



A New Example of the Rare $Wr(a+b-)$ Phenotype and anti- Wr^b

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A new example of the rare $Wr(a+b-)$ phenotype and anti- Wr^b

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BBTS Annual Conference, Harrogate 2016

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Blood and Transplant

Outline

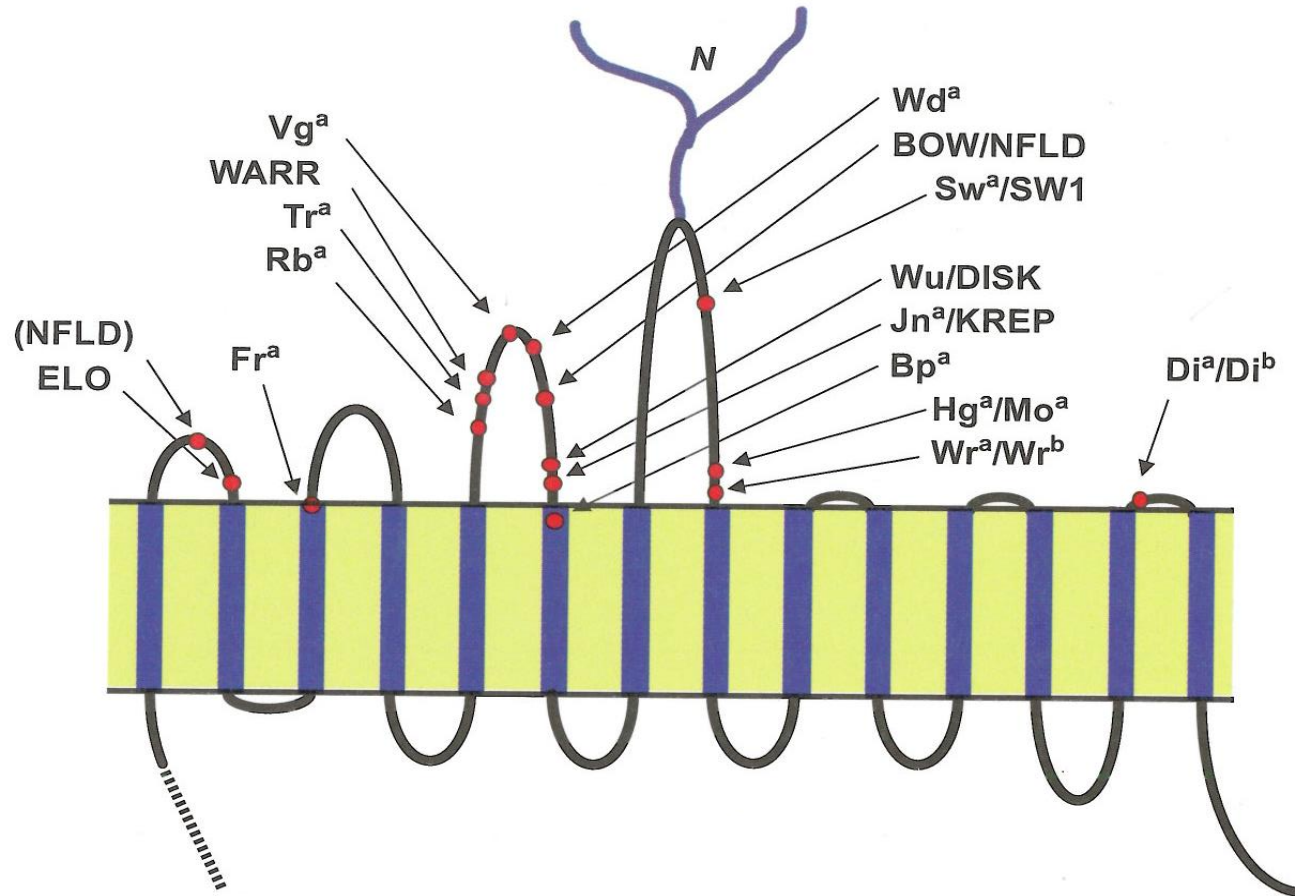
- The Diego blood group system
- Complexity of the molecular bases of the Wr(b-) phenotype
- Case study – referred from Brazil

Diego Blood Group System

ISBT symbol: DI, number: 010

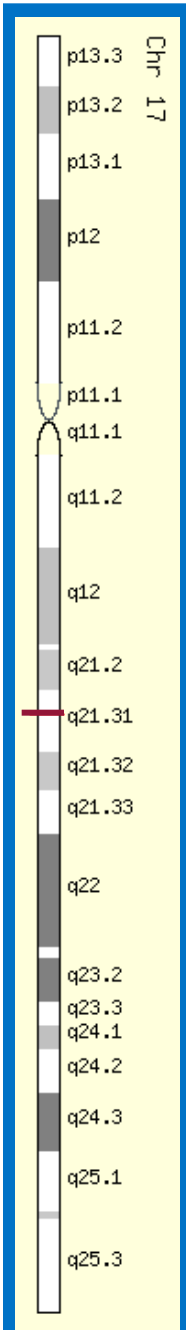
- antigens on red cell membrane protein Band 3 (AE1)
- 22 antigens
- two sets of antithetical antigens: Di^a/Di^b and Wr^a/Wr^b (low/high)
- 19 low frequency antigens
- 3 high frequency antigens

Diego antigens on Band 3



DI Gene

- Band 3 is encoded by a single gene *DI* (*SLC4A1*, *AE1*, *EPB3*)
- *DI* locus on chromosome 17q21.31
- 20 kbp in size
- Organised in 20 exons
- All DI antigens result from a single nucleotide polymorphism (SNP) in the *DI* gene



Glycophorin A and Wr^b

- GPA and Band 3 are closely associated in the red cell membrane
- In the absence of GPA, Wr^b is not expressed
 - En(a-)
 - M^kM^k
 - rare phenotypes due to GPA/GPB hybrid glycoproteins eg. MiV/MiV (GP.Hil)
- Mutations not in *Df* – implications for genotyping
- Not known if GPA is required for Wr^a expression

Case Study

- sample referred from Brazil
- 60 year old male of African origin
- No history of transfusion
- Pre-surgery (prostatectomy) for prostate cancer
- Gp O, D+ C- c+ E- e+, K- k+, Kp(a-b+), M+ N+ S+ s+, Jk(a+b-), Fy(a+b-), Le(a-b+), Lu(a-b+), P1-
- All panel cells positive with plasma
- Auto control negative

Initial Findings

													IAT		18°C
	D	C	E	c	e	S	s	K	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Unt	Pap	Unt
1	+	+	0	0	+	+	+	0	0	+	0	+	2+	3+	0
2	+	0	+	+	0	+	0	0	+	+	+	0	2+	3+	0
3	0	+	0	+	+	0	+	0	+	0	0	+	2+	3+	0
4	0	0	+	+	+	+	+	0	+	+	+	+	2+	3+	0
5	0	0	0	+	+	0	+	0	0	+	0	+	2+	3+	0
6	0	0	0	+	+	0	+	0	+	0	+	0	1+	2+	0
7	0	0	0	+	+	0	+	+	+	+	+	+	2+	3+	0
8	+	0	0	+	+	0	+	0	0	0	+	0	2+	3+	0
Auto	+	0	0	+	+	+	+	0	+	0	+	0	0	0	0

Initial Findings

													IAT		18°C
	D	C	E	c	e	S	s	K	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Unt	Pap	Unt
1	+	+	0	0	+	+	+	0	0	+	0	+	2+	3+	0
2	+	0	+	+	0	+	0	0	+	+	+	0	2+	3+	0
3	0	+	0	+	+	0	+	0	+	0	0	+	2+	3+	0
4	0	0	+	+	+	+	+	0	+	+	+	+	2+	3+	0
5	0	0	0	+	+	0	+	0	0	+	0	+	2+	3+	0
Wr(a+b+)	0	0	0	+	+	0	+	0	+	0	+	0	1+	2+	0
7	0	0	0	+	+	0	+	+	+	+	+	+	2+	3+	0
8	+	0	0	+	+	0	+	0	0	0	+	0	2+	3+	0
Auto	+	0	0	+	+	+	+	0	+	0	+	0	0	0	0

Clue?

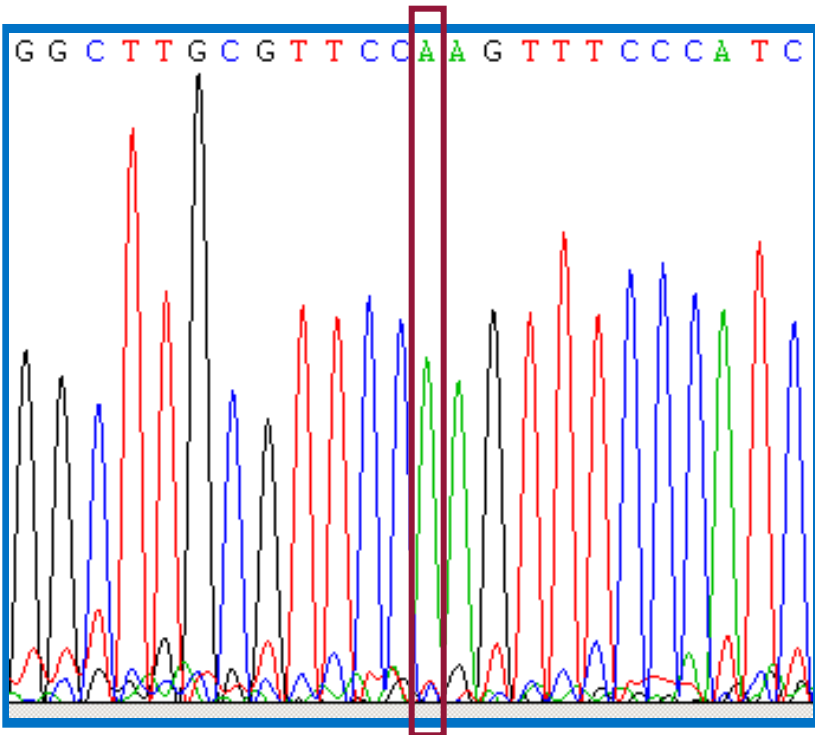
Phenotype

Cells	Anti-							
	Wr ^a	Wr ^b	M	N	S	s	U	En ^a
Patient	4+	0	3+	3+	3+	3+	4+	3+
Pos cont.	3+	3+	3+	3+	3+	3+	4+	3+
Neg cont.	0	0	0	0	0	0	0	0

Wr(a+b-), M+ N+ S+ s+, U+, En(a+)

Genotype

- Sequencing of exon 2-7 of *GYP A* 2,4,5 & 6 of *GYP B*
 - ➡ No mutations
 - ➡ M+N+S+s+ phenotype predicted
- Sequencing of exons 14 and 16 of *DI*



Exon 16
homozygosity for 1972A,
encoding Lys658,
associated with
expression of Wr^a

DI*02.03/DI*02.03

Patient's plasma

- No other Wr(a+b-) available cells for testing

Cells	Unt	Pap
En(a-)	0	0
En(a-)	0	0
M ^k M ^k	0	0
MiV/MiV	0	0
Pos cont.	2+	3+
Patient	0	0

Antibody exclusions

- Patient: R₀, K- k+, M+ N+ S+ s+, Jk(a+b-), Fy(a+b-)

								IAT	
Cells	C	E	K	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Unt	Pap
En(a-)	+	0	0	+	0	+	+	0	0
En(a-)	0	0	0	0	+	0	+	0	0
M ^k M ^k	+	+	0	+	+	0	+	0	0
MiV/MiV	+	0	0	+	0	+	+	0	0
Patient	0	0	0	+	0	+	0	0	0
Excluded	✓	✓	✗		✓		✓		

Clinical Significance of anti-Wr^b

- No report of alloanti-Wr^b causing HTR
 - ➔ Very rare alloantibody
 - ➔ Only three previously described Wr(a+b-) individuals, all had anti-Wr^b
- HDFN: DAT+ but no clinical signs (one case)
- Autoanti-Wr^b relatively common in AIHA patients, has been responsible for fatal intravascular haemolysis (two cases)
- Our patient
 - ➔ no history of transfusion
 - ➔ no siblings or donors available
 - ➔ assessed for autologous donation, two units stored
 - ➔ no transfusion required

Summary

- This is only the fourth $Wr(a+b-)$ individual with alloanti- Wr^b to be described
- The anti- Wr^b was present despite no known immunising event.
- The molecular basis of the $Wr(a+b-)$ phenotype is homozygosity for a SNP in exon 16 of *DI*
- However the $Wr(b-)$ phenotype can result from absence of GPA arising from *GYP A* exon deletions or altered GPA due to *GYP A/GYP B* hybrid genes

Acknowledgements



Rosalind Laundry
Louise Tilley



Immunohaematology Division,
DiaMed Latino America, Brazil

Kennia Maria Duarte
Karina Cruz



Centro de
Hematologia
e Hemoterapia
de Santa Catarina

HEMOSC

Rodolfo J. Ramos
Everaldo J. Shorner

Thank You

