

EXPANSION OF THE RHD DAU CLUSTER: IDENTIFICATION OF TWO ADDITIONAL NOVEL ALLELES

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INTRODUCTION

- The DAU family of RHD alleles is characterized by c.1136C>T (p.Thr379Met).
- The DAU0 allele has this single change, is not associated with depressed or altered D antigen expression, and is the ancestral allele from which other DAU alleles are purported to have evolved.
- In contrast, many DAU family alleles are associated with altered D antigen. Srivastava *et al*¹ recently summarized serologic characteristics of RBC D antigen expression and the incidence of anti-D alloimmunization associated with 18 DAU family alleles.
- Here we investigated two samples with the c.1136C>T change referred with weak D antigen expression.

MATERIALS AND METHODS

Serology testing

- RBC testing was performed by standard tube agglutination with commercial reagents according to manufacturer's instructions.
- Samples were tested with multiple anti-D reagents and with the ALBAclone partial RhD typing kit (Quotient).

DNA testing

- Genomic DNA was isolated from WBCs from peripheral blood and used for testing.
- RHD BeadChip prototype assay (BioArray/Immucor) was performed according to manufacturer's instructions.
- RHD exons 1 to 10 were amplified and Sanger sequenced.

CASES

Sample 1

- 17 year old multiracial female.
- RBCs typed weaker than expected at immediate spin (mi-1+) with multiple anti-D and 3+-4+ in IAT.

Sample 2

- Study sample unlinked for identification.
- RBCs gave variable, weak reactivity by IAT.

SEROLOGY RESULTS

Sample 1

Reactivity with multiple anti-D on immediate spin and IAT (IS/IAT):

	Immucor Gamma clone	Immucor Series 4	Immucor Series 5	Ortho BioClone
Proband	1+ ^s / 3+	1+ ^s / 3+	1+ ^s / 3+	mi+ / 4+

ALBAclone partial D IAT testing results:

Kit ID	Anti-D cell line	Wk D type 1 & 2	DII & DNU	DIII	DIV	DV	DCS	DVI	DVII	DOL	D-FR	DMH	DAR	DAR-E	DHK & DAU4	DBT	R ₀ ^{HLR}	Sample 1
A	LHM76/58	+	+	+	+	+/0	+	+	+	+	+	+	+	0	0	0	(+)/0	3+
B	LHM76/59	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	0	2+ ^s
C	LHM174/102	(+)/0	+	+	0	0	+	+	0	0	+	0	0	0	0	0	0	0
D	LHM50/2B	+	+	+	+	+	+	0	+	+	+	+	+	+	+	0	0	2+
E	LHM169/81	+	+	+	0	0	+	+	+	+	+	+	0	0	0	0	0	2+
F	ESD1	+	+	+	0	+	+	+	+	+	+	+	+	+	+	0	0	2+
G	LHM76/55	+	+	+	0	+	+	+	+	+	+	+	+	+	+	0	0	2+
H	LHM77/64	+	0	+	0	+	+	+	+	+	+	+	+	+	+/0	0	0	2+
I	LHM70/45	(+)/0	+	+	0	0	0	0	+	0	0	0	0	0	0	0	0	2+
J	LHM59/19	+	+	+	+	+	+	0	0	0	0	(+)	0	(+)	+	+	0	2+
K	LHM169/80	+	+	+	+	+	+	0	+	+	+	+	+	+	0	0	0	2+
L	LHM57/17	+	+	+	+	0	0	+	+	0	+	+	0	0	+	0	0	0

- Pattern does not match any partial D identified by these clones.

Sample 2: Testing with multiple anti-D

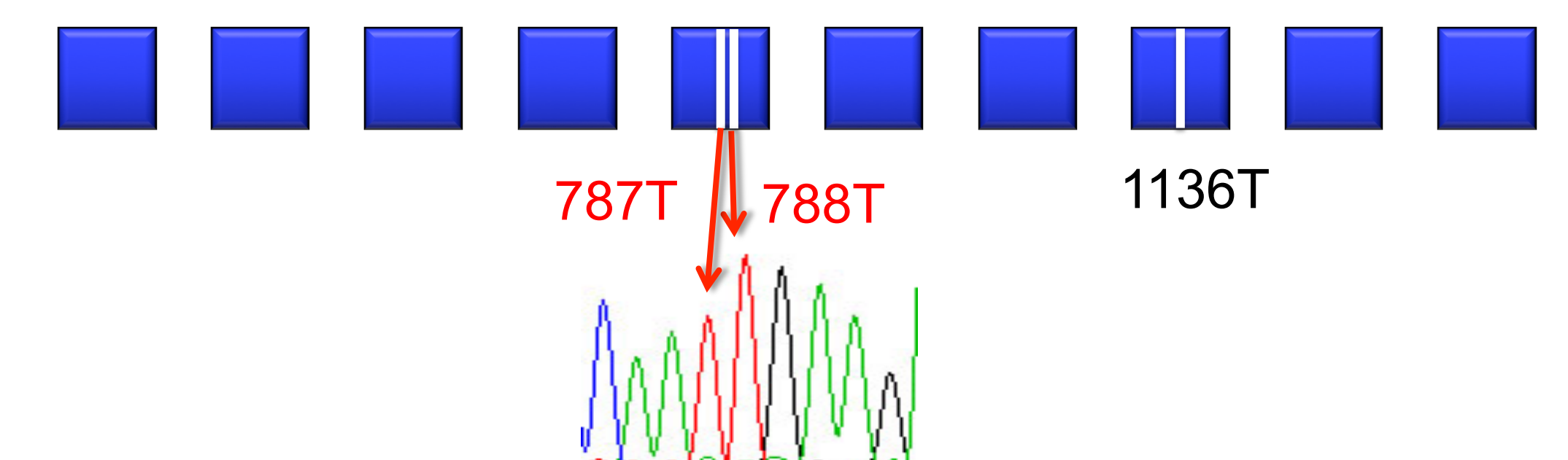
Anti-D Reagent	Direct testing (extended incubation)	IAT/papain IAT
Immucor Gamma-clone	0 (0)	0
Ortho BioClone	0 (0)	mi+/1+ ^s
Immucor Series 4	0 (0)	0
Immucor Series 5	0 (0)	0
Quotient ALBAclone Blend	0 (0)	0
Quotient ALBAclone Alpha	0 (0)	0
Quotient ALBAclone Delta	mi+	NA

- Results suggest a Del-like phenotype

DNA RESULTS

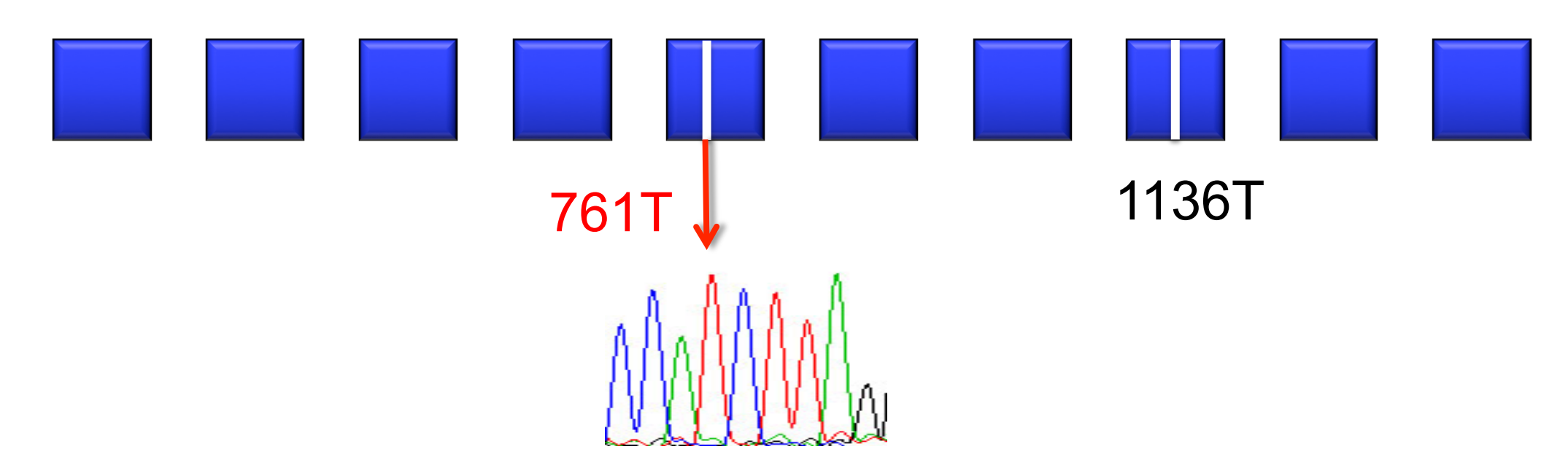
Sample 1

- RHD BeadChip: low signal (LS) for c.787G>A.
- PCR-RFLP: c.1136C>T (p.Thr379Met) – DAU allele.
- RHD Sequencing:
 - Exon 5: two adjacent changes, c.787G>T and c.788G>T (c.787_788delinsTT), encoding p.Gly263Leu.
 - Exon 8: confirmed c.1136C>T.



Sample 2

- RHD BeadChip: no changes.
- PCR-RFLP: c.1136C>T (p.Thr379Met) – DAU allele.
- RHD Sequencing:
 - Exon 5: c.761C>T encoding p.Ser254Leu.
 - Exon 8: confirmed c.1136C>T.



CONCLUSIONS

- We report two new DAU family alleles:
 - **c.787_788delinsTT (p.Gly263Leu)**
 - Non-reactive with clones LHM174/102 and LHM57/17, indicating absence of epitopes and partial D antigen.
 - GenBank#: KY680215
 - **c.761C>T (p.Ser254Leu)**
 - Del-like phenotype.
 - first observed to our knowledge for a DAU allele.
 - GenBank#: KY680216
- While neither of these nucleotide changes are listed in dbSNP database, c.761C>G (p.Ser254Ter) was reported with a D- phenotype in Japanese².
- This study brings the DAU family of alleles to 21.
- The number and diversity of alleles in the DAU cluster supports that the c.1136C>T change is a major ancestral background allele.³

REFERENCES

- Srivastava *et al* (2016). The DAU cluster: a comparative analysis of 18 RHD alleles, some forming partial D antigens. *Transfusion* 56(10):2520-2531
- Ogasawara *et al* (2015). Molecular basis for D- Japanese: identification of novel DEL and D- alleles. *Vox Sang* 2015, 109(4): 359-65.
- Wagner *et al* (2002). The DAU allele cluster of the RHD gene. *Blood* 100(1):306-11.