Health Care
Case Report

Will

HAABB Fall Meeting

Sept 18, 2024

Clinical Scenario

- 11 month old male seen at University Hospital Emergency Department
- Referred from outside hospital with 2 weeks of right arm swelling and redness
- Some nausea and vomiting, diarrhea
- Rubbing his arm like ‘it feels unusual’
- Physical exam confirmed the redness and swelling. Also unable to fully bend the elbow. Distal pulses and capillary refill are intact
- X Ray showed no fracture, but significant soft tissue swelling
- Admitted for further assessment and monitoring

Clinical Scenario

- Elevated WBC count and platelet count, but Hgb 8.4 gm/dL
- Neutrophils and monocytes increased on peripheral smear; no blasts seen
- ESR and C-reactive protein both elevated – inflammation
- MRI – large abscess surrounding the end of the ulna with osteomyelitis extending along proximal to mid-shaft
- Intraoperative cultures – Group A Streptococci

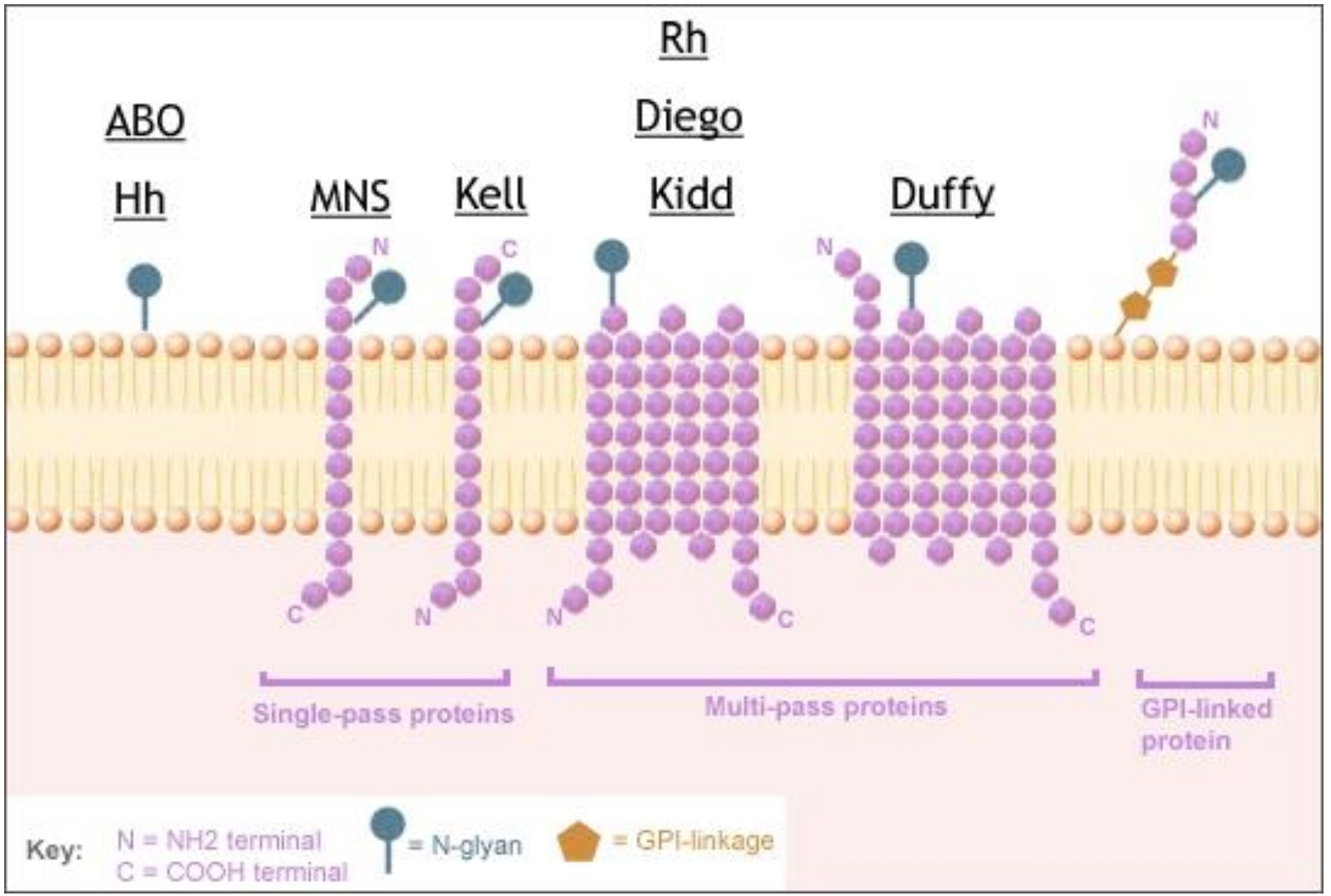
Blood Bank

- Forward type – A, RhD negative
- Reverse type – AB (no reactivity with test cells)
- Occurring on automated analyzer and on benchtop testing at RT
- Repeated by second technician with same results
- First technician able to get weak B reactivity with 4 degrees Celsius incubation x 30 minutes
- Second technician got weak A and B reactivity with similar cold incubation

Blood Bank

- Antibody Screen negative; Negative autocontrol
- Cold Screen panreactive; Positive autocontrol
- Same results upon repeat testing

RBC membrane



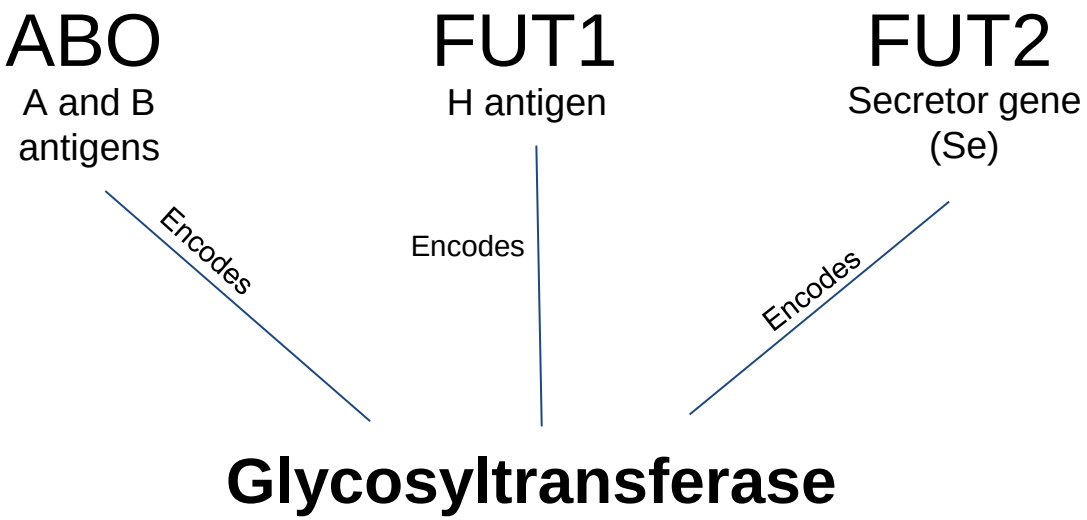
http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=rbcantigen

Carbohydrate Antigens

- Frequently repetitive epitopes
  - Ongoing glycosylation
- Direct B-cell stimulation
  - Ongoing antibody production
  - “Naturally” occuring antibodies
  - Tends to be IgM
- Strong Agglutination at Room Temperature
  - Complement binding and in-vitro hemolysis
- Cold affinity (<<37 degrees Celsius)
  - Uncommonly in vivo hemolysis
    - Exception ABO antibodies

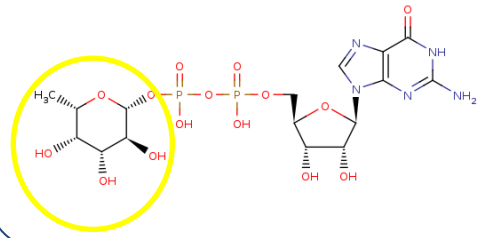
Expression of ABO Antigens

Controlled by 3 genes:

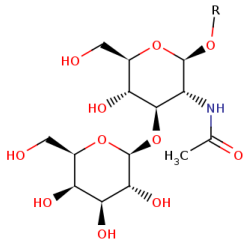


Catalytic Action

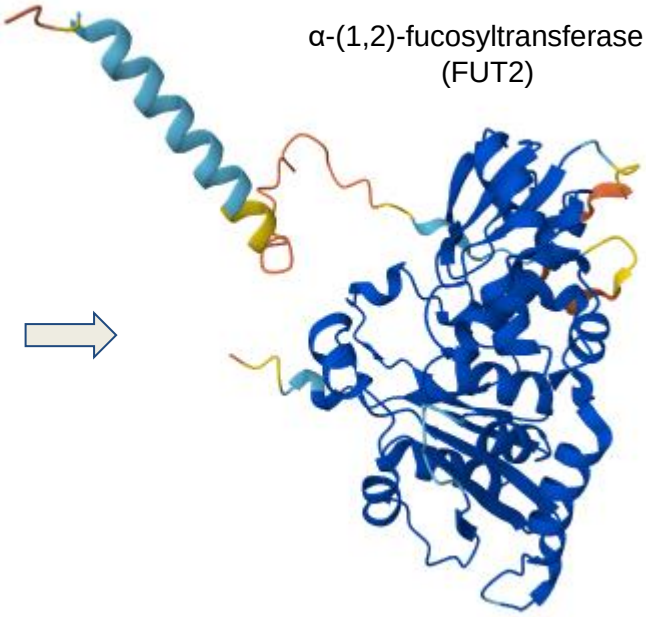
GDP-beta-L-fucose

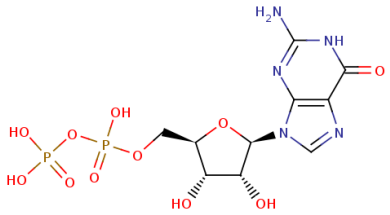


beta-D-galactosyl-(1->3)-N-acetyl- beta-D-glucosaminyl-R

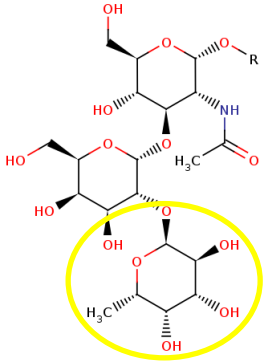


α-(1,2)-fucosyltransferase 2 (FUT2)





GDP



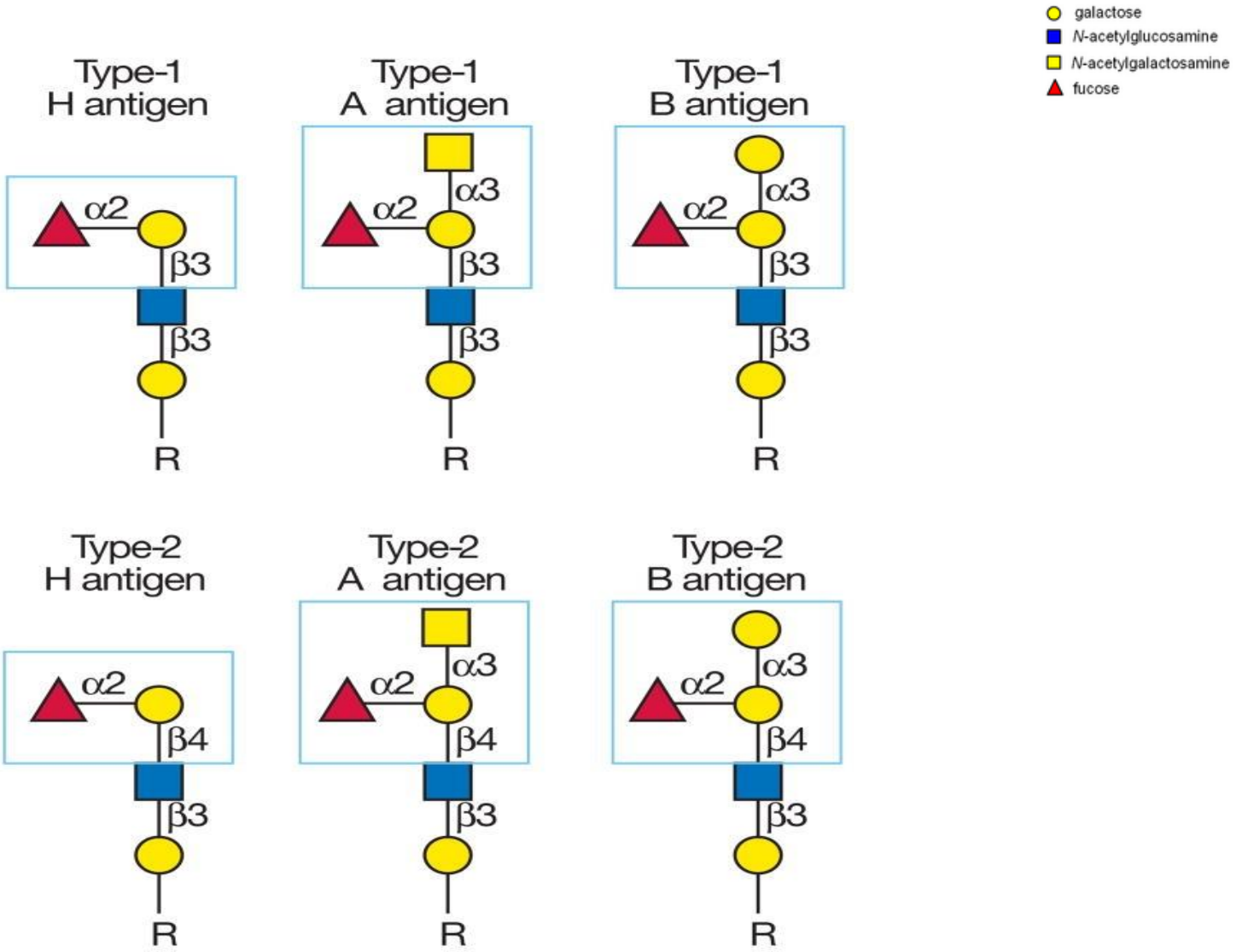
alpha-L-fucosyl-(1->2)-beta-D-galactosyl-(1->3)-N-acetyl-beta-D-glucosaminyl-R

Jumper, J., et al. (2021). Highly accurate protein structure prediction with AlphaFold. Nature, 596(7873), 583-589. https://doi.org/10.1038/s41586-021-03819-2

Varadi, M., et al. (2024). AlphaFold Protein Structure Database in 2024: providing structure coverage for over 214 million protein sequences. Nucleic Acids Research. https://doi.org/10.1093/nar/gkad112

AlphaFold. (n.d.). AlphaFold entry: Q10981. AlphaFold Protein Structure Database. Retrieved from https://alphafold.ebi.ac.uk/entry/Q10981

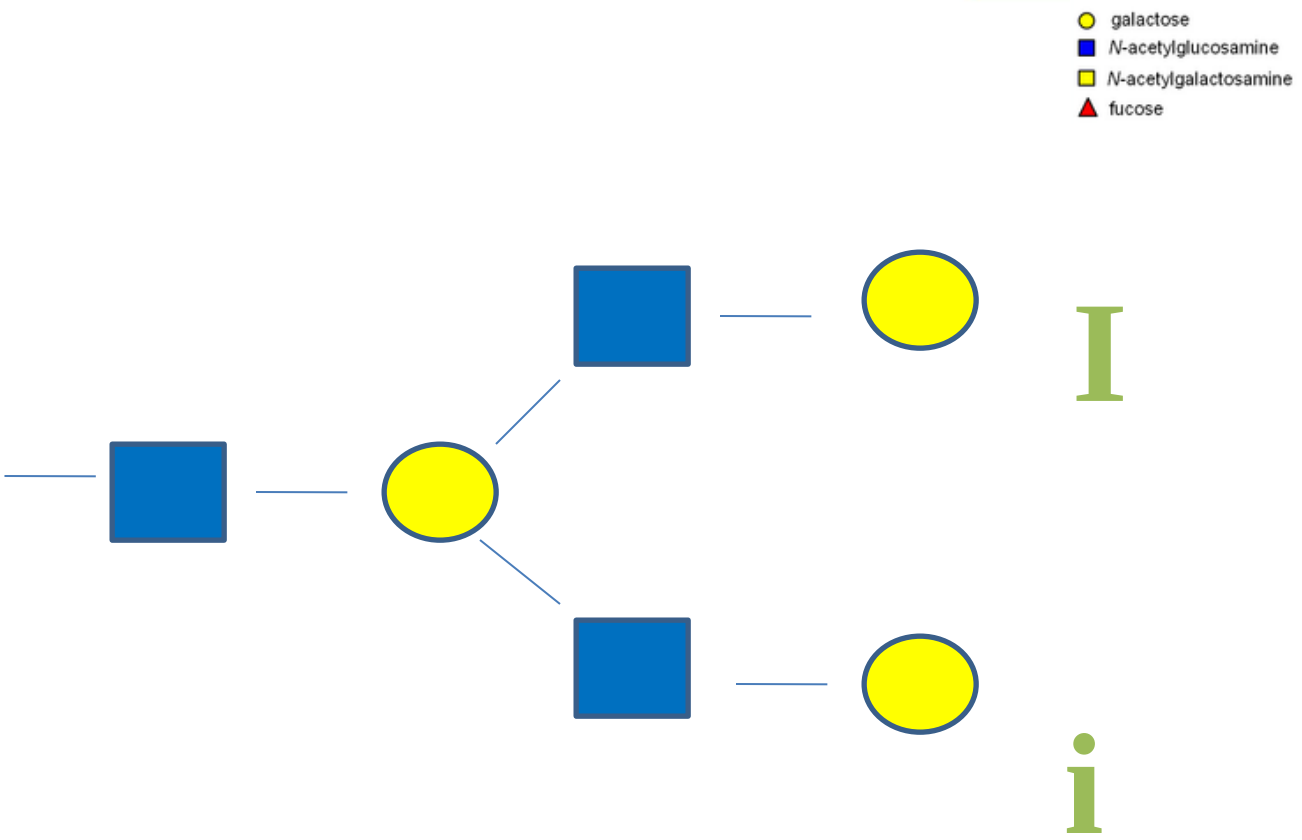
Working Inside Out



I and i antigens

- Precursor substance for H, A, & B
- “Maturation” from little i to big I
  - Adults still have some little i on the RBC – below clinical detection limits
  - Relates to marrow transit time – reticulocytes have the most

I and i antigens



Anti-I/anti-i

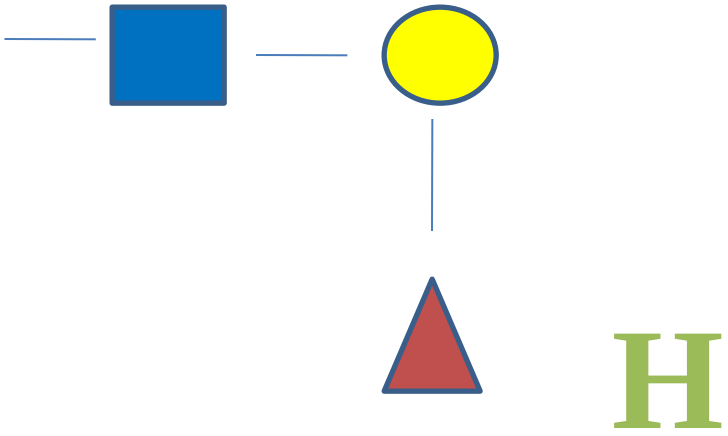
- Auto-anti-I at subclinical levels in many people
- Nuisance antibody at ABO testing
- Cold autoimmune hemolytic anemia
  - Anti-I – Mycoplasma pneumoniae (big people)
  - Anti-i – EBV (infectious mononucleosis – little people)
  - Wide thermal amplitude
    - If you can detect it at 37, it can hemolyze cells
    - Works best at 4 degrees Celsius
  - Cold agglutinin panel will test adult cells (I) and cord cells (i) for specificity

H substance

- (α1-2) fucosyltransferase on 19q13.3 (FUT1)
  - Connects a fucose to the terminal galactose of the I/i chain
  - Fucose moiety is required to build A & B antigen
  - Group O individuals have only H substance as the predominant antigenic oligosaccharide

Ulex europeaus

H substance


galactose
N-acetylglucosamine
N-acetylgalactosamine
fucose
H

Anti-H

- Individuals without FUT1 (hh) produce a strong anti-H which will hemolyze >99% transfused cells (Bombay/O_h)

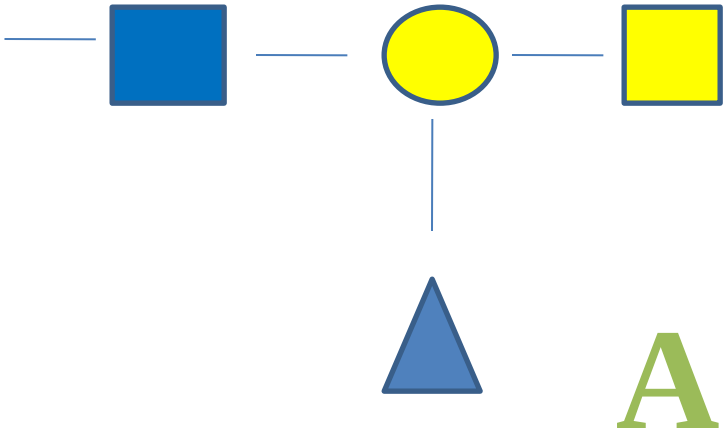
- FUT2 will produce H, A and B on secretions, so can adsorb to RBCs resulting in
  Weak A or B phenotype
- Anti-A or anti-B on eluate

ABO

- H substance glycosylated to form the A or B antigen
  - Requires fucose
  - A and B genes on chromosome 9 code for glycosyltransferases
    - Only differ by four nucleotide residues resulting in four different amino acids
  - No transfer of antigenic sugar – type O
    - Most common is an amorph – premature stop codon due to deletion at nucleotide 261 (AA117)
    - Approximately 50 other identified “O” alleles

A substance

Addition of N-acetylgalactosamine

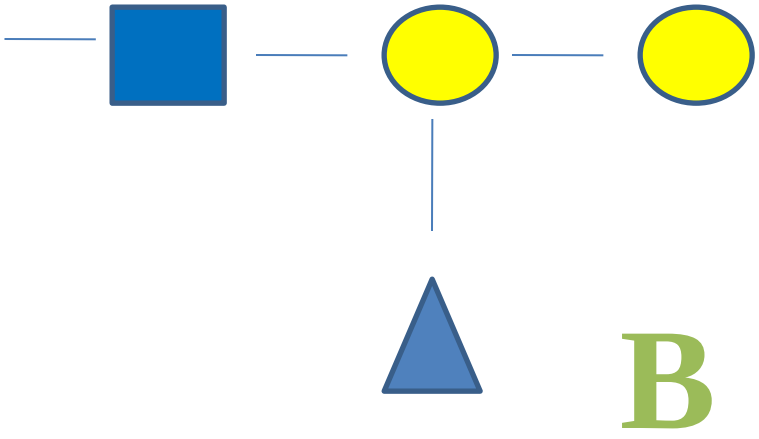


galactose
N-acetylglucosamine
N-acetylgalactosamine
fucose

B substance

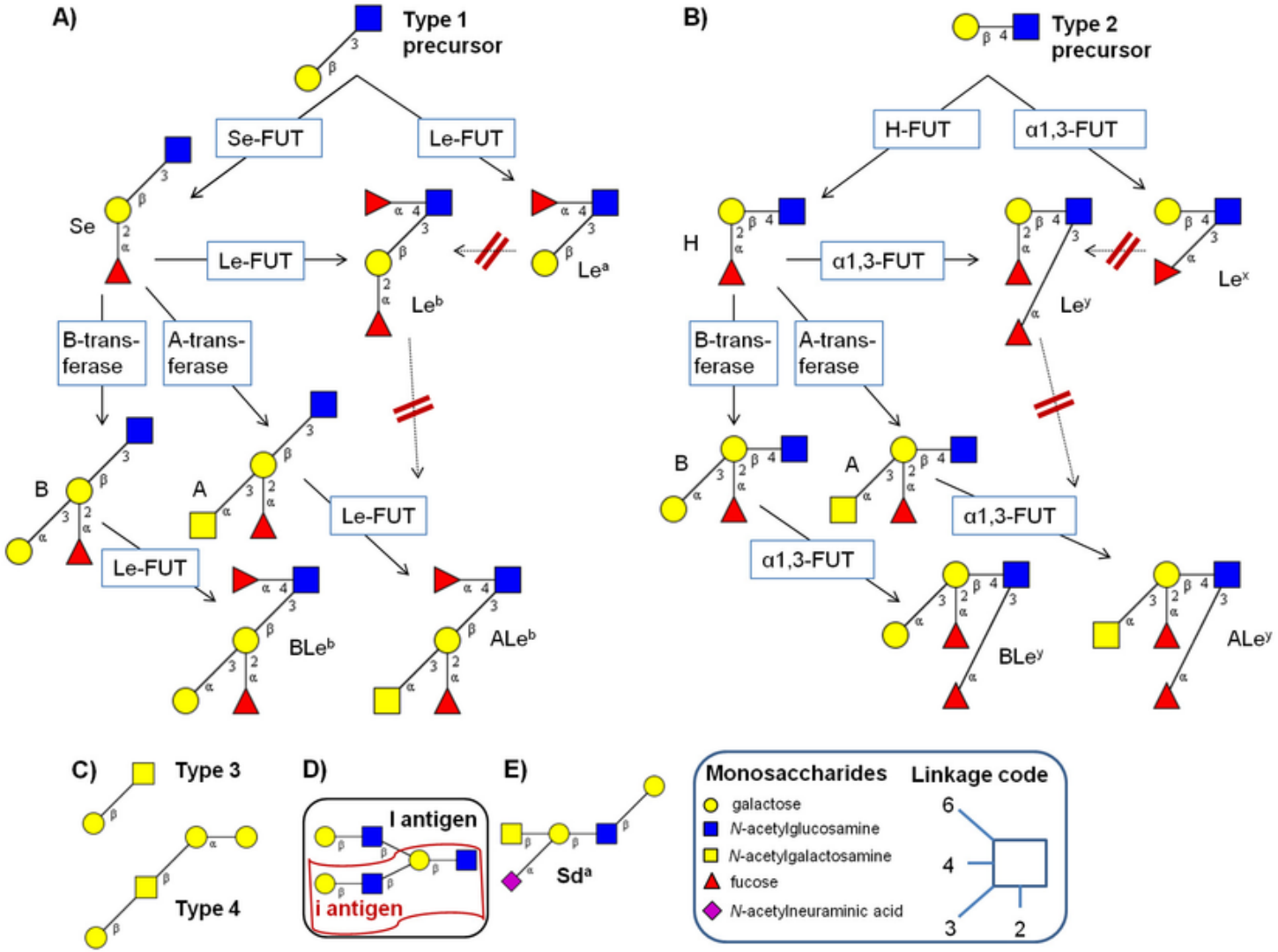
galactose
N-acetylglucosamine
N-acetylgalactosamine
fucose

Addition of Galactose



B

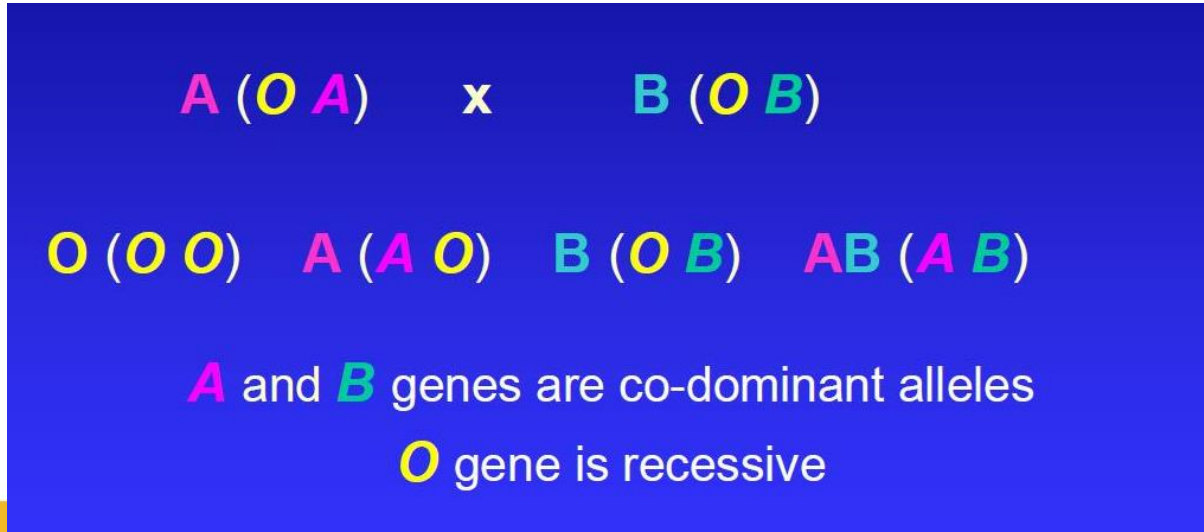
Full Pathway



Dotz, Viktoria & Wuhrer, Manfred. (2015). Histo-blood group glycans in the context of personalized medicine. Biochimica et Biophysica Acta (BBA) - General Subjects. 1860. 10.1016/j.bbagen.2015.12.026.

ABO

Co-dominant in phenotype



ABO

- Although it is considered an RBC antigen, it is not specific
  - Platelets, intestinal cells, vascular endothelium, soluble in secretions and excretions (saliva, milk, urine)
    - Transfusion of ABO incompatible platelets results in approx. 75% of expected recovery at one hour

Anti-ABO antibodies

- “Naturally-occurring”
  - Similarities between bacterial antigens and A/B
- By six months will invariably have antibodies against non-self antigens
  - Peak around age 5-10
  - Wane in elderly – may become clinically undetectable
  - Predominantly IgM
    - Anti-A,B made by Group O individuals is IgG
      - Can pass through placenta and cause HDFN

A and B subgroups

80% of type A individuals have an enzyme with strong activity (A1)

Complex oligosaccharides with internal A epitopes

20% have weaker enzymes (predominantly A2) that can recognize the structure of ‘A1” as foreign

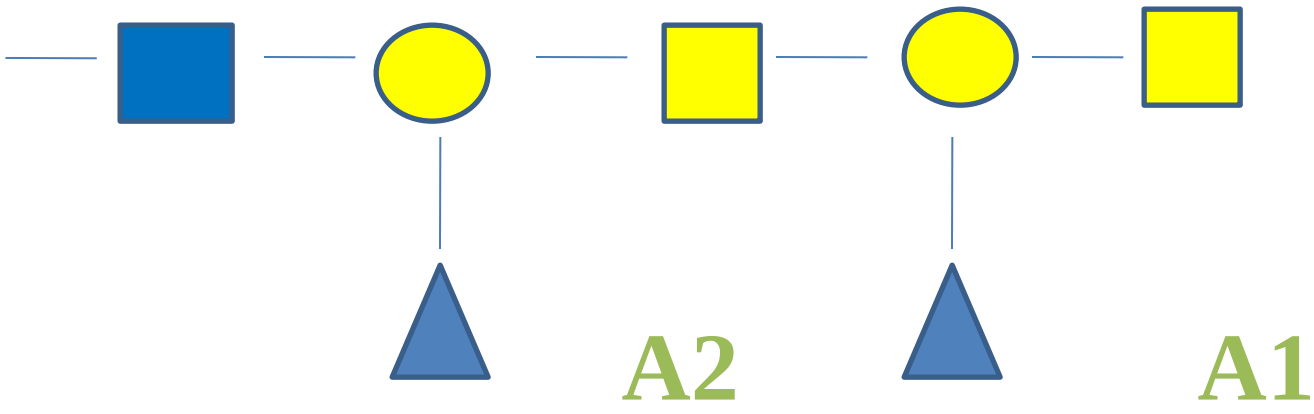
A_3 will produce mixed field reaction (A and O)

A_x will have weak/absent reactivity with anti-A and frequently a weak anti-A1 (funny O)

B antigenic subgroups are similar

A2 and A1

Addition of N-acetylgalactosamine



Anti-A1

- Unexplained reactivity with A1 cells in a type A patient
  - Typing discrepancies
  - 1-2% of A2
  - 25% of A2B

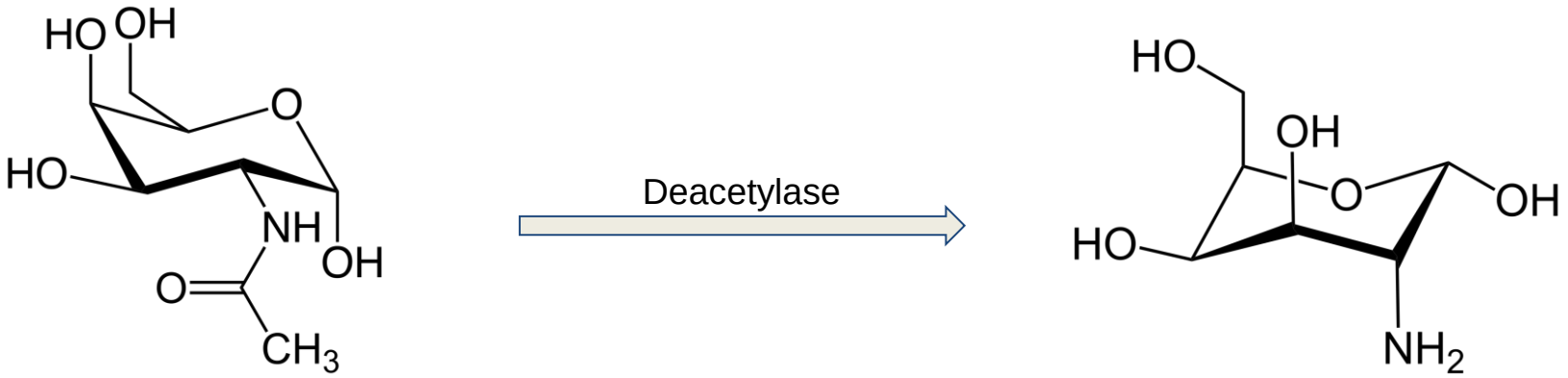
- Resolved with an anti-A1 lectin from *Dolichos biflorus* (horse gram)

Acquired “B” antigen

Gut bacteria have a deacetylase enzyme, which, if bacteremic, can convert N-acetylgalactosamine to galactosamine which has sufficient antigenity to bind anti-B in blood typing

Weak B on forward typing

Anti-B on reverse typing



Cis-AB

- Weak A and B activity inherited together from one parent
- Mostly AB on front type, although A and B have been reported and later confirmed genetically
- ?? Production of anti-A1

Considerations

- Hypogammaglobulinemia (elderly, hematopoietic malignancies resulting in poor antibody production and non-reactivity on reverse typing
  - Bacterial Infection??
- Autoantibodies interfering with hemagglutinins
  - Very weak
- Intestinal/Pancreatic/Biliary/Ovarian malignancies with excretion of soluble A/B antigen which binds up hemagglutinins

Workup

- Quantitative Immunoglobulins were elevated or within normal levels (no hypogammaglobulinemia)
- One allele positive for RAG1 gene associated with autosomal recessive severe combined immunodeficiency (SCID)
  - Could it be that the other allele is not detected by this assay?
    - 71 different variants identified, but only 10% pathogenic
    - Parents did not want further expensive testing

Conclusions

- Uncertain
  - If SCID, why adequate immunoglobulins?
  - Discharged on long term antibiotics to be seen at outside provider

Any Questions?