

Frequency of MBL defective haplotypes in Czech population

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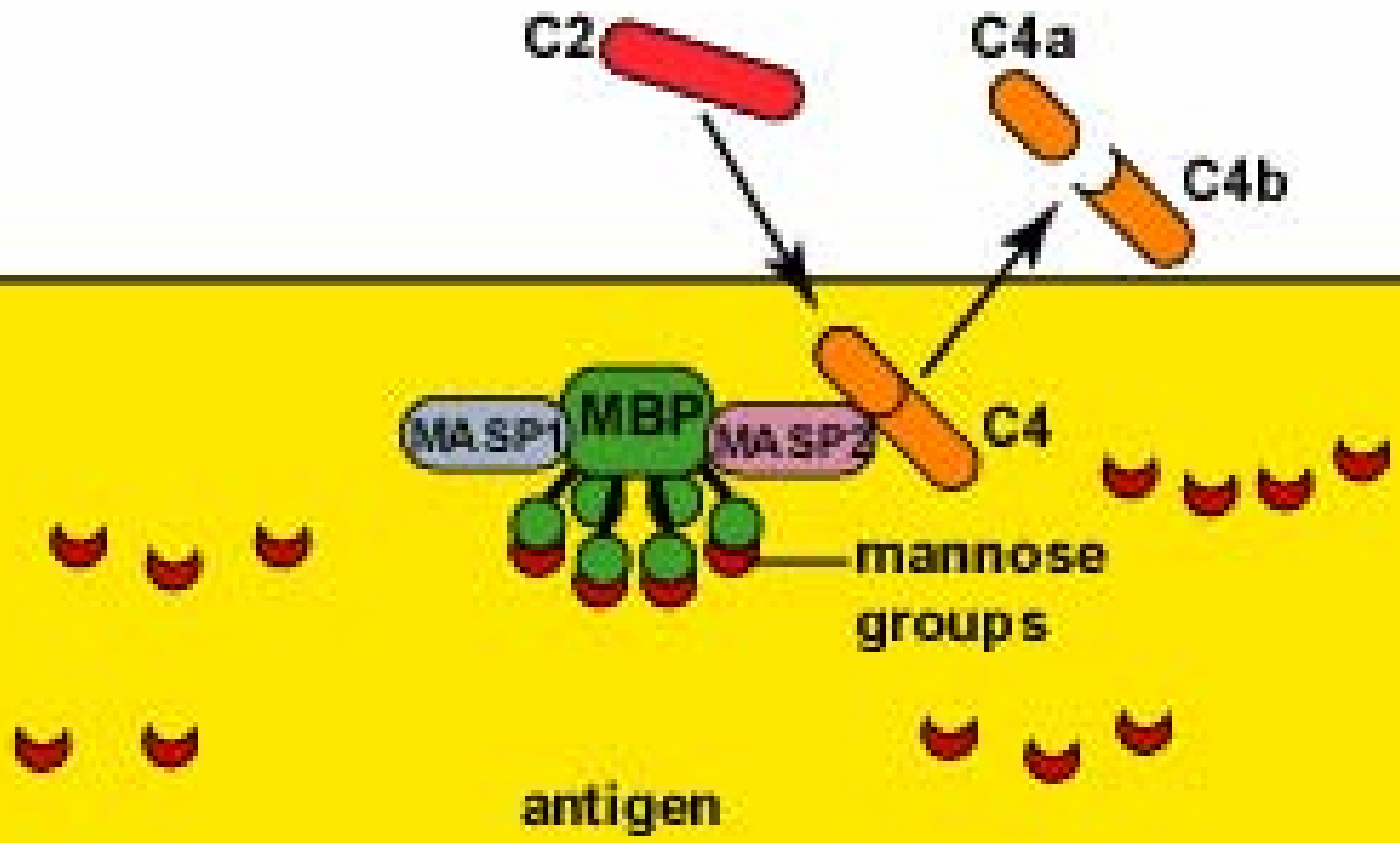
Mannan binding lectin

- ◆ important component of innate immunity
- ◆ binds to polysaccharide structures of bacterial, fungal and viral pathogens
- ◆ significantly higher frequency of more severe and/or recurrent infections in children with MBL deficiency compare to controls

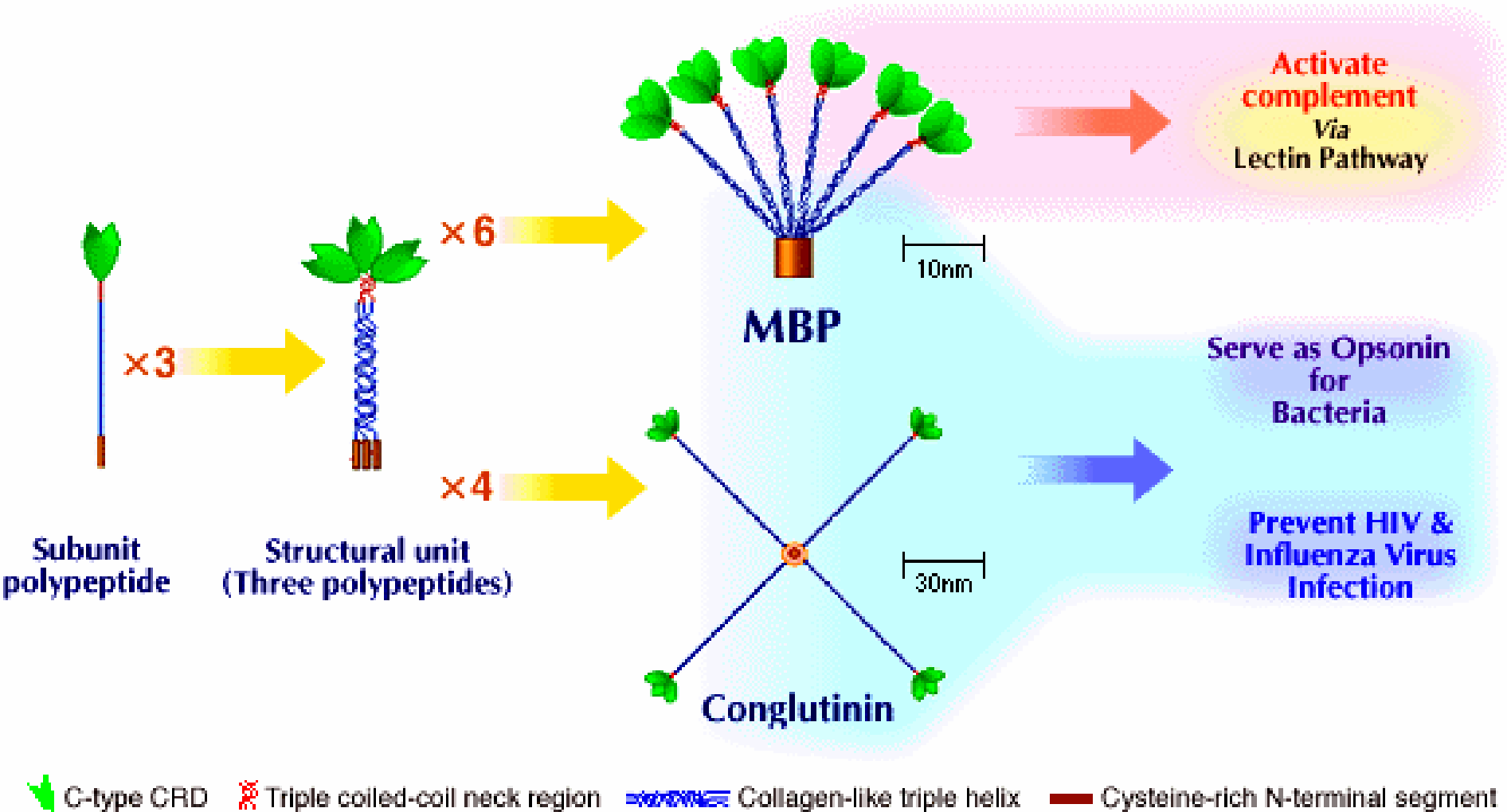
Mannan binding lectin

- ◆ activates lectin (3rd) complement pathway
- ◆ mediates phagocytosis via micro- and macro-pattern recognition

MBL action

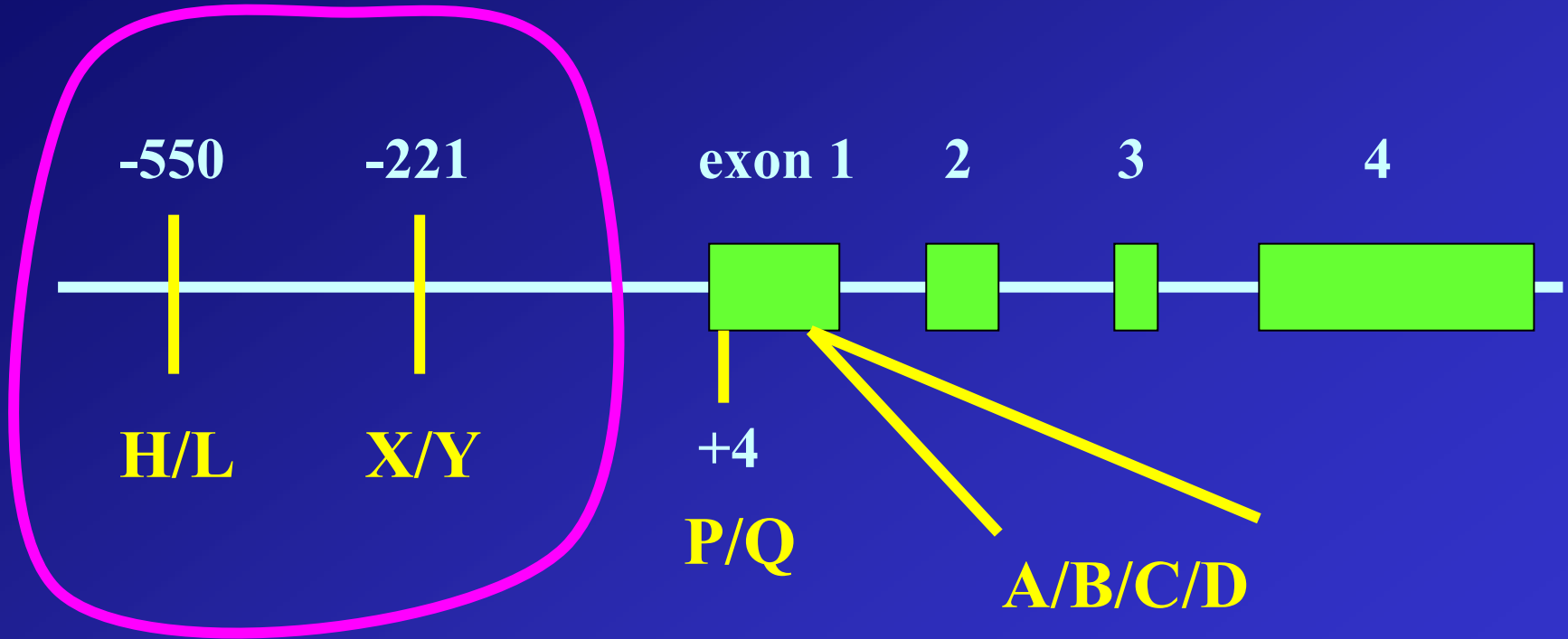


Molecular structures and biological activities of collectins (MBP and conglutinin)



■ G-type CRD
 ⊗ Triple coiled-coil neck region
 ≡≡≡ Collagen-like triple helix
 ■ Cysteine-rich N-terminal segment

PROMOTOR AND 1st EXON MBL POLYMORPHISMS



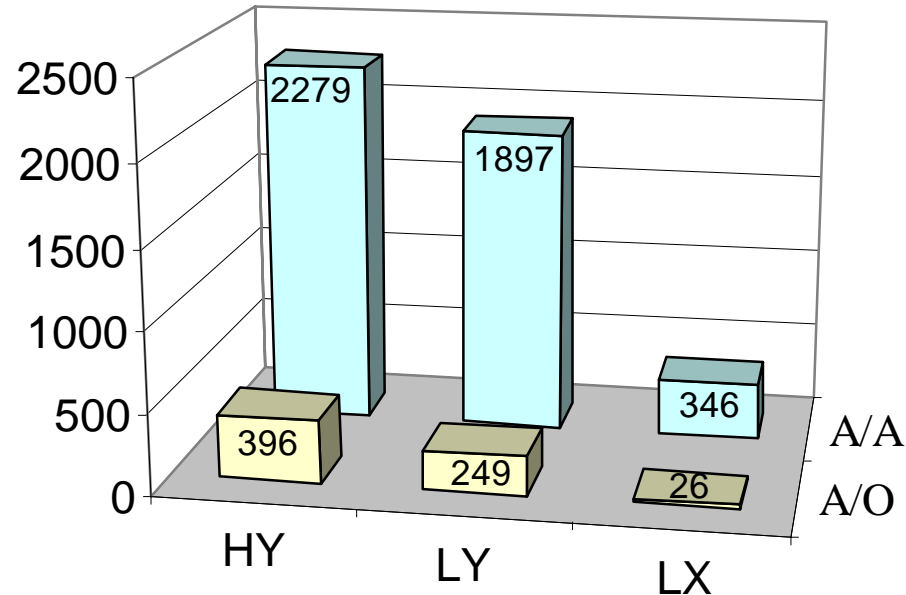
MBL HAPLOTYPES

- ◆ **HYPA**
- ◆ **LYPA, LYQA**
- ◆ **LXPA**
- ◆ **HX. A ... rare**
- ◆ **HYPD**
- ◆ **LY . D ... rare**
- ◆ **LYPB**
- ◆ **LYQC**

**high variability of
serum MBL
concentrations even
among carriers of the
same genotype
(up to 6x)**

MBL plasma concentrations ($\mu\text{g.l}^{-1}$) in relation to MBL genotypes (Caucasian population)

Median of MBL plasma concentrations ($\mu\text{g/l}$)



A/A homozygotes for A allele and particular promoter variant

A/O heterozygotes carrying A allele together with the defective one (B, C, or D),
(only promoter variants on A allele considered)

Mannan binding lectin

◆ low plasma concentrations

- ☒ infections
- ☒ autoimmune diseases (SLE, RA)
- ☒ cystic fibrosis, CVID, recurrent miscarriages, clinical manifestation of atherosclerosis
- ☒ malignancies

◆ high plasma concentrations

- ☒ potentiate a pathogenicity of intracellular microorganisms (facilitate their entry to the cell) (mycobacterium)

MBL

- ◆ Wallis R, Cheng JY: **Molecular defects in variant forms of mannose-binding protein associated with immunodeficiency.**
J Immunol., 1999.
- ◆ Neth O, Hann I, Turner MW, Klein NJ: **Deficiency of mannose-binding lectin and burden of infection in children with malignancy: a prospective study.**
Lancet, 2001.
- ◆ Mullighan CG, Marshall SE, Welsch KI: **Mannose-binding lectin polymorphisms are associated with early age of disease onset and autoimmunity in CVID.**
Scand J Immunol, 2000.

MBL

- ◆ **Mullighan CG et al.: MBL gene polymorphisms are associated with major infection following allogeneic HSCT.**

Blood, 2002.

- ◆ **Peterslund NA et al.: Association between deficiency of MBL and severe infections after chemotherapy.**

Lancet. 2001.

- ◆ **Schmiegelow K et al.: Increased frequency of MBL insufficiency among children with ALL.**

Blood, 2002.

Methods of MBL genotyping

- ◆ PCR – RFLP
- ◆ SSOP
- ◆ ARMS
- ◆ ARMS + SSOP
- ◆ RQ-PCR

MBL promotor genotyping – double ARMS

———— L spec.

———— H spec.

-550



H/L

-221



X/Y

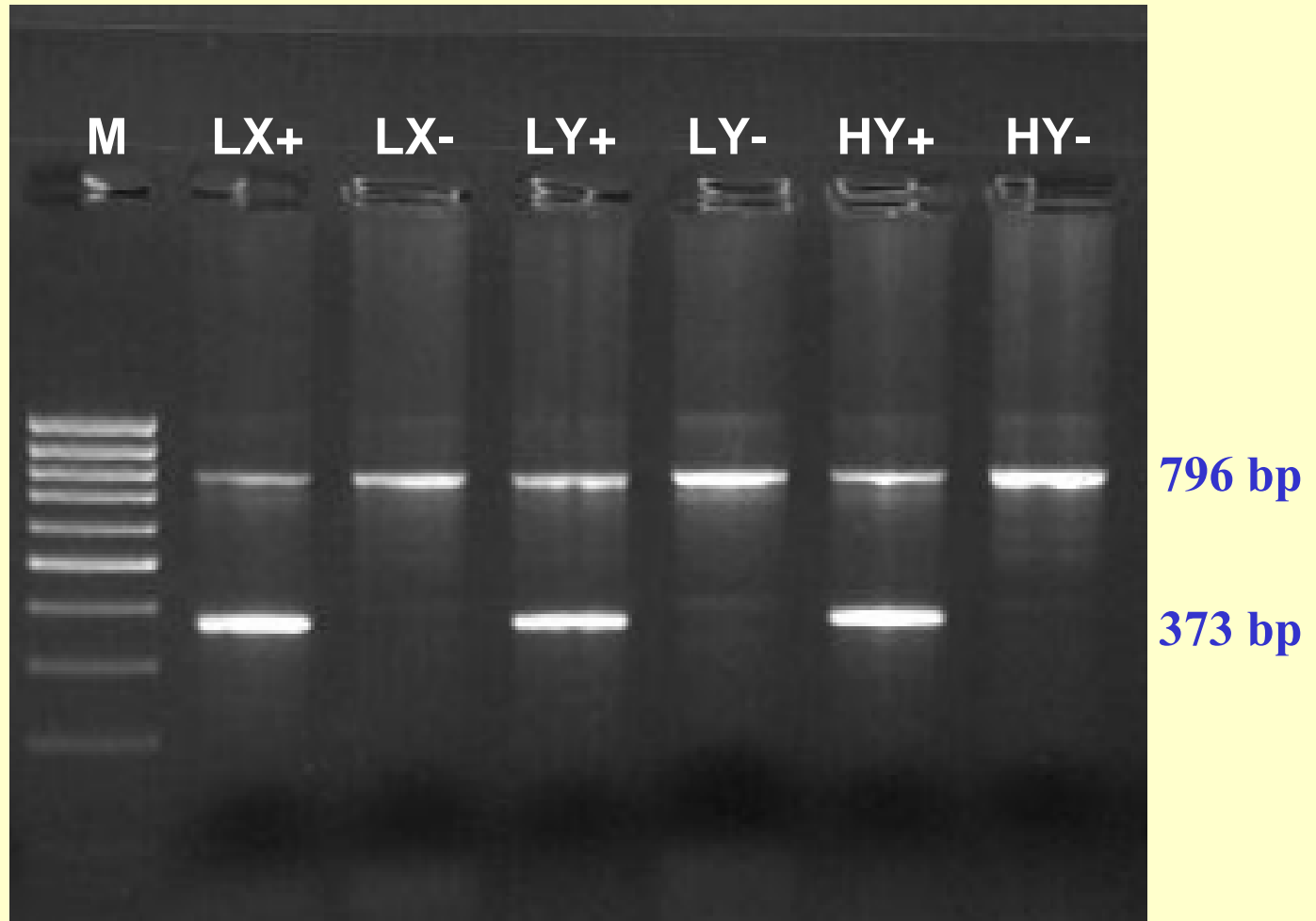
X spec. —————

Y spec. —————

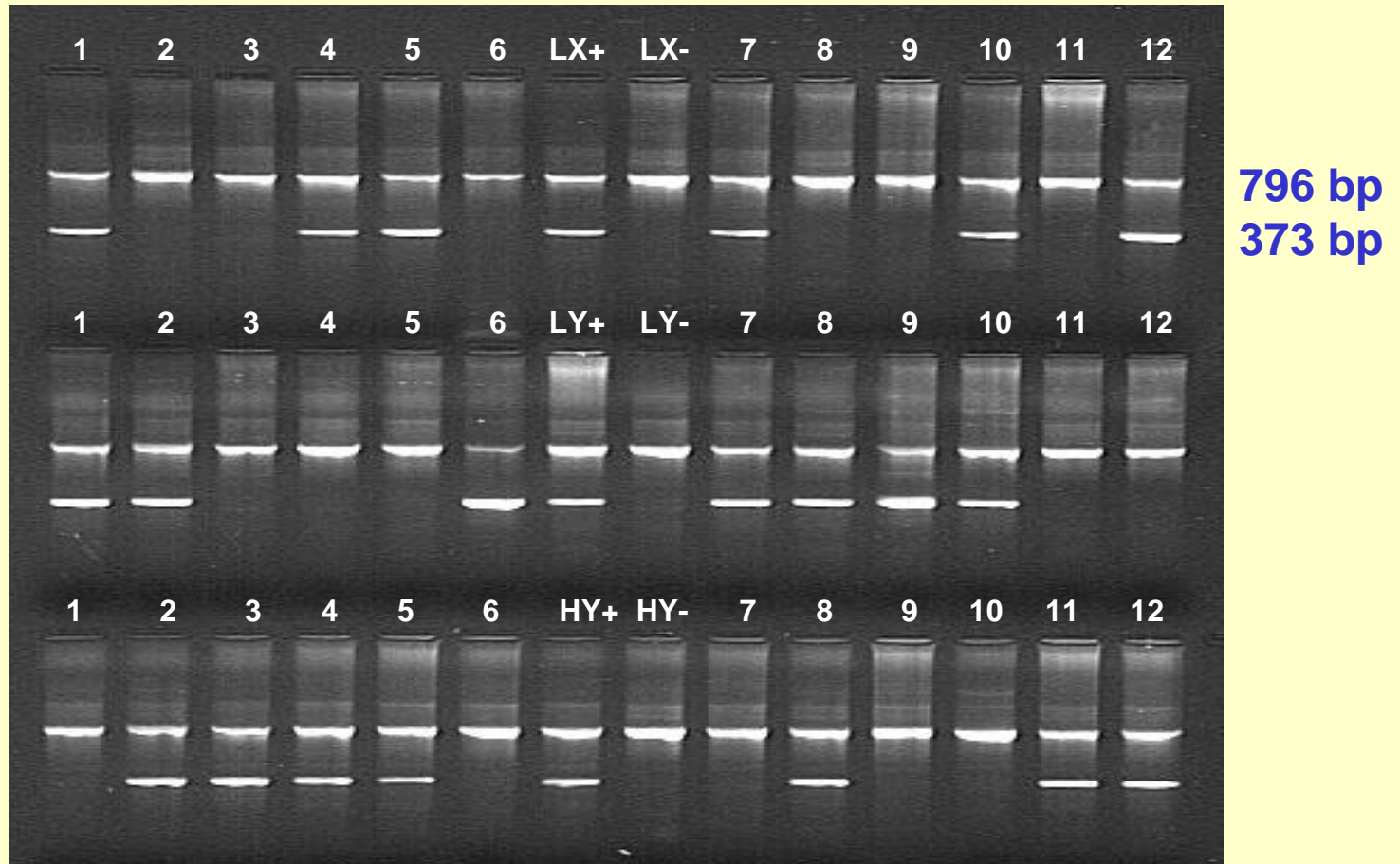
L + X; L + Y; H + Y

373 bp

Promotor genotyping (I)



Promotor genotyping (II)



MBL 1st exon genotyping

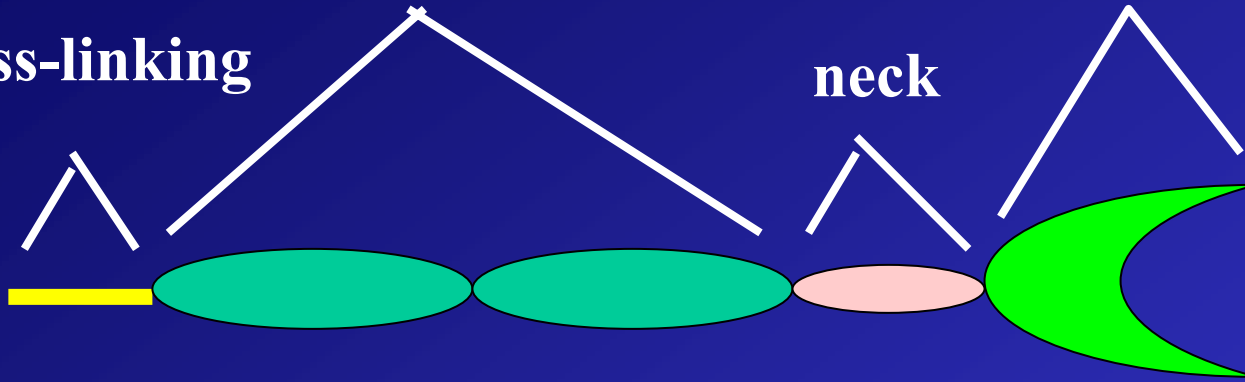
- ◆ multiplex PCR
- ◆ 1 + 1 reactions

collagen-like

carbohydrate recognition

cross-linking

neck



GINGFPGKDGRDGTKGEKGEPGOGLR

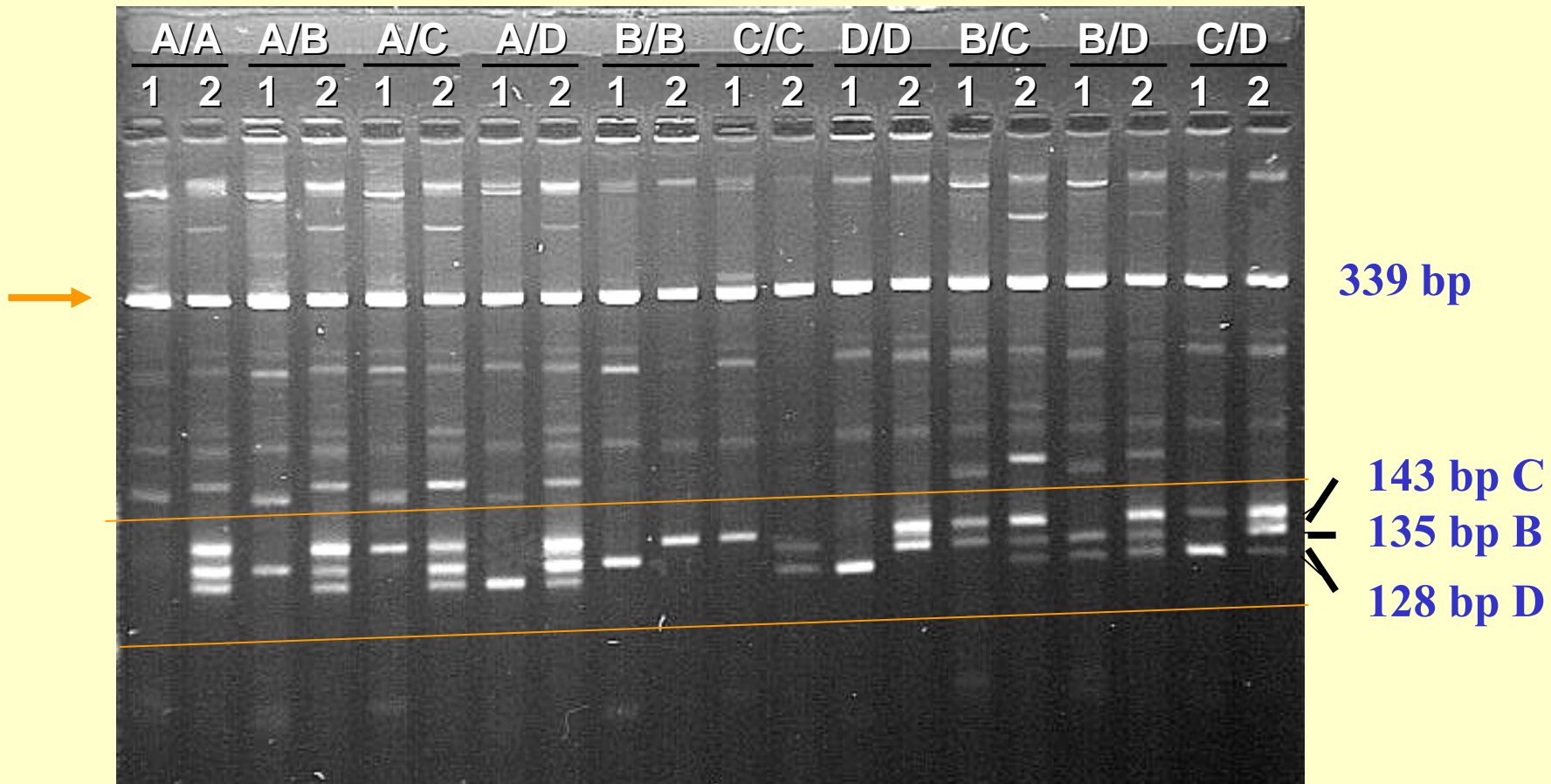
codon 52 R → C ... allele D

codon 54 G → D ... allele B

wild type ... allele A

codon 57 G → E ... allele C

1st EXON GENOTYPING



MBL genotyping in Czech population

◆ 359 individuals

☒ 255 healthy children

☒ 104 cord blood samples

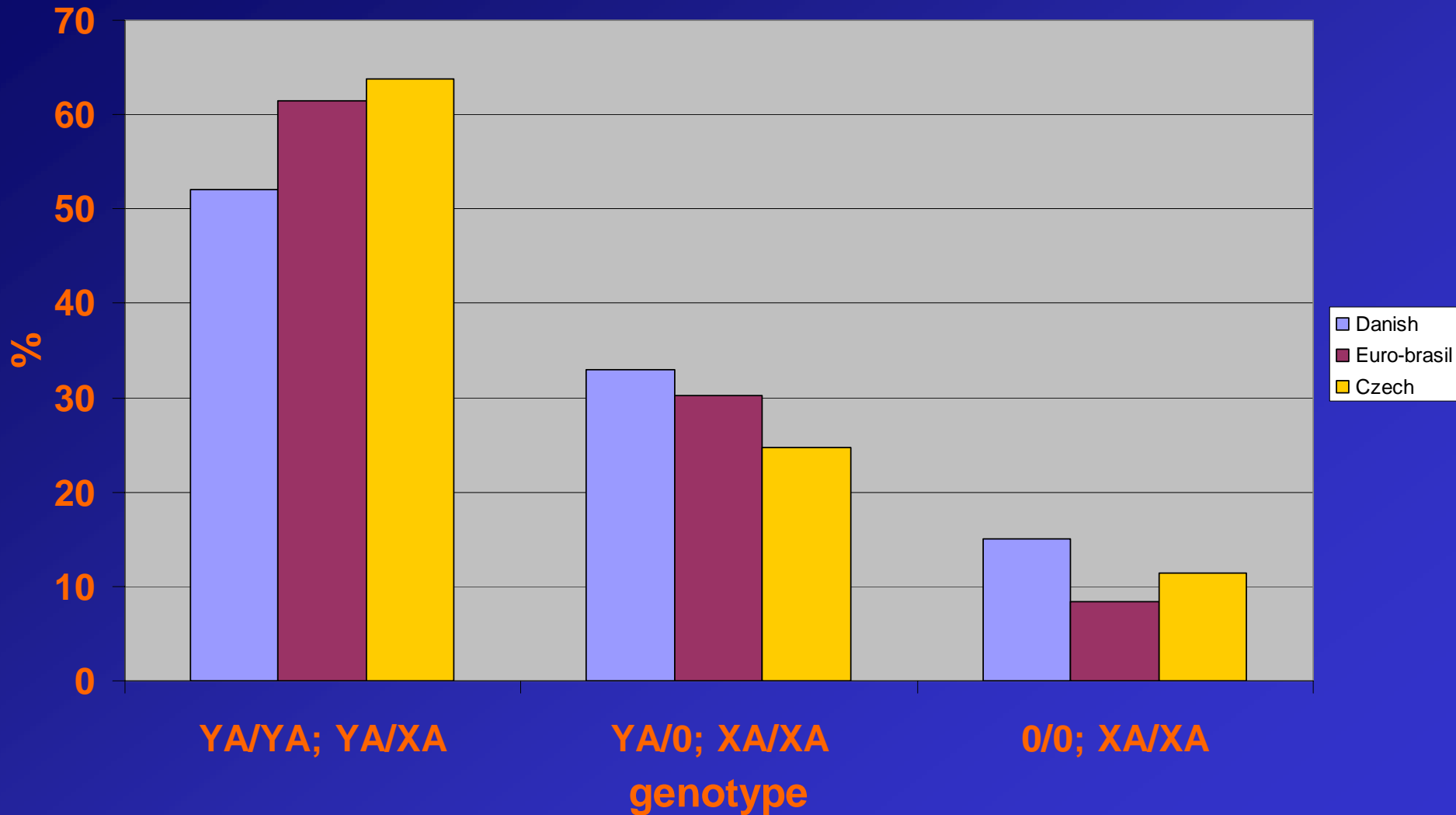
MBL haplotype frequencies in Czech population

| Haplotype | Absolute frequency | Relative frequency \pm SD |
|--------------|--------------------|-------------------------------------|
| HYA | 233 | 0.32 \pm 0.02 |
| LYA | 194 | 0.27 \pm 0.02 |
| LXA | 164 | 0.23 \pm 0.02 |
| LYB | 83 | 0.12 \pm 0.01 |
| LYC | 6 | 0.008 \pm 0.003 |
| HYD | 30 | 0.04 \pm 0.01 |
| LYD | 8 | 0.011 \pm 0.004 |
| Total | 718 | 1.0000 |

MBL genotype frequencies in Czech population

| Genotype | Absolute frequency (n) | Relative frequency (%) | Protein serum concentration |
|-------------------------|------------------------|------------------------|-----------------------------|
| YA/YA; YA/XA | 229 | 63.8 | normal |
| YA/0; XA/XA | 89 | 24.8 | intermediate |
| 0/0; XA/0 | 41 | 11.4 | low |
| Total | 359 | 100.0 | |

MBL genotype frequencies in Caucasians



Conclusion

- ◆ **New rapid and cost effective method of MBL genotyping**
- ◆ **Haplotype and genotype frequencies in general Czech population**
- ◆ **Very rare LYD haplotype was described in 1.1% individuals of general Czech population**

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