The Application of Genotyping for Rare Donor Screening

Nicole Thornton
Head of Red Cell Reference, IBGRL
NHSBT

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Head of Red Cell Reference, IBGRL, Bristol

NHS Blood and Transplant

Outline

- Define “Rare Donor”
- Current screening approach at NHSBT
- Molecular bases of blood groups
- Genotyping for rare donor screening
  - Pros
  - Cons
- Understanding demand
- The future

Definition of a Rare Donor

- Different between countries

Vox Sanguinis (2008) **95**, 236–253

DOI: 10.1111/j.1423-0410.2008.01084.x
Vox Sanguinis

INTERNATIONAL FORUM

Donors with a rare pheno (geno) type

H. W. Reesink, C. P. Engelfriet, H. Schennach, C. Gassner, S. Wendel, R. Fontão-Wendel, M. A. de Brito, P. Sistonen, J. Matilainen, T. Peyrard, B. N. Pham, P. Rouger, P. Y. Le Pennec, W. A. Flegel, I. von Zabern, C. K. Lin, W. C. Tsoi, I. Hoffer, K. Barotine-Toth, S. R. Joshi, K. Vasantha, V. Yahalom, O. Asher, C. Levene, M. A. Villa, N. Revelli, N. Greppi, M. Marconi, Y. Tani, C. C. Folman, M. de Haas, M. M. W. Koopman, E. Beckers, D. S. Gounder, P. Flanagan, L. Wall, E. Aranburu Urtasun, H. Hustinx, C. Niederhauser, E. Massey, A. Gray, M. Needs, G. Daniels, T. Callaghan, C. Flickinger, S. J. Nance & G. M. Meny

Question 1. What is your definition of a rare donor?

- There was no complete consensus concerning the definition of a rare donor

Definition of a Rare Donor

Two categories

1. a donor who lacks a high frequency antigen (99.9% incidence in the population)
   - Rare [Lu(b-), Kp(b-), U-]
   - Very rare [-D-, K_0, O_h, pp]
   - Extremely rare [Rh_{null}, At(a-), McLeod, M^kM^k]
2. a donor who lacks a combination of antigens such that the incidence is 1:200 or less in a population

Current screening program

Serology

- Routine Rh typing (D, C, c, E, e) facilitates the detection of r'r', r"r", Rh_{null}, D--/D-- and other Rh variants.
- O_h donors detected via anomalous ABO grouping results
- K+k-, Lu(a+b-), Kp(a+b-), Jk(a-b-), Fy(a-b-) and S-s-U-, detected through routine extended typing strategy

Current screening program

Serology

- Screening carried out by Tooting RCI since late 1970's
- Moving to Filton under management of IBGRL from 2017

Specificity	Antibody type	Dilution	Technique
Co^a	human	1:20	IAT
Ge	monoclonal	1:20	Saline
I	human	1:20	Saline
k	human	1:20	Saline
Kp^b	monoclonal	1:150	IAT
Lu^b	monoclonal	1:20	Saline
Wr^b	monoclonal	1:80	Saline
Vel	human	1:50	Papain
Lan	human	1:100	IAT
Yt^a	human	1:15	IAT

- Manual microplate method
- Batches of 640 new donors
- Adaptable to patient need

- Additional screening based on ethnicity (tube serology)
  - In^b, Js^b
- All rare donors confirmed by IBGRL
- NHSBT is one of the main rared onor contributors to the IRDP

Molecular bases of blood groups

- Single nucleotide polymorphism (SNP)
- Deletions (nucleotide, exons, gene)
- Insertions
- Intergenic exchanges – hybrid genes
- Some unknown eg. Emm, AnWj, PEL, Er^a

Most of the common clinically relevant antigens (except ABO antigens and D)

Genotyping

- Most genotyping platforms commercially available are based on SNP detection
- Important to remember this when dealing with rare phenotypes – may not be looking in the right place

Example:

- Jk^a/Jk^b encoded by SNP at 838A/G in exon 9 of SLC14A1
- Jk(a-b-) phenotype can arise from homozygosity or compound heterozygosity of at least 15 different alleles, only two of which occur in exon 9, still would be missed
- incorrect phenotype would be predicted

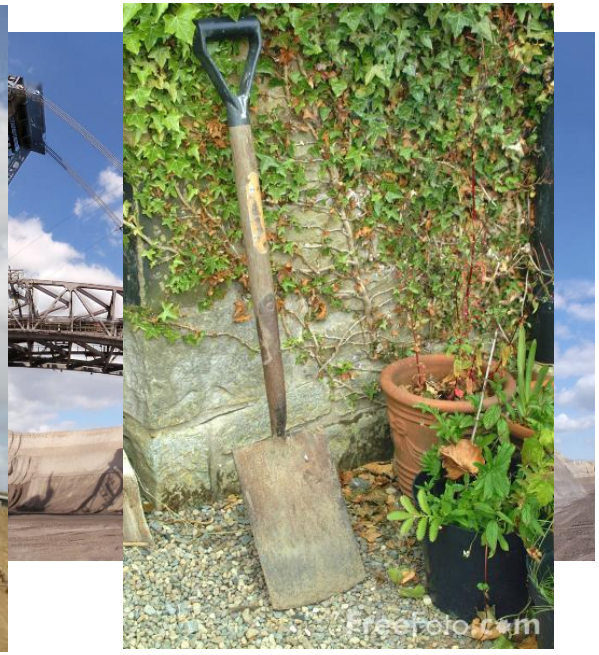
Genotyping

- Genotyping technology has progressed very quickly and is becoming more cost effective
- Many options depending on throughput and budget

  eg. Allelic discrimination by quantitative PCR using Taqman technology, DNA array analysis, microarrays, Luminex, MALDITOF MS, Next generation sequencing or massively parallel sequencing

Genotyping for rare donor screening

- The future – ? full genome established for all donors
- Currently this approach feels like:



Understanding demand

Rarity	Other Requirements	Rarity	Other Requirements
M^kM^k	(O) D-	r"r"	(O) S- Jk(b-)
r'r'	(O)	U-	(O) C- E-
Lan-	(O)	Hr^B-	(A)
Lan-	(B)	K_0	(O)
U-	(O)	Lan-	(A)
Lan-	(O) D- K-	D--/D--	(O)
MAM-	(O)	pp	(O)
Rh_{null}	(O)	Co(a-b-)	(O)
Lan-	(B)	Ge3-	(O)
En(a-)	(O)	Vel-	(A) C^W-
U- or $U+^{var}$	(A) N-	Fy(a-b-)	(O) E- C- N- S- K- Jk(b-)
Hr-	(O) D- or DAR	Co(a-b-)	(O)
U-	(O) K- (CMV- for baby unit)	Rh_{null}	(O)
In(b-)	(AB)	D--/D--	(O)
O_h		U-	(O) E-
Hr^B-	(A) D- S-	k-	(O) c- E- Fy(a-) Jk(a-)

Understanding demand

- Having enough S-s-U- units continues to be problematic in England
- Transfusion dependent haemoglobinopathy patients with multiple alloantibodies, may also include rare antibodies
- Already targeting Black donors for extended typing
- Genotyping these donors in particular is a better approach than serology
- Not enough donors

Photo: EM Unit, UCL Medical School, Royal Free Campus http://wellcomeimages.org/indexplus/image/B0000521.html

Genotyping Pros & Cons

Pros

- No need for rare antibodies eg. Anti-U, -Do^{a}, -Do^{b}, -hr^{S}, -hr^{B} -Js^{a}
- More suited to automation and data transfer
- High throughput capability (especially if package with “routine” donor genotyping)

Cons

- Must know molecular basis
- Some rare phenotypes genetically complex
- ? Serological confirmation
- Development costs

Combined approach

- Use genotyping for rare types that can be predicted confidently by SNP or gene detection
  - K+k-, Kp(a+b-), Js(a+b-), Lu(a+b-), In(a+b-), Vel-, Di(a+b-), Co(a-b+), Yt(a-b+), S-s-U-, Sc:-1, Rh:-51, Fy(a-b-), Hr-, Hr^{B}-, Do(a+b-), Do(a-b+), Wr(a+b-)

- Use serology for rare types that are genetically complex and antibodies are available
  - Lan-, Jk(a-b-), Lu(a-b-), I-, Jr(a-), En(a-) and $M^{k}M^{k}$ [MNSs typing], O_{h}, Rh_{null}, Ge:-2,-3, -D-, K_{0}, McLeod, Co(a-b-), pp, P^{k}

Logistics

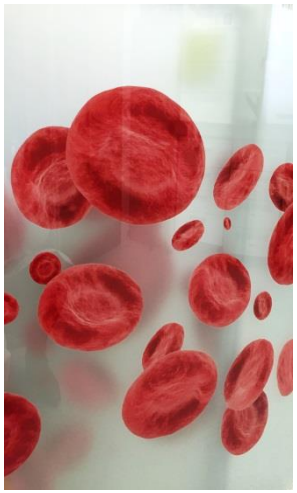
- Only screen repeat donors – likely to come back
- Targeted screening based on demand
- Cost must be justified – rare blood is required rarely
- Rare donor retention – can we do more?
- Actively recruit family members of patients and donors with rare phenotypes to be tested
- Regulatory issues - labelling units with geno results

Summary

- Currently, optimisation of rare donor screening can be achieved through a combined molecular and serological testing approach
- The genetic complexity of some rare blood groups means that we cannot confidently predict phenotype from genotype
- The future is looking very different with individualised medicine predicted and the promise of full genome data to help us achieve this
- However, the boundaries associated with implementing change mean that we must make the best of what we can change now, whilst we wait for…….

The Future

Thank You



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