The rare Wr(b-) phenotype arising from a novel mutation in *GYPA* paired with GP.Hil (Mi.V)

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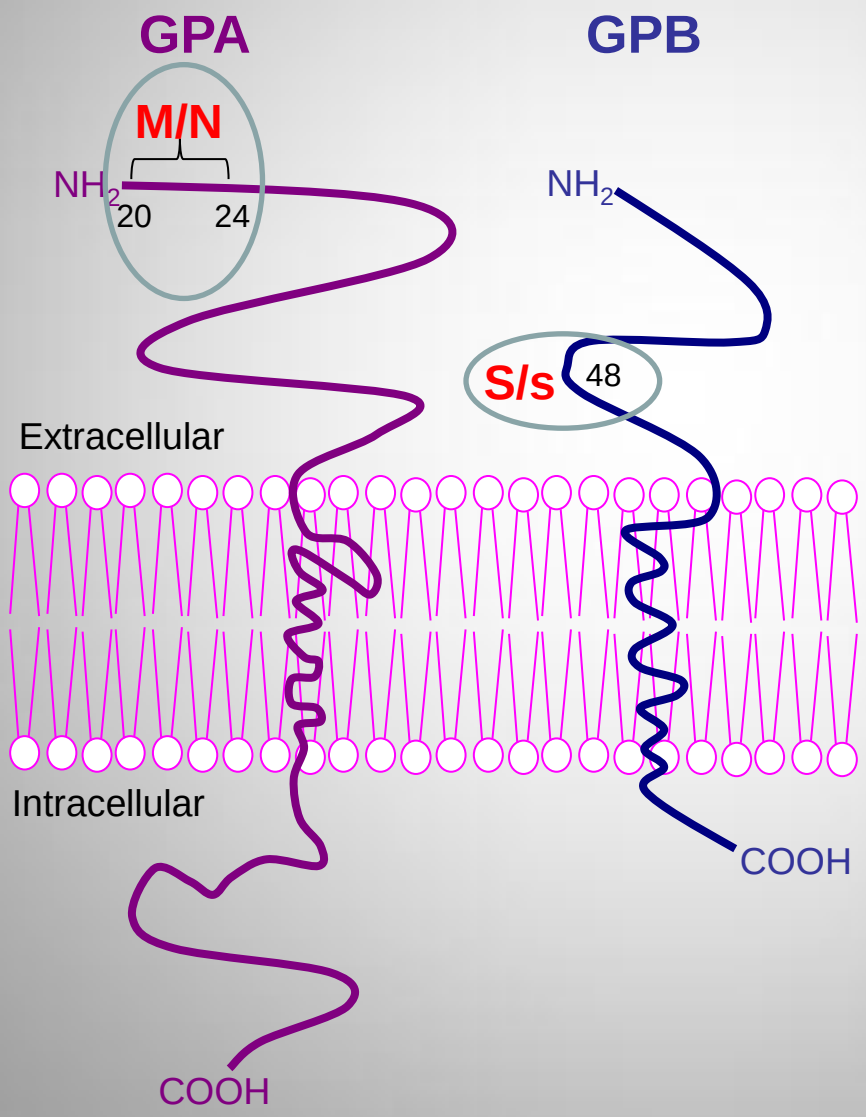
MNS Blood Group System

- MNS antigens carried on red cell membrane sialoglycoproteins GPA & GPB
- Encoded by *GYPA* and *GYPB* genes
- Mature GPA and GPB have 131 and 72 amino acids respectively
- 19 amino acid leader sequence cleaved after membrane insertion
- Numbering based on full translated protein

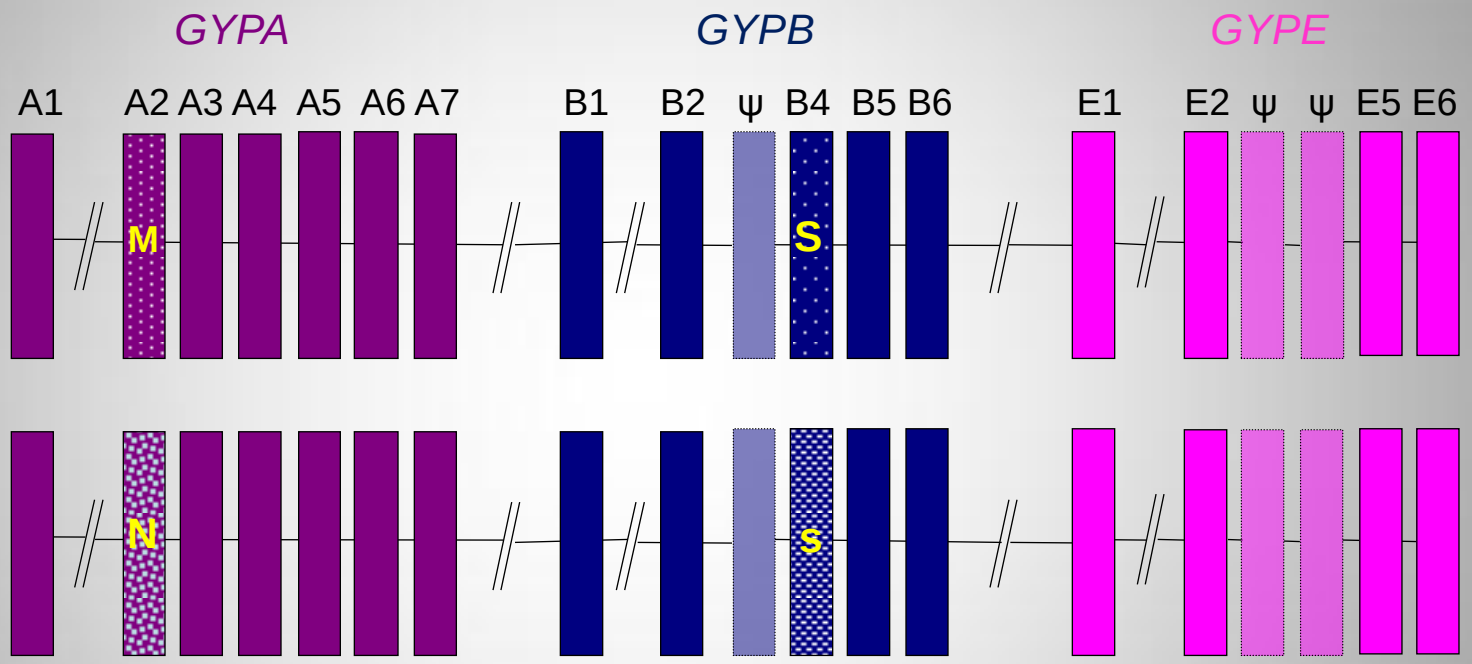
MNS Blood Group System

- M/N carried on GPA, 3 nucleotide changes in *GYPA* exon 2
- M=Ser20, Gly24; N=Leu20, Thr24
- S/s carried on GPB, single nucleotide change in *GYPB* exon 4
- S=Met48, s=Thr48

GPA and GPB


GPA
GPB
M/N
NH2
20
24
NH2
S/s
48
Extracellular
Intracellular
COOH
COOH

GYP Genes



Homologous gene cluster on chromosome 4q28-31

GP.Hil

- Unequal crossing over between *GYPA* and *GYPB* results in hybrid proteins, with parts of both GPA and GPB
- GP.Hil (or Mi.V) variant results from *GYPA(exons 1-3)-GYPB(exons 4-6)* hybrid gene
- Hybrid protein has amino acids 1 to 77 from GPA fused to amino acids 46 to 91 from GPB
- GPB in GP.Hil has Thr48 (s)
- Hybrid protein expresses Hil antigen, resulting from unique amino acid sequence at fusion point of GPA and GPB

Wr antigens

- Wr^a (low incidence) and Wr^b (high incidence) antigens defined by single a.a. substitution in band 3 protein (exon 16 of *SLC4A1*)
- Glu658=Wr^b, Lys658=Wr^a
- Expression of Wr^b dependent on presence of GPA
- Wr(a-b-) phenotype occurs in GPA deletions and some hybrids, despite genetic homozygosity for Wr^b
- GP.Hil phenotype red cells express no Wr^b

Methods

- Whole blood & DNA samples from Wr(b-) Shanghai blood donor
- Serology by standard techniques, using in-house reference antisera
- Periodic Acid Schiff (PAS) staining of sialoglycoproteins after SDS-PAGE of red cell membrane proteins
- Genomic DNA sequencing of *GYPA* exons 2 to 6; *GYPB* exons 2, 4, 5 and 6; and *SLC4A1* exon 16

Serology results

- Wr(a-b-) phenotype confirmed with panel of anti-Wr^b
- Panel of anti-En^a (GPA) gave variable reactions
- Typing for low incidence MNS antigens showed cells Hil+
- Aberrant reactivity with anti-M and anti-N

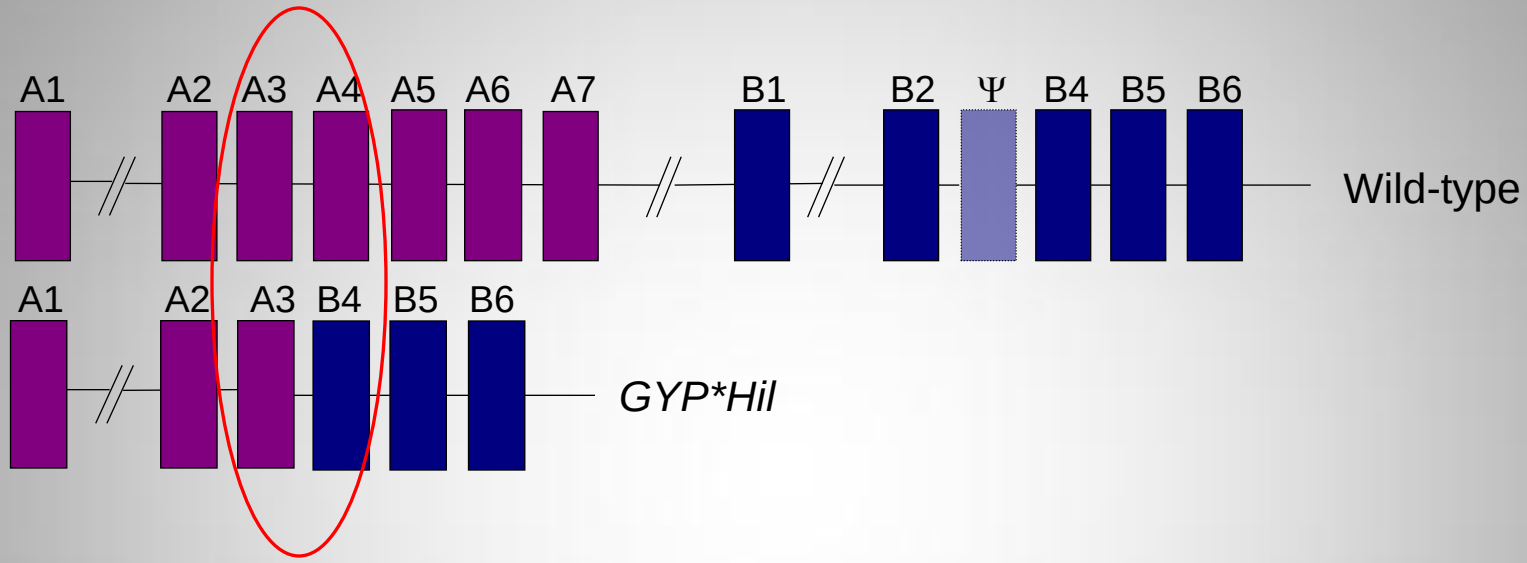
PAS staining

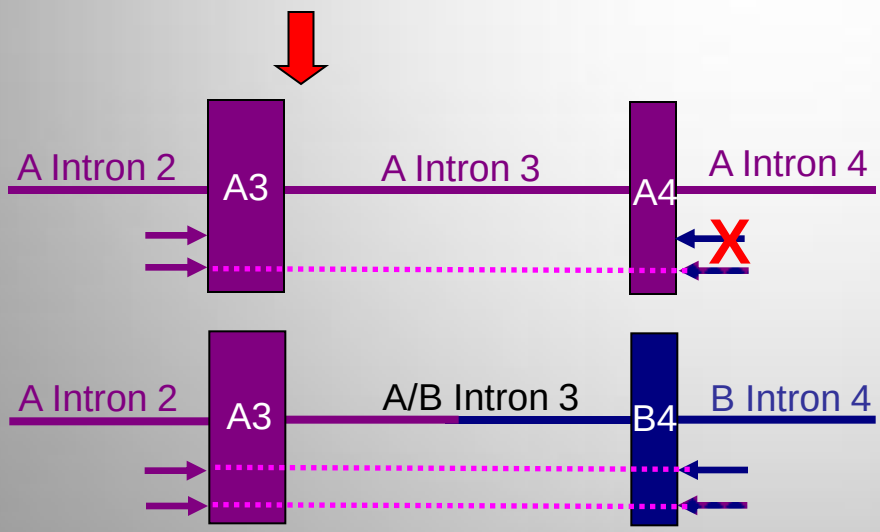
- Presence of GP.Hil hybrid protein
- Weaker staining than GP.Hil homozygous control
- No GPA monomer visible
- GPB staining same as normal control
- Heterozygosity for GP.Hil and abnormal GPA

Molecular Genetics

- *SLC4A1* sequencing: Glu658, Wr(a-b+)
- *GYPA* Exon 2: M+ N+
- *GYPB* Exon 4: S- s+
- *GYP*Hil* confirmed by amplification of PCR product spanning *GYPA* exon 3 to *GYPB* exon 4

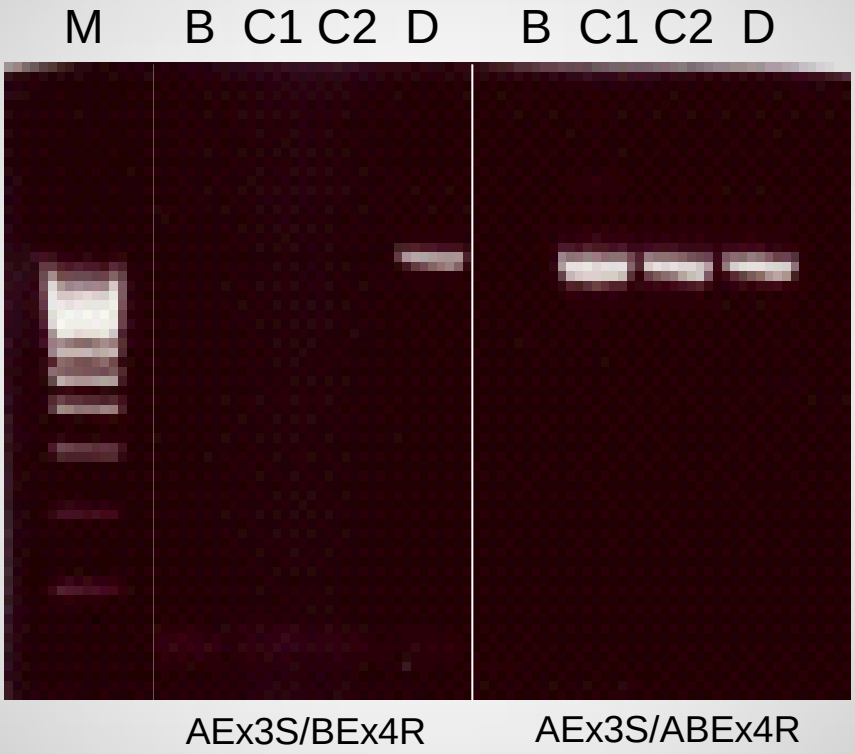
Hybrid PCR





Forward primer	Reverse primer	*GYP*Hil*	Wild-type
AEx3S	BEx4R	✓	✗
AEx3S	ABEx4R	✓	✓

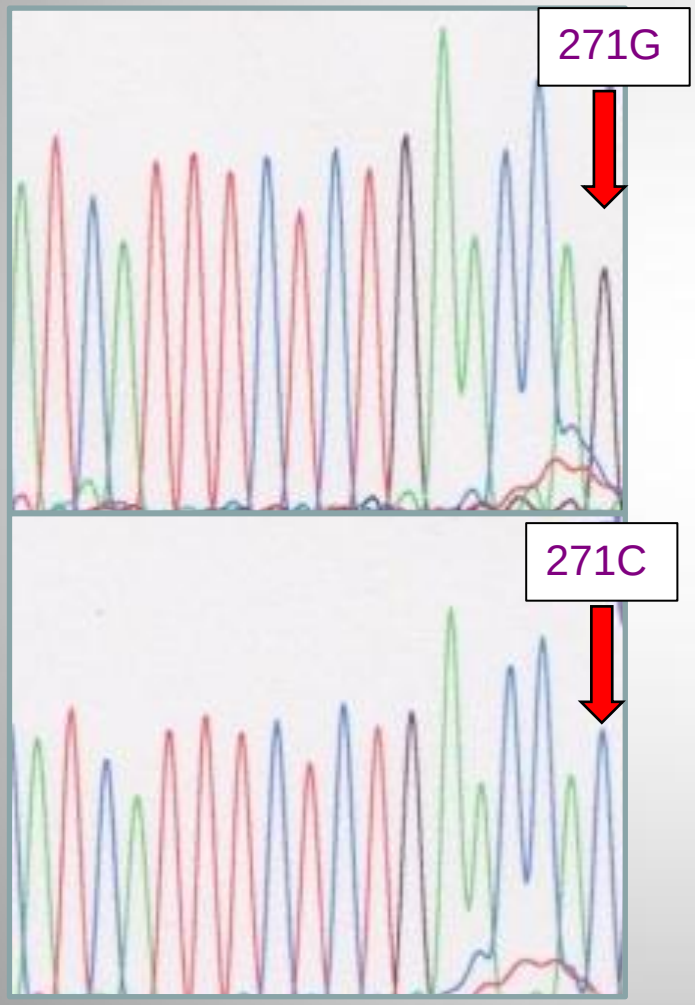
Hybrid PCR



GYP sequencing results

- *GYPB*: No mutations
- *GYPA*: Novel mutation; 271G>C in exon 4

GYPA Exon 4 Sequencing



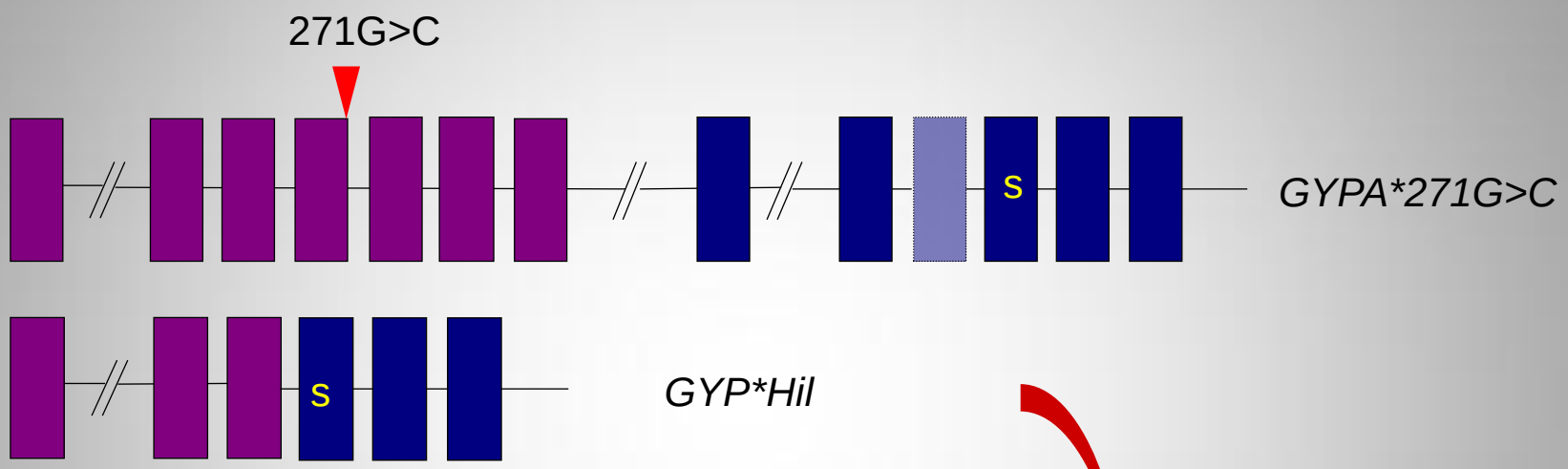
Control

Donor

GYPA mutation

- 271G>C at final nucleotide of exon 4
- Mutation likely alters splicing & expression
- Results in production of little or no full-length GPA (lack of GPA on blotting)
- Glu91Gln substitution
- Located at cell surface, adjacent to membrane-spanning domain
- Region critical for Wr^b expression so abnormal protein unlikely to express Wr^b

Conclusions



Splice site mutation
Little or no full-length GPA
Glu91Gln in GPA region critical for Wr^b expression
=**No Wr^b**

Lacks GPA region critical for Wr^b expression
=**No Wr^b**

Acknowledgements

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