Lucky 13

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Case Study

- 32 year old female blood donor
- Attended for whole blood collection in October 2015
- Lapsed donor (last successful donation in November 2010)

- Automated test results from current donation:
  - Blood Group - A NEG
  - Antibody screen – POSITIVE

- Historical results on NBMS:
  - Group History – A NEG
  - Rh & K – C-E-c+e+ (rr); K-
  - Antibody History - NEGATIVE

Antibody Investigation

- Initial antibody investigation inconclusive
  - 4 of 11 panel cells showed weak reactions by Grifols CAT-IAT

Lot No	Donor No	Rh Type	No	Rh						Kell				Duffy		Kidd		MNSs				P	Lewis		Lutheran		Co		Extra Cell Types				Cell	Results
				D	C	c	E	e	C^w	K	k	Kp^a	Kp^b	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	P_1	Le^a	Le^b	Lu^a	Lu^b	Co^a	Co^b	Wr^a	Vel	Bg^a	Bg^b		CAT
06171.21.3	AR148	R_1R_1	1	+	+	0	0	+	0	0	+	0	+	+	0	+	0	+	0	+	0	0	0	+	0	+		0	0	+	0	0	1	tr
06181.21.3	AR146	R_1R_1	2	+	+	0	0	+	0	+	0	0	+	0	+	+	0	+	+	0	+	+	0	+	0	+		0	0	+	+	0	2	+
06191.21.3	AR003	$R_1^wR_1$	3	+	+	0	0	+	+	0	+	0	+	+	+	0	+	0	+	0	+	+	+	0	0	+		0	0	+	0	0	3	0
06201.21.3	AR218	R_2R_2	4	+	0	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	+	+	+	0	0	+		+	0	NT	0	0	4	0
06211.21.3	AR189	R_2R_2	5	+	0	+	+	0	0	0	+	0	+	+	0	0	+	0	+	0	+	+	0	+	0	+		0	0	+	0	0	5	0
06221.21.3	AR154	r'r	6	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	0	+	+	0	0	0	0	+		0	0	+	0	0	6	wk
06231.21.3	AR224	r''r	7	0	0	+	+	+	0	0	+	0	+	+	0	+	+	0	+	+	0	0	0	0	+	0	+		0	0	NT	0	0	7	0
06241.21.3	AR155	rr	8	0	0	+	0	+	0	+	+	0	+	+	+	0	+	0	+	+	0	+	0	+	0	+		0	0	+	0	0	8	0
06251.21.3	AR153	rr	9	0	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	+	+		+	0	+	0	0	9	tr
06261.21.3	AR156	rr	10	0	0	+	0	+	0	+	+	0	+	+	0	+	0	+	0	0	+	0	+	0	0	+	+	+	0	+	0	0	10	0
06271.21.3	AR149	rr	11	0	0	+	0	+	0	0	+	+	+	+	+	+	0	+	+	+	+	+	+	0	0	+		0	0	+	0	0	11	0
	Auto																																auto	0

Antibody Investigation

- Results remained inconclusive when tested by Bio-Rad CAT-IAT
  - 8 of 11 panel cells showed weak reactions

- Tube technique was performed to obtain further information:
  - All 11 panel cells showed moderate strength reactions by PEG-IAT
  - Auto control negative
  - No reactions observed in SRT phase
  - Possible antibody to high-incidence antigen / HTLA antibody

Reactivity Pattern

	0.8 % Cells	3% Cells	
	Bio-Rad CAT-IAT	Tube 30' SRT	Tube PEG-IAT
Cell 1	$+^{w}$	0	$+^{s}$
Cell 2	+	0	++
Cell 3	0	0	+
Cell 4	0	0	$+^{s}$
Cell 5	tr	0	++
Cell 6	0	0	+
Cell 7	$+^{w}$	0	$++^{s}$
Cell 8	tr	0	$++^{s}$
Cell 9	$+^{w}$	0	++
Cell 10	$+^{w}$	0	++
Cell 11	$+^{w}$	0	++
Auto	0	0	0

Next Steps

- Possible next steps in testing:
  - Papain
  - Titration (? HTLA pattern)

- Before proceeding further, we go back to basics:
  - Check for a patient file
    - **<u>Anti-Lu13 previously detected in 2013!!</u>**

- Completed the current donor investigation:
  - Phenotyping – LU:-13
  - Excluded underlying allo-antibodies using LU:-13 & Lu(a-b-) cells
  - Antibody titration – Neat
  - Referred sample to QLD R&D to confirm LU:-13 status by DNA sequencing

DNA Sequencing Results

- Homozygous for the mutations associated with loss of the Lu13 antigen ⟶ predicted phenotype is LU:-13

The TruSight™ One Sequencing Panel enables targeted DNA sequencing of exonic and 3' untranslated regions of 39 genes related to blood group systems. DNA sequence reads are aligned against the human reference sequence (GRCh37) in order to identify variants relative to the reference sequence.

PATIENT RESULTS:

2 genetic variations related to request identified

GENETIC VARIATIONS RELATED TO REQUEST:

GENE VARIATION	**INTERPRETATION**
● ***BCAM*** **(Lutheran)** c.1340C>T c.1742A>T	These nucleotide substitutions (c.1340C>T and c.1742A>T) lead to missense amino acids (p.S447L and p.Q581L) in the Lutheran blood group protein. These mutations was identified reliably as it was found in 16 of 18 and 27 of 27 DNA sequence reads respectively, consistent with **homozygosity for c.1340C>T and c.1742A>T**. These variants lead to a loss of the high frequency Lu13 antigen on red blood cells and therefore define the *LU*02.-13* allele and the LU:-13 phenotype at a homozygous level[1,2]. The genotype is therefore **LU*02.-13 /LU*02.-13** and the predicted phenotype is **LU:-13.**

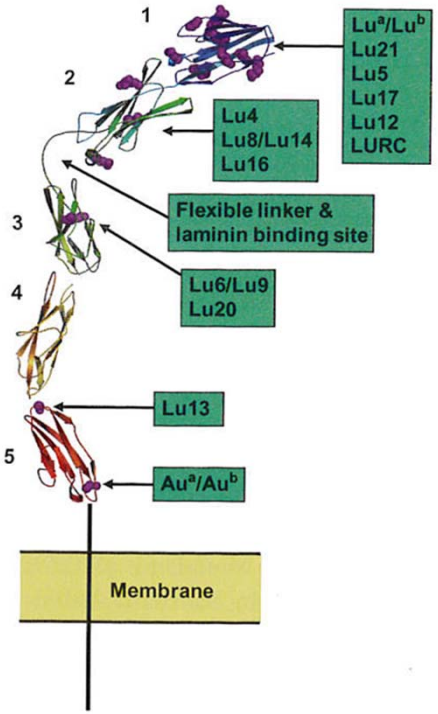
[1]http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology/. [2]Reid, M.E., *et al.* The Blood Group Antigen Facts Book. Elsevier, 3rd Edition, ed. 2012. [3]Xiang, L., *et al.* J Immunol. 1999 Nov 1;163(9):4939-45

Lucky Lutheran 13

- ISBT symbol : LU13
- Obsolete name : Hughes
- First reported in 1983
- Occurrence : 100% in all populations
- Only a few probands have been reported

- Antigen expression is weak on cord RBCs
- Non-detectable serologically on dominant Lu(a-b-) RBCs
- Papain resistant
- Trypsin / α-Chymotrypsin / DTT / AET sensitive

- 2 nucleotide substitutions lead to missense amino acids in the Lutheran blood group protein:
  - c.1340C>T Ser to Leu 447 (in IgSF domain 5 – exon 11)
  - c.1742A>T Gln to Leu 581 (transmembrane domain – exon 13)



Reid ME, Lomas-Francis C, Olsson ML (2012). The Blood Group Antigen Facts Book Third Edition, Elsevier Ltd.

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Clinical Significance – Anti-Lu13

- No specific data is available on the clinical significance of anti-Lu13, due to the extreme rarity of the antibody

- In general, Lutheran system antibodies are considered relatively benign:
  - If at all, they typically only cause mild, delayed transfusion reactions
  - If at all, they typically result in mild HDFN that can be readily treated by phototherapy

- However, despite this experts recommend transfusing Lu(a-b-) blood if specific LU:-13 blood is not available
  - Cryogenic storage of red cells is therefore recommended
  - Siblings should be tested for compatibility

Origin of Donor's Antibody

- Pregnancy?
  - 8 consecutive Negative antibody screen results as a donor between 2008 – 2010
  - First pregnancy in 2011 – Positive antibody screen at 28/40
- Transfusion?
  - Handwritten note on patient referral paperwork suggesting possible transfusion of 6 units of RBC's in 1995
  - This timeframe matches up with notes in the donor's record indicating involvement in a MVA at age 12 (plus an additional MVA at age 18)

The Future

- This donor has since attended for 2 whole blood donations in 2016
  - Both of these red cell components were cryopreserved for future use
- Possible future SCARFE cell
- ? Family studies