HBM4EU project

science and policy
for a healthy future

Genetic variability in human biological monitoring

Vita Dolžan

1st HBM4EU Training School 2018

Summary

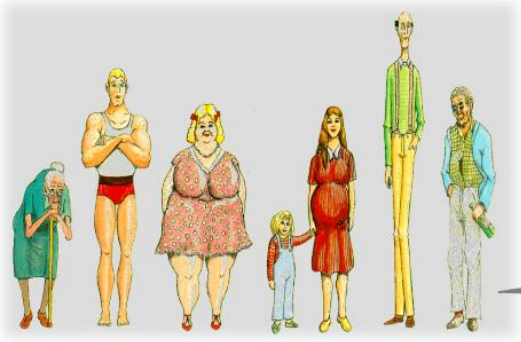
1. Interindividual variability of susceptibility
2. Molecular basis of genetic variability
3. Epigenetics contributes to interindividual variability
4. Is information on genetic variability important?
5. Xenobiotic metabolism and genetic variability
6. Conclusions

Interindividual variability of susceptibility

- individuals can often differ markedly in their qualitative and quantitative responses to chemical/ environmantal exposure
- Such interindividual differences can be genetically mediated, or can be the result of some environmental stressor, lifestyle, disease process or other epigenetic factor.
- These mey lead to different levels of expression of genotypic variations in at risk phenotype
- these interindividual differences can also be usefully employed as biomarkers of individual susceptibility to xenobiotics
- Susceptibility markers are useful because they can partially explain interindividual variation inherent in the general population, and thus provide a biological rationale for investigation of inherent vulnerability prior to exposure to environmental hazards

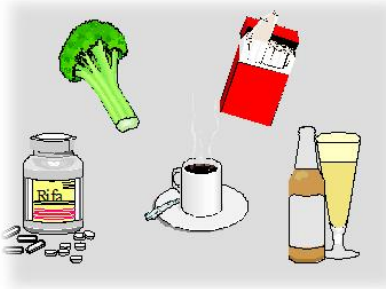
Franko et al., J Biomed Biotechnol, 2009
Franko et al., J Arh Hig Rada Toksikol, 2008
Franko et al., J Biomed Biotechnol, 2011

Interindividual variability of susceptibility

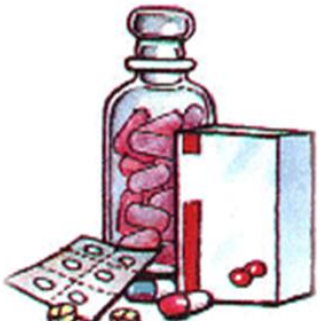


Biologic factors
- gender, age
- constitution
- diseases
- physiology
- hormones

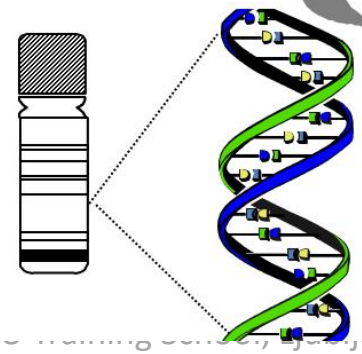
Environmental factors
- drugs (interactions)
- nutrients, smoking
- pollutants
- lifestyle



Interindividual variability



Exposure
- dose
- duration



Genetic factors
- XME
- transporters
- drug targets

Molecular basis of genetic variability

CCT**A**GTTGAC**TG**ATCGCGGGATTCACACACATGG

CCT**G**GTTGAC..ATCGCGGGATT**CACACACACACA**TGG

InDels
Insertions/deletions
>1,000,000

Variable number of tandem repeats - **VNTR**

- **> 1,000,000**
- several alleles
- microsatellities (1-5nt)
- minisatellites (6-100 nt)
- …

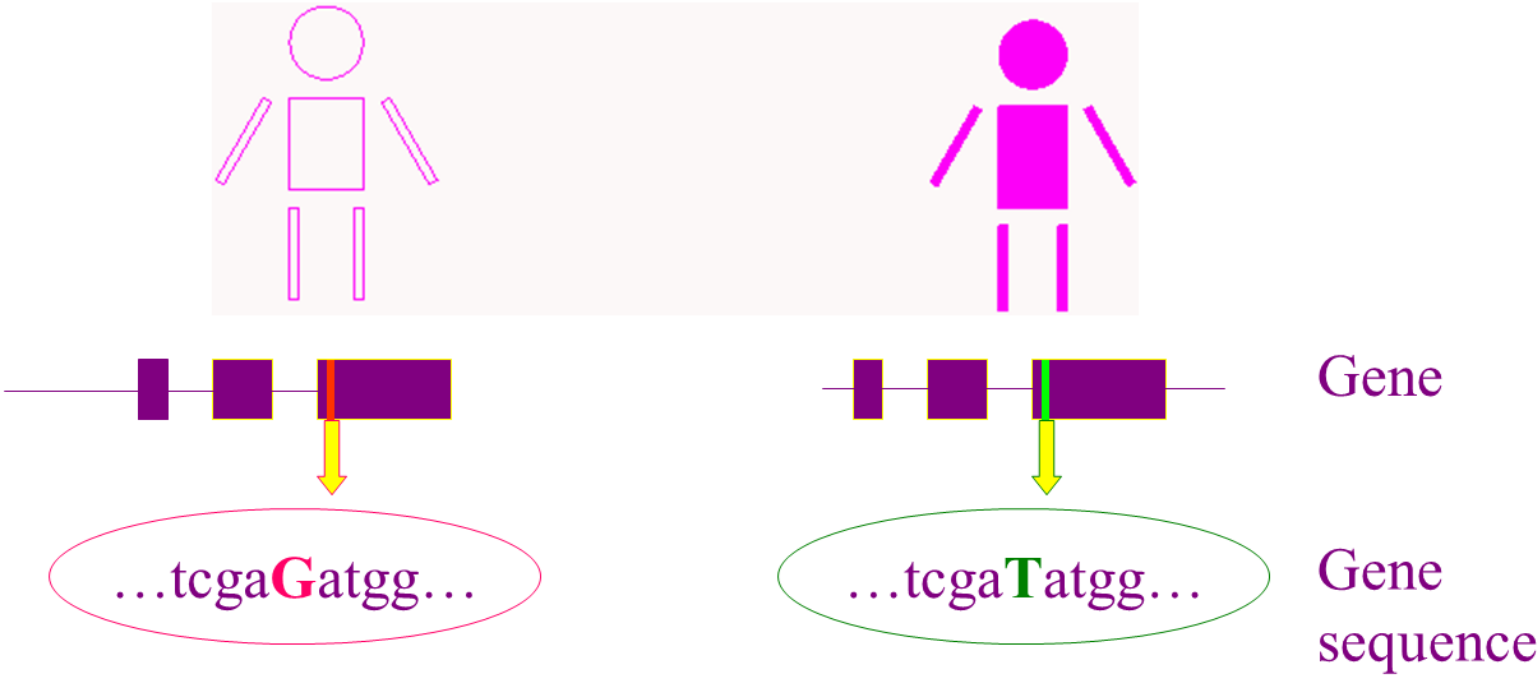
SNP
Single Nucleotide Polymorphisms;

- **> 10 mio**
- **usually biallelic**

Alterations >1000bp – STRUCTURAL VARIATIONS
< 3 million bp – submicroscopic variations
> 3 million bp – microscopic variations

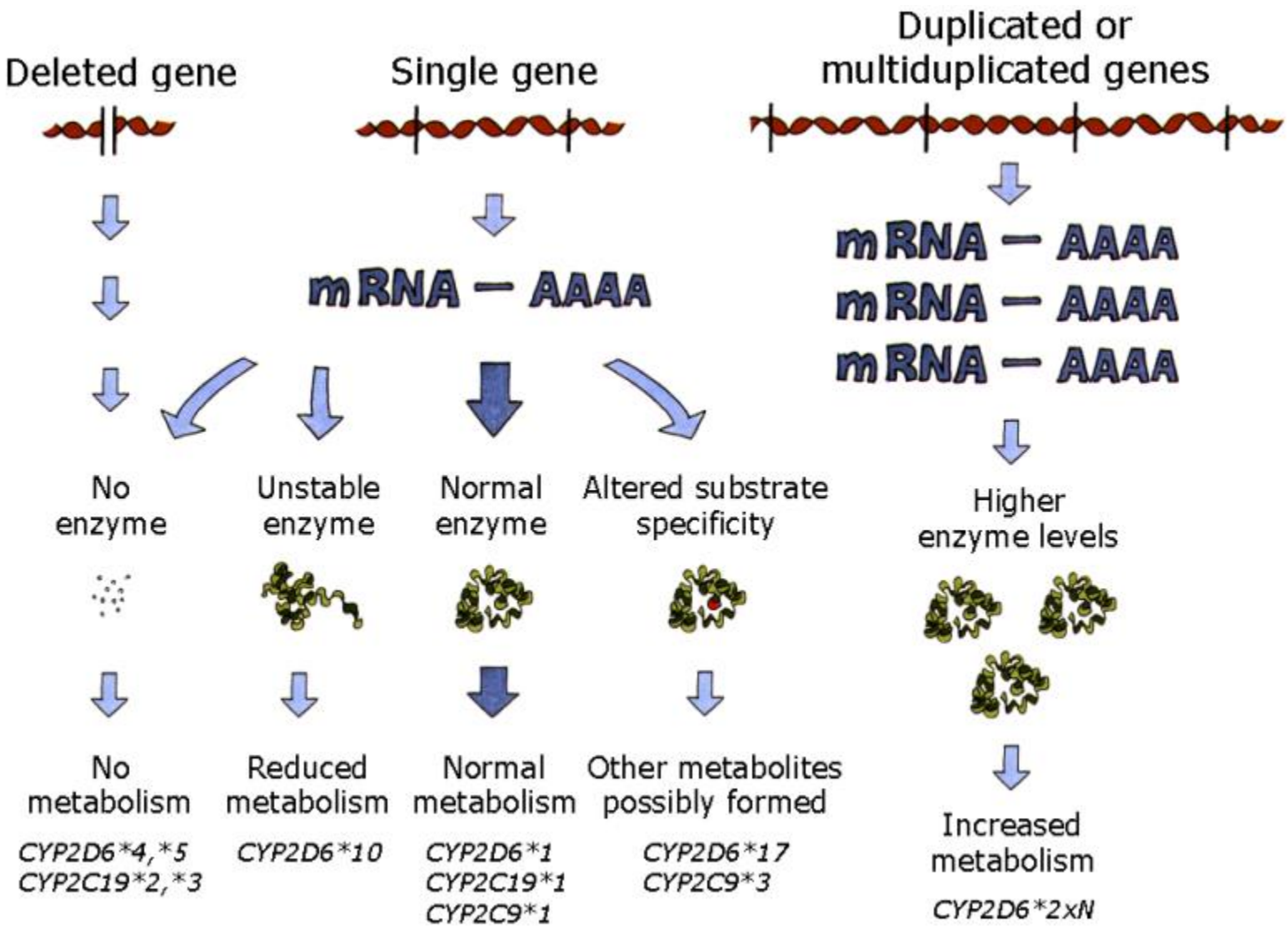
Genetic polymorphisms

- occurrence of two or more alleles at a given locus
- the rare allele has a frequency of at least 1% or more

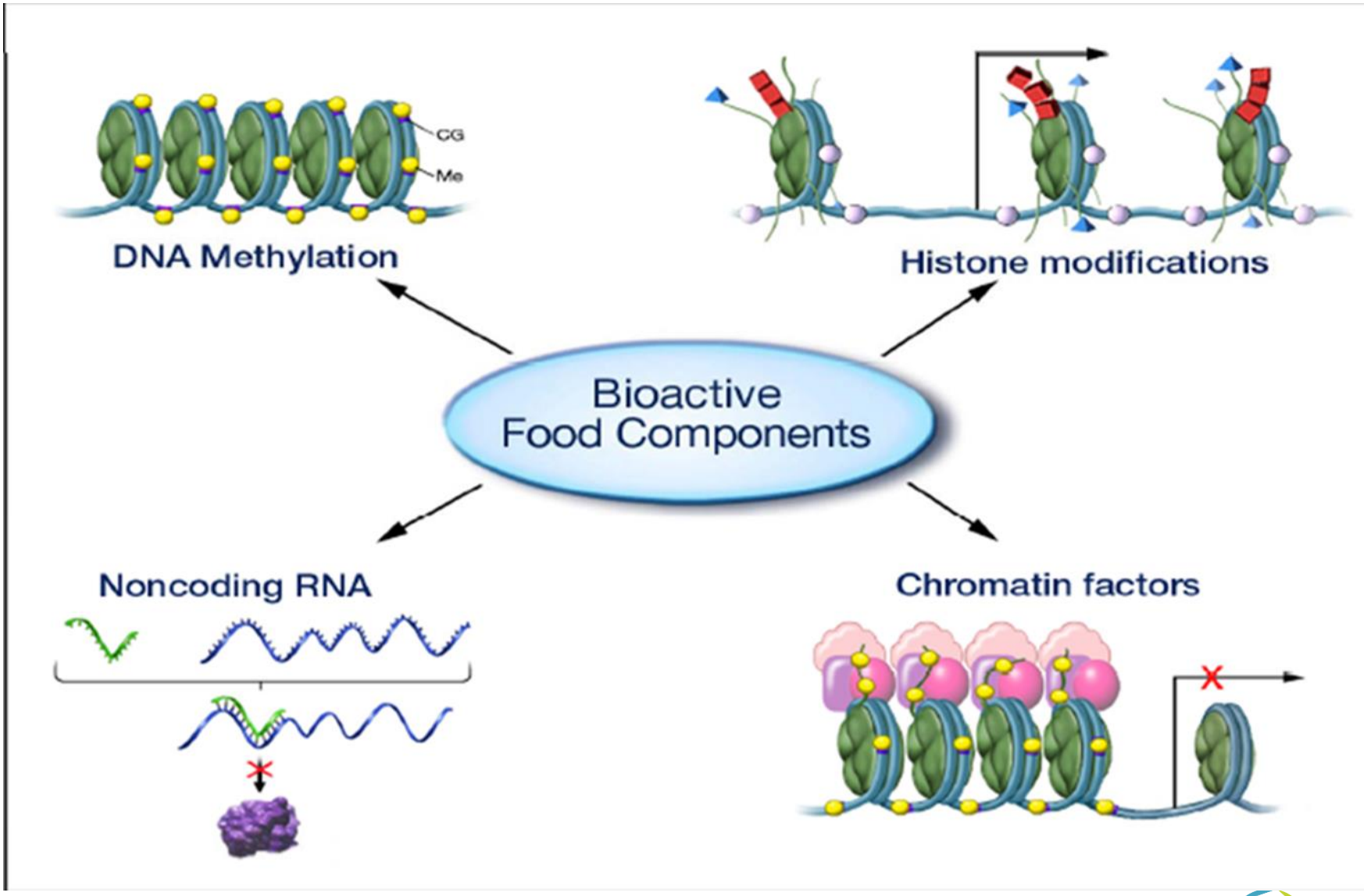


- single nucleotide polymorphisms (SNPs)
- copy number variations (CNVs)

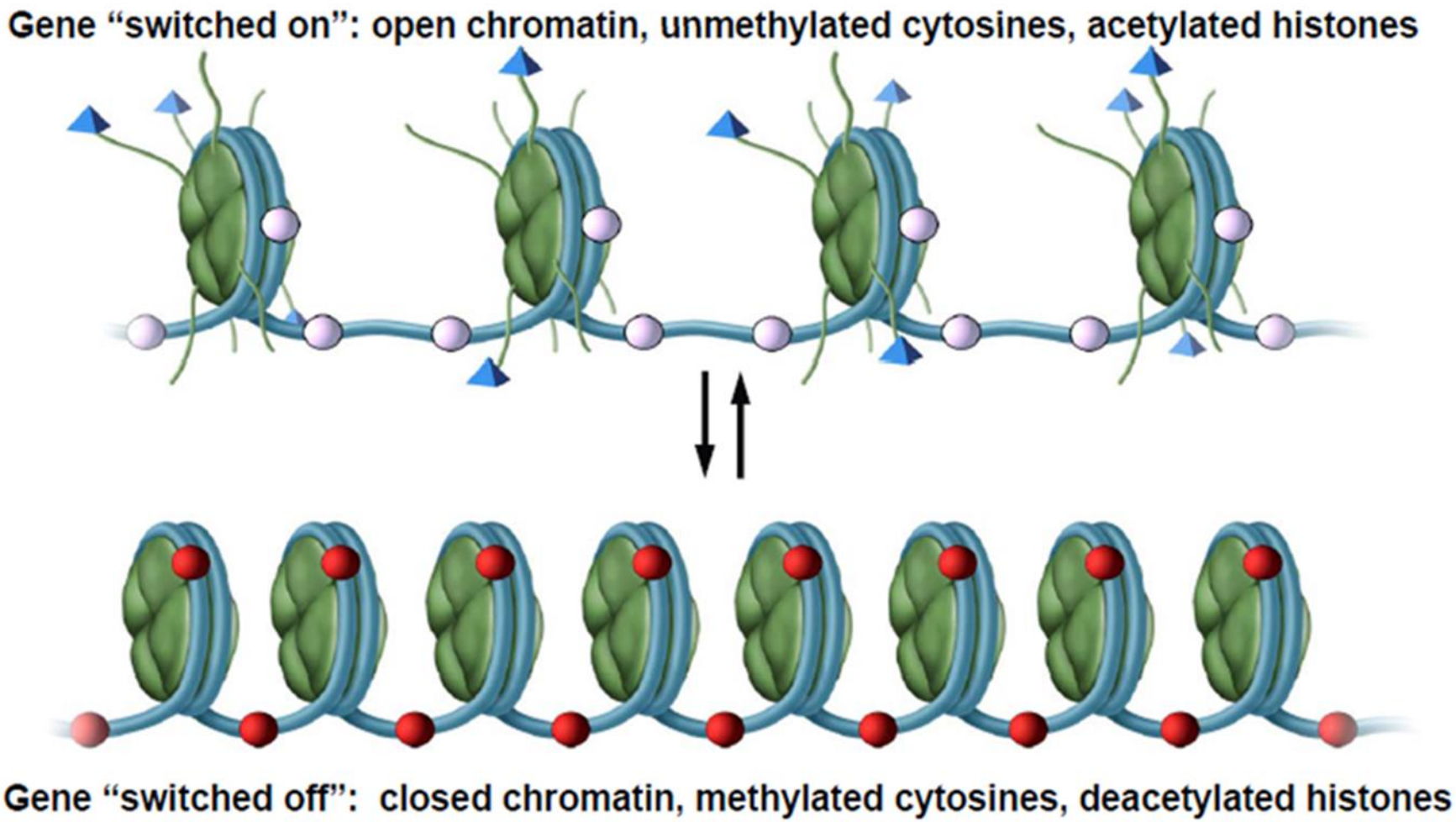
Functional consequences of genetic variability



Epigenetics contributes to variability



Chromatin structure influences gene expression



Non-coding RNA (ncRNAs)

Structural RNA:

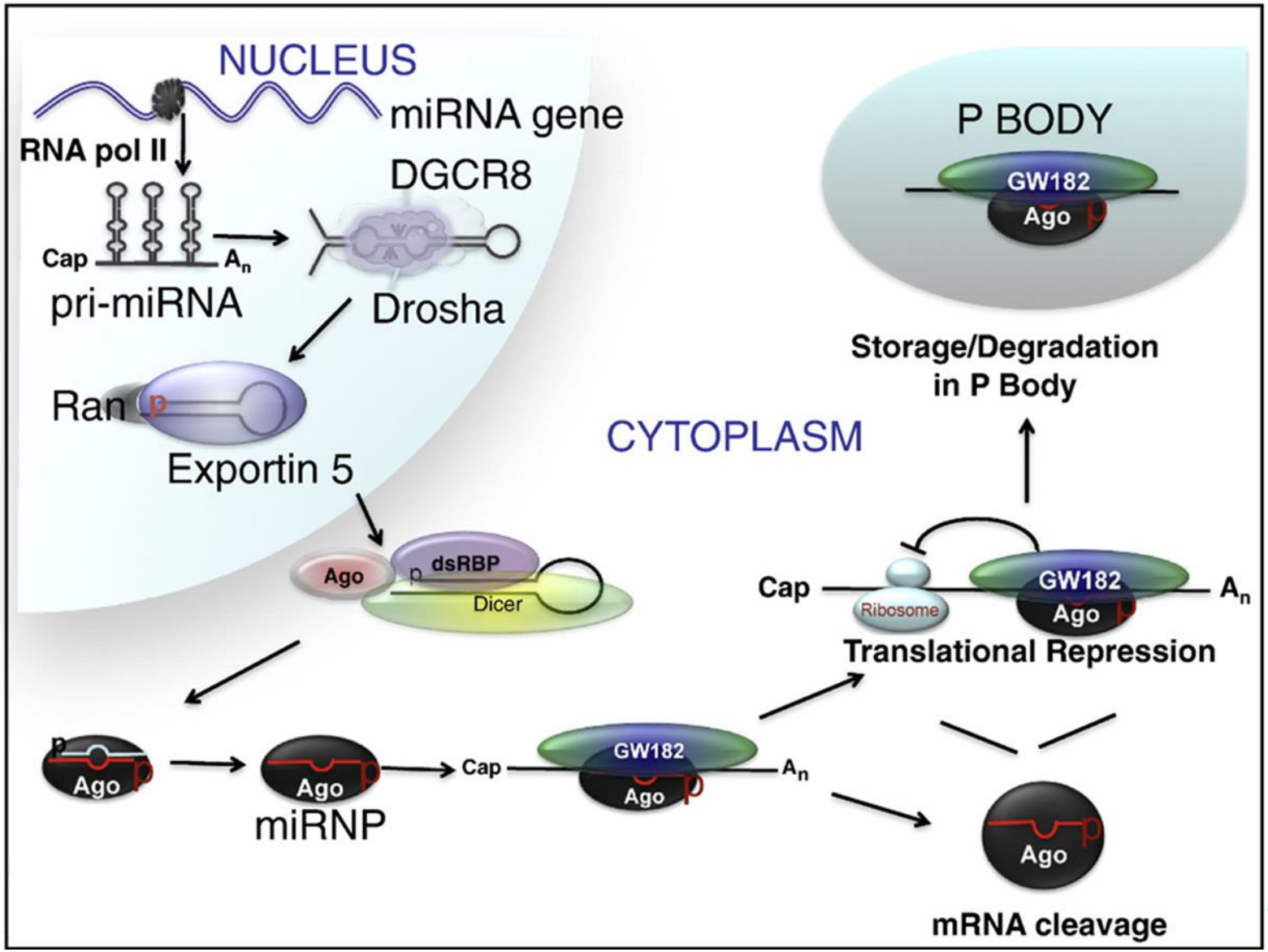
- transfer –tRNA
- ribosomal – rRNA
- small nuclear RNAs (snRNAs)

Regulatory RNAs:

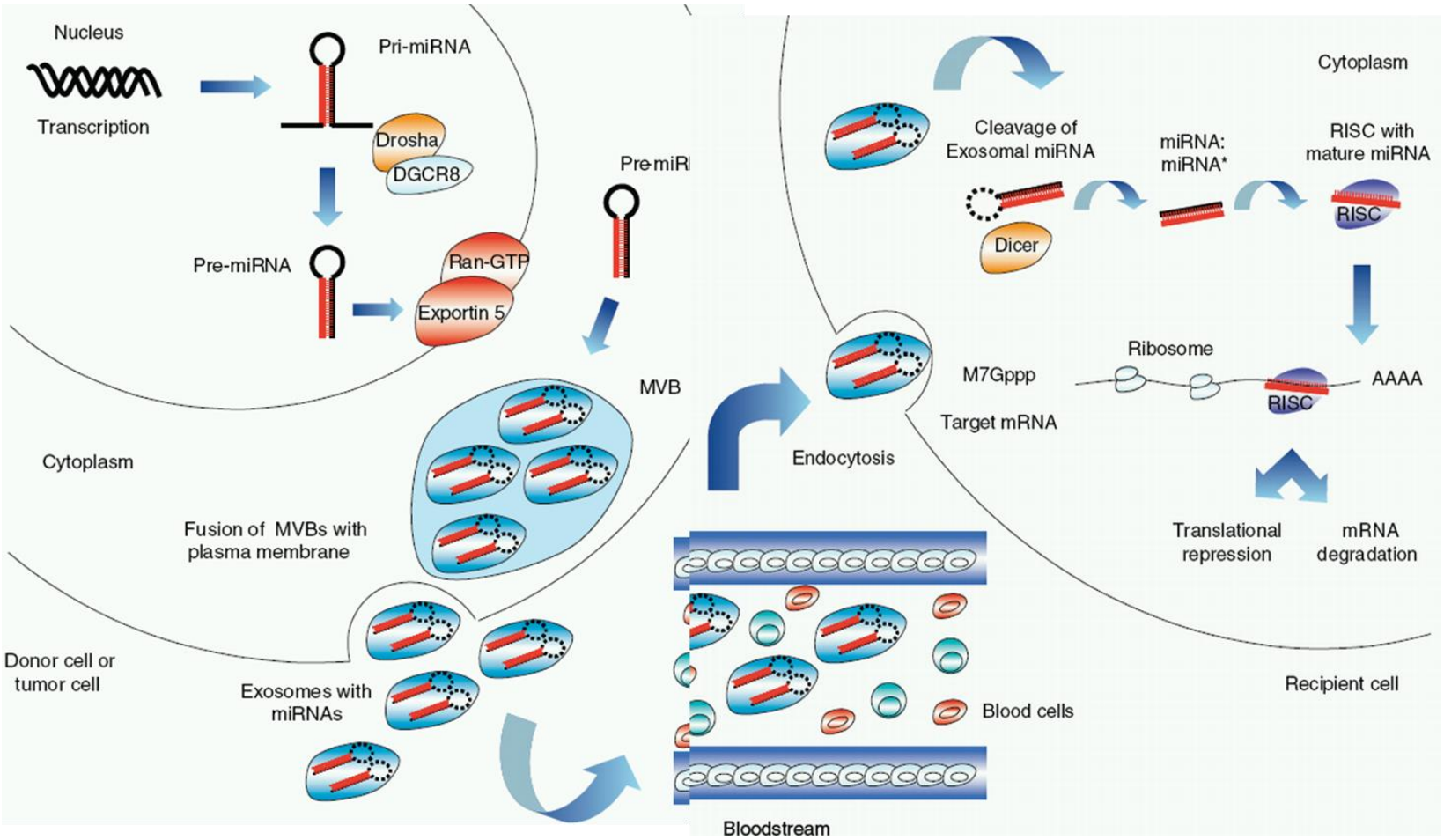
- long (>200 nt) or small (<200 nt) ncRNAs
- only a subset has clearly defined functions
- telomeric ncTNAs (TelRNAs)
- micro RNAs post-transcriptional regulation
- short interfering RNAs (siRNAs) of gene expression
-

micro RNA (miRNAs)

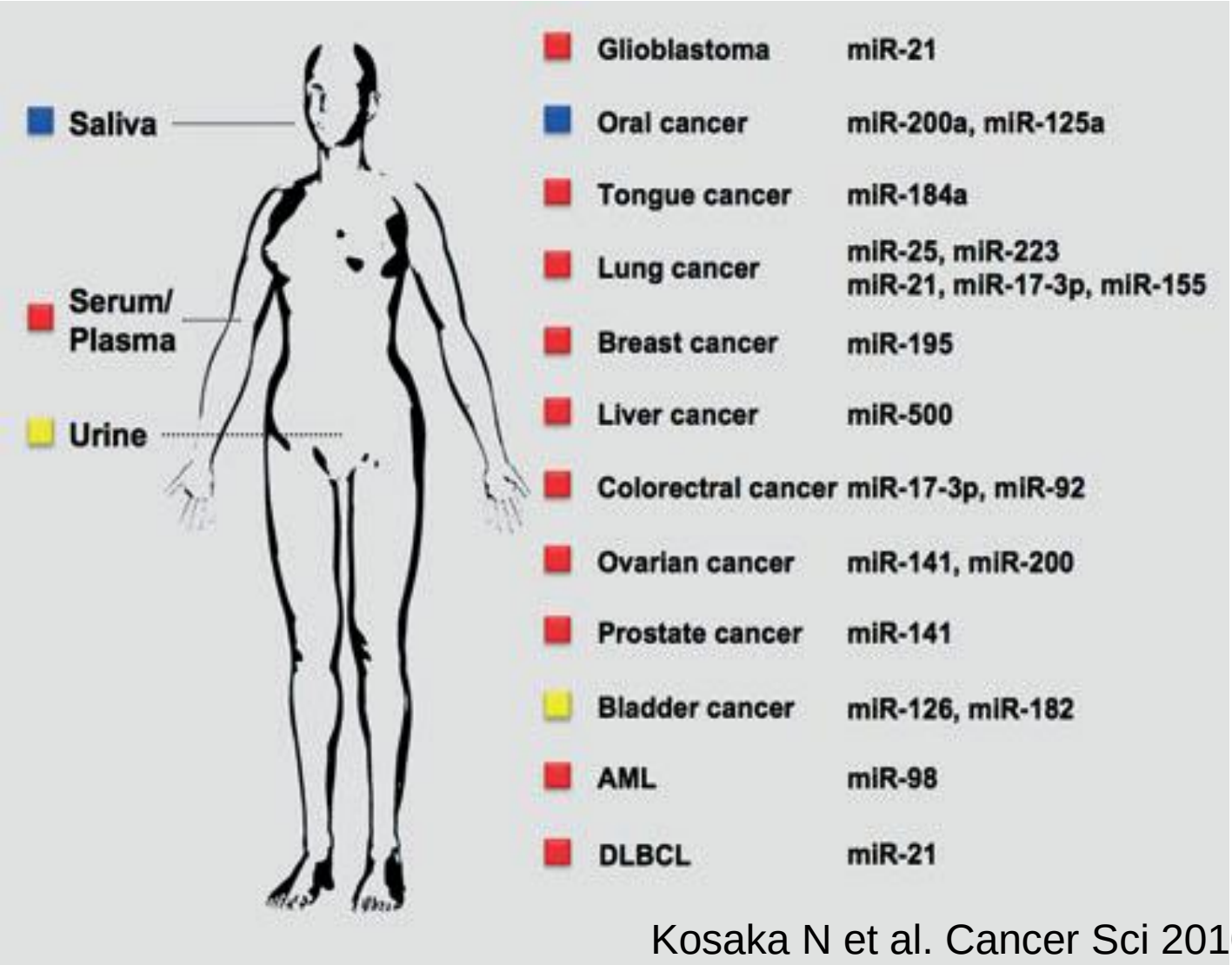
18-25 nt



Circulating miRNA as biomarker



Circulating miRNA – noninvasive early diagnostic cancer biomarker



Kosaka N et al. Cancer Sci 2010; 101:2087

Is information on genetic variability important?

- Genetic variability influences individuals' characteristics

- phenotype
- disease risk
- treatment response

Genes?
Environment?
Both?

Is information on genetic variability important?

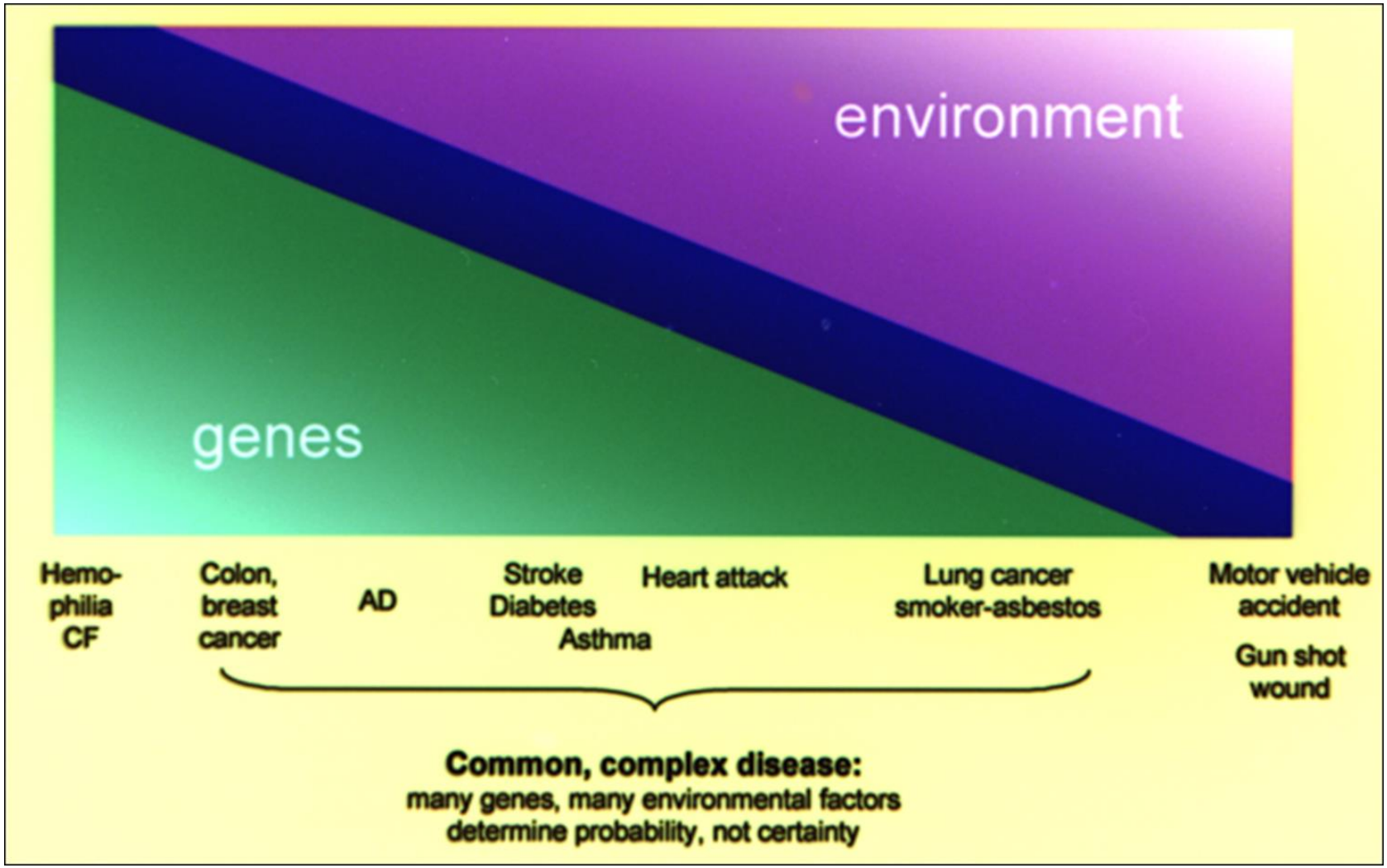
Disease-Marker Association

A marker locus is associated with a disease if the distribution of genotypes at the marker locus in disease-affected individuals differs from the distribution in the general population

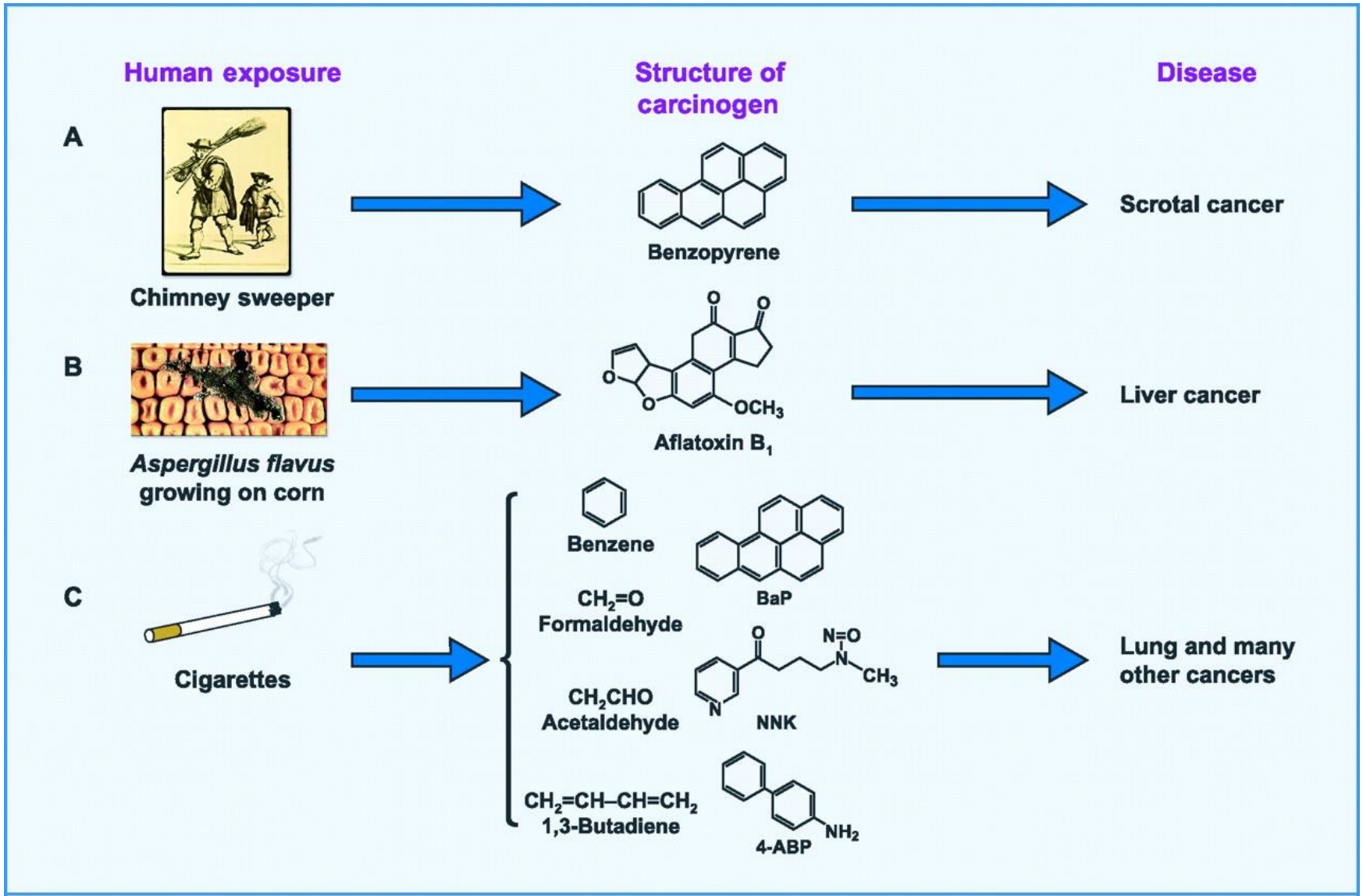
A specific allele/haplotype may be:

- positively associated (over-represented in affected) or
- negatively associated (under-represented)

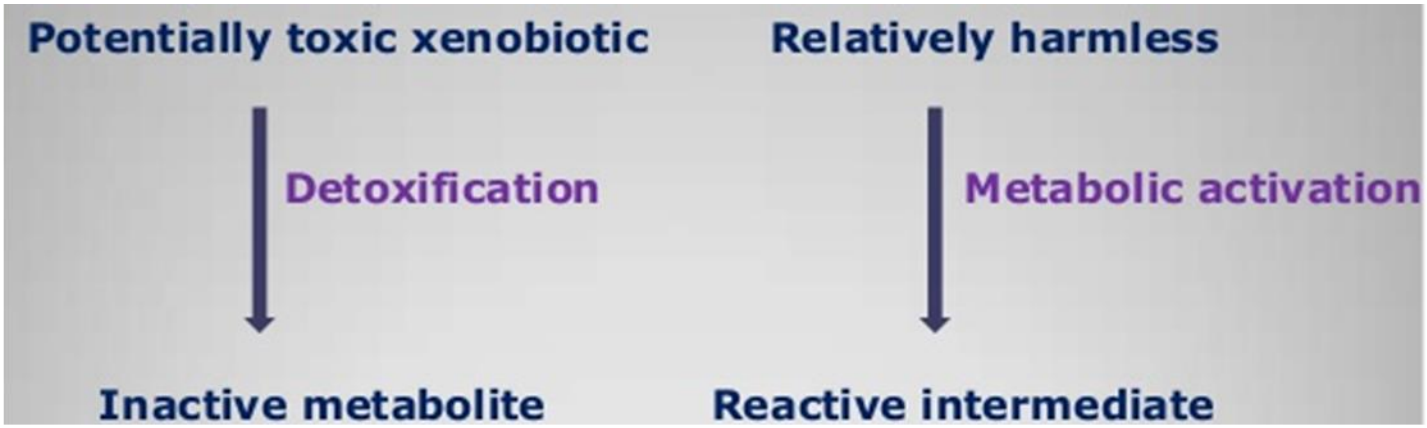
Is information on genetic variability important?

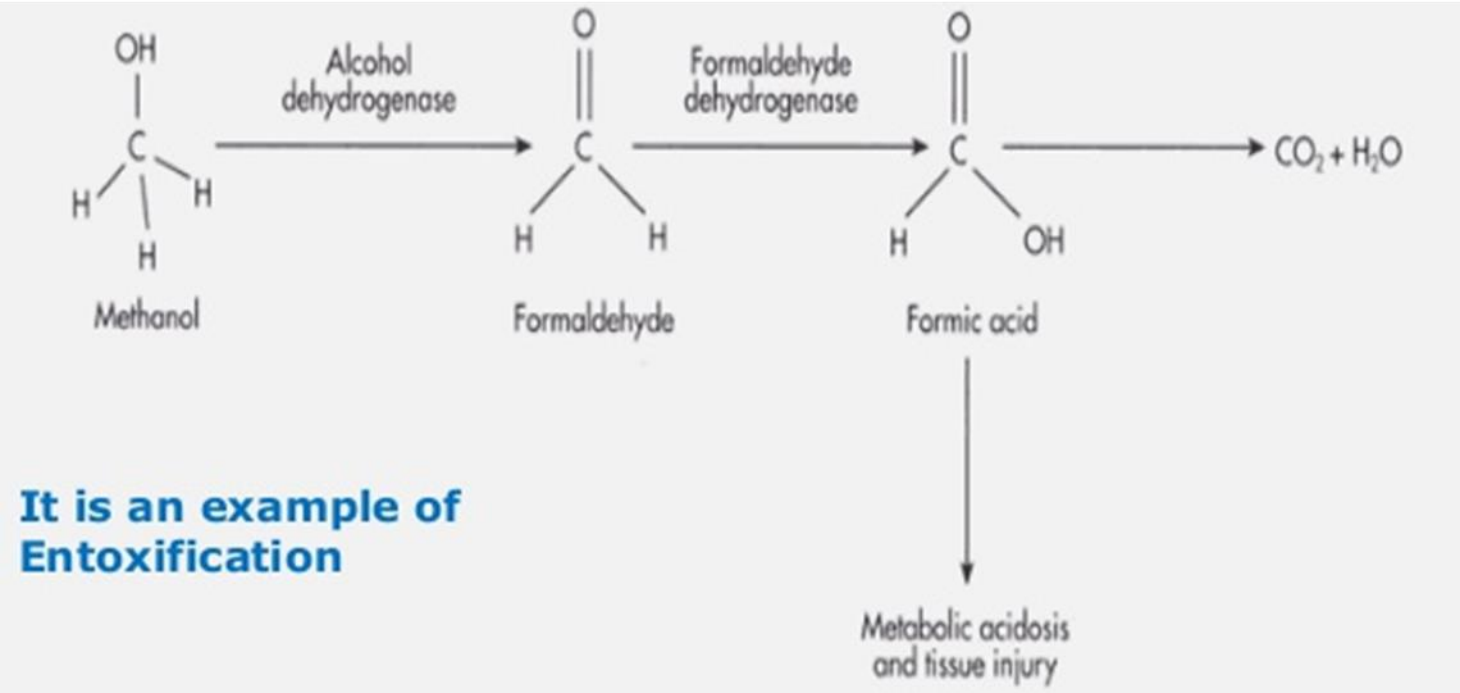


Xenobiotic exposure and carcinogenesis



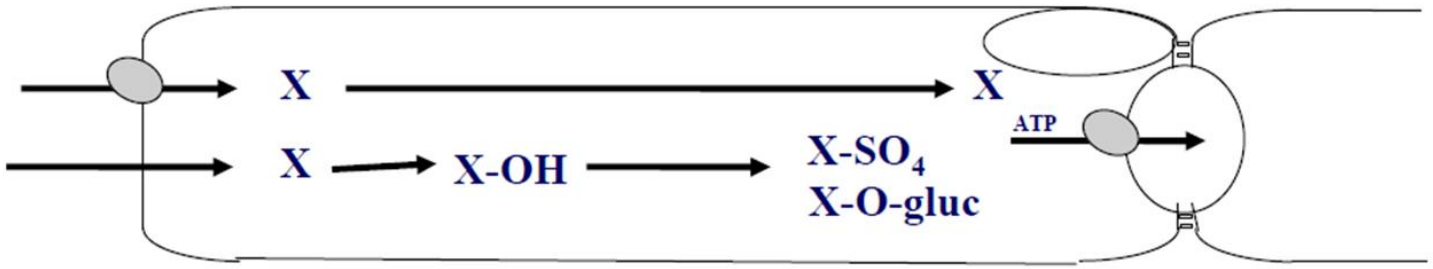
Biotrasformation: detoxification vs toxification





Xenobiotic metabolism

The liver as an example: Interplay between metabolism & transport



Drug > Phase 0 > Phase I > Phase II > Phase III > Excretion

uptake	*functionalization*	*conjugation*	*export*
Oatp	oxidations (CYP450)	glucuronidation (UGT)	P-gp (MDR1)
oct	reductions	sulfation (ST)	TC transporter (cBST)
	hydrolysis	glutathion conjug. (GST)	cMOAT (MRP2)
	isomerizations	methylation	
		acetylation	

J.Huwyler, 11.2008, page 4

Biotrasformation: Human cytochromes P450

> 57 genes (17 families)*

$$NADPH + H^+ + O_2 + RH \rightarrow NADP^+ + H_2O + R\text{-}OH$$

Cytochrome P450 substrates:

endogenous:

steroids
steroide hormones
prostaglandines
lipids
fatty acids

exogenous - xenobiotics - CYP1, 2 and 3:

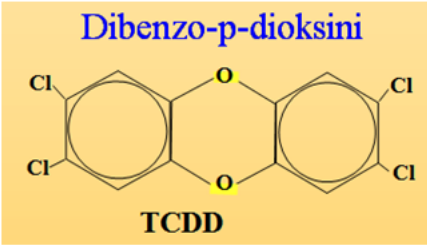
drugs
(1A2, 2C9, 2C19, 2D6, 2E1, 3A4)
carcinogenes
(1A1, 1A2, 1B1, 2A6, 2C9, 2E1, 3A4)

* Nelson DR, Rendić S; DMR 2002ca

CYP1A1

Inducible, polymorphic

- expressed in liver and extrahepatic tissues (skin, lung!)
- substrates:
    - polycyclic aromatic hydrocarbons (PAHs)
    - halogenated PAHs (HAHs)



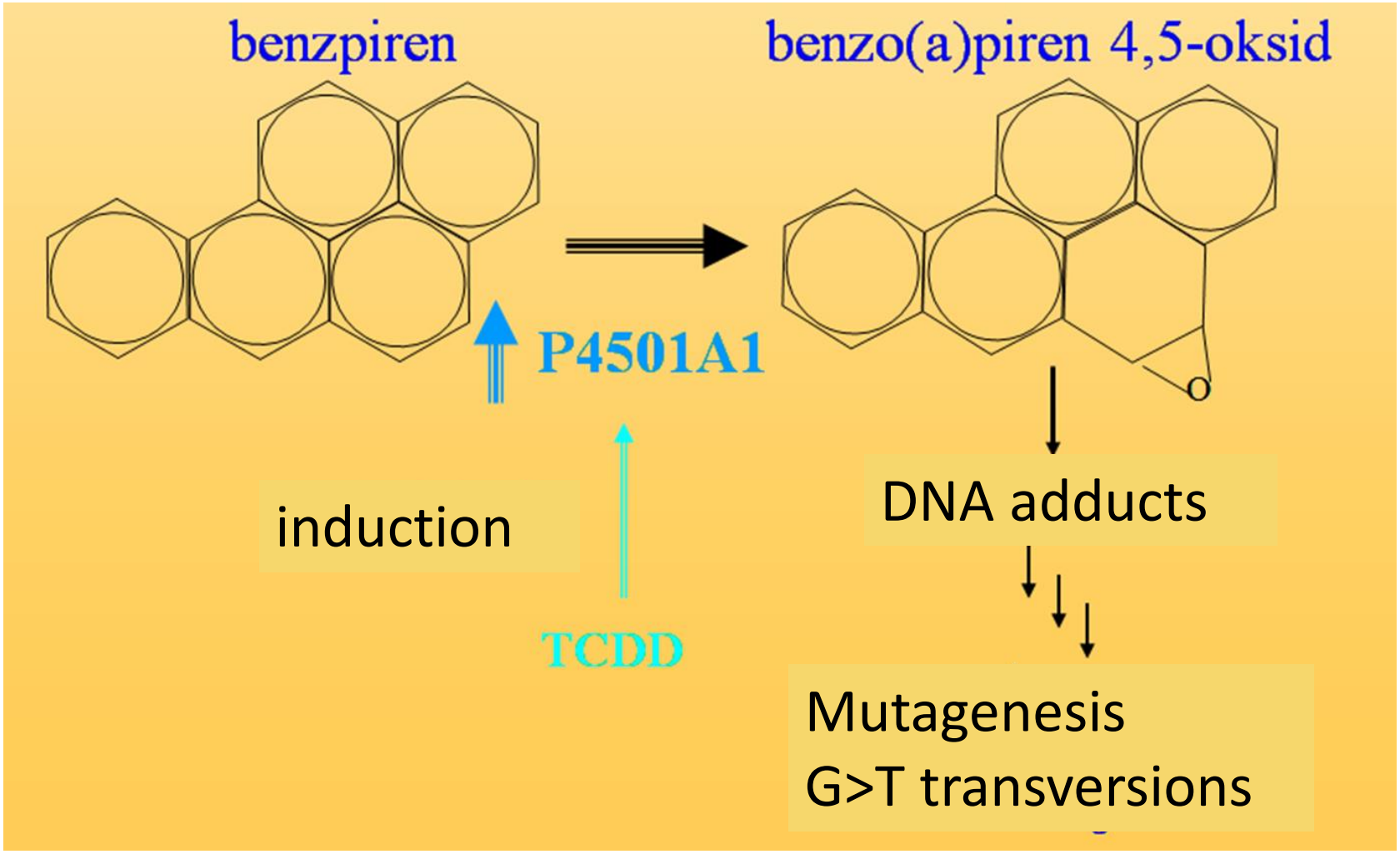
Substrates:	75	135	209
Inductors:	7	10	13

PAH sources:

- burning of fossil fuels, forest fires, waste incineration,
- metallurgy, chemical industry,
- contaminated soil, water
- tobacco smoke

accumulation in the food chain!

CYP1A1 and carcinogenesis



CYP1A1 polymorphisms and human cancer

Author (year)	Ref	Population/ country	Cancer type	Case/ control	*CYP1A1* genotype		Combined genotype	
					Poly-morphism (variant)*	OR (95% CI)	Poly-morphism (variant)*	OR (95% CI)
Cha *et al.* (2007)	17	Koreans	Oral	72/221	**2A* (2)	3.8 (1.9-7.7)	**2A*,(2) *GSTM1* (-)	4.9 (1.5-15.5)
Wang *et al.* (2004)	31	China	Lung	91/138	**2A* (2)	NS†	**2A*,(2) *GSTM1* (-/-)	2.47 (1.03-5.90)
Sreeja *et al.* (2005)	34	South India	Lung	146/146	**2A* (2)	3.178 (1.294-7.803) *P*=0.012	**2A, GSTT1+*	2.106 (1.235-3.592) *P*=0.006
							**2A, GSTT1+, GSTM1+*	2.331 (1.246-4.361) *P*=0.008
Ng *et al.* (2005)	43	Chinese women	Lung	126/162	**2A* (2)	1.7 (0.9-3.3)	**2A, GSTT1*(-/-)	2.7 (1.1-6.9)
							**2A, GSTM1* (-/-)	2.3 (1.0-5.0)
Belogubova *et al.*, (2006)	120	ND‡	Lung	141/246	**2A*	2.27 (1.14-4.52)	**2A+, GSTM1*(-)	3.85 (1.43-10.33)
Yoshida *et al.* (2007)	60	Japan	Colorectal	66/121	**2A* (2)	0.87 (0.31-2.47)	**2A*,(1-2) *NAT2* Rapid (2)	3.18 (0.95-10.6)
					**2C* (2)	1.99 (0.41-9.63)	**2C*,(1-2) *NAT2* Rapid (2)	3.12 (1.15-8.51)
Hou *et al.* (2005)	63	Non-Hispanic Caucasian	Colorectal	725/729	**2C* (2)	No association	**2C*,(2) *NQO1 Ser187* (1-2)	2.2 (1.1-4.5)
Quinones *et al.* (2006)	126	Chile	Prostate	60/117	**2A* (2)	No association	**2A* (1-2), *GSTM1*(-/-)	6.87 (1.68-27.97) *P*=0.007
							**2A* (1-2), *GSTM1* (+)	5.00 (1.47-17.05) *P*=0.01
Lira *et al.* (2006)	128	Italian	Skin cancer in transplant recipients	107/132	**2C*	No association	**2C, GSTM1* (-/-)	4.5 (1.1-21.4) for NMSC
							**2C, GSTM1* (-/-)	6.5 (1.4-34.4) for SCC
D'Alo *et al.* (2004)	130	Italian	Adult AML	193/273	**4*	2.2 (1.3-3.7) *P*=0.006	**4, GSTT1*(-/-)	7.0 (2-24.8) *P*=0.001

AML=Acute myeloid leukemia; NMSC=Non melanoma skin cancer; ‡Not defined; *Number of variant alleles; †Non significant; SCC=Squamous cell carcinoma

Biomarkers of susceptibility beyond XME

In addition to enzymes involved in biotransformation of xenobiotics (XME), other potential susceptibility biomarkers have been explored or proposed in human and animal studies:

- antioxidative defence systems
- DNA repair enzymes
- nuclear and cytoplasmic receptor proteins
- oncogenes and corresponding gene products
- tumor suppressor genes
- humoral and cellular immune system components.

Conclusions

Genetically / epigenetically determined variability in interindividual and interethnic susceptibilities may be related to

- individual expression of clinical signs of chemical toxicity
- biological monitoring data in exposed workers, and
- interpretation of the results of epidemiological or molecular epidemiological studies

However:

- for environmental and occupational fields, the research so far has not led to any routinely usable biomarkers
- genetic based methods cannot easily identify all individuals at risk in a hazardous environment, due to a lack of understanding of the interaction of compensatory genetic and cellular mechanisms and complex environmental influences
- studies of genetic variations associated with hazardous exposures can predict population vulnerability

Univerza *v Ljubljani*

Contact

vita.dolzan[at]mf.uni-lj.si

Contributers

- Assoc Prof Alenka Franko, MD, PhD
- Assistant Katja Goricar, PhD

Speaker's information

Vita Dolžan, MD PhD, spec.med.lab.genet., works as a full professor of Medical biochemistry and molecular genetics at the University of Ljubljana, Faculty of Medicine (UL MF) and is the head of the Pharamacogenetics laboratory at the Institute of Biochemistry UL MF. Her main areas of research interest are pharmacogenetics, molecular genetics and molecular epidemiology. She is collaborating with members of HBM4EU WP2.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733032.