



Red blood cell genotyping to reduce allo-immunisation and improve blood selection for haemoglobinopathy patients

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Haemoglobinopathies

- Sickle cell disease
- Thalassaemia
- Diamond-Blackfan anaemia
- Aplastic anaemia

- 11,000 patients in England
- Multi-transfused
- Accounting for ~3% of all red cell demand

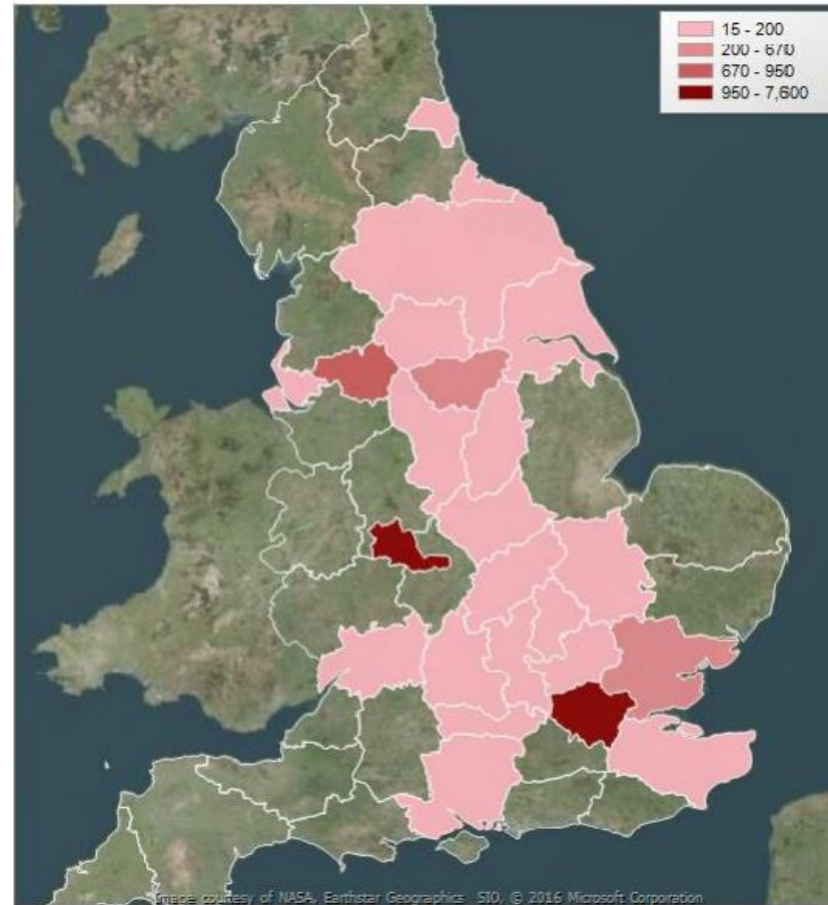


Image: National haemoglobinopathy register

Challenges of transfusing HGP patients

Alloimmunisation risk can be high

Haemolytic transfusion reactions
(Hyperhaemolysis syndrome)



“Untransfusable” patients



Autoimmune haemolytic anaemia

BCSH guidelines : ABO compatible, Rh (D, C, c, E, e) and K antigens

Despite prophylactic matching, alloimmunisation to Rh antigens still occurs

Majority of donors from Caucasian population

Majority of patients from Black Minority Ethnic (BME) population, with Large % of variant blood group alleles



Aim

- Free extended blood group genotype to all Hbopathy patients in England over 1.5/2 years
- Identify individuals with variant $RH^*D/C/e$ alleles
- Better selection of red cells for transfusion
- Ultimately reduce alloimmunisation and improve transfusion therapy



Options

- 10,000 expected samples over a 2 year period
 - Commercial or in house genotyping kits?
 - Consider cost, allele coverage and throughput
- In house Taqman allelic assays were designed - cost effective and high-throughput
 - 22 targets including *RHD* and *RHCE* variants.

Rh targets

SNP	ALLELE 1	ALLELE 2	Variant I.D.
RHD EX7	<i>RHD</i> present	<i>RHD</i> absent	RHD*D, no RHD
RHD int 4	<i>RHD</i> present	<i>RHD</i> absent	RHD*D, no RHD
RHD 674C/T	<i>RHD 674C</i>	<i>RHD psi 674T</i>	RHD*pseudogene
RHD 455A/C	<i>RHD 455A</i>	<i>RHD var 455C</i>	RHD*DIIIa, DIVa
RHD 667T/G	<i>RHD 667G</i>	<i>RHD var 667T</i>	RHD*WkD type 4, DAR, DOL
RHCEint2/ ex2	<i>RHCE*C</i>	<i>RHCE*c</i>	RHCE*C/c
RHCE 667G/T	<i>RHCE667G</i>	<i>RHCEvar667T</i>	ceMO
RHCE676C/G	<i>RHCE*E 676C</i>	<i>RHCE*e 676G</i>	RHCE*E/e
RHCE712A/G	<i>RHCE712A</i>	<i>RHCEvar712G</i>	ceAR, ceEK, ceBl,
RHCE733C/G	<i>RHCE733C</i>	<i>RHCEvar733G</i>	(C)ces, V/VS, hrB neg
RHCE1006G/T	<i>RHCE1006G</i>	<i>RHCEvar1006T</i>	(C)ces, hrB neg



Other targets

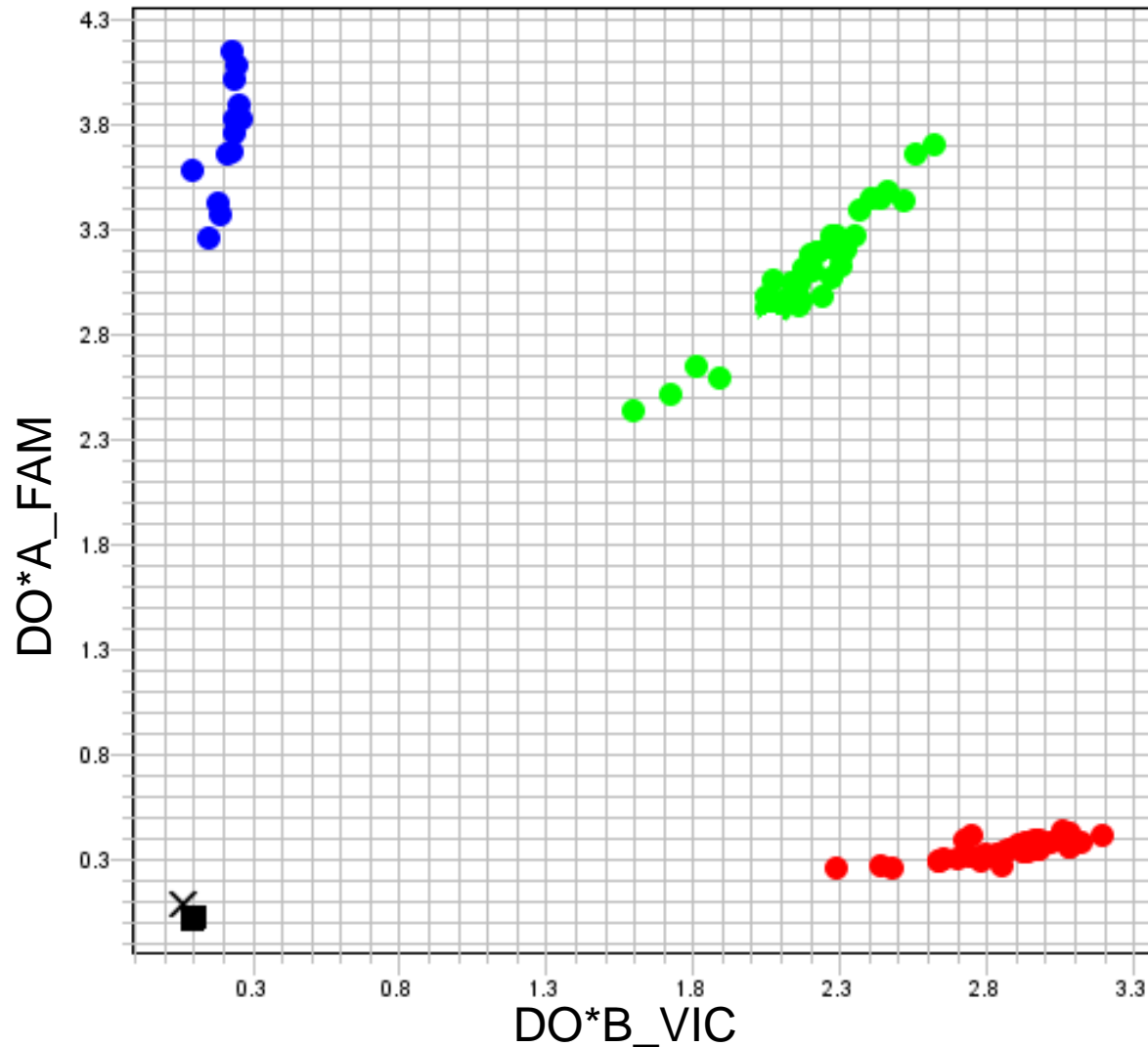
SNP	ALLELE 1	ALLELE 2
K/k	<i>K</i>	<i>k</i>
Jsa/b	<i>JS*A</i>	<i>JS*B</i>
Kpa/b	<i>KP*A</i>	<i>KP*B</i>
Fya/b	<i>FY*A</i>	<i>FY*B</i>
Fy/wildtype	<i>GATA</i> mut+	<i>GATA</i> mut-
Jka/b	<i>JK*A</i>	<i>JK*B</i>
M/N	M	N
S/s	S	s
S silencing 230	Silent S	normal S
S silencing int5+5	Silent S	normal S
Doa/b	<i>DO*A</i>	<i>DO*B</i>

Method

- Whole blood samples
- High-throughput automated DNA extraction
- 8 x 384 well PCR plates/95 patients
- Taqman allelic discrimination
- Software analysis



Allelic Discrimination Plot



Homozygous

DO^*B_VIC/DO^*B_VIC

Homozygous

DO^*A_FAM/DO^*A_FAM

Heterozygous

DO^*B_VIC/DO^*A_FAM

Undetermined



Challenges Gen → Phen

Use allelic discrimination plots to predict phenotype (4 different software programs)
Very complex procedure (SNP → genotype → predicted phenotype)

E/e	e variant SNP sites		
676			
G/G			
C/C			
G/C			
*UND			

***Undetermined**



Technical challenges of the project

Scientific challenges

1. Algorithm challenges
2. Homology of the genes
3. Primer design

Automated equipment

1. Tube sizes
2. Modification of extraction procedure

IT complications

Linking several different softwares together for reporting



Results so far

- Number tested: 3,728 patients / 10 000 expected
- Majority (77%) of patients tested – SCD

11% = 1 or more variant Rh alleles

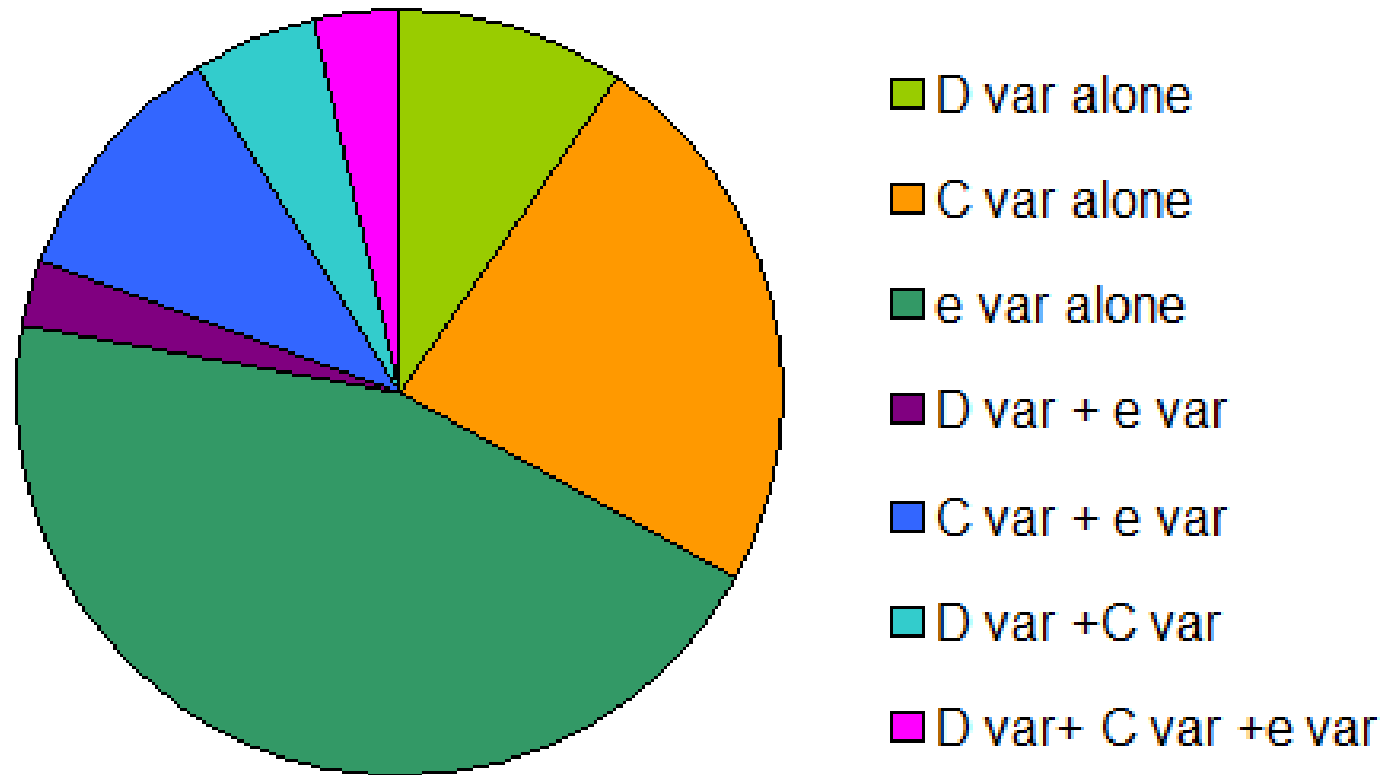
3% = 2 or more variant Rh alleles

0.4% = 3 variant Rh alleles

Further work to be done:

- Analysis of non-*RH* genotypes
- Unresolved samples sent for Sanger Sequencing

Results so far – Rh variant



Impact on product selection

Predicted phenotype	Potential Ab	Product advice without Ab	Product advice with Ab
D+	Auto/allo anti-D	D+	D—
D^{var}	Anti-D	D— *	D—
D+ E+ e^{var}	Anti- hr ^s /-hr ^b	e—	e— (D+ E+)
D+ E— e^{var}	Anti- E, anti-hr ^s /hr ^b	E—	1. E— 2. E— and IVIG cover 3. If unacceptable haemolysis D+ E+ e—
D— E— e^{var}	Anti-D, anti-E, anti-hr ^s /hr ^b	D— E—	1. D— E— 2. D— E— and IVIG cover 3. If unacceptable haemolysis r''r''
D+ C^{var} c+	Anti-C	C—	C—
D— C^{var} c+	Anti-D, anti-C	D— C—	D— C—
D^{var} C^{var} e^{var} E—	Anti-D, anti-C, anti-hr ^s /hr ^b , anti-E	D— C— e+ E—	1. D— C— e+ E— 2. e+ IVIG cover 3. If unacceptable haemolysis r''r''
D^{var} C^{var} e^{var} E+	Anti-D, Anti-C, anti-anti-hr ^s /hr ^b	D— C— e+ E—	1. D— C— e+ E— 2. E— + IVIG cover 3. If unacceptable haemolysis r''r''

* If there is a history of D+ transfusion, without production of anti-D, D+ may be considered.



Conclusions

- Main observation = significant % of these patients have variant Rh alleles
- Have these patients been inappropriately transfused based on their serological phenotype?
- Change in blood selection practice
 - better matching
 - reduction in alloimmunisation
 - reduction in untransfusable patients
- Future : Donor genotyping, increasing stocks of Ro, rr and r''r'' blood



Acknowledgements

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Karen Desay, Olivia Hill, Susan Tovey, Kirstin Finning

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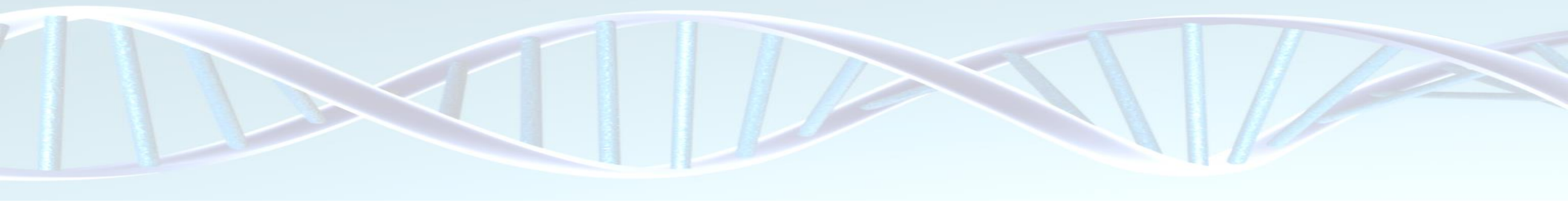
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Dr. Tom Latham



Questions?



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