A novel molecular background and a family study of a Gy(a-) individual with anti-Gy^{a}

V Karamatic Crew[1], N Thornton[1], A McNeil[1],
MI Moser[2], MJ Rodrigues[2], R Pimentel[3], G Daniels[1]

[1]BITS/IBGRL, Filton, Bristol, UK
[2]CST Lisboa/IPST. IP, Lisbon, Portugal
[3]Dr. Joaquim Chaves, Laboratório de Análises Clínicas, Lisbon, Portugal

BBTS 32nd Annual Conference, 2014, Harrogate, UK

Background

Dombrock blood group system

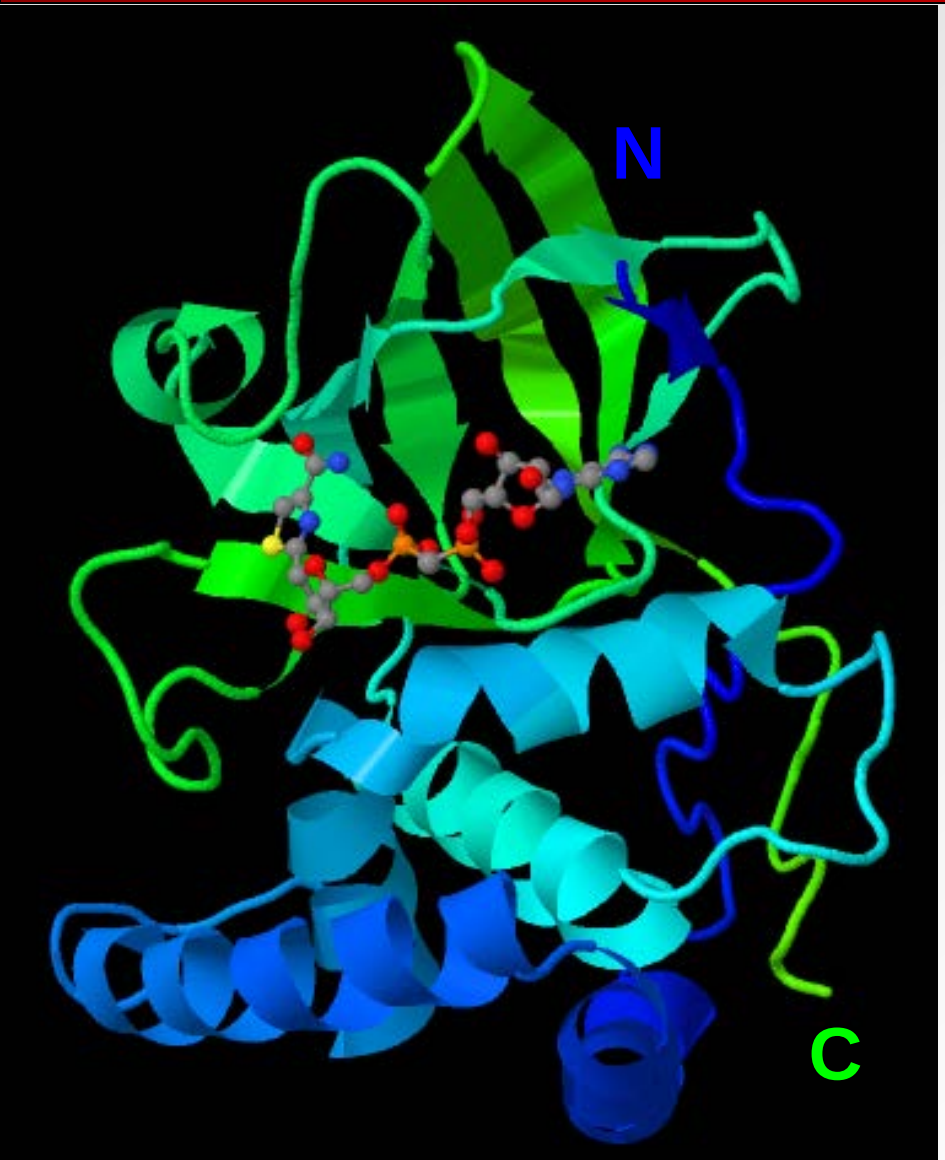
Nine antigens in the system:

- One set of antithetical antigens (Do^a/Do^b)
- Seven high incidence antigens (Gy^a, Hy, Jo^a, DOYA, DOMR, DOLG, DOLC)

Dombrock antibodies have not been implicated in HDFN

Anti-Do^a, -Do^b, -Hy have caused acute and delayed HTR

ART4 (CD297)



- GPI-linked membrane glycoprotein
- 5 N-linked glycosylation sites
- Member of the mono-ADP-ribosyltransferase family
- No enzymatic activity shown on red cells

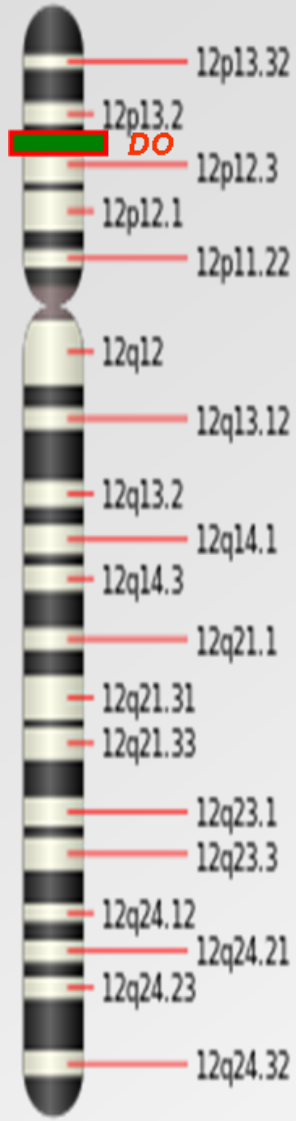
Model based on crystal structure of mono-ADP-ribosyltransferase ART2.2 in complex with TAD (*RCSB PDB*)

Dombrock null phenotype

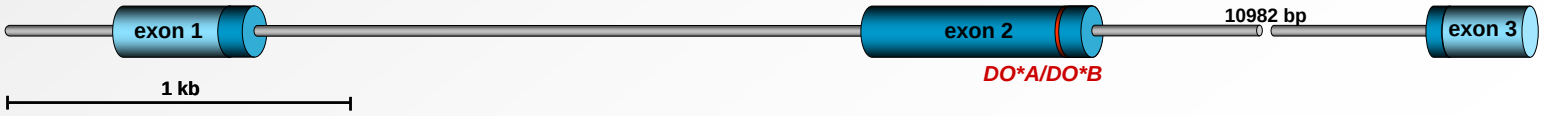
- Gy(a-) is Dombrock null (Do_{null}) phenotype with red cells lacking all Dombrock antigens
- Very rare

- *DO*01N.01*; 442C>T, Gln148stop; USA
- *DO*01N.02*; 343del343-350, fs stop; Reunion Island
- *DO*02N.01*; IVS1-2a>g, fs stop; original case USA
- *DO*02N.02*; IVS1+2t>c, fs stop; Canada
- *DO*02N.03*; 185T>C, Phe62Ser, no protein, USA
- *DO*02N.04*; 555dup555_561, fs stop, Slovenia

DO (*ART4*) gene



- Dombrock is encoded by a single gene *DO, ART4*
- *DO* locus on chromosome 12p13.2-12.3
- 14 kbp in size
- Organised in 3 exons



Case study

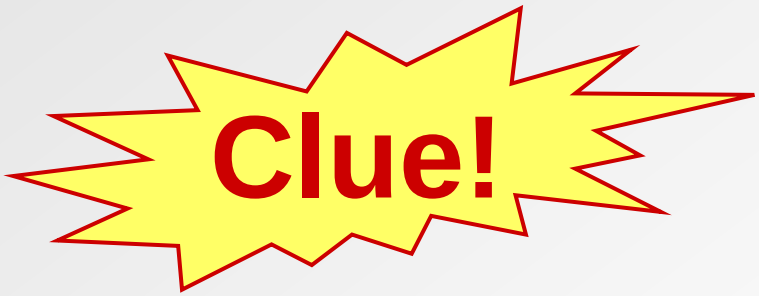
- A 19-weeks pregnant female of Caucasian origin from Portugal
- No known pregnancies, no known transfusions
- A, ccddee (rr), ss, P1+, Lu(a+b+), kk, Kp(a-b+), Le(a-b+), Fy(a-b+), Jk(a+b+)
- Her serum reacted with all panel cells by LISS/IAT and Enz/IAT

Additional tests in Lisbon

Patient's plasma tested against range of cells lacking high incidence antigens:

Rh_{null}, K_o, Lu(a-b-), Gy(a-), Yt(a-), Vel-, In(b-), Sc:-1, Kna-, Sla-, Yka-, Ge-2,-3,4, Lan-, Er(a-)

All strongly positive except for one negative reaction



Only one example of Gy(a-) cells tested, referred to IBGRL for Ab identification

Serology

Initial panel

Cells	IAT Untreated	IAT Papain	18°C
1	3	3	-
2	3	3	-
3	3	3	-
4	3	3	-
5	3	3	-
6	3	3	-
Auto	-	-	-

Next stage

Patient's cells

Typed for Dombrock antigens:

Do(a-b-)
Gy(a-)
Hy-
DOYA-
DOLG-

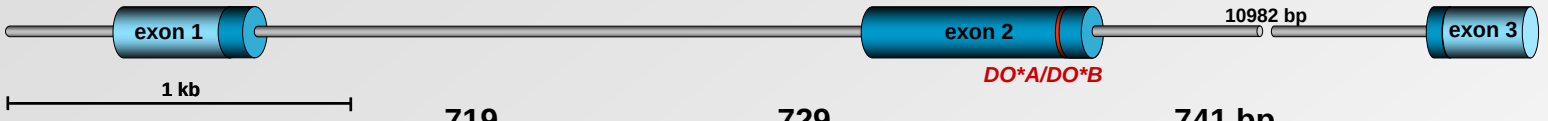
Patient's plasma

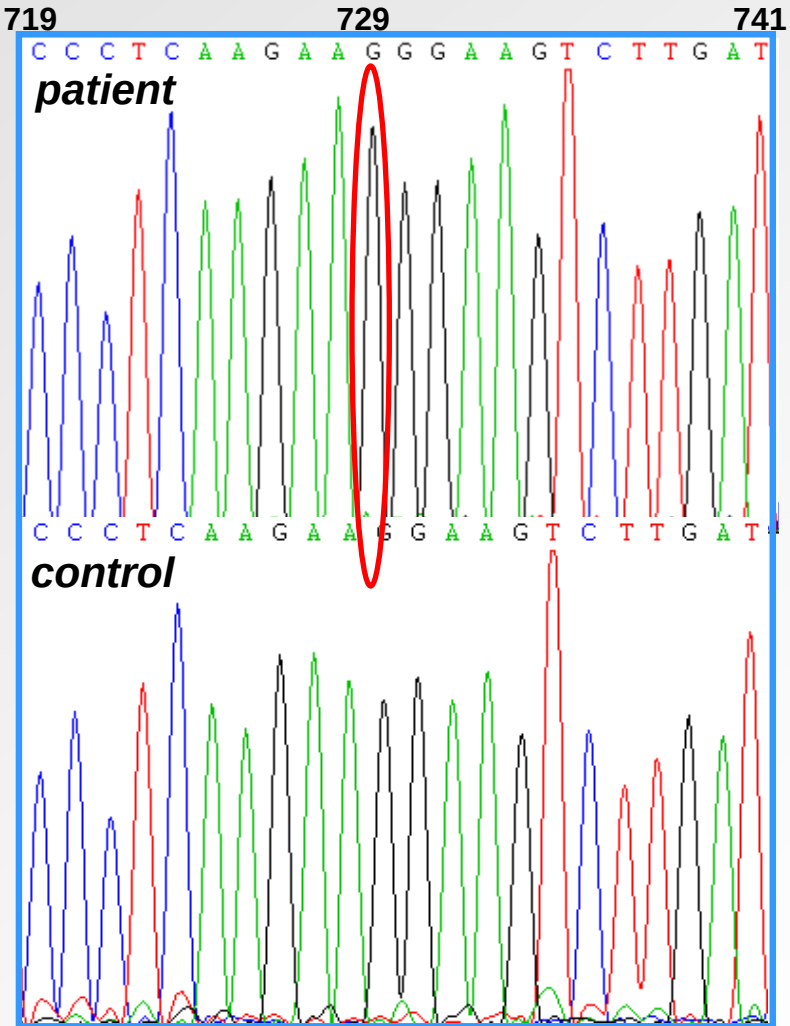
Three additional examples of Gy(a-) cells found to be compatible

One Hy- example very weakly positive

Gy(a-) phenotype with anti-Gy^{a} ?

DO sequencing





***DO*01 (DO*A)*:**

793A; Asn265

silent 378C; Tyr126

silent 624T; Leu208

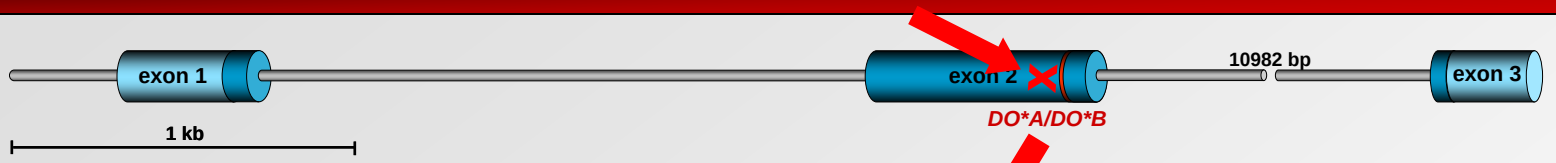
Novel insertion:

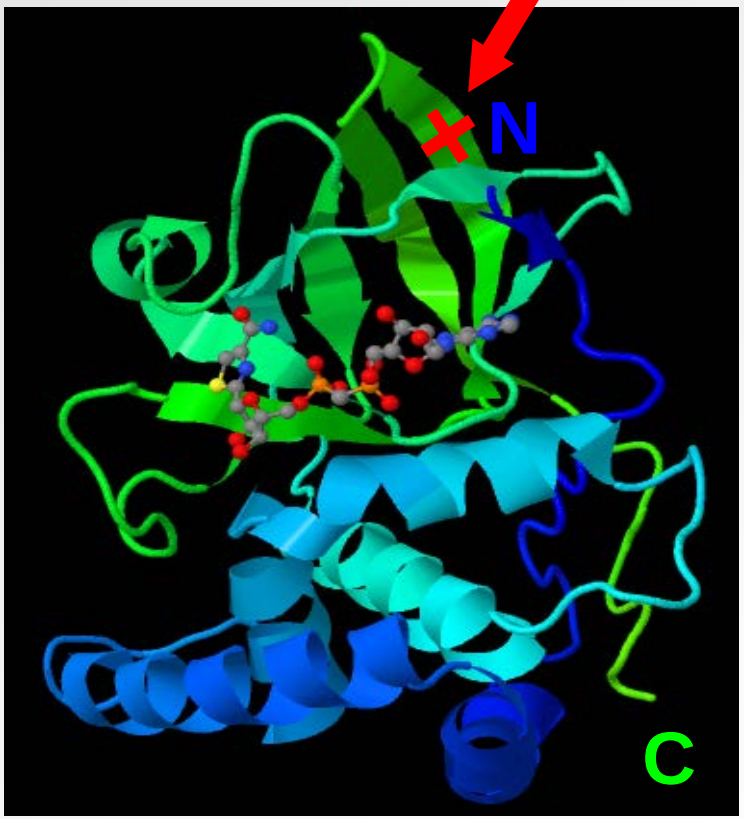
729insG;

frame shift

Glu251stop

c.729insG encodes premature stop

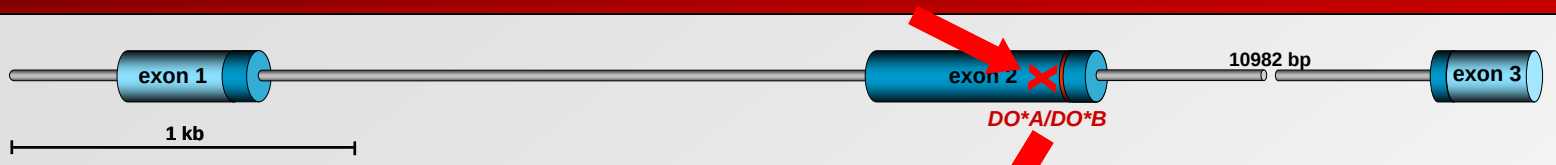




wild type: 243 Lys – Glu – Val – Leu – Ile – Pro – Pro – Tyr – 251 Glu

Patient: 243 Lys – Gly – Ser – Leu – Asp–Pro –Ser – Leu – 251 STOP

c.729insG encodes premature stop



wild type: 243 Lys – Glu – Val – Leu – Ile – Pro – Pro – Tyr – 251 Glu

Patient: 243 Lys – Gly – Ser – Leu – Asp–Pro –Ser – Leu – 251 STOP

Extended family

Sample
Patient
Mother
Father
Brother
Maternal Aunt 1
Maternal Aunt 2
Maternal Uncle
Paternal Aunt 1
Paternal Aunt 2

Serological typing

All family members are Gy(a+)

Cells of Maternal Aunt 1 weaker with routine anti-Gy^a and patient's serum

Cells of Mother, Father, Maternal Aunt 2, Paternal Aunt 1 marginally weaker with patient's serum

Extended family - genetics

Sample	378 T/C; Tyr126	445C>A; Gln149Lys *DO*B-SH-Q149K*	624 C/T; Leu208	793A/G, Asn265Asp *DO*A/B*	729G insert
Patient	C	C	T	A (DO*A)	homozygous
Mother	T/C	C	C/T	A/G (DO*A/B)	heterozygous
Father	T/C	C	C/T	A/G (DO*A/B)	heterozygous
Brother	T	C	C	G (DO*B)	no
Maternal Aunt 1	C	C/A	C/T	A/G (DO*A/B)	heterozygous
Maternal Aunt 2	C	C/A	C/T	A/G (DO*A/B)	heterozygous
Maternal Uncle	T/C	C	C/T	A/G (DO*A/B)	no
Paternal Aunt 1	T/C	C	C/T	A/G (DO*A/B)	heterozygous
Paternal Aunt 2	T	C	C	G (DO*B)	no

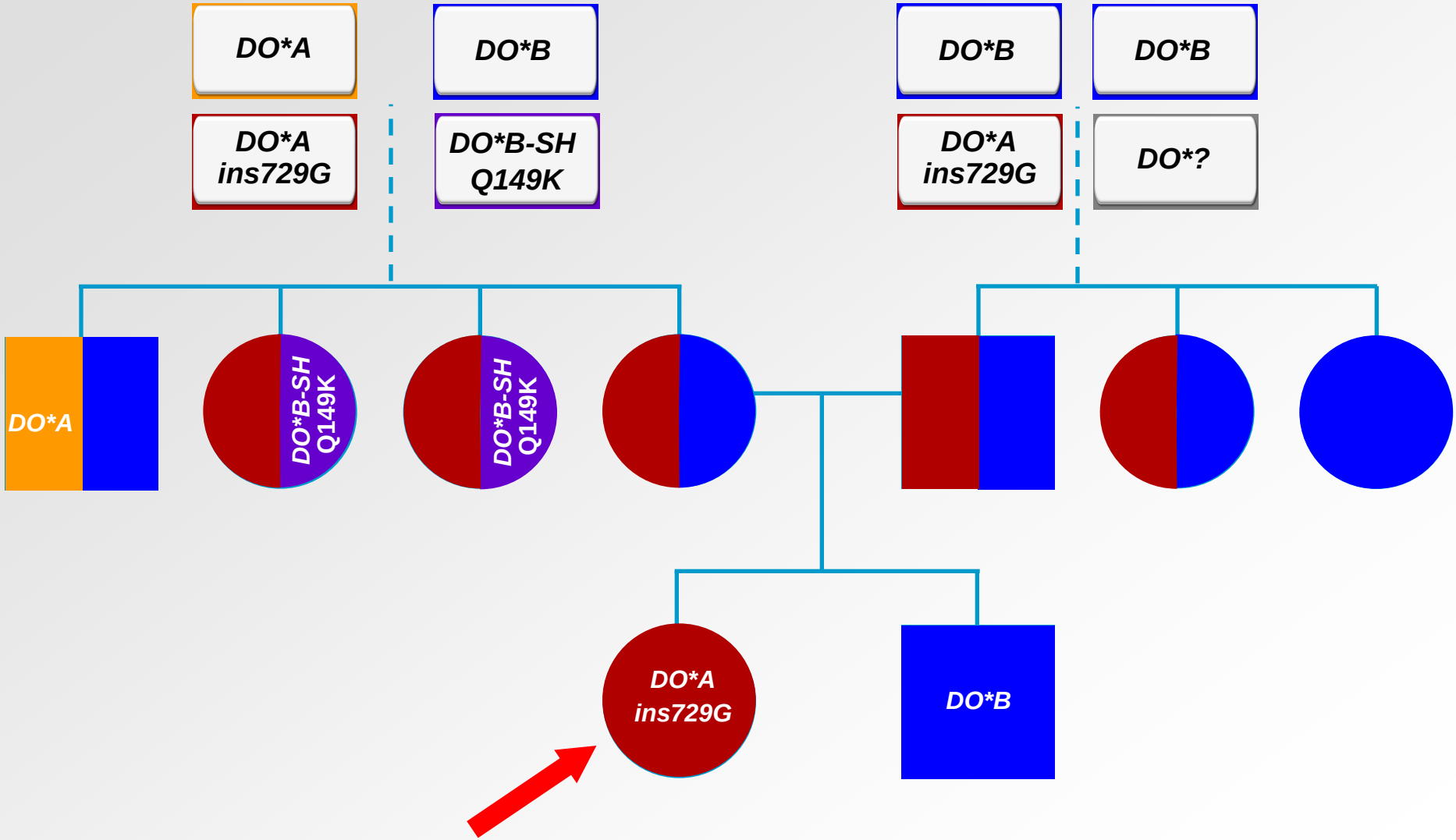
Serological typing

All family members are Gy(a+)

Cells of Maternal Aunt 1 weaker with routine anti-Gy^{a} and patient's serum

Cells of Mother, Father, Maternal Aunt 2, Paternal Aunt 1 marginally weaker with patient's serum

Family tree


DO*A
DO*B
DO*B
DO*B
DO*A ins729G
DO*B-SH Q149K
DO*A ins729G
DO*?
DO*A
DO*B-SH Q149K
DO*B-SH Q149K
DO*A ins729G
DO*B

Conclusions

- We describe a novel molecular background of a Gy(a-) in an individual with anti-Gy^a in her plasma
- This DO_{null} is resulting from an insert c.729insG encoding a reading frame shift and premature termination of protein translation at p.Glu251
- The inheritance of Gy(a-) has been shown through two generations of patient's family
- All of patient's family members tested were shown to be crossmatch incompatible

Acknowledgements

IBGRL/BITS Bristol:

Nicole Thornton

Abigail McNeil

Geoff Daniels

Vanja Crew

CST/IPST.IP, Lisbon:

Maria Inês Moser

Maria José Rodrigues

Dr J. Chaves Lab, Lisbon:

Rui Pimentel

Thank You