Blood Group Genetics

Department of Medical Genetics
Medical University of Warsaw

Classical genetics

Classical genetics laws:

- *Mendel's First Law* – Law of Segregation
- *Mendel's Second Law* – Law of Independent Assortment

- Co-dominance – phenotypic expression of gene's alleles is simultaneous and independent
- Epistasis – phenomenon in which one gene influences the phenotypic expression of another gene, while not being its allele

Blood Group Systems

Blood Group – antigenic determinants on the surface of erythrocytes distinguished by the immune system in individuals without a particular antigen (alloantibodies)

- 285 antigens
- 33 group systems

Group system – consists of one or more antigens controlled at a single gene locus, or by two or more very closely linked homologous genes

Collection – consist of serologically, biochemically, or genetically related antigens, which do not fit the criteria required for system status (200 series)

901 Series – (or 'public') contains antigens with an incidence of greater than 90% and which cannot be included in a system or collectioni

700 Series – (or 'private') contains antigens with an incidence of less than 1% and which cannot be included in a system or collection

Red blood cells group systems

Nr	Nazwa	Symbol	Gen	Chromosom
001	**ABO**	ABO	ABO	9q34.2
002	***MNS***	*MNS*	*GYPA, GYPB, GYPE*	*4q31.21*
003	***P1PK***	*P1PK*	*A4GALT*	*22q13.2*
004	***Rh***	*RH*	*RHD, RHCE*	*1p36.11*
005	***Lutheran***	*LU*	*LU*	*19q13.32*
006	***Kell***	*KEL*	*KEL*	*7q34*
007	***Lewis***	*LE*	*FUT3*	*19p13.3*
008	***Duffy***	*FY*	*DARC*	*1q23.2*
009	**Kidd**	JK	SLC14A1	18q12.3
010	**Diego**	DI	SLC4A1	17q21.31
011	**Yt**	YT	ACHE	7q22.1
012	**Xg**	XG	XG, MIC2	Xp22.33
013	**Scianna**	SC	ERMAP	1p34.2
014	**Dombrock**	DO	ART4	12p12.3
015	**Colton**	CO	AQP1	7p14.3
016	**Landsteiner-Wiener**	LW	ICAM4	19p13.2
017	**Chido/Rodgers**	CH/RG	C4A, C4B	6p21.3

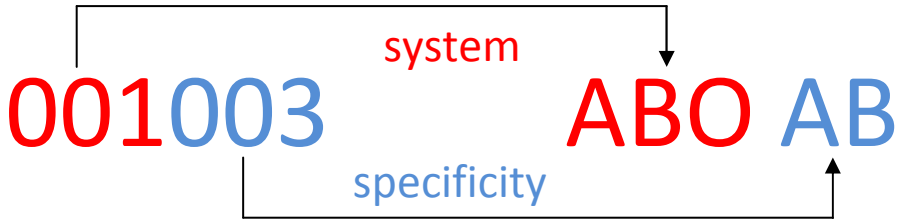
Nr	Nazwa	Symbol	Gen	Chromosom
018	**H**	H	FUT1	19q13.33
019	**Kx**	XK	XK	Xp21.1
020	**Gerbich**	GE	GYPC	2q14.3
021	**Cromer**	CROM	CD55	1q32.2
022	**Knops**	KN	CR1	1q32.2
023	**Indian**	IN	CD44	11p13
024	**Ok**	OK	BSG	19p13.3
025	**Raph**	RAPH	CD151	11p15.5
026	**John Milton Hagen**	JMH	SEMA7A	15q24.1
027	**I**	I	GCNT2	6p24.2
028	**Globoside**	GLOB	B3GALT3	3q26.1
029	**Gill**	GIL	AQP3	9p13.3
030	**Rh-associated glycoprotein**	RHAG	RHAG	6p21-qter
031	**FORS**	FORS	GBGT1	9q34.13
032	**JR**	JR	ABCG2	4q22
033	**LAN**	LAN	ABCB6	2q36

T/Tn (antygeny Th, Tk, Tr, Tx) C1GALT1, C1GALT1C1 7p14-p13
CAD
Sid (Sda)

As of 2013
International Society of Blood Transfusion

Nomenclature

Antigens



Phenotype

MNS:1,-2, 8 (system)

MNS $M^{C}+$

Allele

ABO*012 (system: ABO; allele: 012)

ABO 0

Genotype

ABO*101/103 (allele 1: 101; allele 2: 103)

ABO AA

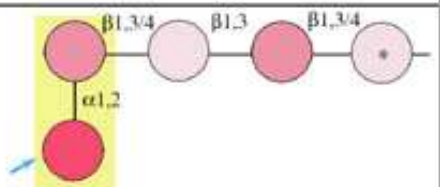
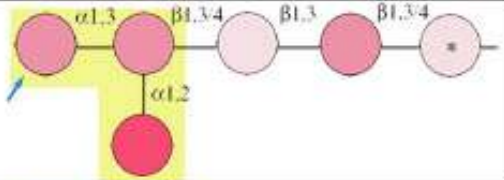
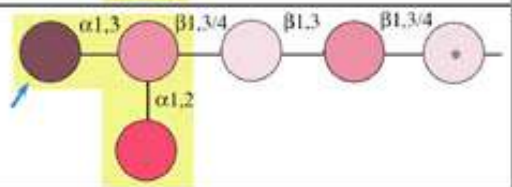
ABO group system

No. of antigens	**4: A1, A2, B, H (=0)**
Nature of antigen	oligosaccharide
Carrier molecule	glycoproteins, glycolipids
Gene	**ABO** 9q34.1-q34.2
Frequencies of antigens	**A:** 43% Caucasians, 27% Blacks, 28% Asians **B:** 9% Caucasians, 20% Blacks, 27% Asians **A1:** 34% Caucasians, 19% Blacks, 27% Asians
Frequencies of phenotypes	Caucasians: group O, 44%; A_1, 33%; A_2, 10%; B, 9%; A1B, 3%; A2B, 1% Blacks: group O, 49%; A_1, 19%; A_2, 8%; B, 20%; A1B, 3%; A2B, 1% Asians: group O, 43%; A_1, 27%; A_2, rare; B, 25%; A1B, 5%; A2B, rare Note: Blood group A is divided into two main phenotypes, A_1 and A_2

ABO Group System

- Basic blood types A, B, AB, O
- Antigens are oligosaccharides modified by gene specific transferases
- Present in most cells, and in secretors in secretions and body fluids
- Antigens appear around 6 week of embryonic life, but their full expression occurs from 6 to 18 months after birth

ABO Group System

Antigen	Structure	Minimal determinant structure
H		Fuc–α1→2–Gal–β1–R
B		Gal–α1→3 ↘ Gal–β1–R Fuc–α1→2 ↗
A		GalNAc–α1→3 ↘ Gal–β1–R Fuc–α1→2 ↗

Gal GalNAc Fuc GlcNAc *: residue could be glucose in case of glycolipids; yellow shade: minimal determinant or core structure; blue arrow: residue added by blood group gene product; examples of type 1 and 2 core structures are illustrated above but they can vary widely, as they can be assembled on at least six possible types of carbohydrate chains; they can reside on a variety of protein or lipid glycan structures containing branches, repeats, etc.

Antigen specificity conditioning:

Antigen A = N-acetylgalactosamine

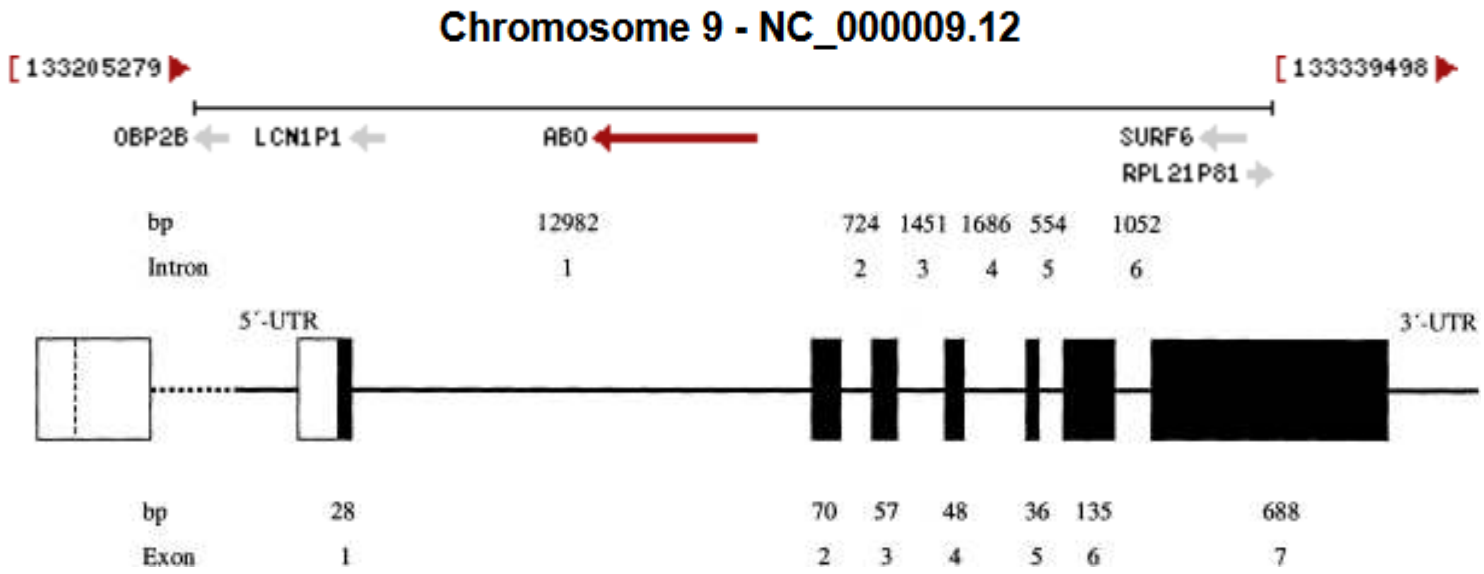
Antigen B = D-galactose

Antigen H = L-fucose

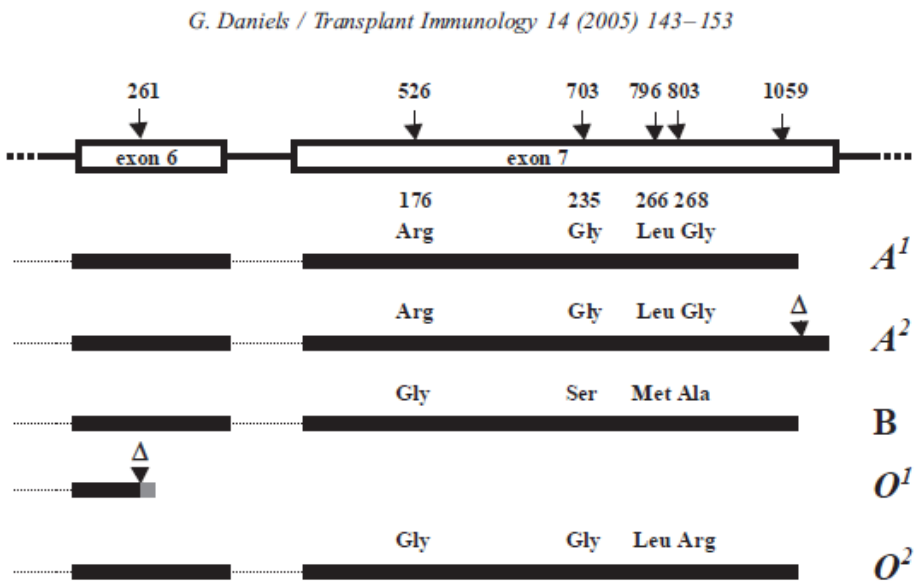
ABO Group System

Gene ABO 9q34.1-q34.2

- 7 exons (28-688 bp, in total 18 kpz), longest 6 and 7
- coding region: 1065 bp = 353 AA, proteins: transferase A, transferase B
- Alleles:
  - A - 110 alleles (9 subgroups), allele 101 is taken as reference
  - B - 46 alleles (6 subgroups)
  - 0 - 62 alleles (most frequent del within exon 6)



ABO Group System



Transferase A vs Transferase B::
7 SNP- 6 (1) and 7 (6) exon, while two substitutions determine substrate specificity (L266M i G268A)

Allele 0
01 - Deletion in codon 117, frameshift, STOP codon
02 – G268A = lack of activity in transferases A and B

```
<< <C> >>
Codon Nr.          87 //  99 // 176 // 219 // 235 // 266 // 268 // 310
ABO*A1.01.01.1 Val // Thr // Arg // His // Gly // Leu // Gly // Leu
ABO*B1.01.01.1 --- // --- // Gly // --- // Ser // Met // Ala // ---
ABO*O.01.01.1  --- // His // ... // ... // ... // ... // ... // ...
<< <C> >>
Codon Nr.          87 //  99 // 176 // 219 // 235 // 266 // 268 // 310
ABO*A1.01.01.1 GTG // ACA // CGC // CAC // GGC // CTG // GGG // CTG
ABO*B1.01.01.1 --- // --G // G-- // --T // A-- // A-- // -C- // --A
ABO*O.01.01.1  --. // --- // --- // --- // --- // --- // --- // ---
```

ABO Group System

Amorphic allele 0 is recessive to A and B

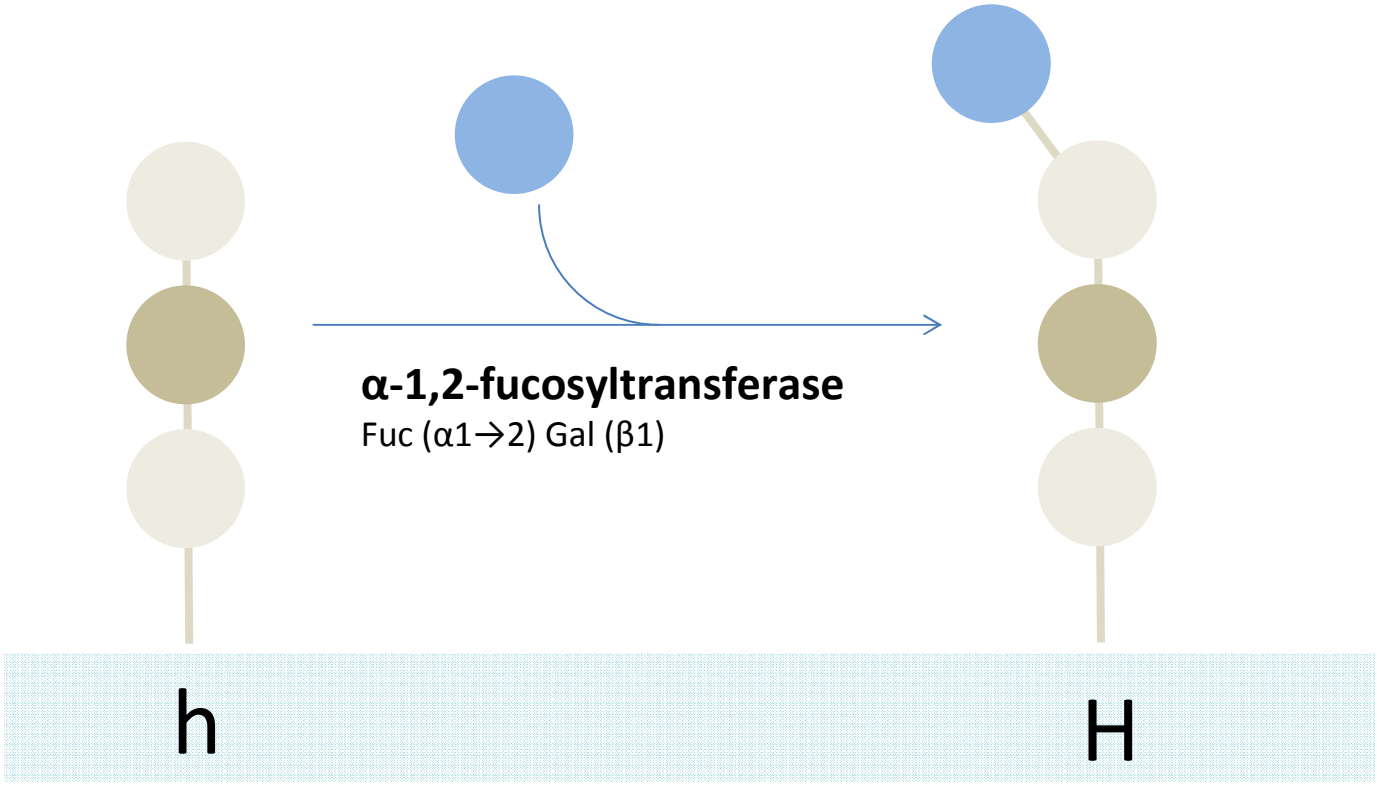
Co-dominance of alleles A and B

ABO genotype in the offspring		ABO alleles inherited from the mother		
		A	B	O
ABO alleles inherited from the father	A	A	AB	A
	B	AB	B	B
	O	A	B	O

H Group System

No. of antigens	1 (antigen H)
Nature of antigen	oligosaccharide
Carrier molecule	glycoprotein, glycolipid
Genes	FUT1 (H) α-1,2-fucosyltransferase FUT2 (Se) α-1,2-fucosyltransferase (Sec1 – pseudogene located 5' of FUT2 within a 30 kb region; homologous to FUT1 and FUT2)

H Group System



D-galactose

N-acetylgalactosamine

L-fucose

N-acetylgalactosamine

D-galactose

H Group System

19q13.3 FUT1 and FUT2

70% homology, 35 kbp distance, LD

FUT1	FUT2
H (h)	Se (se)
RBC Erythoid tissues, vascular endothelium and primary sensory neurons of PNS	Exocrine secretors Epithelia, saliva
fucosyltransferase1	fucosyltransferase2
8 exons (365 AA)	2 exons (343 AA)

Allele se – Trp143Ter

H Group System

Frequent genotypes

	Secretors	Nonsecretors
H antigen on RBC	present	present
H antigen in saliva	present	absent
Ig anti-H	absent	absent
Genotype	H/H or H/h Se/Se or Se/se	H/H or H/h se/se

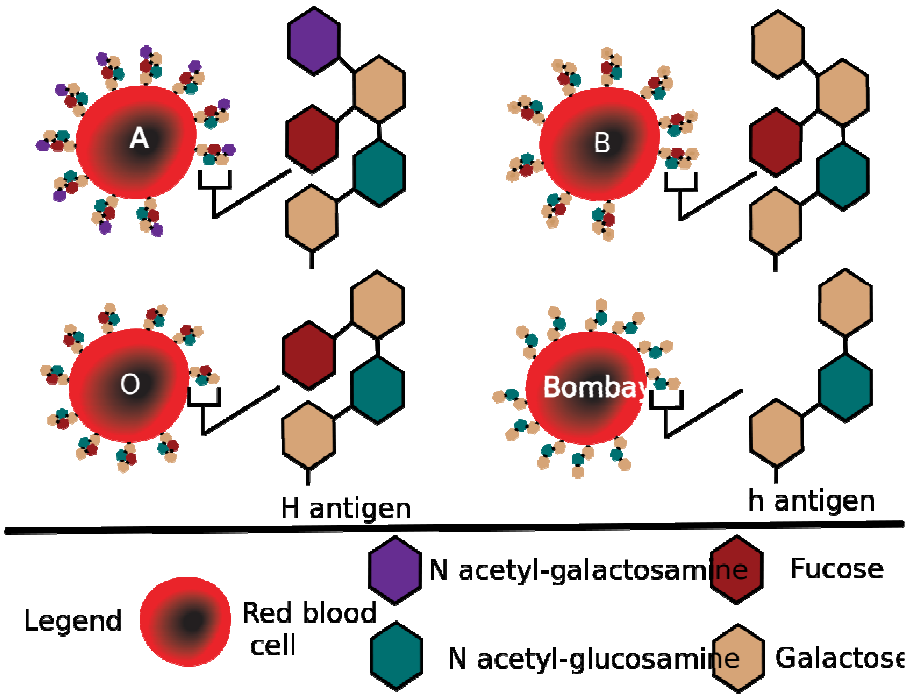
H Group System

Bombay blood group (h/h blood group or O_h or ABH_{null})

– 0 blood type of AB0 system in individuals with ABO gene functional alleles

- Classical Bombay phenotype FUT1 Tyr316Ter (hh)
  - India 1:10 000
  - Taiwan 1:8 000
- Caucasians numerous FUT1 non-sens mutations
  - Europe 1:1 000 000

H Group System



Apparent group 0.
Despite the existence of A and B group genes, the blood in contact with calibration sera is not agglutinating, but other than usually found in the plasma of blood group 0 anti-A and anti-B, in Bombay phenotype there are also anti-H.

H Group System

Rare genotypes

	Bombay	para-Bombay
H antigen on RBC	absent	absent
H antigen in saliva	absent	present
Ig anti-H	present	present
Genotype	h/h se/se	h/h Se/Se or Se/se

Rh Group System

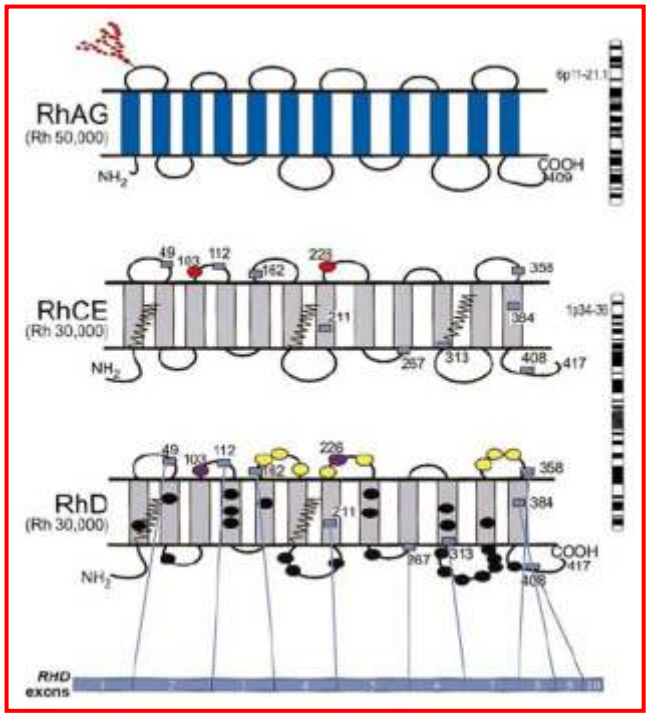
No. of antigens	49, including D, C, E, c, e
Nature of antigen	Protein
Carrier molecule	Protein with unknown function
Genes	RHD (D) RHCE (C, c, E, e)
Frequencies of antigens	D: 85% Caucasians, 92% Blacks, 99% Asians C: 68% Caucasians, 27% Blacks, 93% Asians E: 29% Caucasians, 22% Blacks, 39% Asians c: 80% Caucasians, 96% Blacks, 47% Asians e: 98% Caucasians, 98% Blacks, 96% Asians
Frequencies of phenotypes	Rh haplotype DCe: most common in Caucasians (42%), Native Americans (44%), and Asians (70%) Rh haplotype Dce: most common in Blacks (44%) Rh D-negative phenotype: most common in Caucasians (15%), less common in Blacks (8%), and rare in Asians (1%)
Expression	Erythroid tissues

Rh Group System

- Membrane proteins (12-transmembrane domains),
- 35 aa difference between antigen D and C/c or E/e
- RhD 30 epitopes
- Epitopes Rh C/c (S103P) and RhE/e (P226A) located in 2 and 4 loop, respectively;
  the rest not relevant

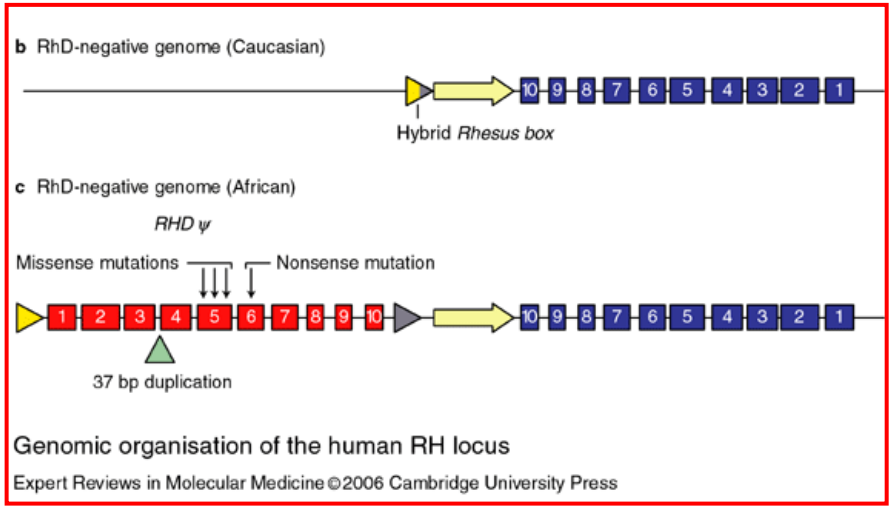
Protein function:

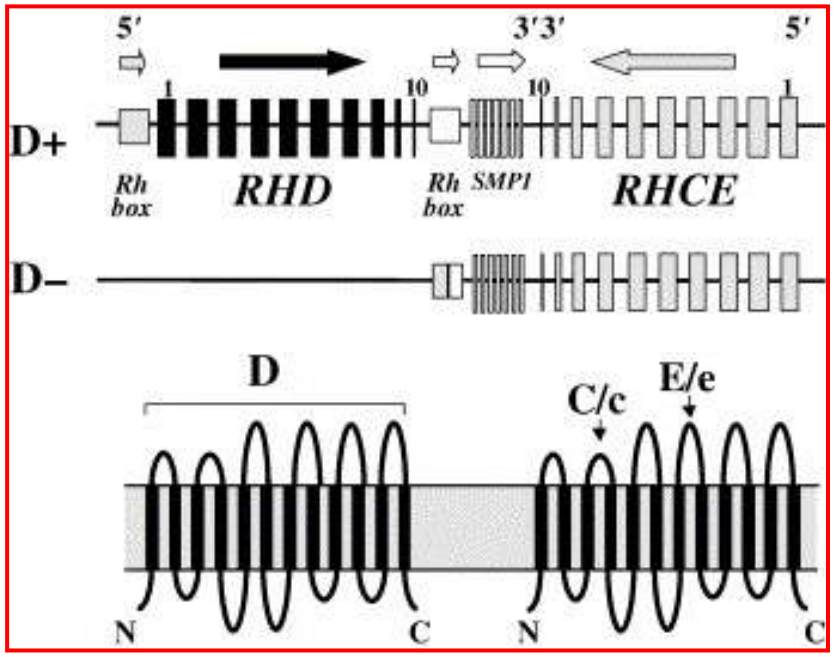
- Maintenance of RBC membrane integrity
- *NH4+ transport*



Rh Group System

- 1p36-p34, 97% homology
- 10 exons, ok. 75 kbp (416 AA)
- *Rhesus box*: 9 kbp, flanking RHD, high homology
- Rhd (dd, Rh-):
  - RHD deletion (Caucasian population)
  - RHDψ pseudogene (African population)

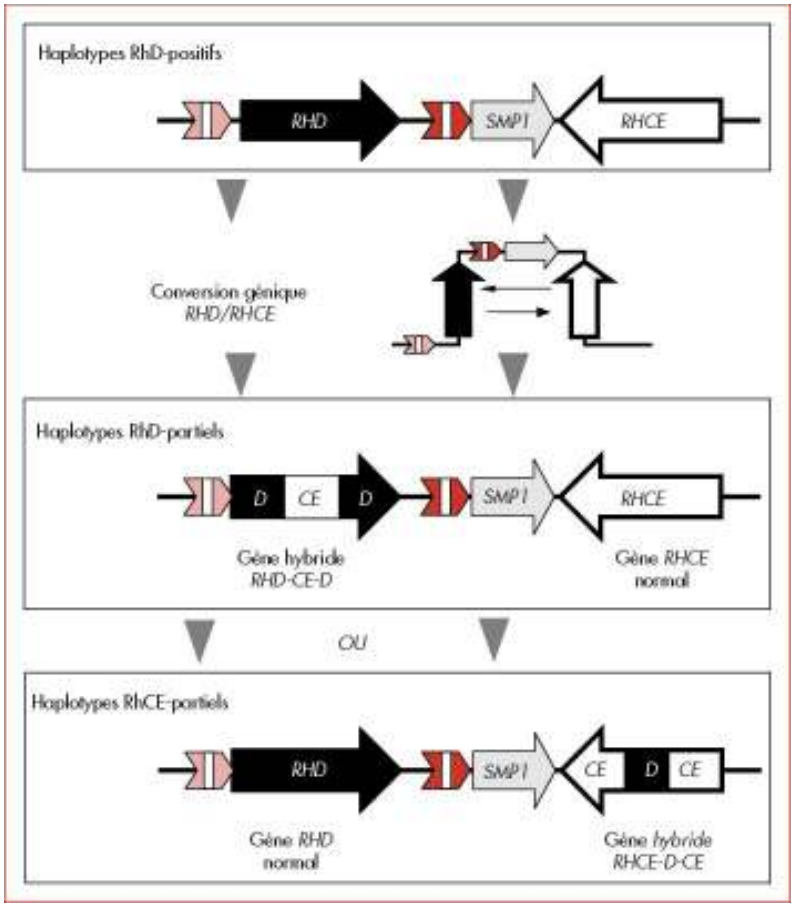


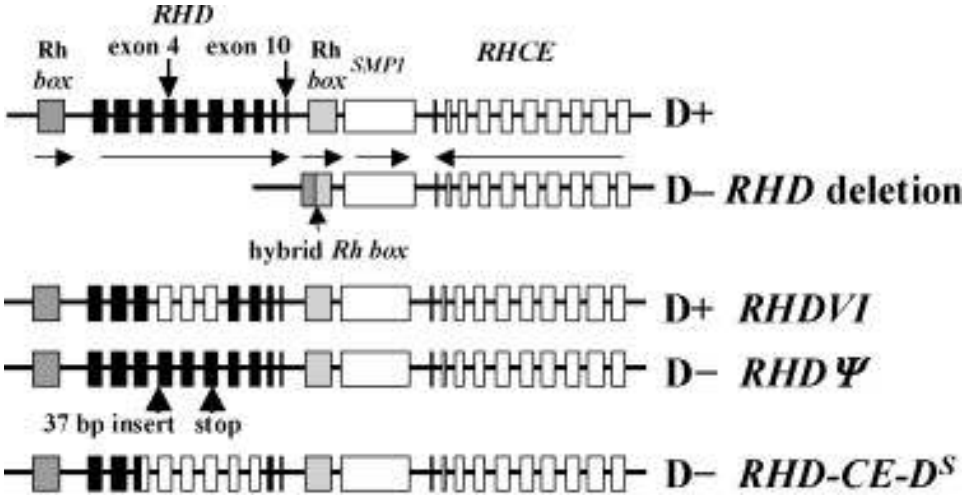


D/d: del RHD (~ 15% of population)
C/c: four nonsynonymous SNPs, while (S103P) determines specificity, 109 bp difference in intron 2
E/e: SNP (676G→C) A226P.

Rh antigens are inherited as haplotypes consisting of 3 alleles

Rh Group System





Rh and RHAG Group Systems

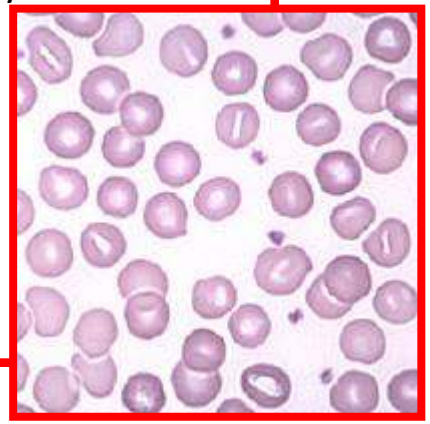
Rh_{null} phenotype

Rh deficiency syndrome

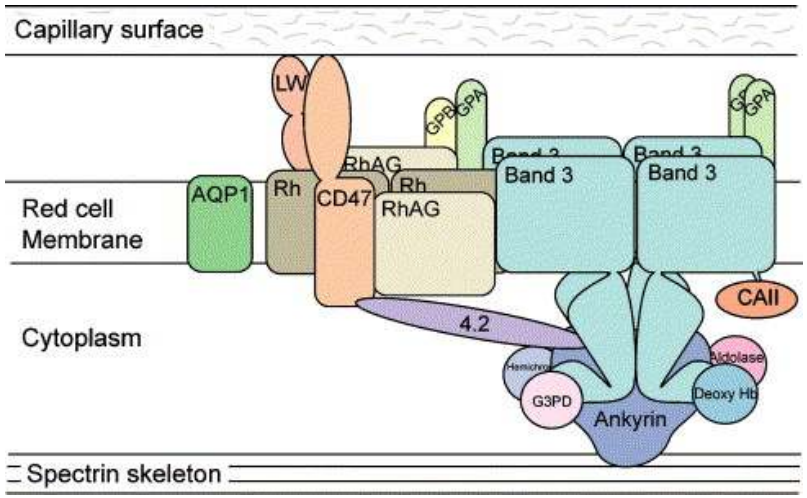
Type *amorph*, *regular*, *mod*

Missense mutations, splicing sites, deletion within exon

- Increased osmosensitivity, shortened RBC life → hemolytic anemia
- Spherocytes, stomatocytes
- Transfuzje – obecność przeciwciał



- RhAG proteins in RBC membrane are necessary for proper location of Rh system antigens
- Glikoproteina związana z Rh wykazuje 35% homologię w sekwencji aminokwasów z białkami układu Rh
- 6p12.3, 10 exons
- No polymorphisms
- It is not Rh antigen (separate RHAG group system)



Rh Group System

D antigen 30 epitopes

Rare Rh phenotypes

- Weak D: all epitopes are present, but in smaller amount
  1% of Caucasian population, a change of one aa causes problems with D integration into the membrane. Transfusiology: no anti-D; as a donor – Rh+, as a recipient – Rh−
- Partial D: lack of some epitopes, but normal expression of present ones
  RHD and RHCE hybryde, difficult indentification, risk of hemolytic disease after blood transfusion

Lewis Group System

No. of antigens	4
Nature of antigen	oligosaccharide
Carrier molecule	glycoprotein, glycolipid
Genes Chr. 19	FUT3 α-1,3-1,4-fucosyltransferase FUT6 (FUT2 (Se) α-1,2-fucosyltransferase)
Notes	lack of expression in erythroid tissues, antigens adsorbed on RBC

	FUT3 (Le/…)	FUT3 (le/le)
FUT2 (Se/…)	Le (a-b+)	Le(a-b-)
del FUT2 (se/se)	Le (a+b-)	Le(a-b-)
FUT2 (385A>T)	Le (a+b+)	Le(a-b-)*

FUT3 encodes transferase attaching fructose to N-acetylgalactosamine with glycosidic bond in configuration 1,4 (Le^a, Le^b) or 1,3 (Le^x, Le^y)

Expression: exocrine epithelial cells, mostly of endodermal origin, digestive track (pancreas, colon). Variable level of Le antigens depends on age, physiological and pathological state.

Kell and XK Group Systems

KEL	
No. of antigens	25
Nature of antigen	protein
Carrier molecule	glycoprotein with catalytic properties enzyme converting endothelin-3
Gene Chr. 7	KEL
Frequency of antigens	~100%: **k, Kp^b**, Ku, **Js^b**, K11, K12, K13, K14, K18, K19, Km, K22, K26, K27 **K** antigen: 9% in Caucasians (2% in Blacks, , up to 25% in Arabs) ~2%: **Kp^a**, U1^a ~0.01%: **Js^a** (0.01% in Caucasians, 20% in Blacks), Kp^c, K23 Others: K17 (~0.3%), rare: K24, VLAN, K16
Frequency of phenotypes	**K-k+** in 91% Caucasians and 98% Blacks **K+k-** in 0.2% Caucasians and is rare in Blacks **K+k+** in 8.8% Caucasians and 2% Blacks **Kp (a-b+)** in 97.7% Caucasians and 100% Blacks **Js (a-b+)** in 100% Caucasians and 80% Blacks

Kell and XK Group Systems

KEL

- 7q33, 19 exons, 21kbp
- high polymorphism

KEL

732 aa, 5 glycosylation sites, one transmembrane domain

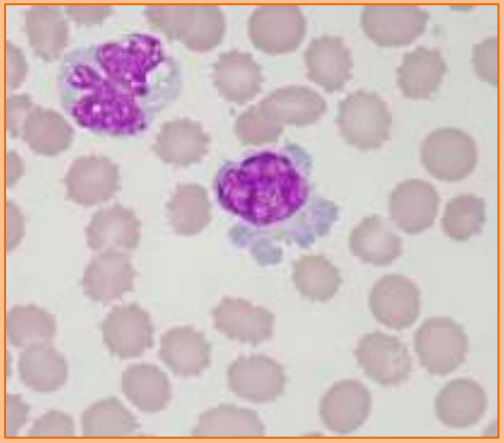
Most frequent antigens of Kell system:

• k	T193M	K*	*lack of glycosylation aa191
• Kp^a	R281W	Kp^b	
• Js^b	L597P	Js^a	

Kell and XK Group Systems

McLeod syndrome (XR)

Kel protein is linked by a single disulfide bond to a RBC integral membrane protein XK (Xp21.1)



Features:

- Onset between 40-50 years of age
- Neuropathy, cardiomyopathy, myopathy, hemolytic anemia, dementia, epileptic seizures
- Acanthocytes (abnormal erythrocytes)

MNS

No. of antigens	43, including M, N, S, s
Nature of antigen	protein
Carrier molecule	glycophorin
Genes Chr. 4	GYPA (co-dominant M and N) GYPB (co-dominant S and s, C and c) GYPE
Frequency of antigens	**M**: 78% Caucasians, 74% Blacks **N**: 72% Caucasians, 75% Blacks **S**: 55% Caucasians, 31% Blacks **s**: 89% Caucasians, 93% Blacks
Frequency of phenotypes	**M+N+S+s+: 24% Caucasians,** 13% Blacks **M+N+S-s+:** 22% Caucasians, **33% Blacks** **M-N+S-s+:** 15% Caucasians, 19% Blacks **M+N-S+s+:** 14% Caucasians, 7% Blacks **M+N-S-s+:** 8% Caucasians, 16% Blacks **M-N+S+s+:** 6% Caucasians, 5% Blacks **M+N-S+s:** 6% Caucasians, 2% Blacks Less common phenotypes are M+N+S+s- (4% Caucasians, 2% Blacks) and M-N+S+s- (1% Caucasians, 2% Blacks).

MNS

4q28.2-q13.1, 97% homology

GYPA: 7 exons, 60 kbp,

MNS1(M) and MNS2(N) Ser1Leu, Gly5Gln

GYPB: 5 exons, 58 kbp

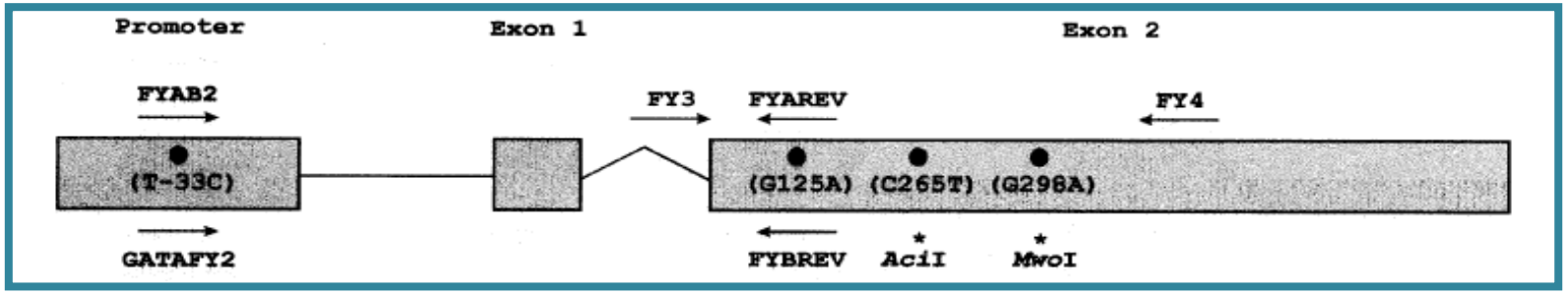
MNS3 (S) and MNS4(s) Met29Thr

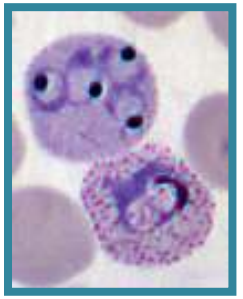
Duffy

No. of antigens	6: Fy^a, Fy^b, Fy3, Fy4, Fy5, Fy6
Nature of antigen	protein
Carrier molecule	glycoprotein, chemokine receptor, receptor for Plasmodium
Genes Chr. 1	FY (Duffy blood group gene)
Frequency of antigens	Fy^a: 66% Caucasians, 10% Blacks, 99% Asians Fy^b: 83% Caucasians, 23% Blacks, 18.5% Asians Fy3: 100% Caucasians, 32% Blacks, 99.9% Asians
Frequency of phenotypes	Fy(a+b+): 49% Caucasians, 1% Blacks, 9% Chinese Fy(a-b+): 34% Caucasians, 22% Blacks, <1% Chinese Fy(a+b-): 17% Caucasians, 9% Blacks, 91% Chinese Fy(a-b-): ~0% Caucasians, 68% of Blacks
Expression	Duffy antigens are expressed on many different types of cells. Even Fy(a-b-) individuals who do not produce Duffy antigens on their RBCs do express Duffy antigens elsewhere, including endothelial cells that line blood vessels, epithelial cells of kidney collecting ducts, lung alveoli, and Purkinje cells of the cerebellum. Duffy antigens are also expressed in the thyroid gland, the colon, and the spleen.

Duffy

- FY, 1q22-q23, exon 155 bp, exon 1038 bp
- Fy (a+), Fy (b+)
  - 2 main alleles FYA i FYB (G125A) encode antigens Fy^a and Fy^b (Gly42Asp)
- Fy(a-b-):
  - FYB^{ES}: -33T→C FYB within GATAbox (antigen present in different tissues!); subsaharian region (and USA) around 70% of population
  - FYAO or FYBO: nonsense mutation in exon (no antigen in different tissues); vary rare
- Fy^x [Fy(b+^{x})]:
  - FYB^{WK} 265C→T (Arg897Cys) linked with 298G→A (Ala100Thr); Cau and Afr ca.2%





Diego

No. of antigens	**21: Di^a, Di^b, and Wr^a**
Nature of antigen	protein
Carrier molecule	glycoprotein: band 3
Gene Chr. 17	**SLC4A1 (**17q21-22)
Frequency of antigens	**Di^b ~100%** **Di^a** 36% South American Indians, 12% Jpn, and 12% Chn, 0,01% Cau i Afr
Frequency of phenotypes	**Di(a-b+) >99.9% Cau iAfr oraz >90% Azjaci** Di(a+b+) <0.1% of Caucasians and Blacks i in 10% Asians Di(a+b-) <0.01% Caucasians, Blacks, i Asians. Di(a-b-) 1 case
Expression	RBC, kidneys

In Poland 6 HDFN cases have been reported

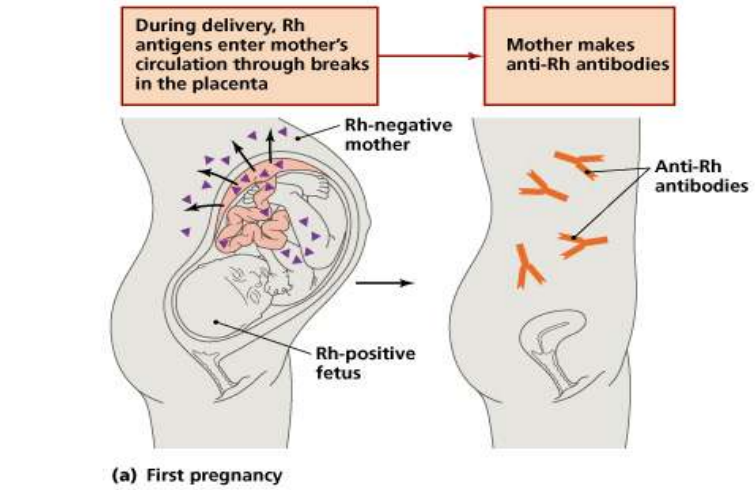
Kidd

No. of antigens	**3: Jk1 (Jk^a), Jk2 (Jk^b) and Jk3**
Nature of antigen	protein
Carrier molecule	glycoprotein - urea transporter
Gene Chr. 18	**SLC14A1** 18q11-q12
Frequency of antigens	**Jk^a**: 77% Caucasians, 92% Blacks, and 73% Asians **Jk^b**: 74% Caucasians, 49% Blacks, and 76% Asians **Jk3**: 100% in most populations, >99% in Polynesians
Frequency of phenotypes	**Jk(a+b+): 50% Caucasians**, 41% Blacks, **49% Asians** **Jk(a+b-):** 26% Caucasians, **51% Blacks**, 23% Asians **Jk(a-b+):** 23% Caucasians, 8% Blacks, 27% Asians **JK(a-b-):** Rare in most populations, found in **0.9% Polynesians**
Expression	RBC, kidneys

Hemolytic disease of the newborn (HDN)

- Most common in RHD and Kell (less frequent in AB0)
- In contrast to Ig anti-rhd, Ig anti-kell apart from hemolysis cause also suppression of erythropoiesis
- Modifying factors:
  - Simultaneous incompatibility in AB0 and Rh systems
  - Isotype of produced IgG
  - Dose of antigen

Hemolytic disease of the newborn (HDN)



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Immunization – producing anti-D (IgM, IgG - pass through the placenta) – HDFN

Symptoms:
-hemolytic anemia
-extramedullary hematopoiesis
-multiorgan damage
-respiratory distress
-hemolytic jaundice

Prophylaxis:
Mother (Rh-) – IgG anti-D

Blood group genotyping

- Genotyping : D, C, c, E (Rh) and K (Kell)
- Advantages:
  - HDFN prevention
  - Limiting prenatal anti-D profilaxis only to RhD- women pregnant with RhD+ child, around 60 %
- Techniques:
  - PCR, sequencing, real-time PCR

Material for genotyping

Invasive	Non-invasive
Chorionic villus sampling (CVS)	(from mother's blood)
Amniocentesis	fetal cells
Percutaneous Umbilical Cord Blood Sampling (PUBS)	fetal free DNA

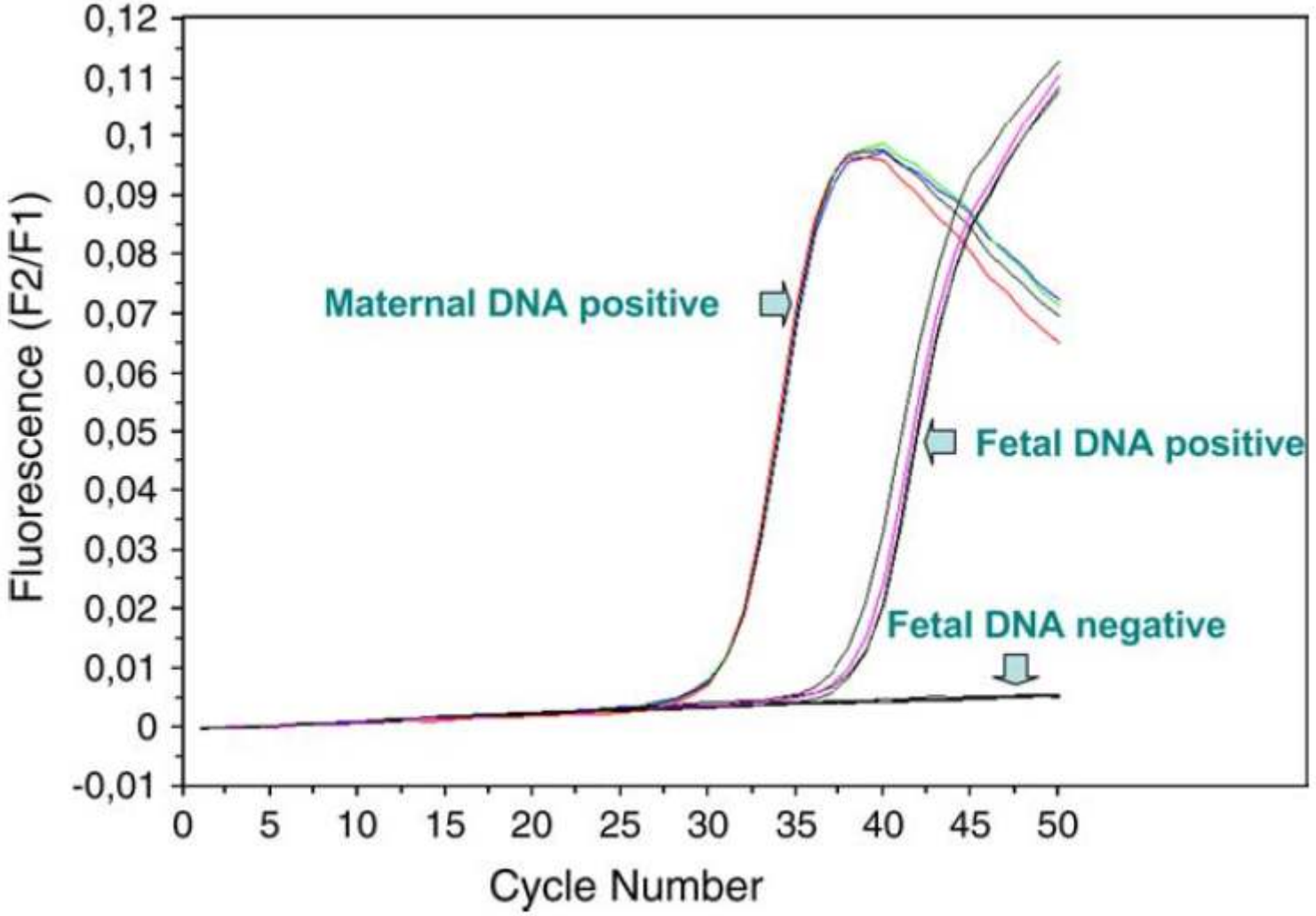
Flaws:

- risk of miscarriage
- risk of mother's immunization
- risk of harming the foetus

Flaws:

- low concentration
- false positive results

Real-time PCR



Non-invasive genotyping

- Prevention from false results:
  - three PCR repeats
  - two independent DNA extractions
- Used controls:
  - positive:
    - of DNA extraction (ALB, GAPDH, CCR5 beta-globina, DNA of corn)
    - of fetal DNA presence (SRY, STR, indel, **met**RASSF1A, **met**SOX14, **met**TBX3
    - PCR
  - negatywne
- Aim of testing: immunoprophylaxis vs risk estimation, costs

Determining RHD zygosity

- Linkage analysis
  Amplification of polymorphic loci linked with RHD deletion
- qPCR
  quantitative PCR , real-time PCR, reference gene
- Rhesus box based amplification

Genotyping and serologic testing of blood types

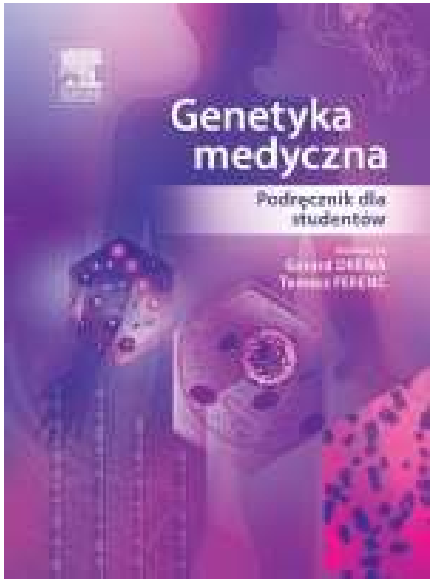
- 0^1 vs A^1 delG261
- 0^2 vs A^1 6 SNPs including G802A,
- 0^3 vs A^1 insG 804
- 0^4 vs A^1 insG88
- 0^5 vs A^1 C322T
- 0^6 vs A^1 G542A

Genotyping and serologic testing of blood types

Indications:

- testing the foetus
- individuals after transfusions
- blood donors for alloimmunized recipients
- donors' screening to identify rare blood types
- determining frequencies of blood groups polymorphisms in population
- determining paternal heterozygosity in foetuses with the risk of HDFN
- genotyping patients with autoimmune hemolytic anemia

Literatura obowiązkowa



Genetyka medyczna

Podręcznik dla studentów medycyny

Gerard Drewa, Tomasz Ferenc (red.)

Wydawnictwo Elsevier Urban & Partner

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