The European Perspective:
Current genotyping in the Czech Republic

MUDr. Martin Písačka

Reference laboratory for immunohematology
ÚHKT Prag

37.Jahreskongress DGTI, 24.September 2004, Mannheim

ÚHKT

„Pre-genotyping“ period

- up to 1996 no genotyping for blood groups was performed in the Czech Republic

- samples with atypical serological reactivity were referred to IBGRL in Bristol
  - contribution to several important publications:
    - molecular background of D VI (Avent, Blood)
    - Fy(null) in caucasians (Mallinson, BJH)
    - new GP(A-B-A) hybrid KI (Hil+,MINY-)

Beginning of genotyping in CR

- in 1996 exon-scanning PCR-SSP designed by Christoph Gassner was introduced in our laboratory in UHKT Prague
- first primer-mixes were obtained from University of Innsbruck
- later this technique was extended to other blood groups (AB0, Kell, Kidd, Duffy, HPA)
- commercialized kit from INNO-TRAIN were used
- more new kits in last years (weak D, D zygosity, MNSs)

Current genotyping in CR

- commercial kits:
  - INNO-TRAIN
  - BAGene („CE“ marked kits)

- 2003 - non-invasive foetal *RHD* and *RHCE* genotyping from maternal plasma was introduced in Faculty Hospital Motol in Prague

- our laboratory is participating in EU project BloodGen (mass genotyping on microarrays)

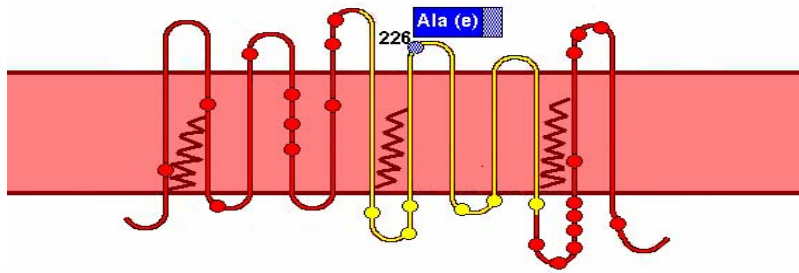
Indications for genotyping in CR

- serological discrepancies in blood grouping
  - weak and variant antigens
  - when serology is nonconclusive (multitransfused patients, DAT positive blood, etc.)
- foeto-maternal incompatibility (HDN, NAITP)
- pregnant women with weak reactions in RhD typing (evaluation whether to use anti-D prophylaxis)

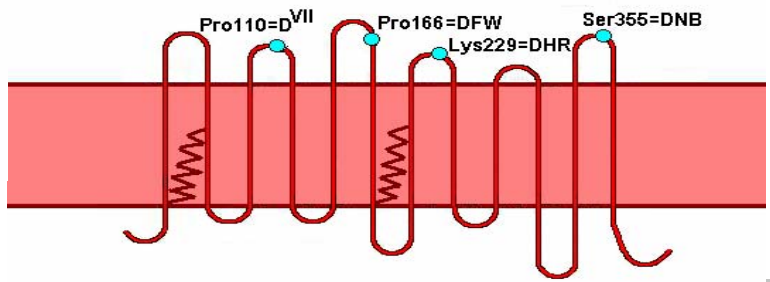
Rh varianty se změnami v *RHD*

D^{II}		**A354D** *1061 C->A*
D^{IIIa}		**N152T...T201R...F223V** *455 A->C...602 C->G...667 T->G*
D^{IIIb}	G-	*RHD-CE(2)-D*
D^{IIIc}		*RHD-CE(3)-D*
D^{III} - IV		**L62F...A137V...N152T** *186 G->T...410 C->T...455 A->C*
D^{IVa} - I	Go^{a} +	**L62F...N152T...D350H** *186 G->T...455 A->C...1048 G->C*
D^{IVb} - II	Evans+	*RHD-CE(7:* **D350H** *-9)-D*
D^{IVb} - III		*RHD-CE(6-9)-D*
D^{IVb} - IV		*RHD-CE(7:* **D350H...A354N** *)-D*
D^{IVb} - V		*RHD-CE(7-9)-D*
D^{Va} - I	D^{W}+	*RHD-CE(5:* **F223V...E233Q** *)-D*
D^{Va} - II	D^{W}+	*RHD-CE(5)-D*
D^{V} - III	Partial E	*RHD-CE(5:* **F223V...226P...V238M** *)-D*
D^{Va} - IV	D^{W}+	**E233Q** *697 G->C*
D^{V} - V		**E233K** *697 G->A*
D^{V} - VI		*RHD-CE(5:* **F223V...V238M** *)-D*
D^{V} - VII		*RHD-CE(5:* **F223V...V245L** *)-D*
D^{VI} - I		*RHD-CE(4-5* **226P** *)-D*
D^{VI} - II	BARC+	*RHD-CE(4-6)-D*
D^{VI} - III	BARC+	*RHD-CE(3-6)-D*
D^{VII}	Tar	**L110P** *329 T->C*
DAR (ARRO-1)		**T201R...F223V...I342T** *602 C->G...667 T->G...1025 T->C*
DBT - I	Rh32+	*RHD-CE(5-7)-D*
DBT - II	Rh32+	*RHD-CE(5-9)-D*
DCS		*RHD-CE(5:* **F223V...226P** *)-D*
DFR - I	FPTT+	*RHD-CE(4:* **M169L...L172F** *)-D*
DFR - II	FPTT+	*RHD-CE(4)-D*
DFW		**H166P** *497 A->C*
DHMi		**T265I** *848 C->T*
DHMii		*RHD-CE(3-5)-D*
DHR		**R229L** *686 G->A*
DIM		**C285Y** *854 G->A*
DMH		**L54P**
DNB		**G355S** *1063 G->A*
DNU		**G353R** *1059 G->A*
DOL		**M170T...F223V**
D+G-		**S103P** *307 T->C*

Varianta D^{VI}-II (hybridní protein RhD-C/ce-D)



Příklady variant s bodovými mutacemi



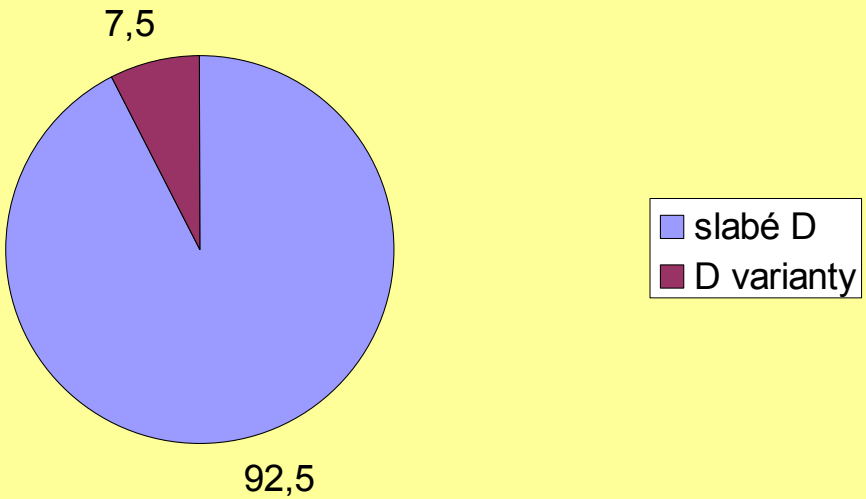
Variant and weak RhD antigens (2000 - 09/2004)

(n=593)

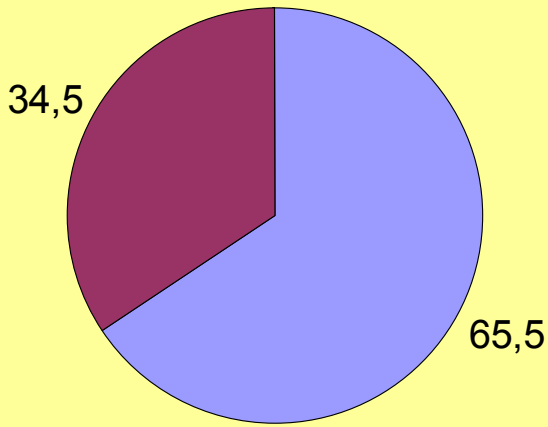
D IIIc	1	*(allo-anti-D)*
D IV type 4	1	
D VI type 1	**10**	
D VI type 2	**5**	
D VII	**7**	(*1x allo-anti-D*)
DFR	**10**	
DCS	**3**	
DNB	1	(*1x allo-anti-D*)
DHMi	1	
DOL	1	
RoHar	1	
DYO	1	
D-“W“(not class.)	1	
Variant D total	**53**	
Weak D	**540**	

Distribution of RhD variants in R1r and R2r phenotypes in Czech Republic

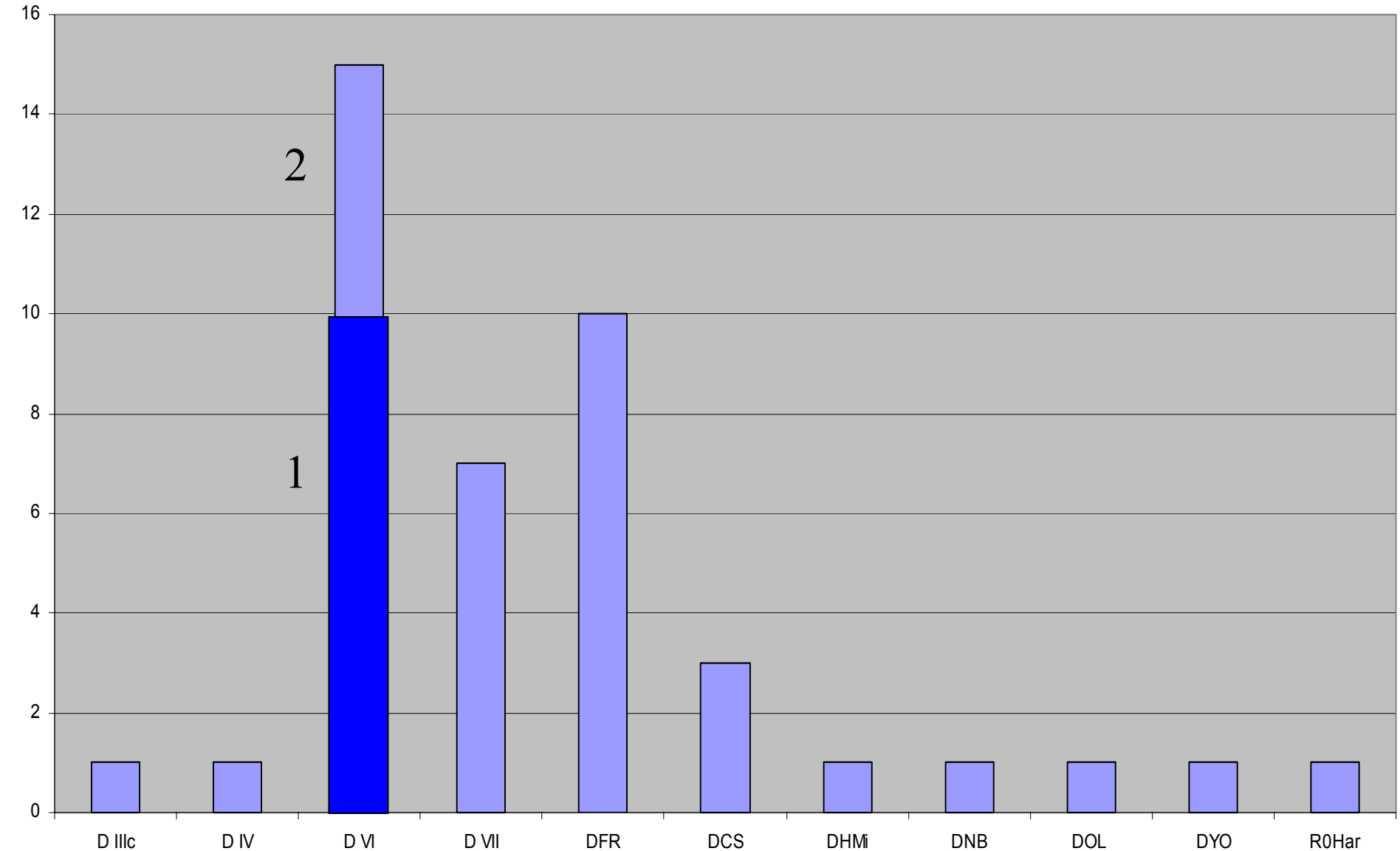
R1r



R2r



RhD variants in CR 2000-2004

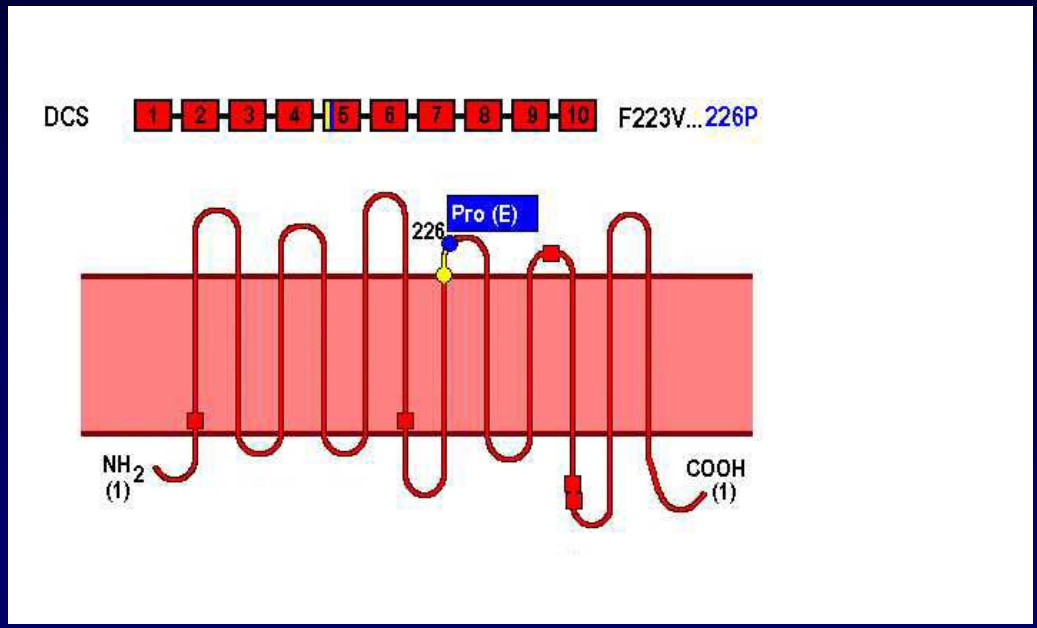


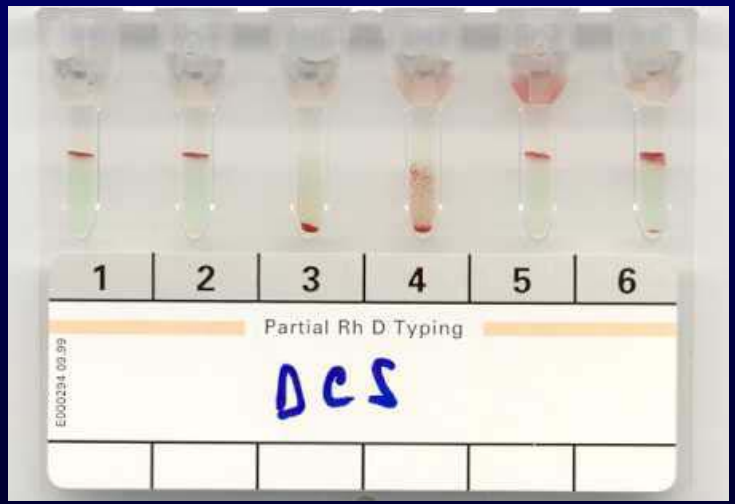
ÚHKT

Partial RhD DCS

- found early after introduction of CDE-SSP PCR
- serologic pattern did not fit into those previously published
- in exon-scanning PCR reaction of exon 5 was missing
- exon 5 was sequenced: sequence differed from *RHD* with two substitutions: T667G and G676C. These nucleotides are typical for the common *RHCE* allele coding for the E+ phenotype
- the nucleotide sequence found in cDNA was confirmed be sequencing the ten *RHD* exons (Dr.Flegel,Dr.Wagner,Ulm). Exon 5 showed T667G and G676C nucleotide substitutions in the common *RHD* nucleotide sequence. The other nine exons were shown to be identical to the common *RHD* allele (deposited in the EMBL/GenBank/DDBJ data bases under the accession number AJ131502).

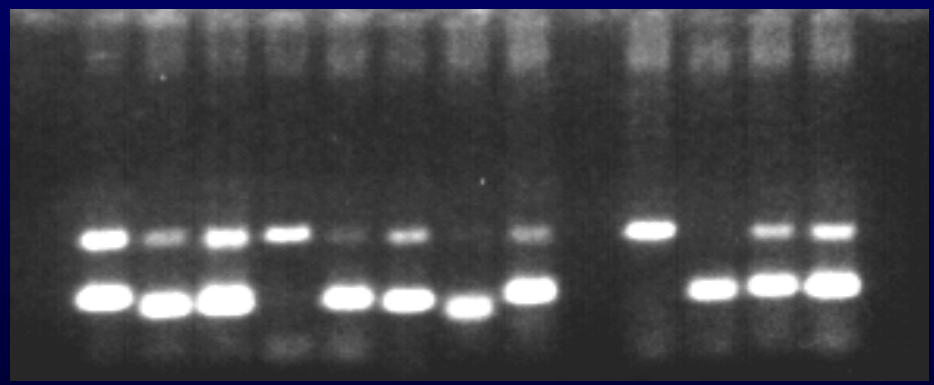
Partial RhD DCS



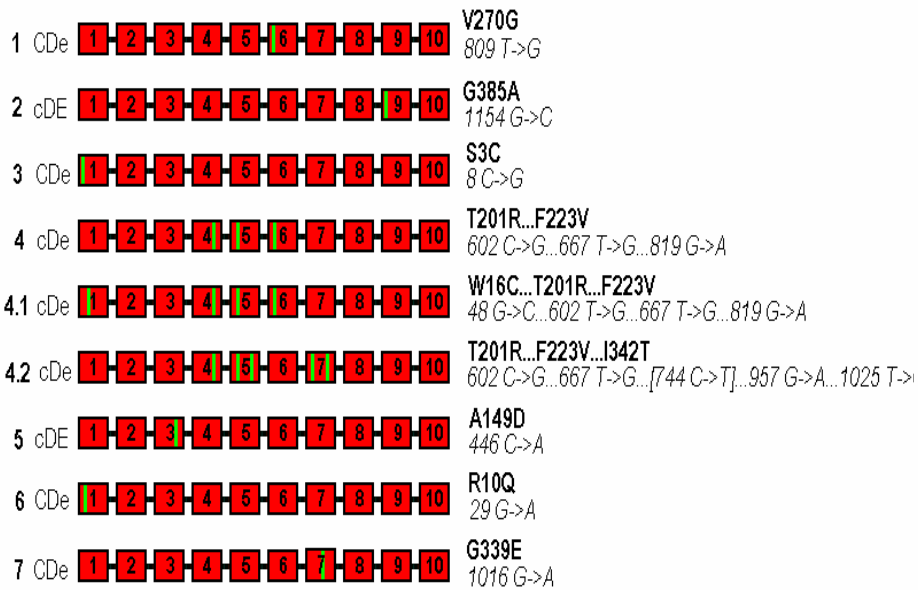


RhDCS

ccD exon 5. negative Ee

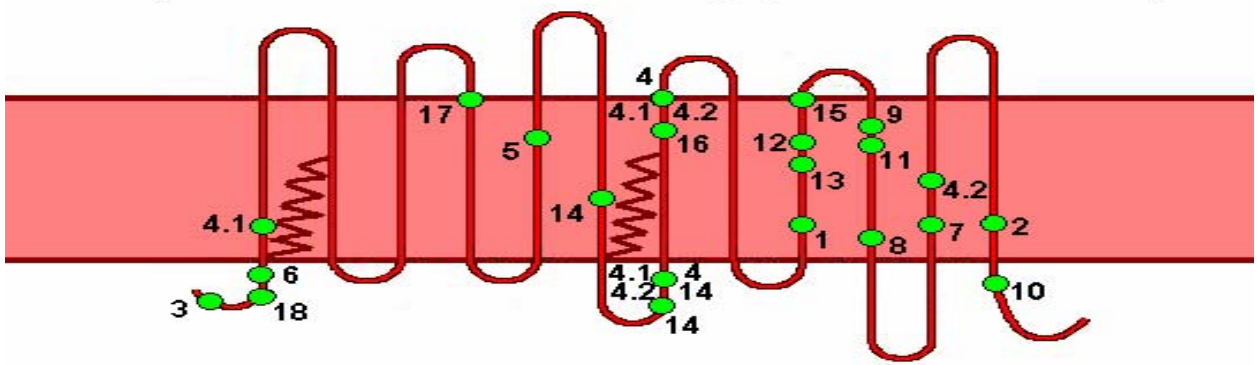


Mutace *RHD* u slabých D antigenů



Rh protein u slabých D antigenů

(mutace v transmembranozní a cytoplasmatické lokalizaci)



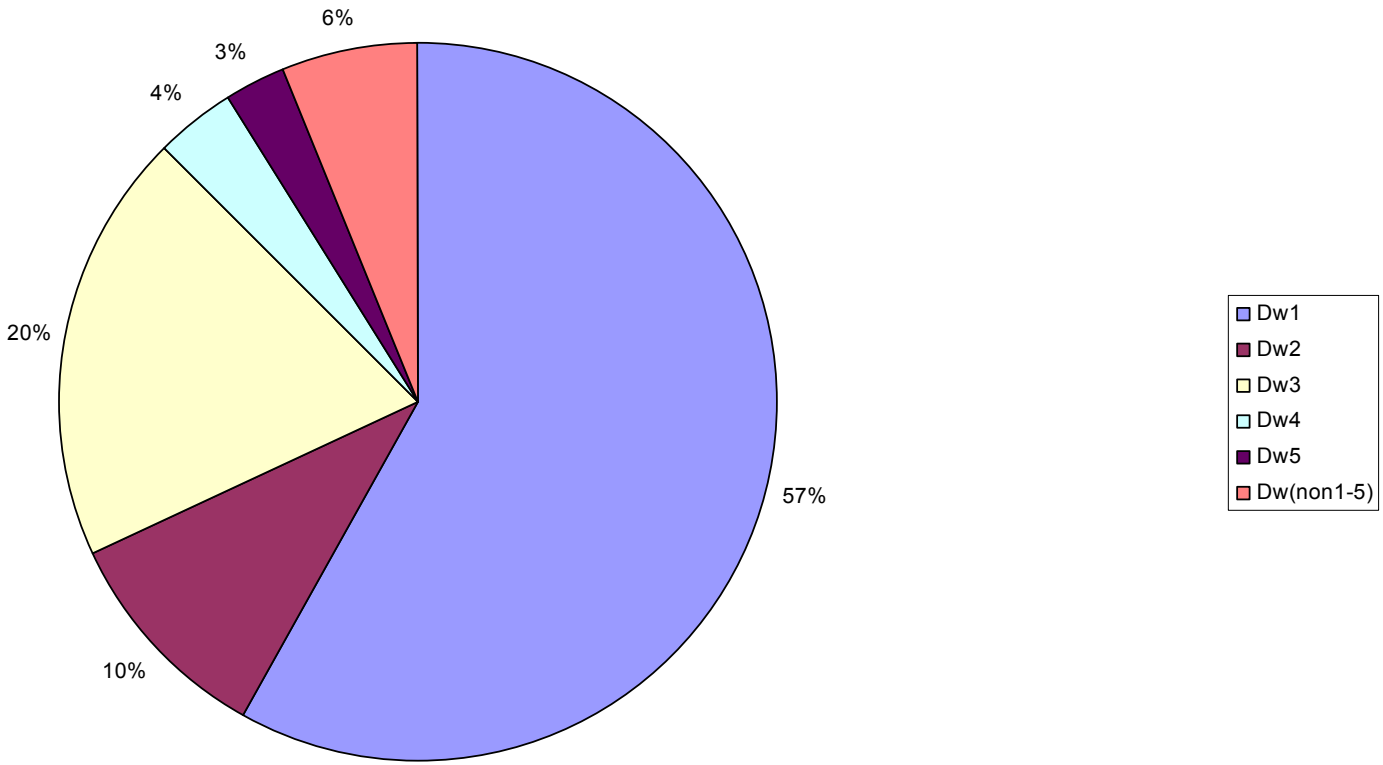
ÚHKT

Weak RhD genotypes (2002 - 09/2004) (n=169)

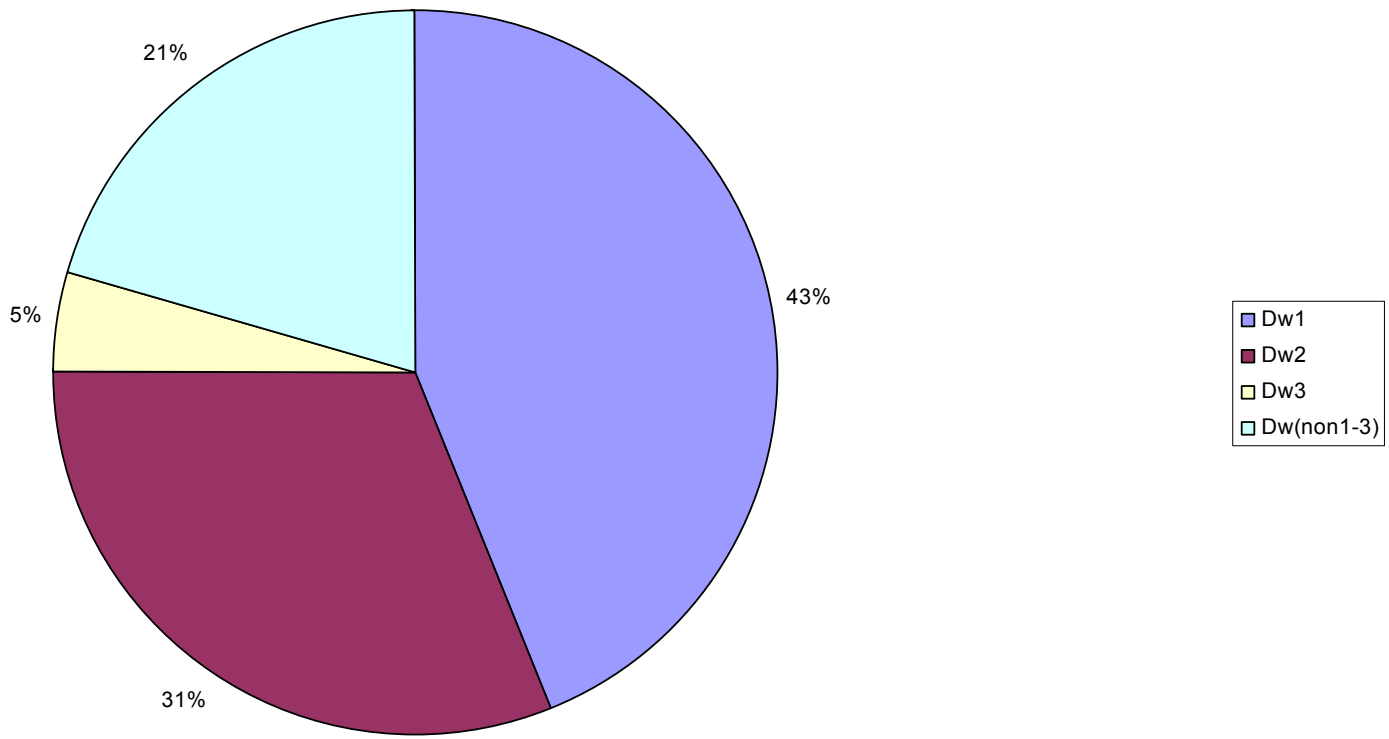
Weak D

type 1	**98**
type 2	**17**
type 3	**33**
type 4	**6**
type 5	**5**
„non 1-5“	**10**

Weak D types in the Czech Republic (n=169)

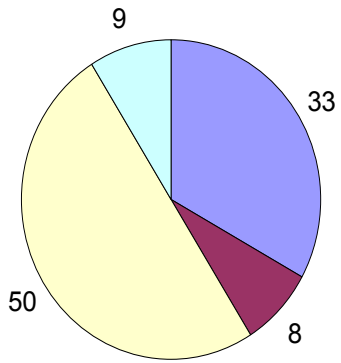


Weak D in France (caucas.) (n=68)

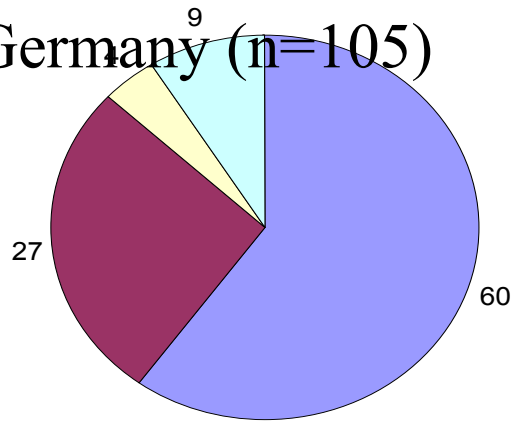


Ansart-Pirenne et al., Transfusion 2004; 44:1282-1286

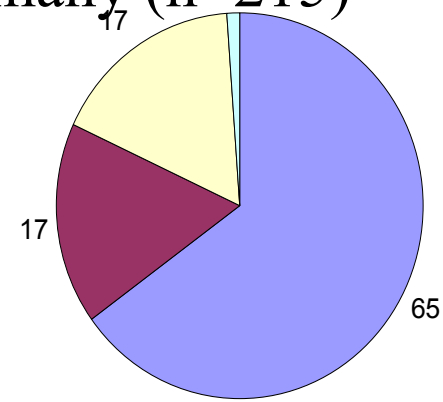
Tyrol (n=109)



S-w Germany (n=105)



N Germany (n=215)



Muller et al., Transfusion 2001;41:45-52

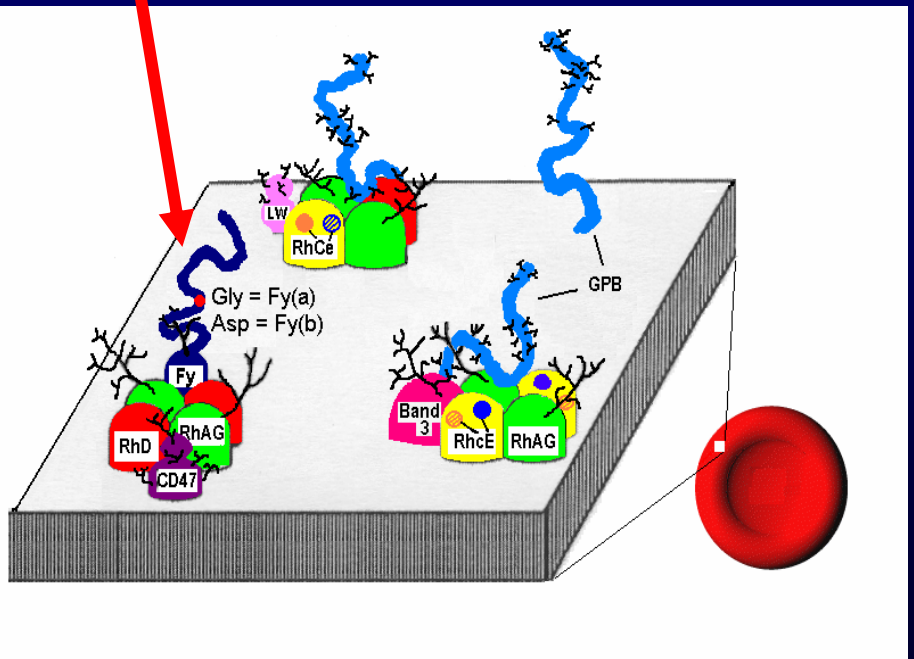
Duffy(null) genotyping in CR

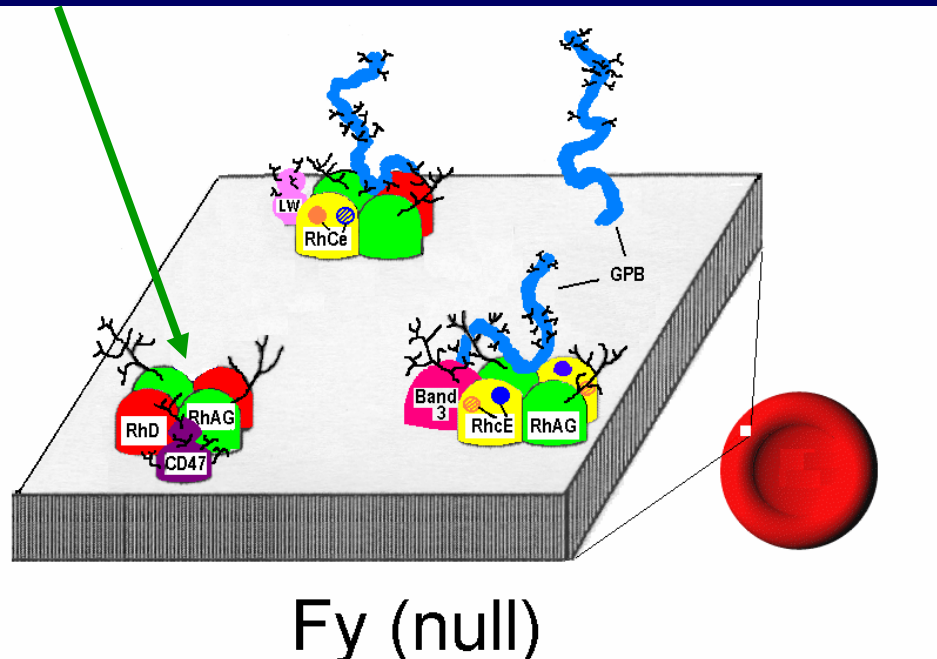
- Fy(a-b-) phenotype is common in individuals of African descent
- high frequency of this phenotype in Africans is related with the resistance to *Plasmodium vivax* infection

- Molecular background: a single-point mutation T-33C in the GATA-1 binding motif for the erythroid promoter of the *FYB* gene causes that the Duffy glycoprotein is absent on red cells while present on other tissues (carriers of this phenotype do not produce anti-Fy(b) nor anti-Fy3

Duffy phenotypes

Fy(a+b+)	47%(c)…1%(b) … 9-28%(a)
Fy(a-b+)	34%(c)…22%(b) … 0,3-3%(a)
Fy(a+b-)	19%(c)…9%(b) … 69-90%(a)
Fy(a-b-)	0%(c) … 68%(b) … 0%(a)





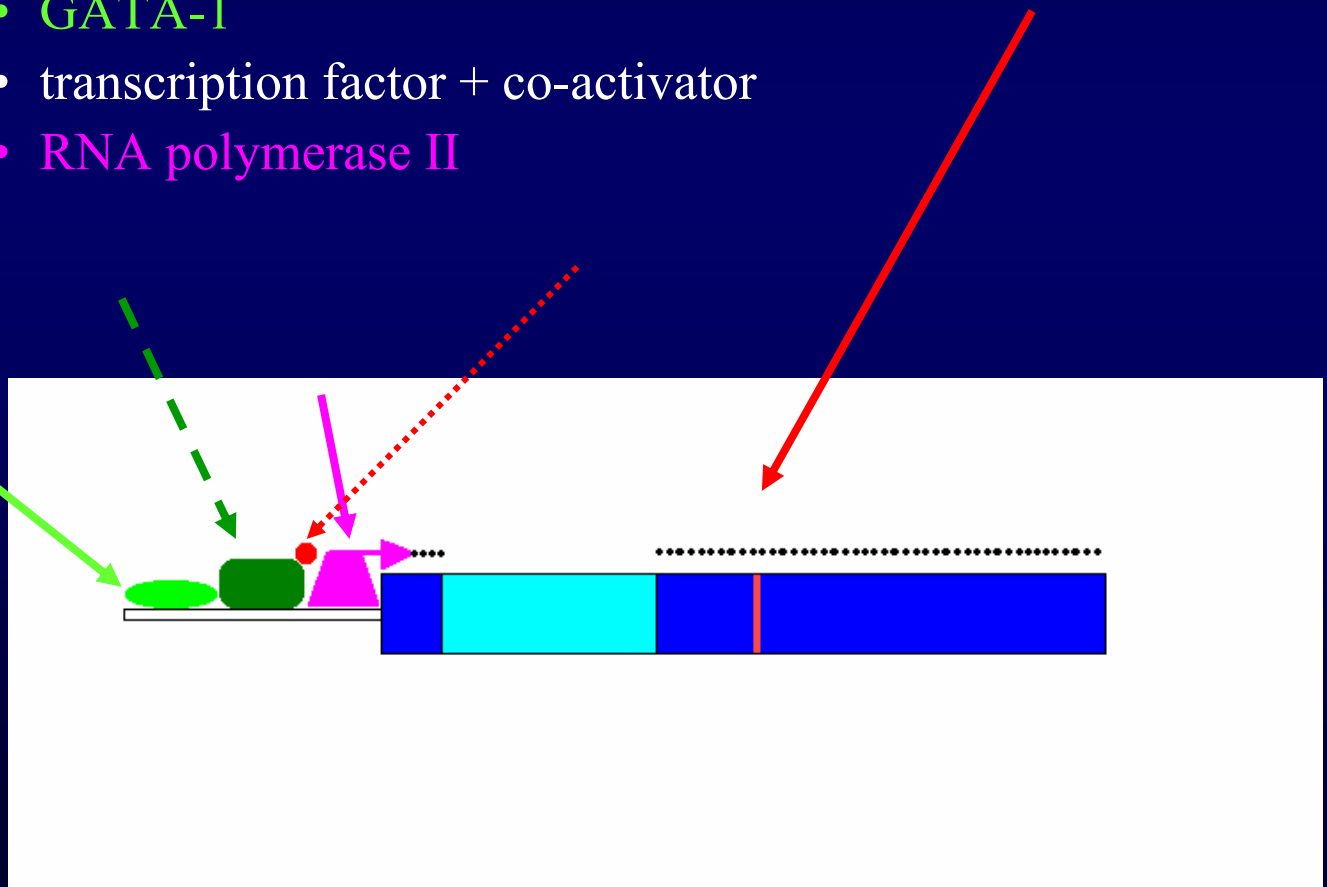
Fy (null)

Duffy gen 1q22-q23

exon 1 (55bp) …intron …exon 2 (1572bp)

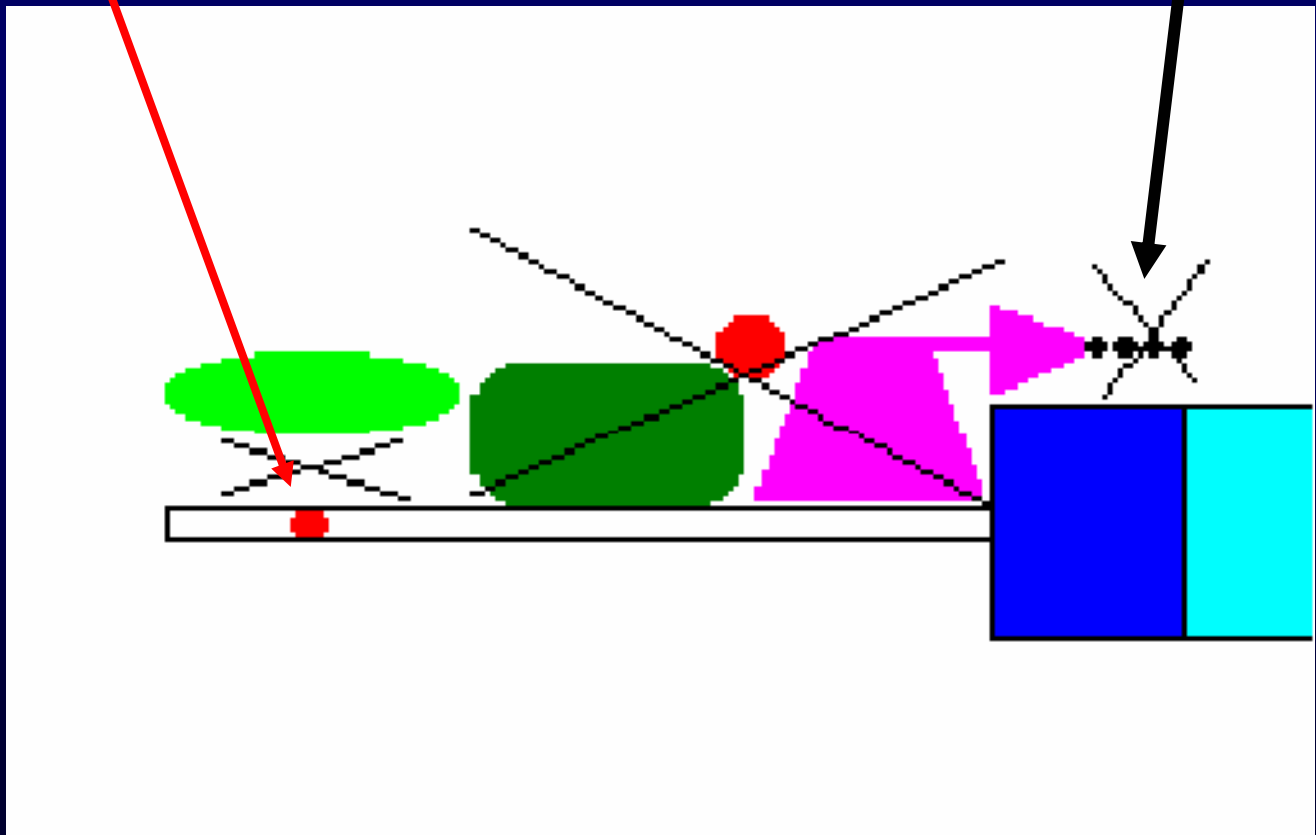
Fy(a/b) polymorphism = G306A

– regulation of erythroid transcription:
  - GATA-1
  - transcription factor + co-activator
  - RNA polymerase II



Duffy-erythroid-silent gen

- mutation T-33C in GATA-1 binding motif - no rbcs-Duffy protein



Duffy(null) in Caucasians

- Fy(a-b-) phenotype is extremely rare in Caucasians and Asians - estimated frequency 1 in a million

- Molecular background:
  - rare individuals have defective *FY* gene with a stop-codon caused by deletion or non-sense mutation - such people can produce anti-Fy3 antibody (a white Australian, Canadian Cree family)
  - GATA-1 box mutation in populations with African admixture (Arabs, Jewish) and in one Caucasian (Swiss and Scottish ancestry)

Duffy(null) in Czech/Slovak Rep.

- Fy(a-b-) phenotype was found in several Czech and Slovak gypsies (Hrubiško et al, Vnitřní lékařství 1976; Libich et al, Vox Sang 1978)
  - *a nation calling themselves Roma, migrated from India in 8th century*
  - *cca 7-9 millions of people dispersed in various european countries, migrating now also to USA and Canada*
  - *less than 1 million of Roma live in Czech and Slovak Republic*
- Molecular background:
  - was not known yet

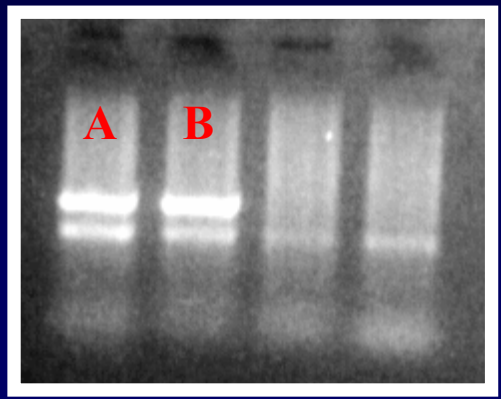
Material and Methods

- Rbcs and genomic DNA from 3 unrelated individuals of gypsy (Roma) ethnic
- Duffy phenotyping - standard serological technique
- PCR-SSP, detecting *FY*A, FY*B, FY*X a FY*null01* alleles - kit KKD (Inno-Train, Germany)

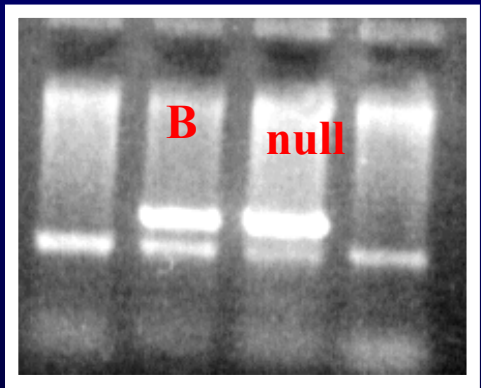
Results (1)

	Phenotype	*Genotype*
I.	Fy(a-b-)	*FY*null01/FY*null01*
II.	Fy(a-b-)	*FY*null01/FY*null01*
III.	Fy(a-b+)	*FY*null01/FY*B*

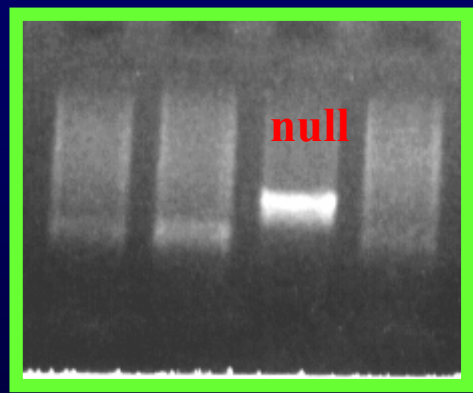
Results (2)



Heterozygote Fy A/B



Heterozygote Fy B/null



Homozygote Fy null

Conclusion

- In the gypsy (Roma) ethnic minority the Fy(a-b-) phenotype is not so infrequent as in other Czech and Slovak populations
- This phenotype is associated with T-33C mutation in the GATA-1 binding motif
- Thus the molecular backgroung of this phenotype is probably the same as in African population
- This fact should be considered in pretransfusion testing (safe use of Fy(b+) blood) and in paternity cases

Other rare genotypes in Czech Republic

- Colton(null) – patient with anti-Co3; sequenced in Ulm
- Kell(null) – two patients with anti-Ku; not sequenced yet
- McLeod phenotype – sequenced in Bristol

Future genotyping in Czech Republic

- PCR-SSP routine for special indications
  - kits „CE“ marked (BAGene) are IVD which will be used in direct human diagnostics (not only for research)

- Mass genotyping using microarrays (BloodGen chip)
  - Potential source of new and more complete informations about random and rare alleles in the Czech population

Thank you for your attention.

ÚHKT