Insights from new blood group systems

31 May 2024
14.30 – 15.00

45th SIMTI, Rimini, Italia (onsite)

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Disclaimer & Disclosure

Disclaimer

- The views expressed do not necessarily represent the view of the National Institutes of Health, Department of Health and Human Services, or U.S. Federal Government.

Disclosure

- No conflicts of interest.

Off-Label Usage

- Some red cell genotyping assays are not FDA approved or CE labeled, particularly for the ‘new’ blood group systems.

Objectives

- What are recent developments in blood group antigens?
  - Antigen types, antibodies, and timeline
- How are antigens summarized in blood group systems?
  - Definition and molecular structures
- What can transfusion medicine contribute?
  - Patient perspective: impact on practical care and patient safety
  - Establish database for biologic variability and pathophysiology

45 blood group systems

- 1900 – 1989 21 systems

- 1990 – 1999 26 systems
- 2000 – 2009 30 systems (270 antigens, total 308)
- 2010 – 2019 36 systems (322 antigens, total 360)
- 2020 – 2023 45 systems (356 antigens, total 384)

45 blood group systems

- 1900 – 1989 21 systems in 90 years

- 1990 – 1999 26 systems
- 2000 – 2009 30 systems (270 antigens, total 308)
- 2010 – 2019 36 systems (322 antigens, total 360)
- 2020 – 2023 45 systems (356 antigens, total 384)

in 35 years: 24 more systems

45 blood group systems

- 1900 – 1989 21 systems

- 1990 – 1999 26 systems + 5
- 2000 – 2009 30 systems (270 antigens, total 308) + 4
- 2010 – 2019 36 systems (322 antigens, total 360) + 6
- 2020 – 2023 45 systems (356 antigens, total 384) + 9

45 blood group systems

- 1900 – 1989 21 systems

- 1990 – 1999 26 systems
- 2000 – 2009 30 systems (270 antigens, total 308)
- 2010 – 2019 36 systems (322 antigens, total 360)
- 2020 – 2023 45 systems (356 antigens, total 384)

- ISBT Barcelona June 2024: next updates
  - "Blood groups – new discoveries and old mysteries solved"

What is on the horizon?

- > 500 distinct proteins in the red cell membrane
  - http://rbcc.hegelab.org/sources
  - Each one could be target of antibodies, if variable.
  - Only 45 are documented = as blood group systems.
- There is a lot of room to be explored.
- A full description defines the ‘space’ for disease, pharmacology, and clinical studies
  - such as: unexplained hemolysis after red cell transfusions

Routinely tested in pharmocogenomics and present in the red cell membrane

Gene	Red cell membrane confidence threshold*
ABCC1	High
ABCC4	High
ABCC5	High
ABCG2	High
SLC16A1	High
SLC19A1	Medium
SLC29A1	High
CYP4F3	Medium
CFTR	High
FLOT1	High
ATP7A	High
EPHX1	High

- Among 1,191 genes tested
  - PharmacoScan platform
- 12 genes are red cell expressed
- all may qualify as blood groups and implicated in hemolysis:

Vox Sanguinis (2021) **116**, 141–154

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- Among 1,191 genes tested
  - PharmacoScan platform
- 12 genes are red cell expressed
  - 4 known today as blood groups
    - 043 – ABCC1 in 2020
    - 040 – PEL in 2020
    - 032 – JR in 2012
    - 036 – AUG in 2015
- all may qualify as blood groups and implicated in hemolysis:

Vox Sanguinis (2021) **116**, 141–154

Since 2011: genetic basis is known for all blood group systems

- 1900 – 1989 21 systems: none with molecular basis
- 1990 – 1999 26 systems: for all, except DO, SC, P1Pk
- since ~ 2000 molecular basis must be known
  for all new systems and antigens

- 2003 SC – last of 22 known protein-based systems
- 2011 P1Pk – last of 6 known sugar-based systems
  at the time

Milestones of genetic basis

- 1990 ABO – Yamamoto, Clausen, White, Marken, Hakomori
- 1990 RH – Cherif-Zahar, Blanchard, Cartron, Colin
- 2003 SC – Wagner, Flegel
- 2011 P1Pk – Thuresson, Westman, Olsson

ISBT: Antigen to form new blood group

- Antigen must be defined by a human alloantibody
  - Antigen must be an inherited character
- Its gene must have been identified and sequenced
  - Its chromosomal location must be known
  - Gene must be different from all other genes
    - encoding antigens of existing blood group systems
    - not a closely-linked homologue

- https://www.isbtweb.org/isbt-working-parties/rcibgt/blood-group-terminology.html

Molecular basis of blood group systems
for a total of 45 systems

- 42 systems
  - 1 gene
- 2 systems
  - 2 genes
- 1 system
  - 3 genes

- All 11 ‘new’ systems since 2014 are coded by only 1 gene each.
- 004 – RH
  - *RHD – RHCE*
- 017 – Chido/Rodgers
  - *C4A – C4B*
- 002 – MNS
  - *GPA – GPB – GPE*

Molecular basis of blood group systems
for a total of 45 systems

- 42 systems
  - 1 gene
- 2 systems
  - 2 genes
- 1 system
  - 3 genes

- All 11 ‘new’ systems since 2014 are coded by only 1 gene each.
- 004 – RH 1939
  - *RHD – RHCE*
- 017 – Chido/Rodgers 1967
  - *C4A – C4B*
- 002 – MNS 1917
  - *GPA – GPB – GPE*

11 ‘new’ blood group systems since 2014

Protein (multi-pass)

- 036 – AUG
- 039 – CTL2
- 040 – PEL
- 041 – MAM
- 043 – ABCC1
- 044 – ER
- 045 – CD36

GPI-linked (protein)

- 035 – CD59
- 037 – KANNO

GPI linker (sugar)

- 042 – EMM

Sugar

- 038 – SID

Only 4 are ‘new’ blood group antigens

blood group antigen known – blood group defined

Protein (multi-pass)

- 036 – AUG 1967 – 2015
- 039 – CTL2 new – 2019
- 040 – PEL 1980 – 2020
- 041 – MAM 1993 – 2020
- 043 – ABCC1 new – 2020
- 044 – ER 1982 – 2020
- 045 – CD36 new – 2023

GPI-linked (protein)

- 035 – CD59 new – 2014
- 037 – KANNO 1991* – 2019

GPI linker (sugar)

- 042 – EMM 1987 – 2020

Sugar

- 038 – SID 1967 – 2019

* not formally recognized in 901 series before 2019

7 of 11 ‘new’ blood group systems are large multi-pass proteins

Protein (multi-pass - segments)

- 036 – AUG 11
- 039 – CTL2 10
- 040 – PEL 12
- 041 – MAM 4
- 043 – ABCC1 17
- 044 – ER 36
- 045 – CD36 2

Protein (single-pass)

- none

GPI-linked (protein)

- 035 – CD59 only extracellular
- 037 – KANNO only extracellular

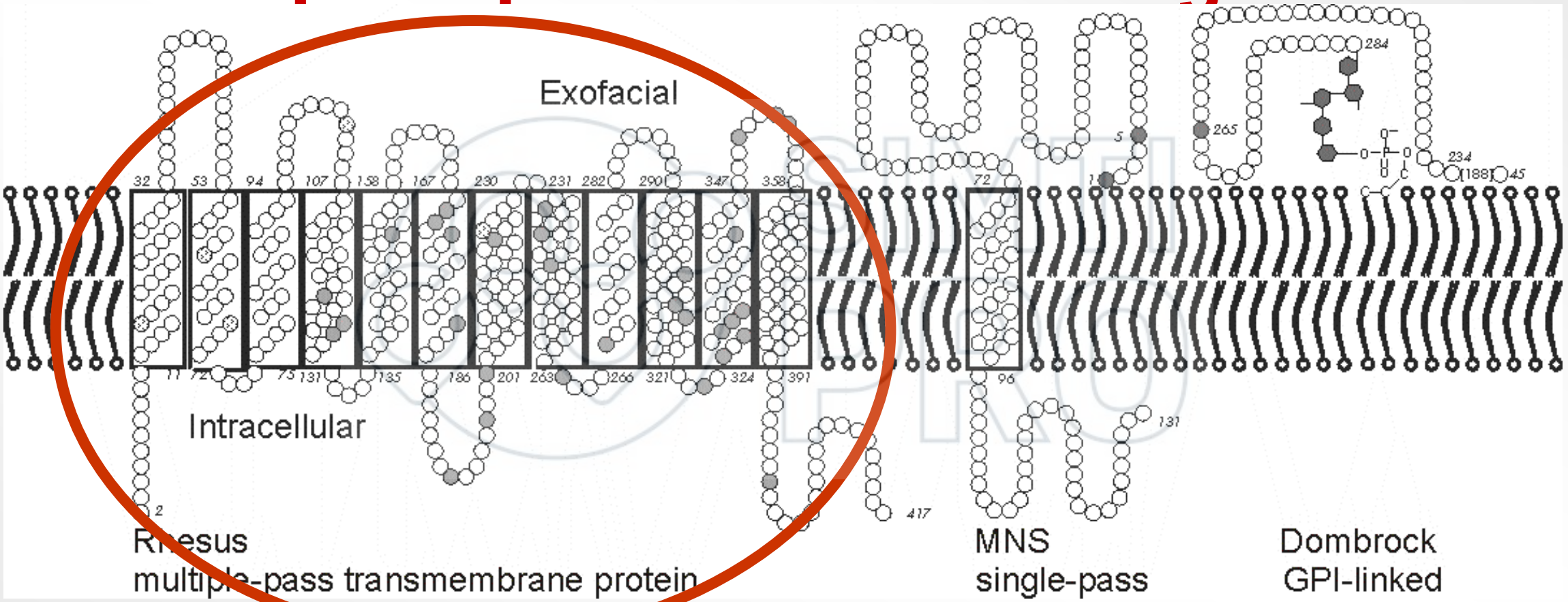
GPI linker (sugar)

- 042 – EMM glycosyltransferase

Sugar

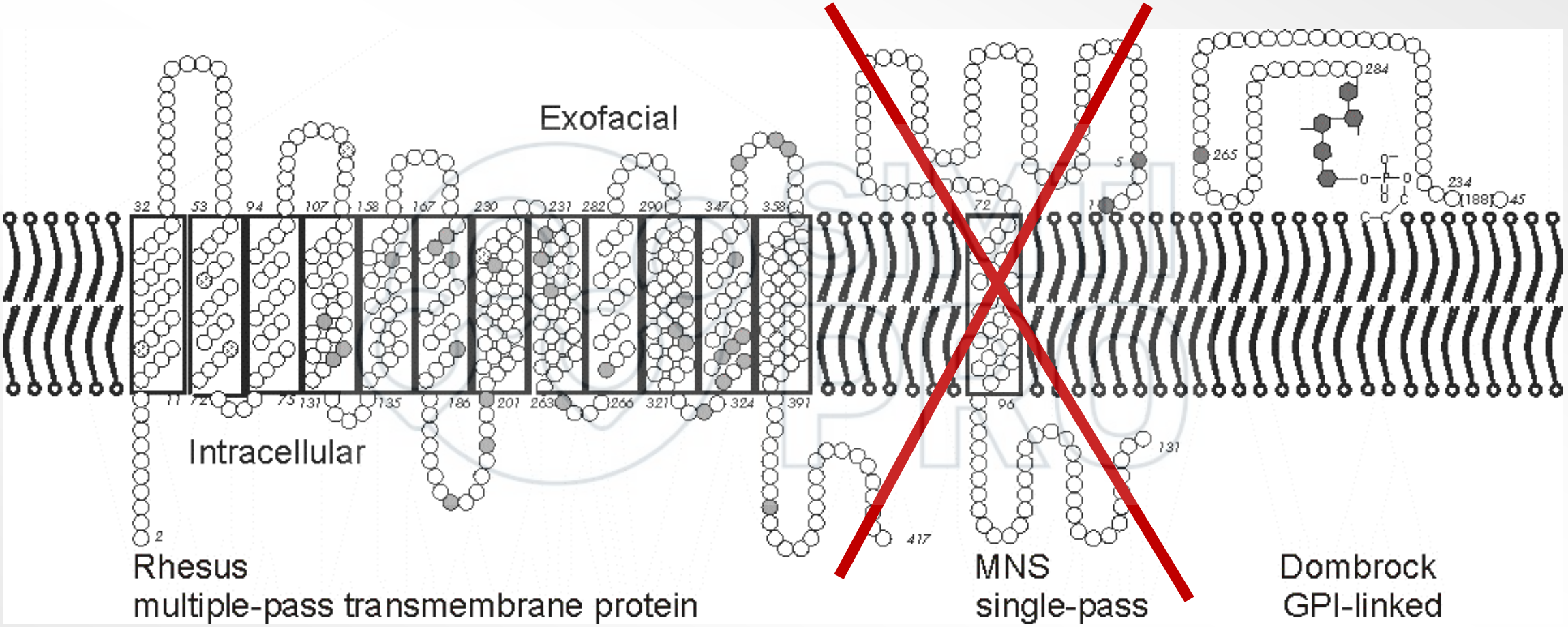
- 038 – SID glycosyltransferase

Proteins in the red cell membrane
7 multi-pass proteins: 2 - 36 segments



Transf Apher Sci 2011(44)81-91

No single-pass protein



Transf Apher Sci 2011(44)81-91

2 ‘new’ blood group systems are located only at the red cell surface

Protein (multi-pass - segments)

- 036 – AUG 11
- 039 – CTL2 10
- 040 – PEL 12
- 041 – MAM 4
- 043 – ABCC1 17
- 044 – ER 36
- 045 – CD36 2

Protein (single-pass)

- none

GPI-linked (protein)

- 035 – CD59 only extracellular
- 037 – KANNO only extracellular

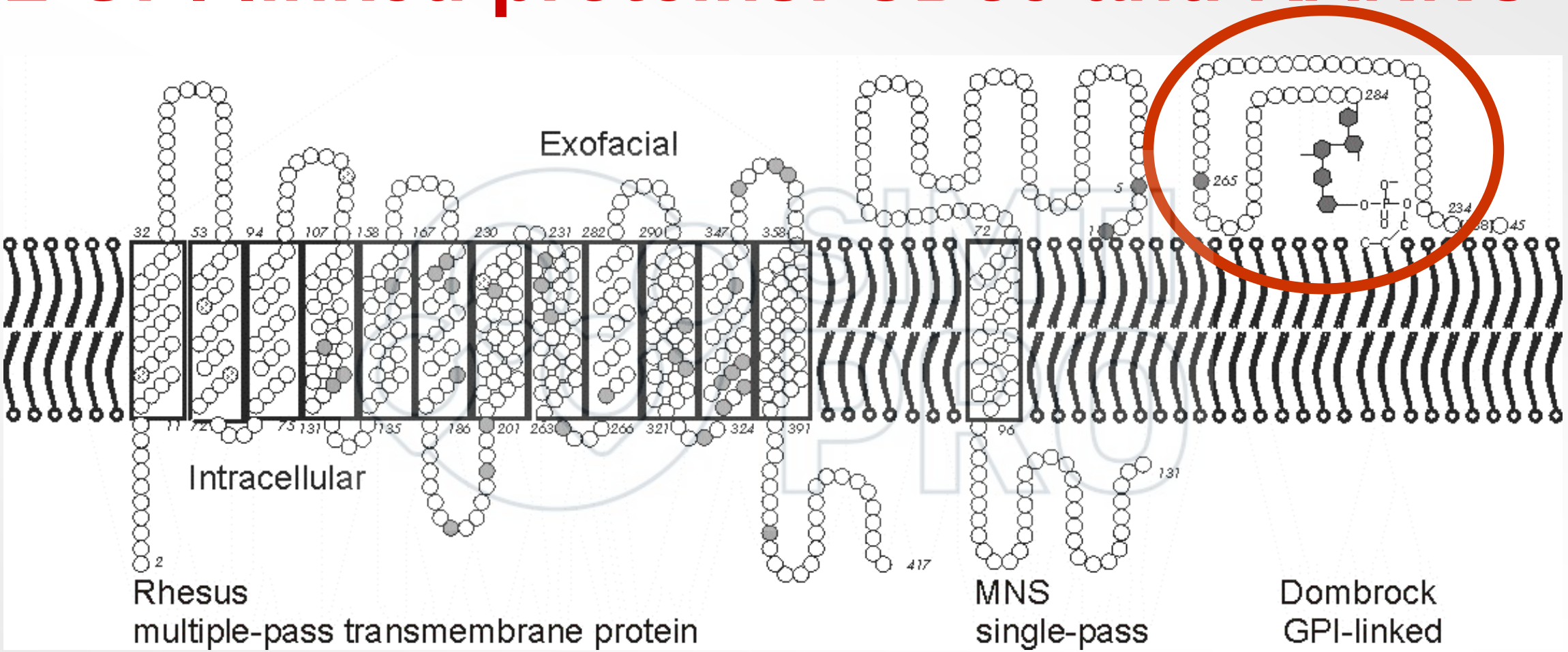
GPI linker (sugar)

- 042 – EMM glycosyltransferase

Sugar

- 038 – SID glycosyltransferase

2 GPI-linked proteins: CD59 and KANNO



Transf Apher Sci 2011(44)81-91

1 ‘new’ blood group system EMM has a new structural feature

Protein (multi-pass - segments)

- 036 – AUG 11
- 039 – CTL2 10
- 040 – PEL 12
- 041 – MAM 4
- 043 – ABCC1 17
- 044 – ER 36
- 045 – CD36 2

GPI-linked (protein)

- 035 – CD59 only extracellular
- 037 – KANNO only extracellular

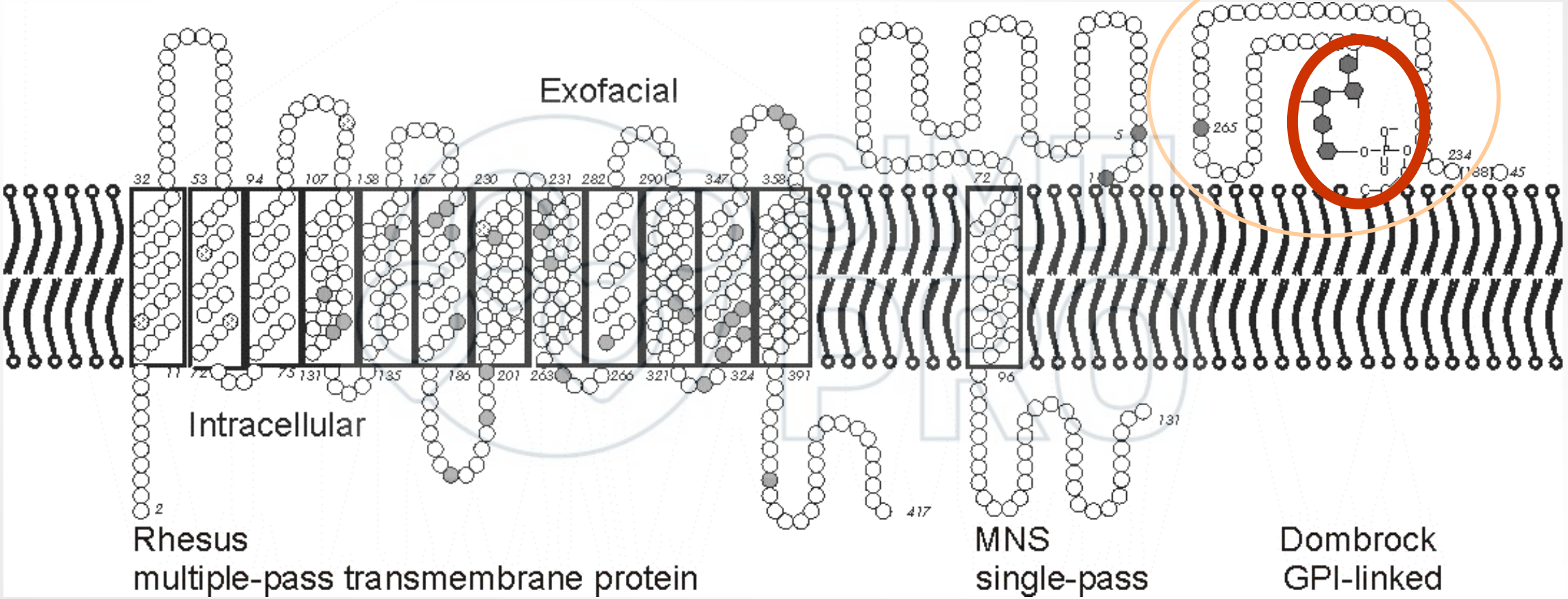
GPI linker (sugar)

- 042 – EMM glycosyltransferase

Sugar

- 038 – SID glycosyltransferase

1 GPI linker (sugar): EMM new structural feature



Transf Apher Sci 2011(44)81-91

1 ‘new’ blood group systems is a typical sugar antigen (like ABO)

Protein (multi-pass - segments)

- 036 – AUG 11
- 039 – CTL2 10
- 040 – PEL 12
- 041 – MAM 4
- 043 – ABCC1 17
- 044 – ER 36
- 045 – CD36 2

GPI-linked (protein)

- 035 – CD59 only extracellular
- 037 – KANNO only extracellular

GPI linker (sugar)

- 042 – EMM glycosyltransferase

Sugar

- 038 – SID glycosyltransferase

Antigens per blood group system mostly 1

Protein (multi-pass)

- 036 – AUG 4
- 039 – CTL2 2
- 040 – PEL 1
- 041 – MAM 1
- 043 – ABCC1 1
- 044 – ER 5
- 045 – CD36 1

GPI-linked (protein)

- 035 – CD59 1
- 037 – KANNO 1

GPI linker (sugar)

- 042 – EMM 1

Sugar

- 038 – SID 1

Antigens & prevalence
mostly 1 and mostly high-prevalence

Protein (multi-pass)

- 036 – AUG 4 – 100% (3) <0.1% (1)
- 039 – CTL2 2 – 100%
- 040 – PEL 1 – 100%
- 041 – MAM 1 – 100%
- 043 – ABCC1 1 – 100%
- 044 – ER 5 – >99% (4) <0.1% (1)
- 045 – CD36 1 – 100%

GPI-linked (protein)

- 035 – CD59 1 – 100%
- 037 – KANNO 1 – 100%

GPI linker (sugar)

- 042 – EMM 1 – 100%

Sugar

- 038 – SID 1 – 97%

Unlikely to encounter in routine clinical practice frequently.

If antibodies are found, patients are difficult to manage.

Protein (multi-pass)

- 036 – AUG severe HDFN
- 039 – CTL2 hemolysis, (TRALI)
- 040 – PEL hemolysis
- 041 – MAM fetal Hb↓, HDFN
- 043 – ABCC1 unknown
- 044 – ER unknown
- 045 – CD36 fetal Hb↓, PLT↓, HDFN

GPI-linked (protein)

- 035 – CD59 hemolysis
- 037 – KANNO unknown

GPI linker (sugar)

- 042 – EMM unknown

Sugar

- 038 – SID unknown

Antibodies in 6 blood group systems: clinically relevant for transfusion.

Terminology: names, names, names

Protein (multi-pass)

- 036 – AUG AUG1, At^{a}, ATML, ATAM
- 039 – CTL2 VER, RIF
- 040 – PEL PEL
- 041 – MAM MAM
- 043 – ABCC1 WLF
- 044 – ER Er^{a}, Er^{b}, Er3, ERSA, ERAMA
- 045 – CD36 CD36.1

GPI-linked (protein)

- 035 – CD59 CD59.1
- 037 – KANNO KANNO1

GPI linker (sugar)

- 042 – EMM Emm

Sugar

- 038 – SID Sd^{a}

VER is the first antigen of the CTL2 blood group system

Protein (multi-pass)

- 036 – AUG AUG1, At, ATML, ATAM
- 039 – CTL2 VER, RIF
- 040 – PEL PEL
- 041 – MAM MAM
- 043 – ABCC1 WLF
- 044 – ER Er^a, Er^b, Er3, ERSA, ERAMA
- 045 – CD36 CD36.1

GPI-linked (protein)

- 035 – CD59 CD59.1
- 037 – KANNO KANNO1

GPI linker (sugar)

- 042 – EMM Emm

Sugar

- 038 – SID Sd^a

VER is the first antigen of the CTL2 blood group system

- 039 – CTL2 VER, RIF
- named after patients' birthplaces

- Choline transporter-like 2 protein
- 10 transmembranous segments
- Implicated in TRALI
- also expressed on granulocytes
  = HNA-3 (anti-HNA-3a vs. anti-HNA-3b)
- Close nomenclature coordination with the platelet & neutrophil working parties

VER is the first antigen of the CTL2 blood group system

- 039 – CTL2 VER, RIF
- named after patients' birthplaces
- Verona
- Rif = region in Morocco

- Choline transporter-like 2 protein
- 10 transmembranous segments
- Implicated in TRALI
- also expressed on granulocytes
  = HNA-3 (anti-HNA-3a vs. anti-HNA-3b)
- Close nomenclature coordination with the platelet & neutrophil working parties

5 blood group systems are associated with diseases.

Protein (multi-pass)

- 036 – AUG severe HDFN
- 039 – CTL2 hemolysis, hearing loss
- 040 – PEL hemolysis
- 041 – MAM fetal Hb↓, HDFN
- 043 – ABCC1 unknown
- 044 – ER unknown
- 045 – CD36 fetal Hb↓, PLT↓, HDFN

GPI-linked (protein)

- 035 – CD59 hemolysis, severe disability
- 037 – KANNO no HDFN, prion protein

GPI linker (sugar)

- 042 – EMM unknown, development↓

Sugar

- 038 – SID unknown, cancer↑, infection↓

2 blood group systems associated with neurologic & developmental deficits

Protein (multi-pass)

- 036 – AUG severe HDFN
- 039 – CTL2 hemolysis, hearing loss
- 040 – PEL hemolysis
- 041 – MAM fetal Hb↓, HDFN
- 043 – ABCC1 unknown
- 044 – ER unknown
- 045 – CD36 fetal Hb↓, PLT↓, HDFN

GPI-linked (protein)

- 035 – CD59 hemolysis, severe disability
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GPI linker (sugar)

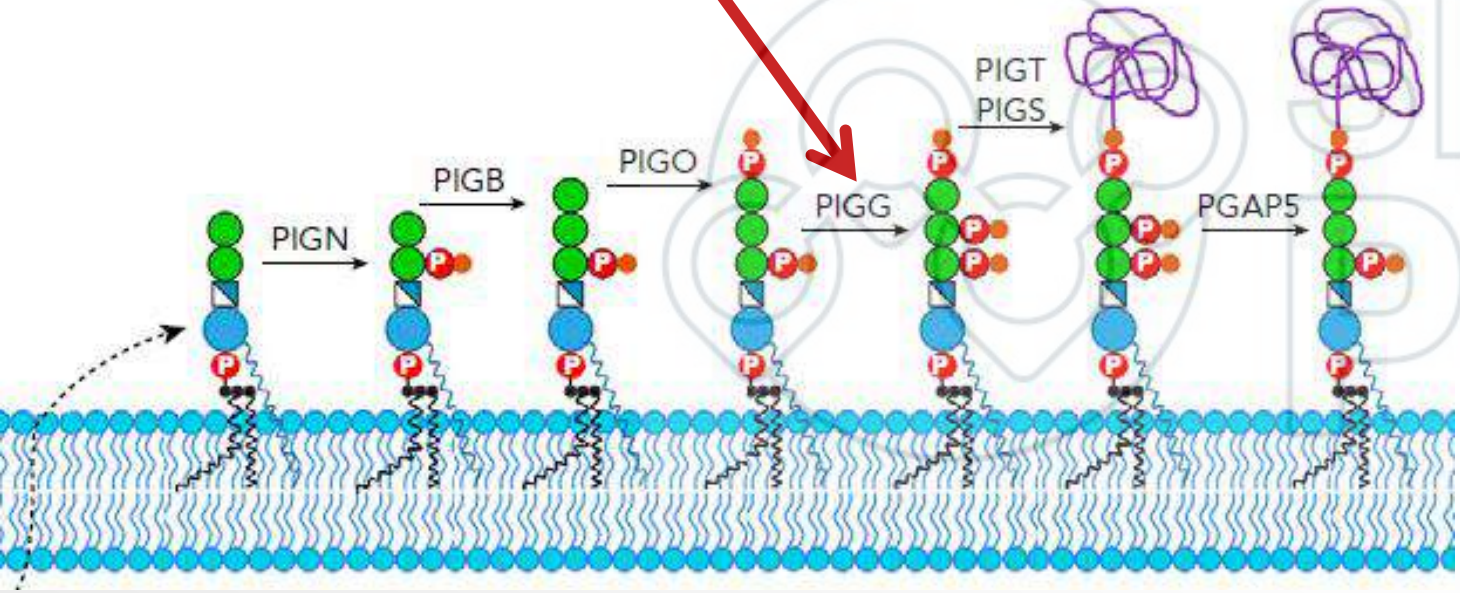
- 042 – EMM unknown, development↓

Sugar

- 038 – SID unknown, cancer↑, infection↓

042 – EMM and 035 – CD59 both involved in developmental deficits

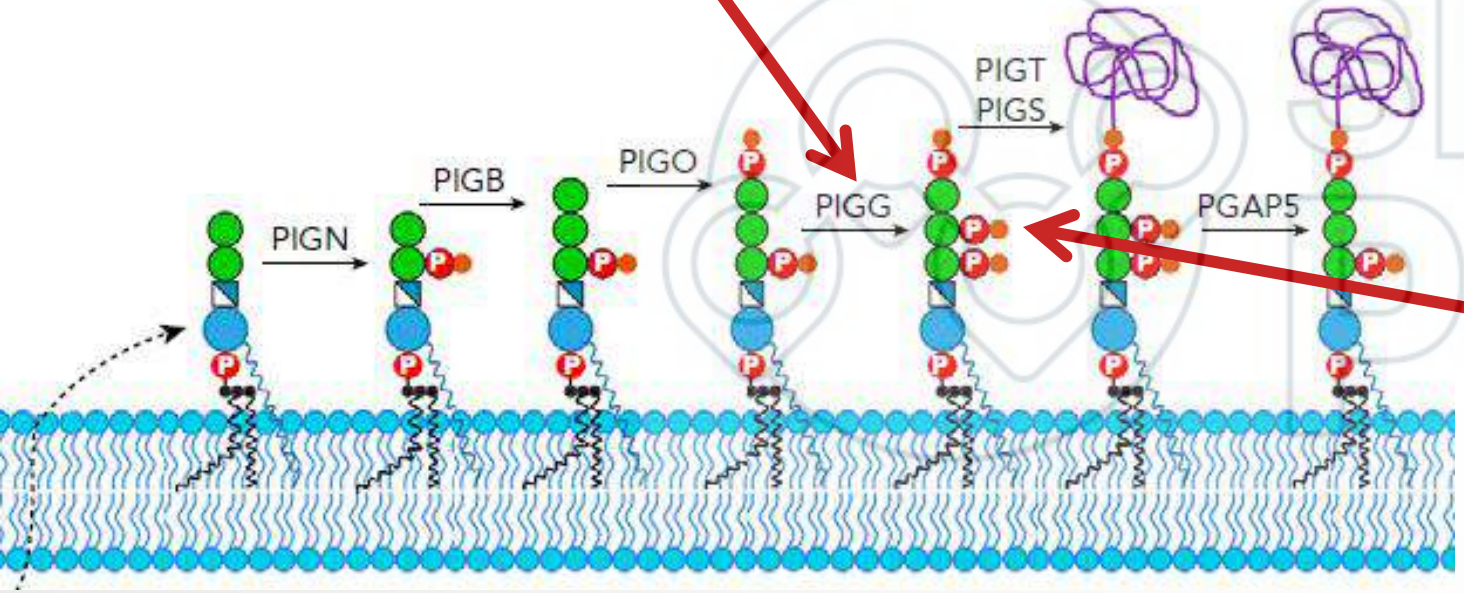
- *PIGG* defective in EMM-



- Failure to synthesize the Ethanolamine-phosphate in 2nd position

042 – EMM and 035 – CD59 both involved in developmental deficits

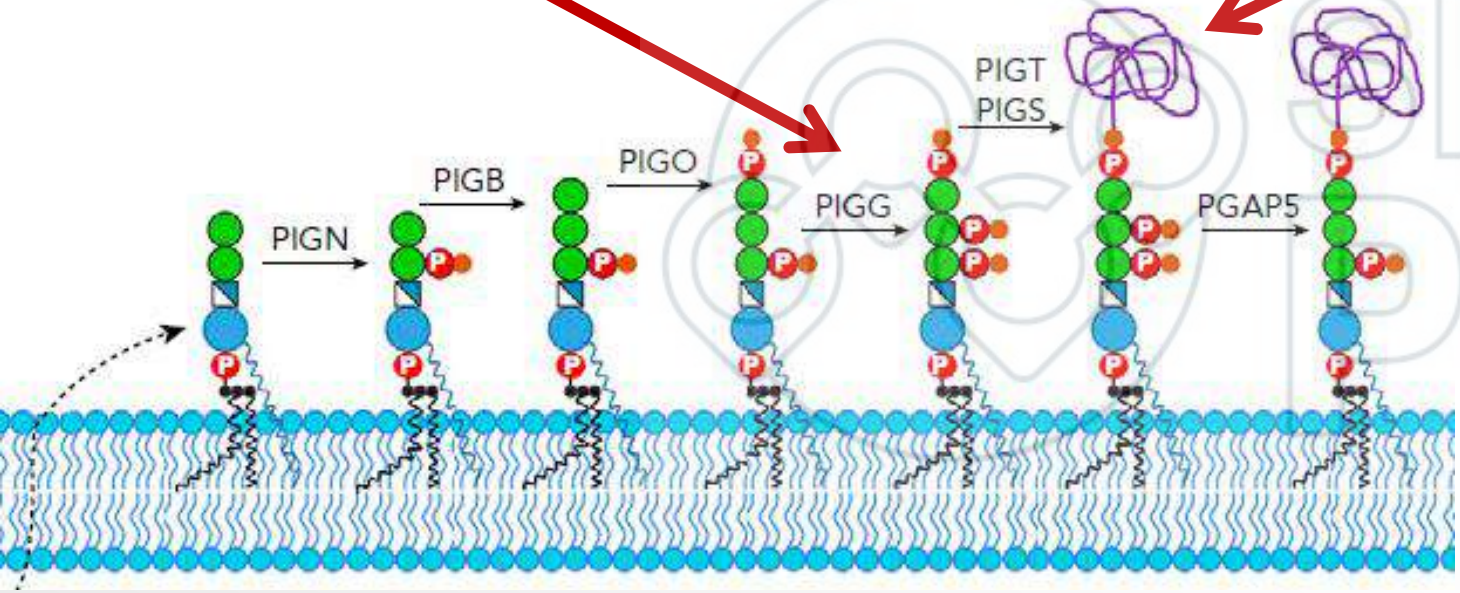
- *PIGG* defective in EMM-



- Failure to synthesize the Ethanolamine-phosphate in 2nd position
- EtNP = precursor to binding all GPI-linked proteins
- could be expected to result in failure of binding GPI-linked proteins

042 – EMM and 035 – CD59 both involved in developmental deficits

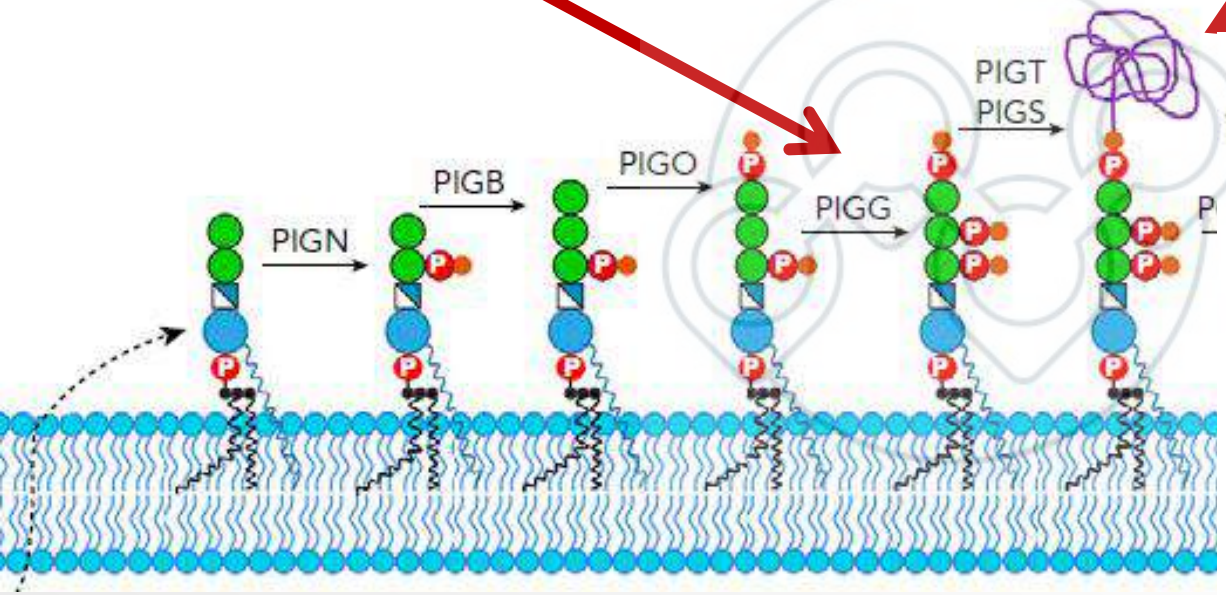
- *PIGG* defective in EMM-

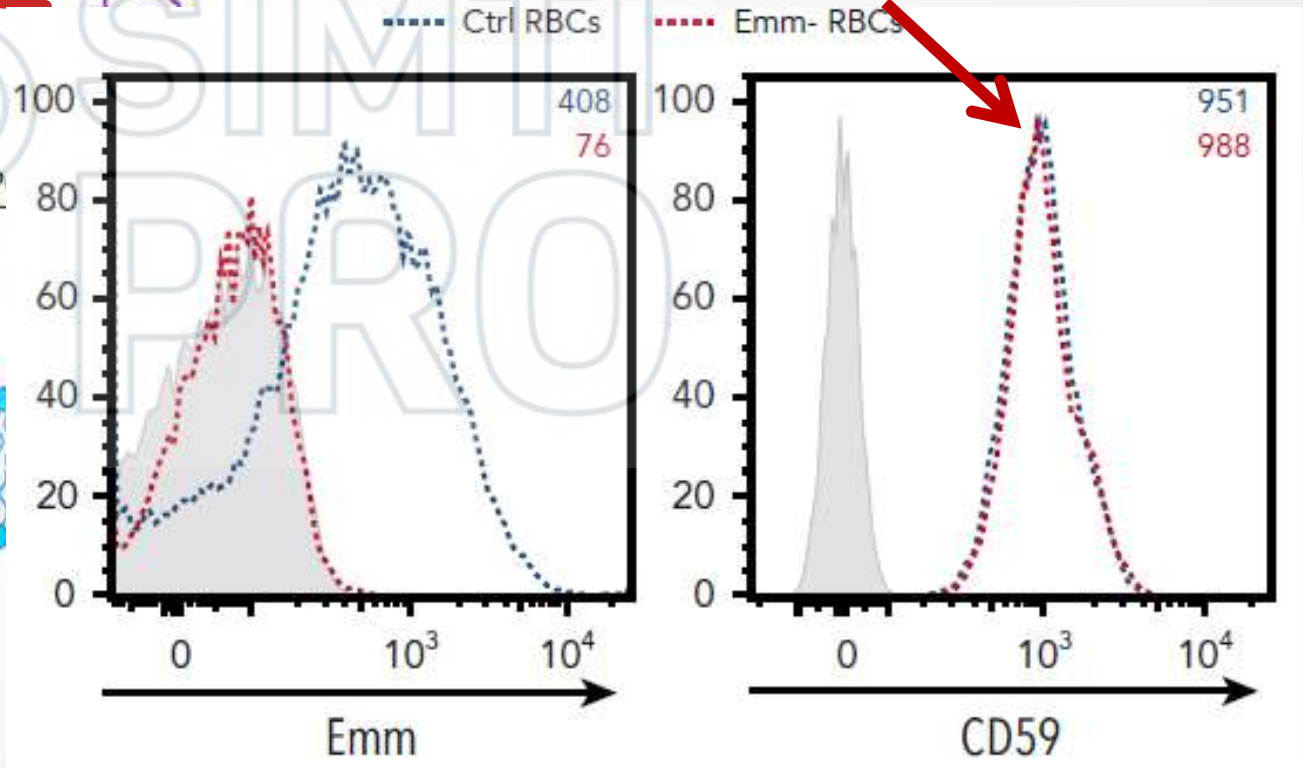


- GPI-linked proteins
  - 011 YT – 1956
  - 014 DO – 1965
  - 021 CROM – 1990
  - 026 JMH – 2000
  - 035 CD59 – 2014
  - 037 KANNO – 2019
- and >150 other proteins attached to the red cell surface

042 – EMM and 035 – CD59 both involved in developmental deficits

- *PIGG* defective in EMM-
- Why is CD59 expression on red cells normal?





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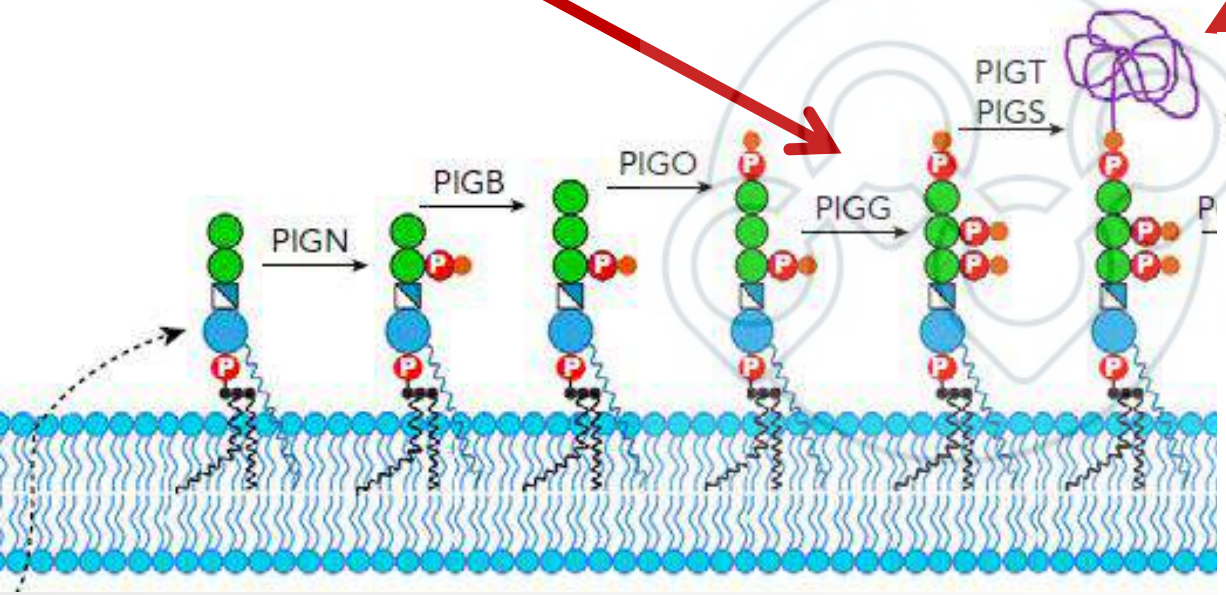
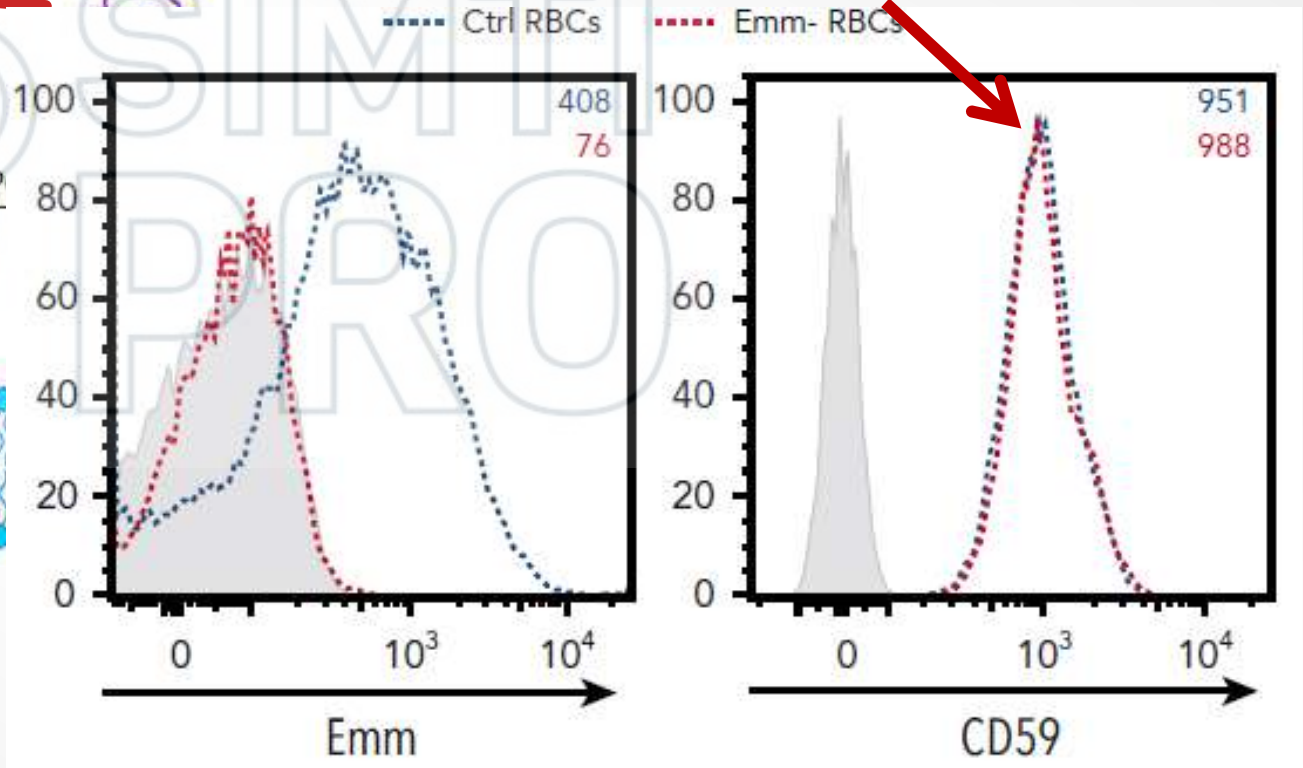
- Why is CD59 expression on red cells normal?





- Alternative mode of GPI attachment in nerves & brain?

035 – CD59 = 1 antigen

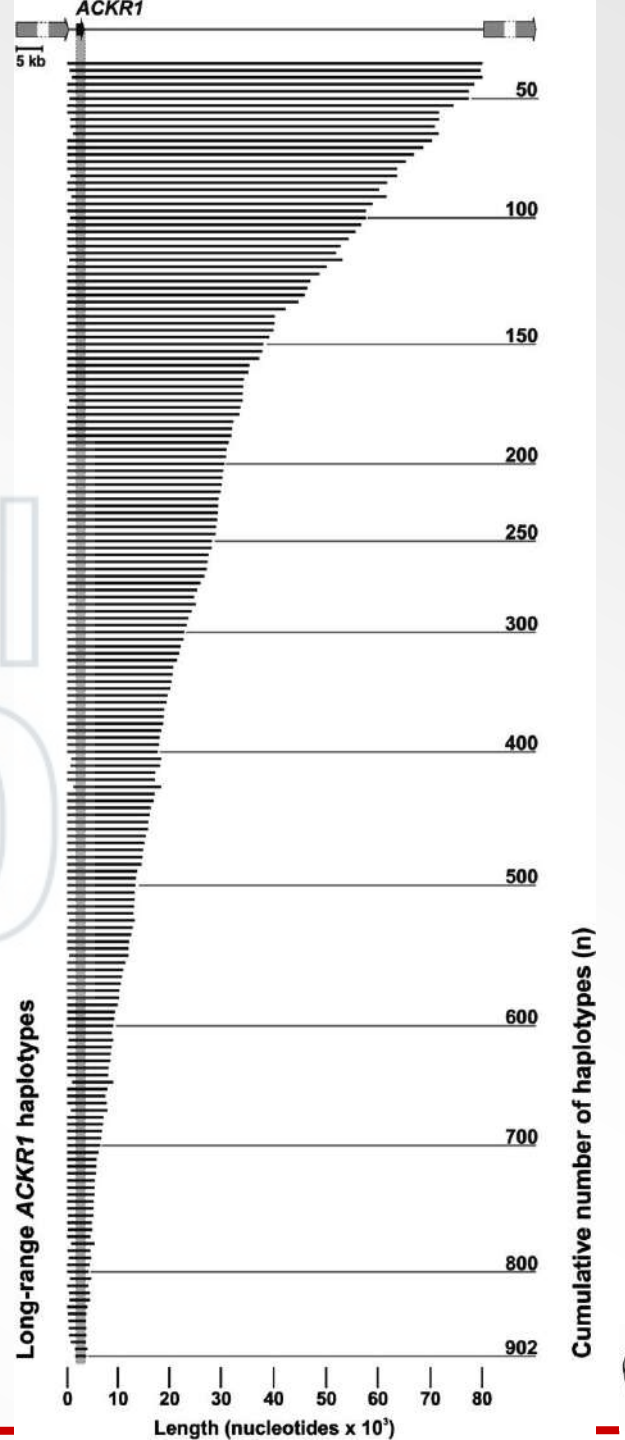
Study cohort	Individuals n	Chromosomes	Nucleotides	Single nucleotide variation (SNV)	Gene variants (alleles)
FDA	53	106	2,408,108	133	70
Ethiopia	60	120	2,726,160	163	80
Total	113	226	5,134,268	216	143

Little antigen variability: n = 1
Enormous genetic variability: more alleles than individuals

Expression patterns in various tissues affects pathophysiology.

Extract nucleotide sequences from genome databases

- 902 distinct sequences for the *FY* gene
  - 1,901 nt to 80,584 nt length
- extracted from the 1000 Genomes Project
  - based on 2,504 unrelated individuals
- with bioinformatic algorithms
  - experimentally confirmed (error free)

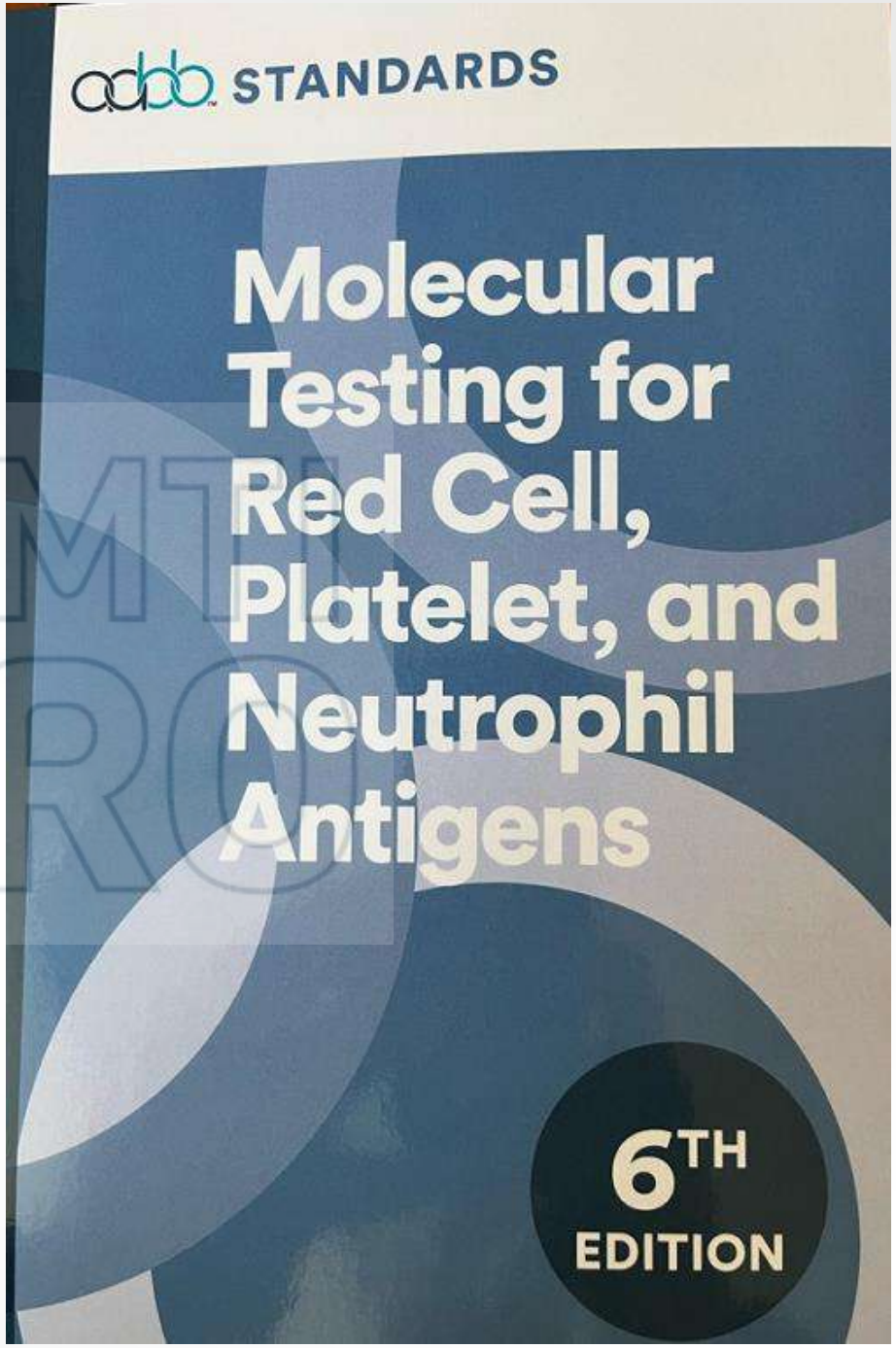


BMC Bioinformatics (2021) 22:273

BMC Bioinformatics 2021; 22: 273

Resources

- 7th Edition: draft currently in review for public comments (for free)
- Many new features covering all blood group, platelet and neutrophil genes.
  - For example, *CD59*:
    - Transcript NM_203330.2
    - RefSeq Gene NG_008057.1
    - Chromosome NC_000011.10
    - rs number rs587777149
    - CD59 negative



Practical benefits of ‘new’ antigens for patient care and patient safety

- Allow to determine, and often exclude, the clinical relevance of high-prevalence antibodies
  - crossmatch positive with all test and donor cells
  - eventually be done by red cell genotyping
- Enable to investigate transfusion reactions
  - unexplained hemolysis following transfusions
- Guide to study protein variants of drug transporter genes
  - pharmacogenomics with red cells

New approaches in transfusion science

- Data science (bioinformatics, biostatistics, big data)
  - Multifactorial analyses
- Application of artificial intelligence
- Implementation of machine learning
- We need to generate the systematic large datasets that are required for these new approaches.

The intricate path from genetic variant to clinical interpretation

- There are few simple answers.
- Most matter is complex.
- It’s not going to be easy.
- Be patient.
- Tolerate costs.
- Accept a pragmatic approach.

- An incremental improvement is better than no progress at all.

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