based on the question being asked***

Some applications of DNA analysis

- To predict antigen type of recently transfused patient
- When RBCs are coated with IgG (+DAT)
- To distinguish allo from auto antibodies
- To detect weakly expressed antigens (e.g., Fy^b with Fy^X phenotype); where patient is unlikely to make antibodies to transfused antigen-positive RBCs
- Determine origin of engrafted leukocytes in a stem cell recipient
- Determine origin of lymphocytes in patient with graft-versus-host disease

More applications of DNA analysis

- **Determine zygosity, particularly *RHD***
- **Resolve discrepancies, e.g., A, B, D, C, c, e**
- **To aid in the resolution of complex serological investigations**
- **To fill a reagent void to determine antigen type of patient or donor when an antibody is weak or not available, e.g., anti-Do^{a}, anti-Do^{b} anti-Jsa, anti-V, anti-VS**
- **To identify molecular basis of unusual serological results, especially Rh variants**

DNA analysis often is the key to unlocking the mysteries of serological testing

Both DNA analysis and serology are essential to put the puzzle together