D variants in obstetrics: positive or negative?

Joint Meeting of UK NEQAS (BTLP) and
BBTS Blood Bank Technology SIG, Birmingham, November 2017

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NHS
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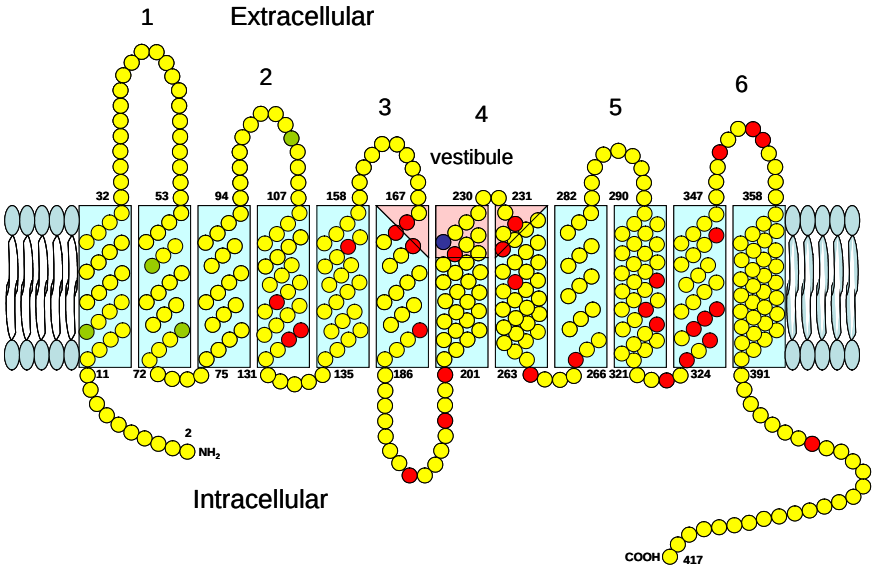
Outline

- Definition of “D variant”
- The importance of D in pregnancy
- Antenatal and postnatal management
- Complex case study

D

Not a simple antigen!

- Not derived from an amino acid polymorphism but from presence of RhD protein



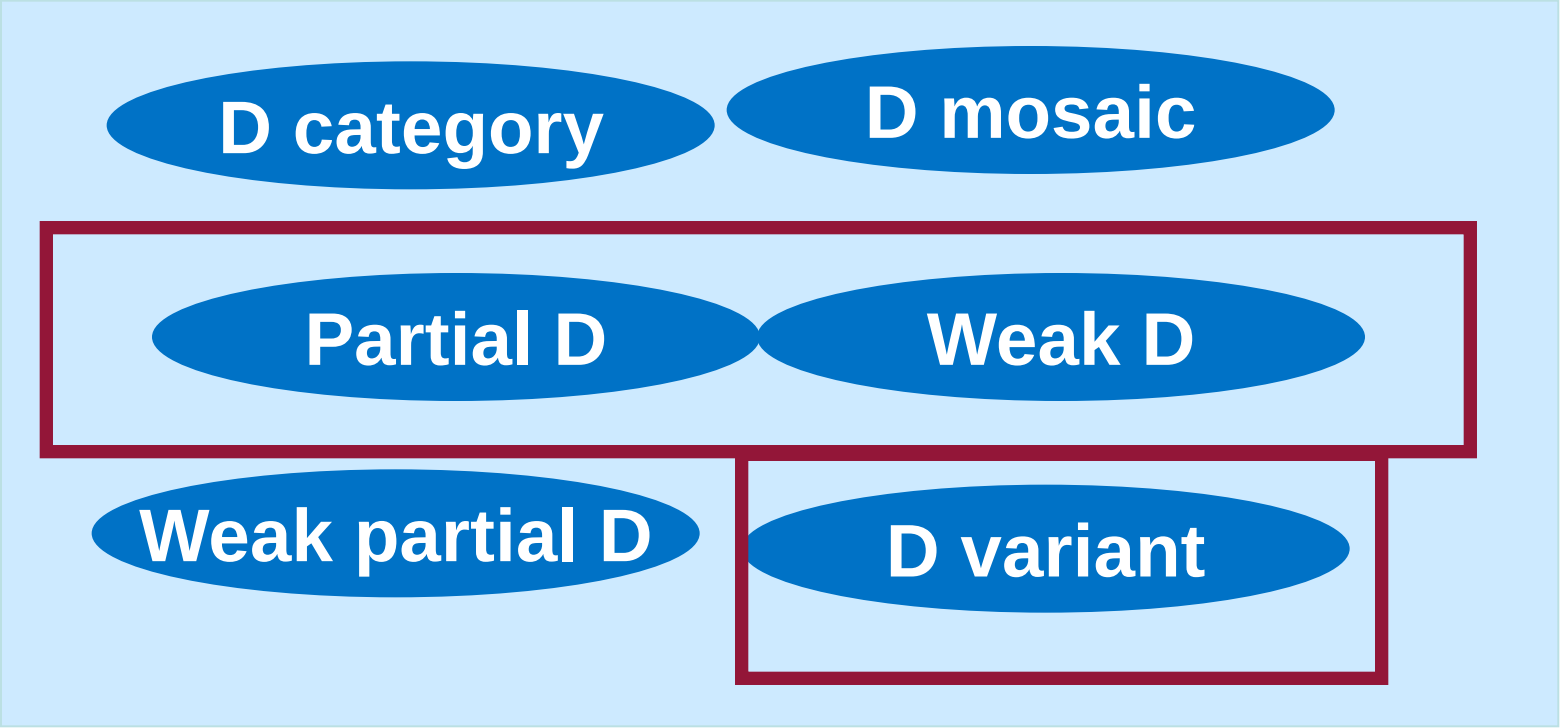
- Antigen expression varies quantitatively and qualitatively
- Genetically complex - D variants arise from point mutations and gene conversion

Image ref: Geoff Daniels. Human Blood Groups. 2013. 3rd ed. Wiley-Blackwell.

Complexity of D

Nomenclature

- D+



- D-

D variants – confusing!

Partial D

- Missing one or more D epitopes
- Might be weak, strong or negative with anti-D
- May produce alloanti-D to the missing epitopes

Weak D

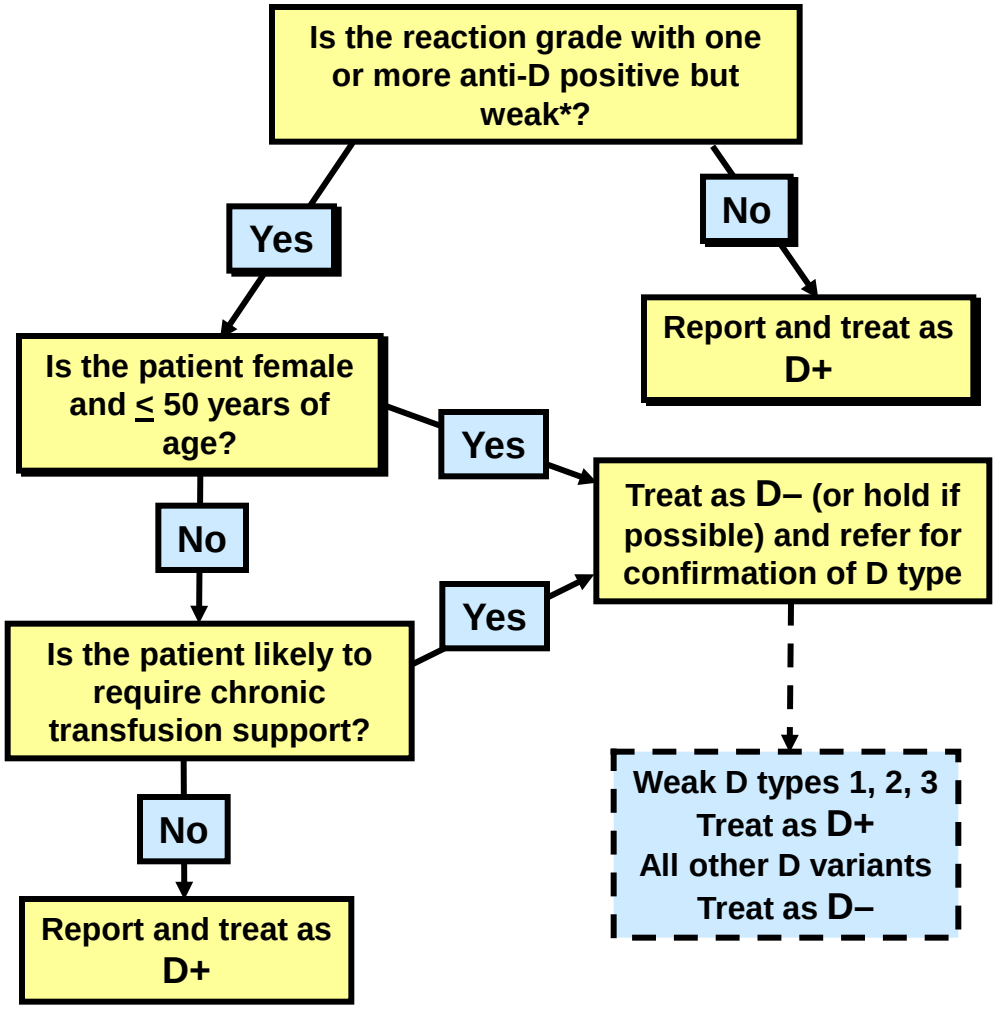
- Types 1, 2 and 3 most common in Caucasians
  - ➲ unlikely to make alloanti-D
- Alloanti-D reported in some less frequent types
  - ➲ Weak D types 4, 11, 15, 21, 57

Historically terms described phenotype, but now may be assigned to indicate predicted location of aa change

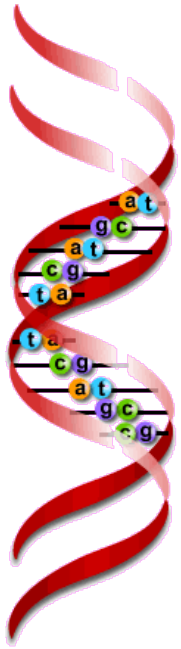
D Typing – BSH Guidelines

Patients & Pregnant Women

What should we do when there are weak or discrepent results?



D Genotyping



- Weak D types 1,2 and 3 sufficient for following the recommendations for transfusion
- All result from simple SNPs in the *RHD* gene
- ‘In house’ multiplex platforms straightforward
- Commercial kits available
- Shortage of reference labs providing this testing service

The Importance of D

Clinically Significant

- Most important antigen in Rh system
- Highly immunogenic (second only to ABO)
  - >30% D– make anti-D after receiving D+ blood
  - D+ red cells not usually transfused to D– patients
  - D+ cells never given to D– girls & premenopausal women
- Anti-D, most important after anti-A and anti-B
  - causes IHTRs
  - causes severe HDFN

Haemolytic disease of the fetus and newborn (HDFN)

- Deaths in England & Wales:

19701.2 per thousand births

Introduction of immunoprophylaxis with anti-D immunoglobulin

19890.02 per thousand births

- Severity varies
- Most severe: fetal death at or after week 17
  Hydrops; jaundice; kernicterus usually leading to death or permanent brain damage

Fetal Genotyping

Cell-free fetal DNA in maternal plasma

- 10–20 weeks: 10–15% cell-free DNA = fetal

  Range: 3–30%

  >21 weeks: increases by ~1% per week

- DNA isolated from maternal plasma

  85-90% maternal DNA

  No *RHD* (mother D-neg)

  10-15% fetal DNA (fetal fraction)

  RHD present if fetus D-pos

  No *RHD* if fetus D-neg

Antenatal & postnatal management

If woman is weak D type 1, 2 or 3:

- treat as D+
  - no proph anti-D required

If woman is any other D variant and does not have alloanti-D :

- treat as D−
  - BUT non-invasive fetal *RHD* screen and/or genotype not suitable
  - Give proph anti-D during pregnancy
  - ? Paternal sample
  - Determine D type of newborn (cord sample)
  - If baby is D− then post delivery proph anti-D not required
  - If baby is D+, weak D type 1, 2 or 3, any other D variant, or D status can not be determined within 72 hrs, then post delivery proph anti-D should be given

Antenatal & postnatal management

If woman is any other D variant and has alloanti-D:

- treat as D−
  - BUT non-invasive fetal *RHD* genotype not suitable
  - ? Paternal sample
  - Treat as would a D− woman with alloanti-D and a D+ fetus
  - Determine D type of newborn (cord sample)
  - If baby is D− then no problem
  - If baby is D+, weak D type 1, 2 or 3, any other D variant, or D status can not be determined then appropriate monitoring will have taken place

D variant neonate

If mother has alloanti-D:

- D− red cells should be selected
  - these should be IAT crossmatch compatible with maternal plasma (or neonatal plasma if maternal not available)
  - Once the antibody screen and DAT on the neonatal sample are negative then do not need to crossmatch against maternal plasma

If mother does not have any IgG alloantibodies and neonatal DAT is negative:

- Weak D type 1, 2 or 3 ➲ treat as D+
- Any other D variant or if D type cannot be determined ➲ treat as D−

Case Study

Background

- 30 year old pregnant (14/40) patient of African origin
- Second pregnancy and history of transfusion
- Previously detected anti-E but now her plasma reacting with all cells except autologous control
- strongest reactions seen with papain treated cells
- ? Anti-E plus antibody to high incidence antigen
- e typing showed some weakness with one anti-e, ?e variant
- D+, C-, c+, E-

Rh Typing

	C 1	C 2	C^W	c 1	c 2	D 1	D 2	D 3	D 4	E 1	E 2	e 1	e 2
Test	–	–	–	5	4	4	–	4	3	–	–	4	3
Pos cont	4	4	4	5	4	4	4	4	4	4	4	4	4
Neg cont	–	–	–	–	–	–	–	–	–	–	–	–	–

C- C^W- c+ D+?var E- e+*

*slightly weaker ?variant

Anti-D Panel

Check our website for the most up-to-date version of the reaction profile, supplementary information and recent findings. www.albabioscience.co.uk

Kit ID	Anti-D Cell Line	Weak D Type 1 and 2[ø]	DII & DNU	DIII	DIV	DV*	DCS	DVI	DVII	DOL	DFR	DMH	DAR†	DAR-E	DHK‡ & DAU-4	DBT	Ro^{Har} §	Test Results: Pos Cont.	Neg Cont	Patient
A	LHM76/58	+	+	+	+	+/0	+	0	+	+	+	+	+	0	0	0	(+)/0	4	0	4
B	LHM76/59	+	+	+	0	+	+	+	+	+	+	+	+	+	+	0	0	4	0	4
C	LHM174/102	(+)/0	+	+	0	0	+	0	+	0	0	+	0	0	0	0	0	4	0	1
D	LHM50/2B	+	+	+	+	+	+	0	+	+	+	+	+	+	+	0	0	4	0	4
E	LHM169/81	+	+	+	0	0	+	0	+	+	+	+	0	0	0	0	0	4	0	1
F	ESD1	+	+	+	0	+	+	+	+	+	+	+	+	+	+	0	0	4	0	4
G	LHM76/55	+	+	+	0	+	+	+	+	+	+	+	+	+	+	0	0	4	0	4
H	LHM77/64	+	0	+	0	+	+	+	+	+	+	+	+	+	+/0	0	0	4	0	4
I	LHM70/45	(+)/0	+	+	0	0	0	0	+	0	0	0	0	0	0	0	0	4	0	0
J	LHM59/19	+	+	+	+	+	+	0	0	0	0	(+)	0	(+)	+	+	0	4	0	1
K	LHM169/80	+	+	+	+	+	+	0	+	+	+	+	+	+	0	0	0	4	0	4
L	LHM57/17	+	+	+	+	+	0	0	+	+	0	+	+	0	0	+	0	4	0	4

[ø]Refer to website for supplementary information.

*Within the DV category there are currently 8 different types. The majority of DV's used in the evaluation of this kit were categorized as DVa. Other DV's may vary in their reaction profile with this kit.

†With kit components C, E and J, DAR homozygotes have been shown to give positive reactions.

‡DHK may also be referred to as DYO.

§ Possible R_o^{Har} samples can be confirmed by testing against Alba Bioscience anti-D alpha, product code Z031.

Bibliography

Wagner *et al.* (2000) Weak D alleles express distinct phenotypes. *B*

Reid, M.E. and Antigen Facts Compa. Pub

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Date of Issue

06 March 2007

For further information or advice please contact your local distributor.

Conclusions / Further Testing Required:

Signature: Date:

Z293PI/04

†With kit components C, E and J, DAR homozygotes have been shown to give positive reactions.

Antibody Investigation

	LISS IAT		37ºC direct agg.	
Cells	**UNT**	**PAP**	**UNT**	**PAP**
R_1R_1	4	5	0	5
R_2R_2	4	4	0	4
r'r	4	5	0	5
r''r	4	4	0	4
rr	4	5	0	5
D--/D--	0	0	0	0
Rh_{null}	0	0	0	0
Self	0	0	0	0

- Rh-related antibody/ies
- Auto control negative ➲ alloantibody/ies
- Presence of anti-D excluded

DNA Sequencing

RHD*DAR, RHCE*ceAR/ceAR

D+^{var} C- E- c+ e+^{var}
hr^{S}- Hr-

Rh alloantibodies the patient could make:
Anti-D, -C, -E, -hr^{S}, -Hr

Alloantibodies present:
Anti-E, -hr^{S}, -Hr

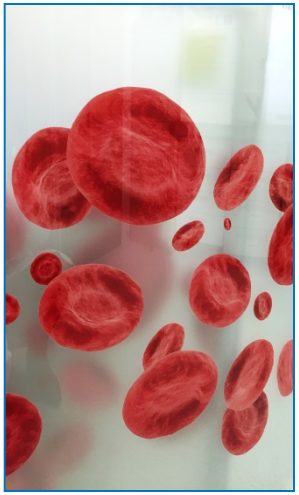
Management

- Paternal sample tested and found to be D+ (R_0r) , determined hemizygous by *RHD* zygosity test, therefore giving a 50% chance that the *RHD* gene would be passed onto his offspring
- Routine antenatal proph anti-D given to mother
- Blood for transfusion – problem!
  - Cell salvage to be used during scheduled c-section
  - Rh_{null} -extremely rare, not available
  - Other RHD*DAR, RHCE*ceAR homozygotes -rare, no suitable donors
  - D--/D-- frozen units available -risks explained but deemed low due to past history of patient receiving D+ units and not forming anti-D.
  - The baby would be heterozygous DAR, not relevant if male and/or inherits D antigen from father but if female and no D inherited from father then theoretical risk of being immunised, deemed low risk due to neonates in general less likely to make alloantibodies

Outcome

- Rare D--/D-- units were thawed to be available to cover the patient's scheduled c-section. No blood was required.
- Healthy baby girl was delivered with pos DAT, no clinical signs of HDFN. Only anti-hr^S detected in eluate.
- Tiny paediatric sample, not sufficient for DNA extraction. Limited serological typing, but confirmed D variant, probable DAR.

Thank You



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