A novel high Incidence antigen of the Indian blood group system INRA (IN5)

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2: Lok Samarpan Blood Bank Research Center, Surat, India

Lok Samarpan Raktdan Kendra, Surat.

NHS Blood and Transplant

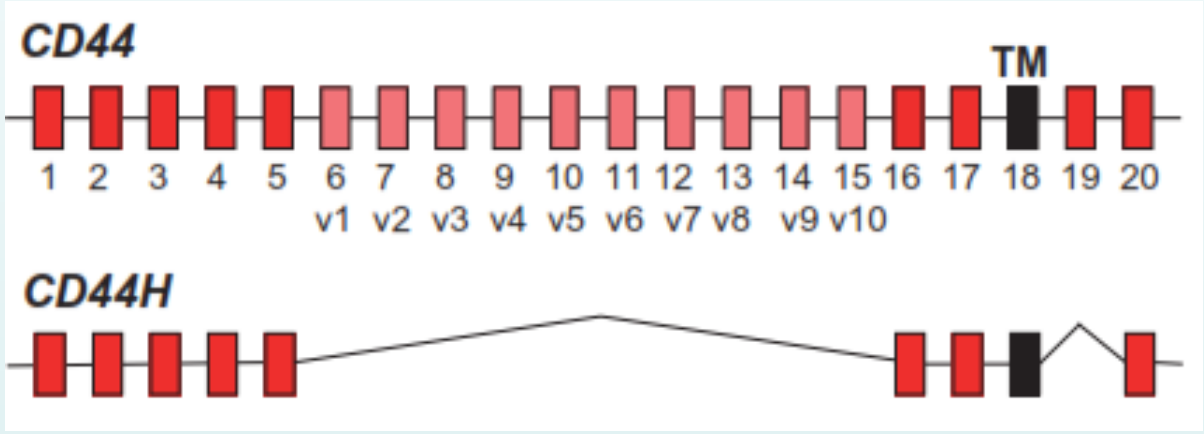
Referral

- 40 year old patient from Surat
- AB D+
- They found an alloantibody reacting with all cells by; saline (18ºC) and column IAT's (37ºC), <u>but not with papain treated cells</u>

- Cross matched with over >50 random donors
- Brother was compatible
- Antibody and geographical location - important clues

Indian System

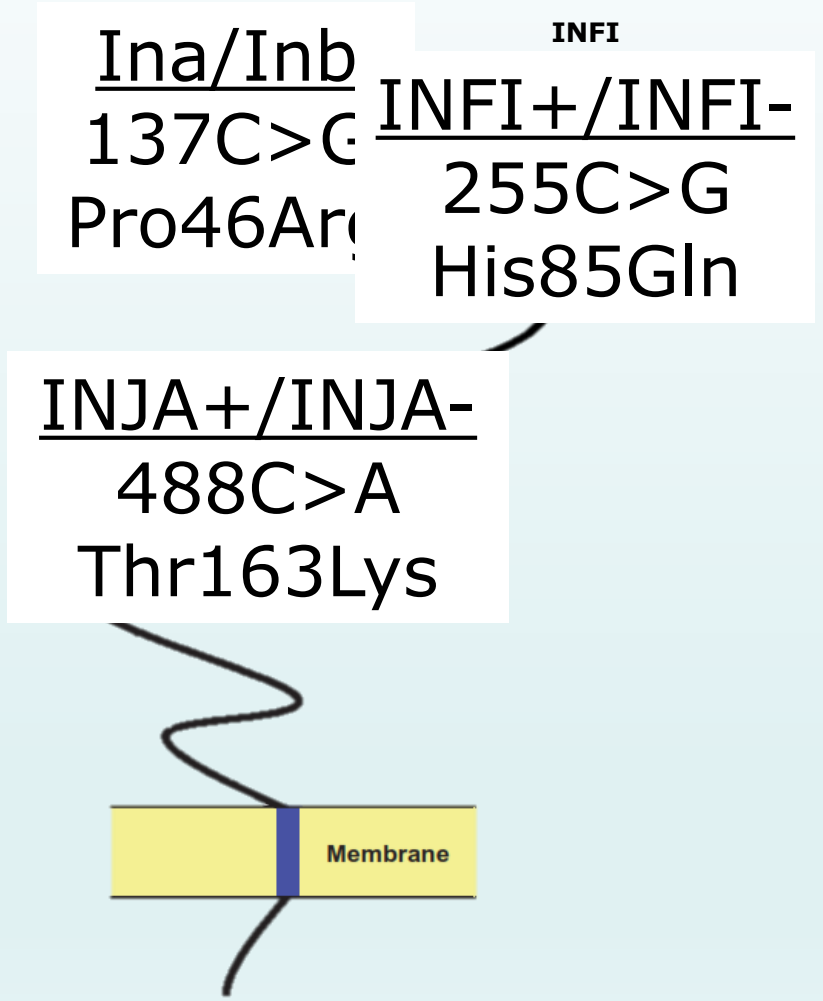
- Indian Antigens (Ags) are located on CD44 ubiquitous glycoprotein
- On Chrom 11p13
- 20 exons, *CD44H* isoform lacks all of the alternatively spliced exons



- CD44 binds hyaluronan, a major component of the ECM and present on cell surfaces
- Plays a regulatory role in erythropoiesis in RBCs

Indian Antigens and Antibodies

- Low Frequency In^a(IN1) antithetical to High Frequency In^b(IN2)
- INFI(IN3)
- INJA(IN4)
- Destroyed by proteases & AET/DTT
- Direct agglutination & IAT



Initial Investigation

- We detected a strong alloantibody;
  - LISS IAT
  - direct agglutination 37ºC
  - direct agglutination 18ºC
- Typed positive for high frequency antigens (HFAs), including papain sensitive Ags;
  - In^b
  - Xg^a
  - Yt^a
  - CD99
- Moved to working with plasma

Serology continued

- Plasma was reactive with;
  - IN:-3 and IN:-4 cells.
  - Null cells
- InLu cells were reacted weaker by IAT and direct techniques
- InLu shown to have reduced (variable) CD44 expression

Cell Type	IAT	Direct (37)
IN:-3	++++	+
IN:-4	++++	++
JMH-	++++	+
CD99-	++++	+
Rhnull	+++	++
M^kM^k	++++	++
Fy(a-b-)	++++	++
Gy(a-)	++++	+
InLu	++	+
InLu	++	(+)
InLu	++	(+)

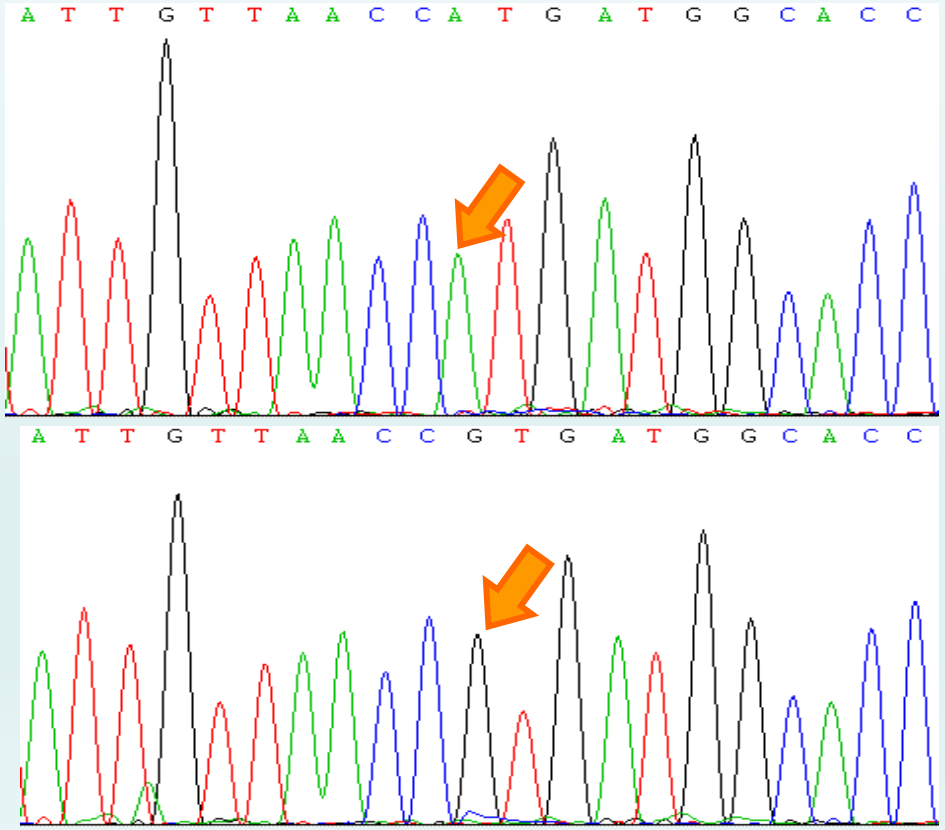
Final Serology

	Unt	Papain	Trypsin	Chymo	DTT
CD44	+	-	-	-	-
Patient	+	-	+	-	wk

- Trypsin and DTT positive
- Alloadsorptions = successful removal & no antibodies to common blood group antigens
- IN3 & IN4 positive
- *CD44* sequencing

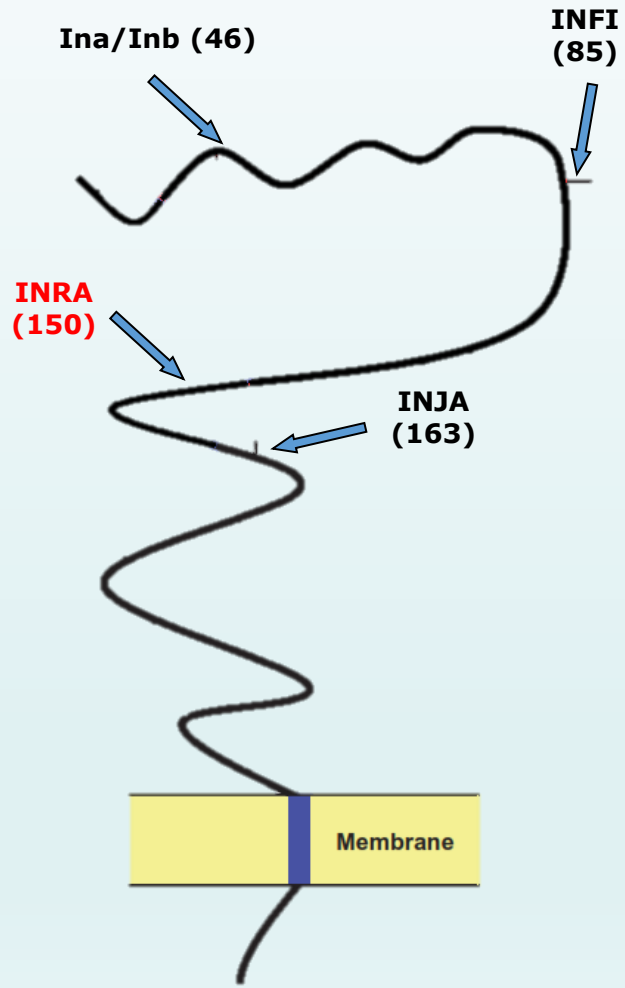
CD44 Sequencing

- Sanger sequencing of the of *CD44*
  - ➢ *IN*B* homozygous
  - ➢ novel homozygous synonymous mutation c.255C>T in exon 3
- Exon 5 novel missense mutation c.449G>A. Encoded a p.Arg150His amino acid substitution Lack of a new high incidence Ag!



INRA (IN5)

- An alloantibody to a new high incidence Ag of the Indian system
- Sequencing results conclusive
- INRA
- Submitted the new allele to GenBank – (KX639826)
- Applied to ISBT for consideration of antigen status and ISBT number (IN5)



Further Investigation

- Family studies
- The monoclonal antibody immobilization of erythrocyte antigens (MAIEA)
- Expression studies to prove the novel mutation causes the lack of a CD44 epitope
- Protein Modelling

Thank you

Dr. Vanja Crew, Nicole Thornton

&

Dr. Joshi and his colleagues from Surat, India

Questions?

Lok Samarpan Raktdan Kendra, Surat.

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