HAEMOGLOBINOPATHY PATIENT GENOTYPING

Optimising clinical care

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Background

- NHSBT mission statement: ‘to save and improve lives’, includes the haemoglobinopathy patient genotyping initiative
- Inherited diseases collectively known as haemoglobinopathies., include sickle cell disease (SCD) and thalassemia.
- 10,000 people with sickle cell disease and around 800 with β-thalassaemia.
- The majority of these patients receive (exchange) red cell transfusion therapy on multiple occasions during their life-time.
- For some patients, it becomes impossible to identify fully compatible UK donors.
- The ethnic disparity between the these patients and the UK blood donor populations means that a high proportion become sensitised, forming antibodies to multiple blood groups.
- Several blood services worldwide are adopting genotyping technologies to improve the provision of extensively-typed blood for multi-transfused patients.

Alloimmunisation in SCD patients

- 1996 BCSH deemed matching for C,c,E,e, K “desirable” for SCD patients
- Evidence that despite matching, alloimmunisation rates are still high
- The majority of alloantibodies formed are against Rh and Kell system antigens

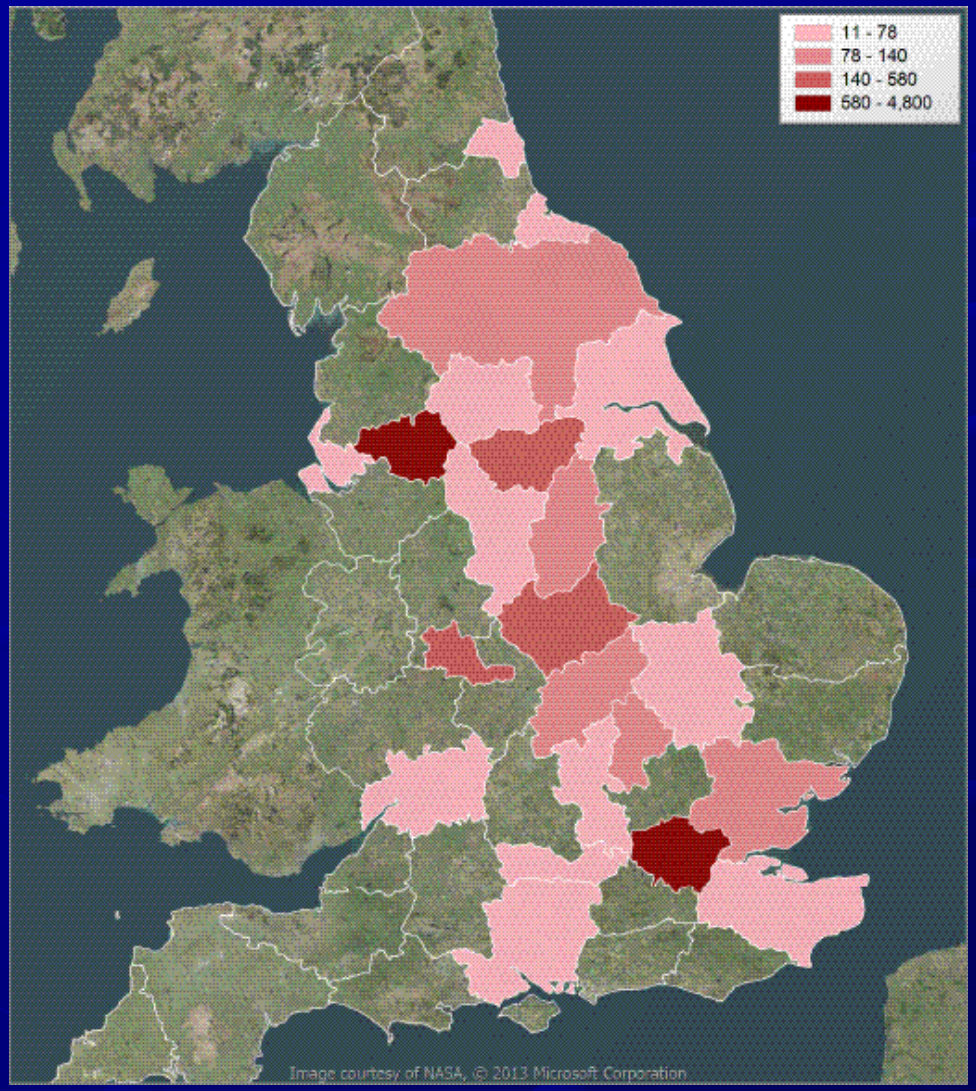
Why are the rates so high?

- Proportion will be already immunised
- Emergency / crisis transfusions not matched
- Patients move around – may receive transfusion abroad or in centre not experienced for SCD
- Variant Rh genes more frequent in black African individuals
- Majority of donors are from Caucasian ethnic background

RCI haemoglobinopathy patient genotyping project

- Proposed project start December 2014 (for 2 years)
- Provide a **free** genotype to haemoglobinopathy patients
- Red cell phenotypes to be available nationally as part of an initiative to provide these patients with optimal transfusion therapy.

SCD patient demographics



- Most SCD patients are in London region, NW and Midlands

-80% SCD patients will be supported by Colindale and Tooting's HTLs

- Project to include patients who are already immunised and those without antibodies

- Urgent clinical cases to use the existing (chargeable) service route

How many haemoglobinopathy patients are there?

Thalassemia 942

Sickle Cell 7690

Other 363

Total 8995

Registry entry requires consent, therefore no accurate estimate of the real number of SCD patients

Estimate testing 10K-14K patients over 2 years

Project Methods

- Nov 2014: NHSBT communications to hospitals etc advising of new test availability
- Begin patient blood sample collection, samples taken at clinics, GPs etc, sent to local RCI centre.
- Samples sent via NHSBT transport to IBGRL, Filton
- Batched sample DNA extraction.
- DNA analysed using real-time PCR allelic discrimination
- Automated transfer of results (predicted phenotypes) to Hematos and available to hospitals via sp-ICE.

Testing coverage (Rh)

SNP	ALLELE 1	ALLELE 2	Variant identification (not defining)
RHD EX7	RHD present	RHD absent	RHD, no RHD
RHD int 4	RHD present	RHD absent	RHD, no RHD
RHD 674C/T	RHD 674C	RHDpsi 674T	RHD pseudogene
RHD 455A/C	RHD455A	RHDvar 455C	DIIIa, DVIa
RHD 667T/G	RHD667T	RHDvar 667T	Weak D type 4, DAR, DOL
RHCEint2/ ex2	RHCE*C	RHCE*c	
RHCE 667G/T	RHCE667G	RHCEvar667T	ceMO
RHCE676C/G	RHCE*E 676C	RHCE*e 676G	
RHCE712A/G	RHCE712A	RHCEvar712G	ceAR, ceEK, ceBI,
RHCE733C/G	RHCE733C	RHCEvar733G	(C)ce^{s}, V/VS, hr^{B} neg
RHCE1006G/T	RHCE1006G	RHCEvar1006T	(C)ce^{s,} hr^{B} neg

Testing coverage (other groups)

SNP	ALLELE 1	ALLELE 2
K/k	K	k
Jsa/b	Jsa	Jsb
Kpa/b	Kpa	Kpb
Fya/b	Fya	Fyb
Fy/wildtype	Gata mut+	Gata mut-
Jka/b	Jka	Jkb
M/N	M	N
S/s	S	s
S silencing 230	Silent S	normal S
S silencing int5+5	Silent S	normal S
Doa/b	Doa	Dob

Aims / expected benefits

1. *Better Compliance* with existing guidelines (for Rh and K matched blood). The project will deliver the rapid access to patient genotypes required to support this, but this will need to be supplemented with education & support directed to hospital transfusion committees and laboratory staff, supported by NHSBT consultants and transfusion practitioners.
2. *Better Selection* of blood, especially for patients with Rh variants identified via DNA-based typing. The project will need to ensure that data presented via SpICE is supplemented with appropriate donor-selection advice (which can be complex).
3. *Better Matching* of blood for non Rh antigens, tentatively agreed as K, Fy (inc GATA mutation), Jk, MNSU, Js, Do, V, VS The project will enable the selection of antigen-negative blood for high risk patients - probably those who have already made an antibody.

Future

- After 2 year period continue prospective genotyping of new patients (c.300 per annum)
- Likely that full benefit only realised if (some) donor genotyping also performed

British Blood Transfusion Society

Genotyping – Optimising Patient Care

Shane Grimsley
British Blood Transfusion Society
Red Cell Special Interest Group
2014

University of BRISTOL

NHS Blood and Transplant

Genotyping
The Silver Bullet?

- Genotyping
- Sequencing
- Next Generation Sequencing

- How can genotyping improve clinical care of Haemoglobinopathy patients, specifically in relation to Rh?

Haemoglobinopathy Patients – Current Situation

- Matching
  - ABO, D, C, c, E, e and K
  - HbS negative

Haemoglobinopathy Patients – Immunisation Occurs

- Why?
  - Match ABO, D, C, c, E, e and K: ~10% (1,500)
  - Not following procedure: ~10-20% (1,500-3,000)
  - Pregnancy
  - Exposure (8 units / 6 weeks = 70 units pa)
  - Responders
- Immunisation to common blood group Antigens

Haemoglobinopathy Patients – Extended Donor Typing

- Matching
  - ABO, D, C, c, E, e and K
  - HbS negative
  - Antigen negative for any clinically significant antibody present
- Extra typing of donations:

  Fy^a, Fy^b, Jk^a, Jk^b, M, S, s, C^w, Lu^a, k, Kp^a

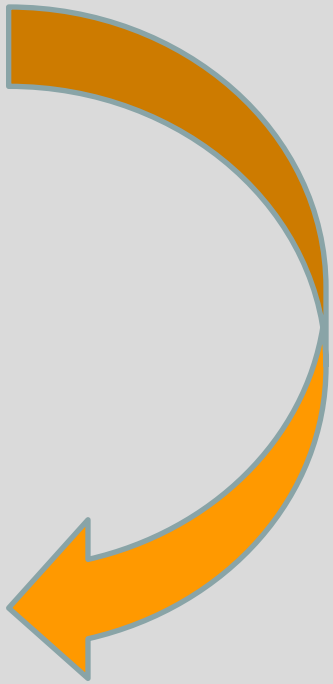
  ~ 500 donations per day
- Sufficient to supply suitable blood for the majority of cases

Haemoglobinopathy Patients – Immunisation Occurs

- Why?
  - Match ABO, D, C, c, E, e and K: ~10%
  - Not following procedure: ~10-20%
  - Pregnancy
  - High exposure
    (8 units every 6 weeks = 70 units p.a.)
  - Responders
  - BEM patients, Caucasian donors
- Immunisation to other blood group Antigens (Rh and Kell systems)

Haemoglobinopathy Patients – The Problem Cases

- Antibodies to Caucasian Antigens
  - hr^S, hr^B, Js^b
- Then transfuse with BEM blood
  - Not enough!
    - Currently 10,000
    - Require 200,000



- ‘Untransfusable’ patient

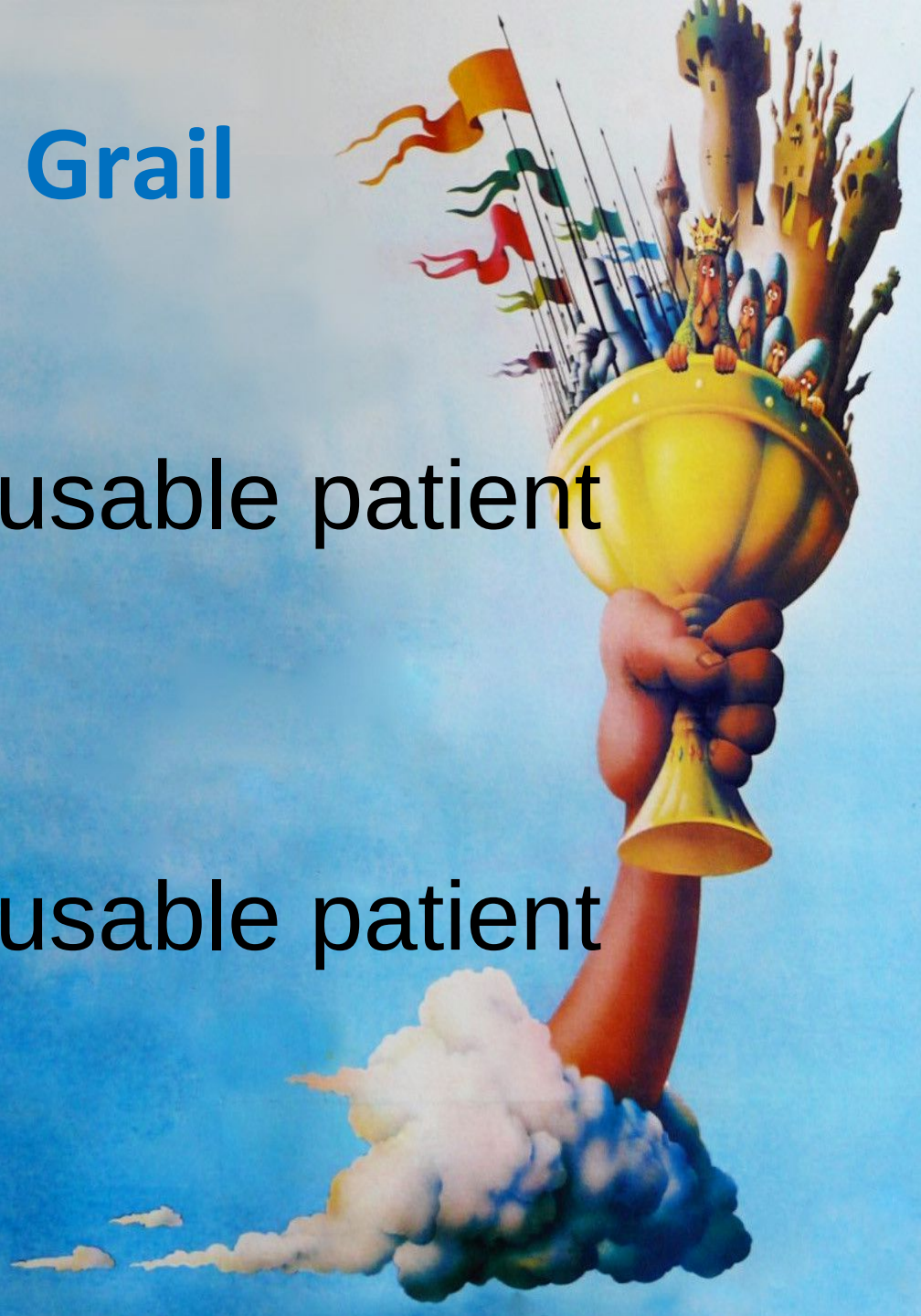
The Holy Grail

- Prevent

  the untransfusable patient

- Manage

  the untransfusable patient



Haemoglobinopathy Patients – Prevent the 'Untransfusable'

1. Procedure

2. More BEM donors

3. More detailed analysis of problem blood group genes
   - *RHD* and *RHCE*

Haemoglobinopathy Patients – Rh

- RhD variants
- RhCcEe variants
- Hybrids

  Are all more common in BEM
- Some negative for ‘HFA’s
- Some are not identified by routine serology

Haemoglobinopathy Patients – *RH* variants – What to look for?

Variants that:

- Are BEM associated
- Produce a clinically significant antibody
- Produce an antibody that makes transfusion problematic
- Are missed by routine phenotype

Haemoglobinopathy Patients – D variants

- D variants can appear D+ by routine serology
  - DIIIa, DIVa, DV, DOL and DAR
- All can make anti-D
- Identify
  - SNP detection, 455A>C, 667T>G
- Give D– blood (before anti-D)

Haemoglobinopathy Patients – D variants

- D variants and hybrids can appear D+ by genotype
  - *RHD*psi*, d(C)ce^{S}, DVI
- All can make anti-D
- Identify
  - SNP detection, 674C>T *(RHD*psi)*
  - Target multiple *RHD* regions (hybrids)
- Give D– blood (before anti-D)

Haemoglobinopathy Patients – D variants

SNP	ALLELE 1	ALLELE 2	Variant identification (not defining)
RHD EX7	*RHD* present	*RHD* absent	*RHD*D*, no *RHD*
RHD int 4	*RHD* present	*RHD* absent	*RHD*D*, no *RHD*
RHD 674C/T	*RHD* 674C	*RHD* psi 674T	*RHD**pseudogene
RHD 455A/C	*RHD* 455A	*RHD* var 455C	*RHD*DIIIa, DIVa*
RHD 667T/G	*RHD* 667T	*RHD* var 667T	*RHD*WkD type 4, DAR, DOL*

- If there is some *RHD* gene missing or altered – give D–

Haemoglobinopathy Patients – *RHCE* variants

- RhCcEe variants can appear antigen positive by routine serology
  - d(C)ce^{S}
- Can make anti-C
- Identify
  - SNP detection, 733C>G, 1006G>T
- Give C– blood (before anti-C)

Haemoglobinopathy Patients – *RHCE* variants

- RhCcEe variants and hybrids can appear normal antigen positive by genotype
- Important to target variant alleles
  - ceMO, ceAR, ceEK, ceBI, ce^{S}
- All can make anti-e-like
- Identify
  - 667G>T, 712A>G, 733C>G
- Give...

Haemoglobinopathy Patients – *RHCE* variants

- Like D:

  Variant *RHCE* alleles can be detected
- Unlike D:

  There is no RhCcEe negative blood
- Matching SIGNIFICANTLY more difficult

Haemoglobinopathy Patients – *RHCE* variants

SNP	ALLELE 1	ALLELE 2	Variant identification (not defining)
RHCEint2/ ex2	RHCE*C	RHCE*c	
RHCE 667G/T	RHCE667G	RHCEvar667T	ceMO, hr^S neg, hr^B neg
RHCE676C/G	RHCE*E 676C	RHCE*e 676G	
RHCE712A/G	RHCE712A	RHCEvar712G	ceAR, ceEK, ceBI, hr^S neg
RHCE733C/G	RHCE733C	RHCEvar733G	d(C)ce^s, V/VS, hr^B neg
RHCE1006G/T	RHCE1006G	RHCEvar1006T	d(C)ce^s, hr^B neg

- Options for transfusing are both limited and challenging

Haemoglobinopathy Patients – *RHCE* variants

- For example:
  - *RHCE*ceAR/ceAR*
  - Transfuse *RHCE*ce/ce*
  - Produces anti-hr^{S} (anti-e-like)

1	2	3
Give *RHCE*ce/ce*	Give *RHCE*cE/cE*	Give *RHCE*ceAR/ceAR*
Accept risk of anti-hr^{s}	Accept risk of producing anti-E	Where from?

Haemoglobinopathy Patients – Prevent the 'Untransfusable'

1. Procedure

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3. More detailed analysis of problem blood group genes
   - *RHD* and *RHCE*

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Thank You!

Questions?

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