Vliv dlouhodobé expozice nanočásticím na integritu a funkci genomu u lidské populace

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Oddělení genetické toxikologie a epigenetiky

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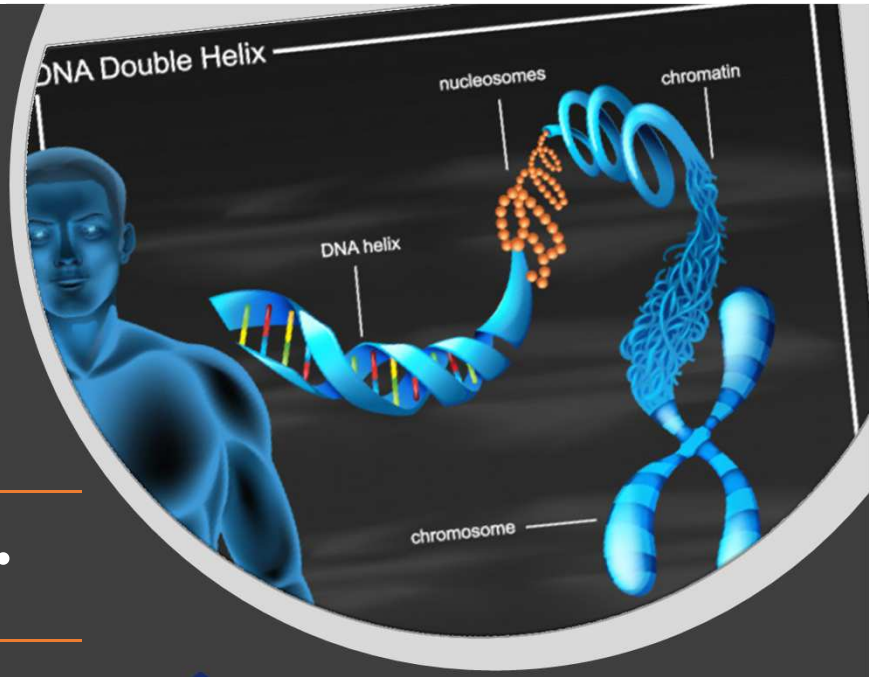
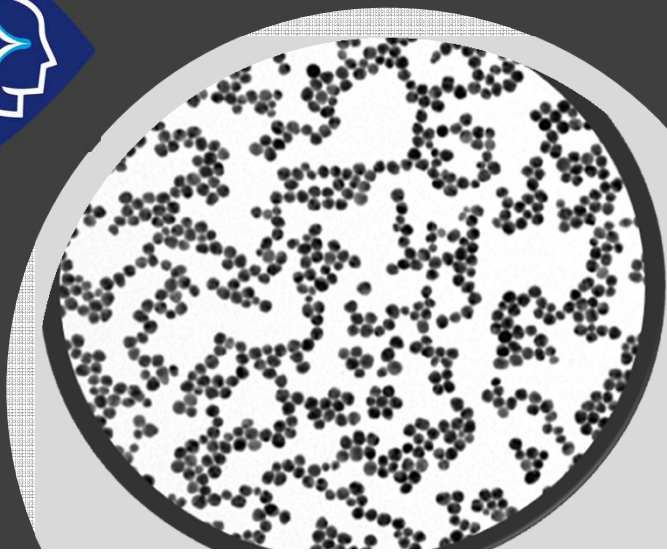




Teisingerův den průmyslové toxikologie, 8. června 2022

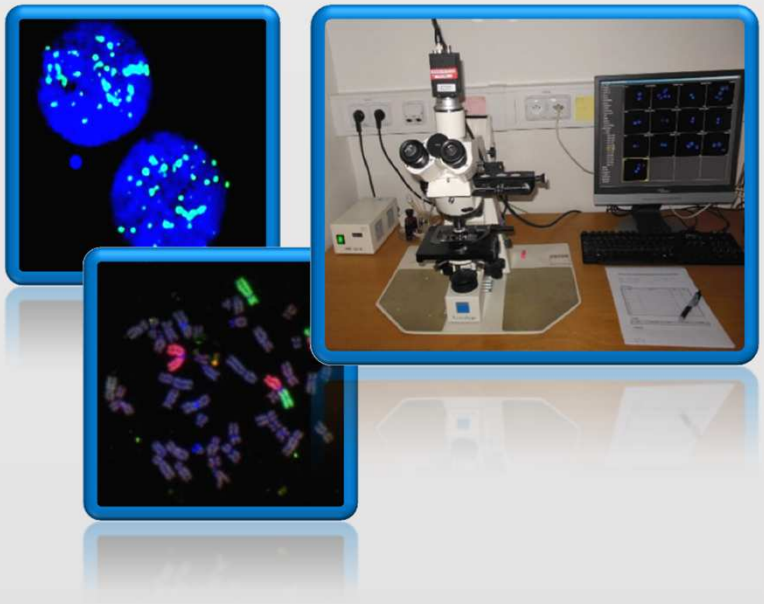
Outline of presentation

Part I: Introduction to the study

1. Background and aim(s)
2. Cohorts
3. Monitoring of exposure (including NPs)

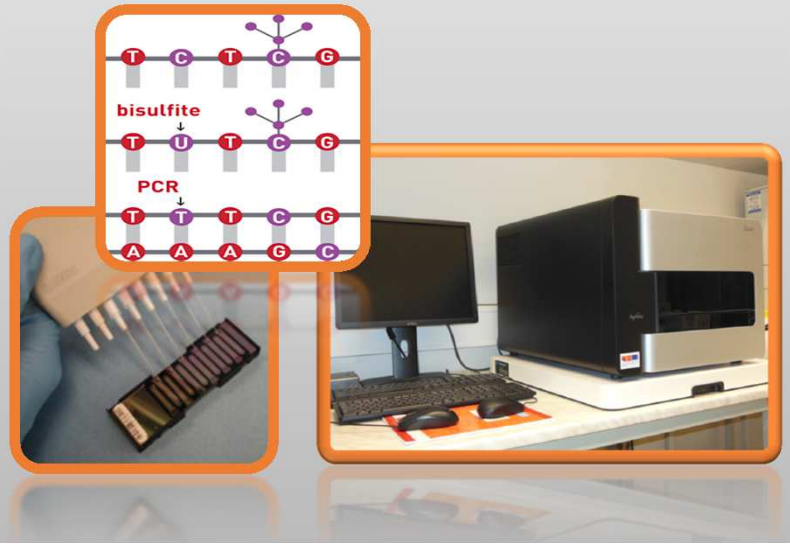
Part II: Cytogenetic part of the study

1. Methods - background (micronuclei, FISH)
2. Methods - microscopic analysis
3. Results (micronuclei, WCP-FISH)



Part III: Epigenetic part of the study

1. Method - background
2. Method - iScan system (array analysis)
3. Results

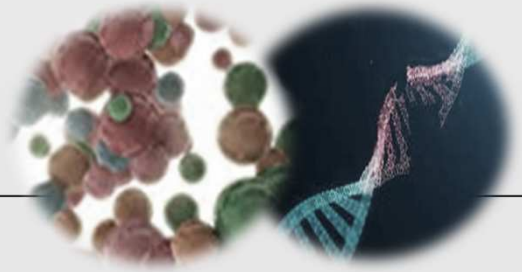


Part IV: Results and theory of adaptation

1. Previous data
2. New data
3. General conclusion

Part I: Introduction to the study

Background and aim(s)



Background:

- The **use of nanomaterials** has been rapidly **increasing** during the last decade in many areas of human life. This phenomenon is accompanied by **increased risk of exposure to nanoparticles.**
- Despite many toxicological *in vitro* studies, the number of human ***in vivo*** **studies is still limited.**

The general aim(s):

- to study the **impact of chronic (and acute) exposure to nanoparticles in a human population** on the **structural** and **functional** **DNA changes,** including possible **mechanisms of adaptation** after long-term exposure.

Investigation of structural changes
by cytogenetic methods
(micronuclei and FISH)

Investigation of functional changes
by epigenetic methods
(global and gene specific DNA methylation)

Part I: Introduction to the study

Cohorts

Sampling:

- September 2015 (pilot)-2016-2017-2018-2019-2020)
- 20 exposed with chronic exposure history+20 controls
- Males (75%) + Females (25%)
- Twice per day (pre-shift and post-shift)
- 2 workshops
- Blood samples for cytogenetic and epigenetic methods

Studied groups	2016		2017		2018	
Group	EXP	CON	EXP	CON	EXP	CON
Number (N)	20	21	20	20	20	20
Males/Females (N)	15/5	15/6	13/7	13/7	14/6	15/5
Age (years: mean±SD)	42±11	39±9	39±11	40±7	39±11	45±12
Chronic exp. (years)	18±10	0	12±9	0	14±9	0

Exposure in sampling day: 30-270 min (50% increase than in a common day).

Workshop 1

- MAG welding and smelting
- Processing of Mild steel S355J2

Workshop 2

- Machining (grinding and milling)
- processing of new nanocomposite materials – epoxide resin with SiO_2 NP

Part I: Introduction to the study

Monitoring of exposure

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Stationary monitoring (2016-2020):

- By **off-line** Berner Low Pressure Impactor (BLPI)-10 stages + gravimetry, ion chromatography + scanning electron microscopy (SEM) to analyze the elemental composition.

- By **on-line** approaches [Scanning Mobility Particle Sizer (SMPS) + by Aerodynamic Particle Sizer (APS)], total data from 32 size classes/decade were available (range from nano-fraction <25 nm to 10 µm).

Personal monitoring:

- New pilot sampling of nano-fraction starting in 2019 by PENS – PErsonal Nanoparticle Sampler (3 parts).

Zdimal, V. et al. Monitoring reports **2016 – 2020**.
Ondrackova, L. et al. XX. výroční konference ČAS **2019**, 100-101.

Examples of relative elementary composition 2019 (from nanofraction)

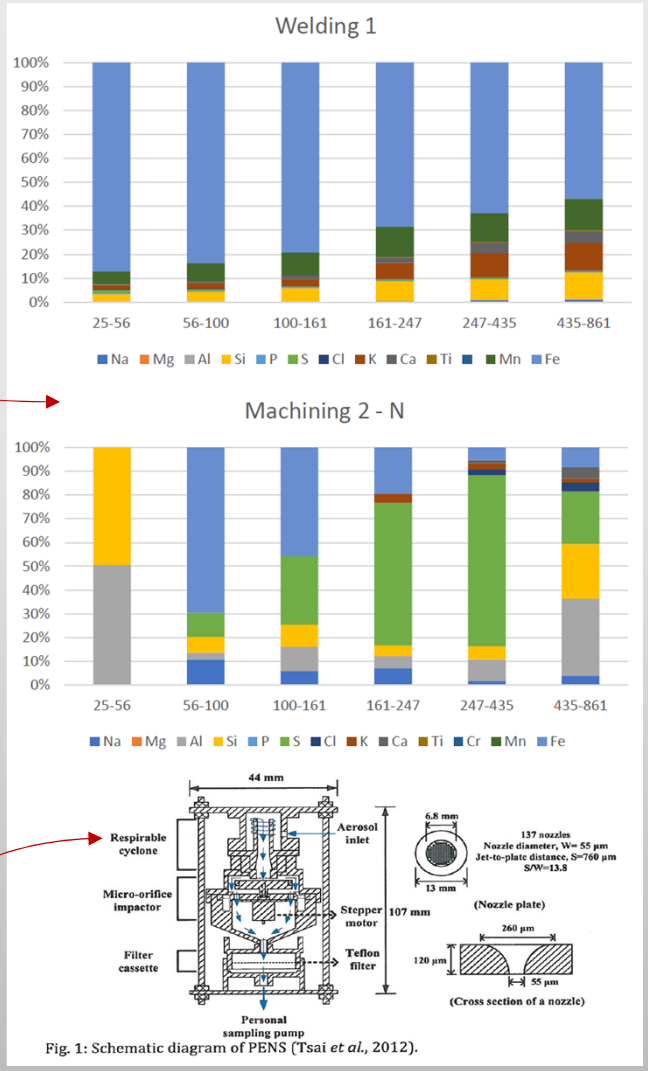


Fig. 1: Schematic diagram of PENS (Tsai *et al.*, 2012).

PENS

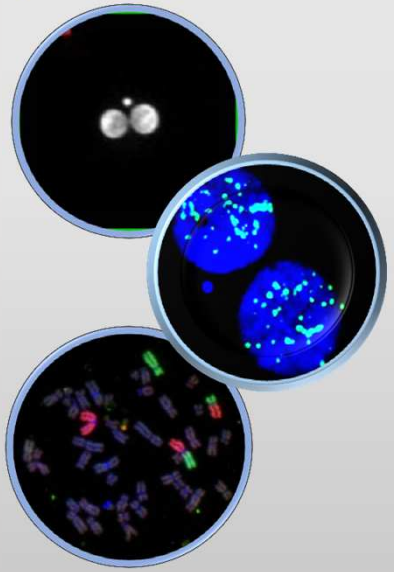
Part II: Cytogenetic part of the study

Methods (MN + FISH)

- 2 basic cytogenetic methods were used

(effect of exposure to various environmental factors: air pollution exposure, various chemicals, smoking, alcohol, diet, radiation, stress...nanoparticles exposure)

1. MicroNucleus test (MN) + FISH modifications with centromere staining



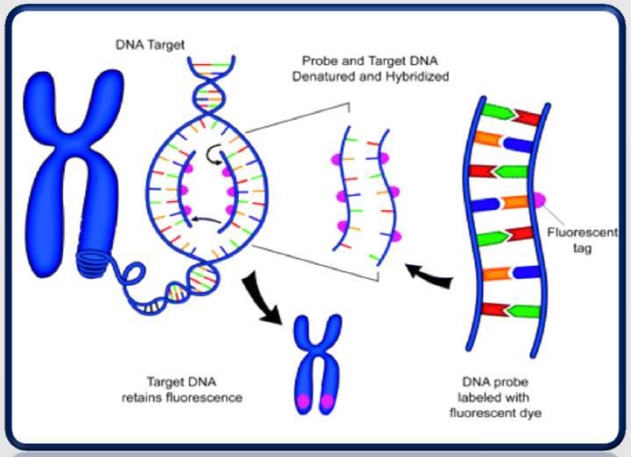
2. Fluorescence *In Situ* Hybridization (FISH) of whole chromosomes

- Chromosomal aberrations are mostly analyzed in human Peripheral Blood Lymphocytes (PBL)

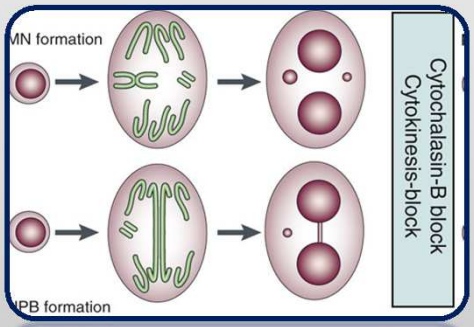
Part II: Cytogenetic part of the study

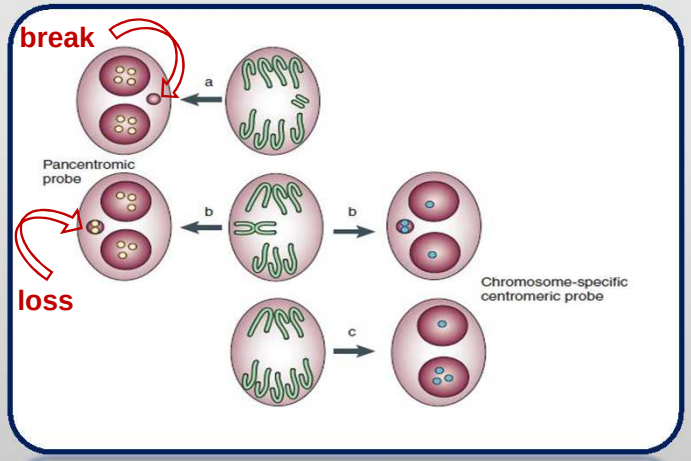
Methods (MN + FISH) and microscopic analysis

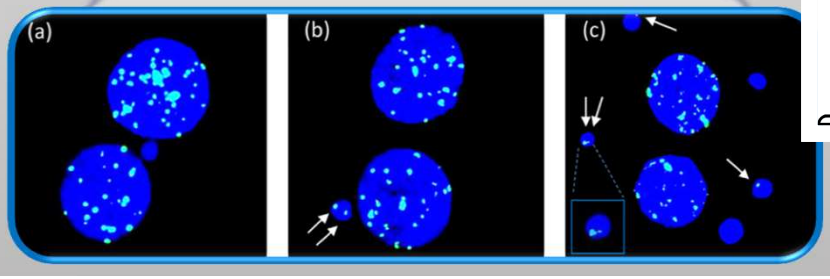
- **ANALYSIS OF MICRONUCLEI IN BINUCLEATED CELLS (main aim)**
- Most frequently used cytogenetic method in molecular epidemiology reflects exposure to agents with clastogenic or aneugenic modes of action (cytochalasin-B is used to inhibit cytokinesis).
- „Basic“ variant of MN assay was **improved** by application of centromeric probes (FISH method) with the aim to recognise chromosome loss (effect of aneugens) or break (effect of clastogens).



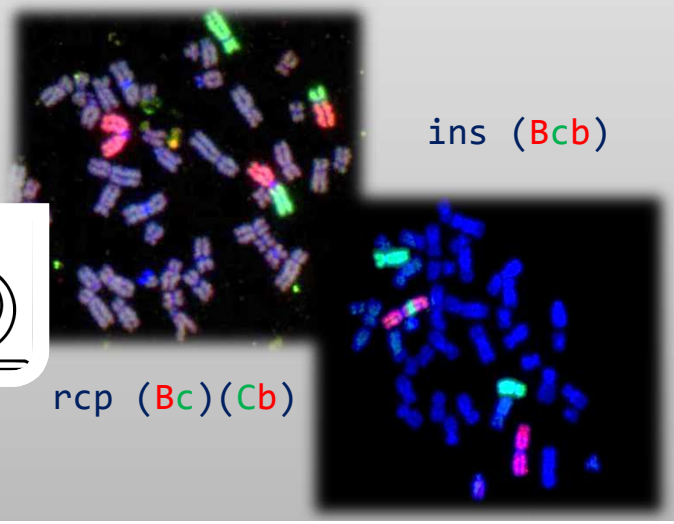
Target DNA (**whole chromosome, centromere**….) and fluorescently labeled DNA probe are denatured and simultaneously hybridized







(a)BNC with 1 MN (CEN-)
(b)BNC with 1 MN (CEN+ 2s.)
(c)BNC with 5 MN (1x CEN+ 2s, 2x CEN+, 2x CEN-)



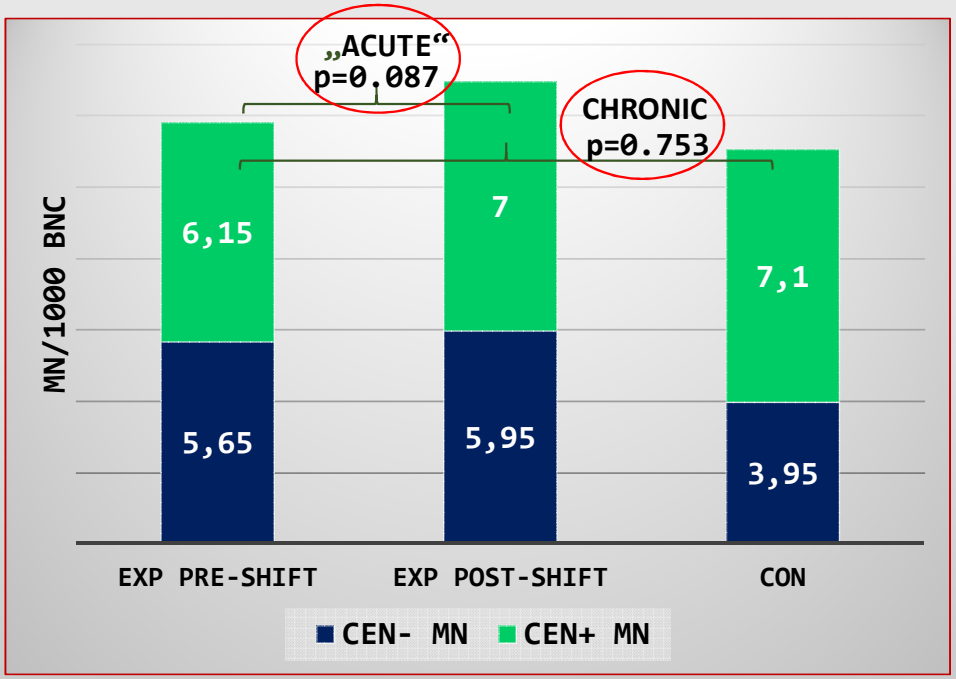
ins (Bcb)

rcp (Bc)(Cb)

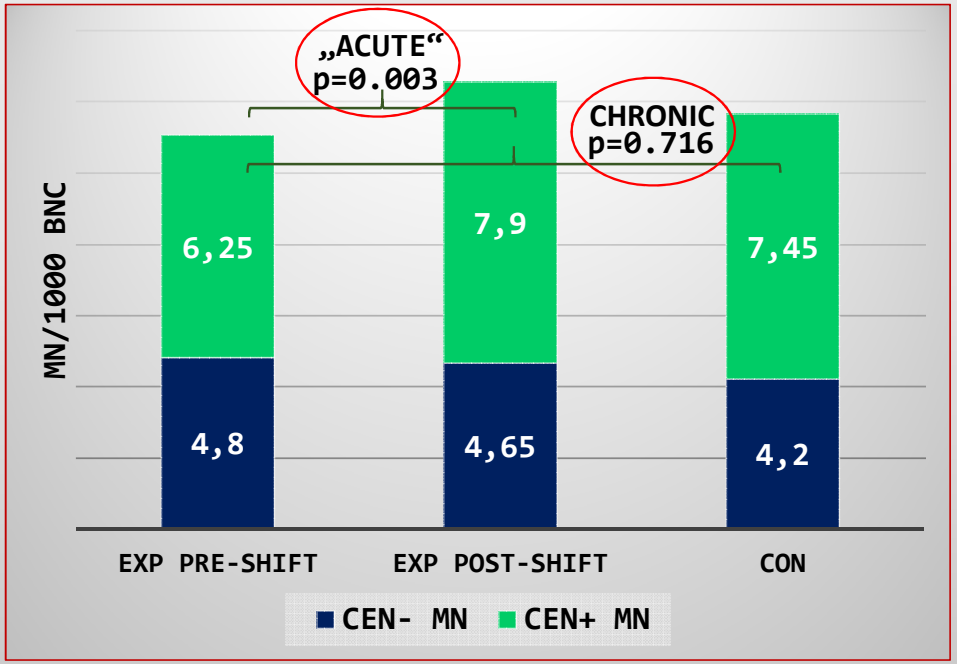
Part II: Cytogenetic part of the study

Results, MN frequency

Absolute values of **total**, CEN+ and CEN- MN/1000 BNC
2016



Absolute values of **total**, CEN+ and CEN- MN/1000 BNC
2017



- In contrast to acute exposure, chronic exposure to NP does not affect the frequency of total **MN.**
- **Gender**-related DNA damage differences were observed by MN analysis → detail WCP FISH.

Rossnerova, A. et al. Mutagenesis **2019**, 34, 253-256.

Rossnerova, A. et al. In NANOCON **2018**, 554-559.

Part II: Cytogenetic part of the study

Rossnerova, A. et al. In *NANOCON* **2019**, in press.

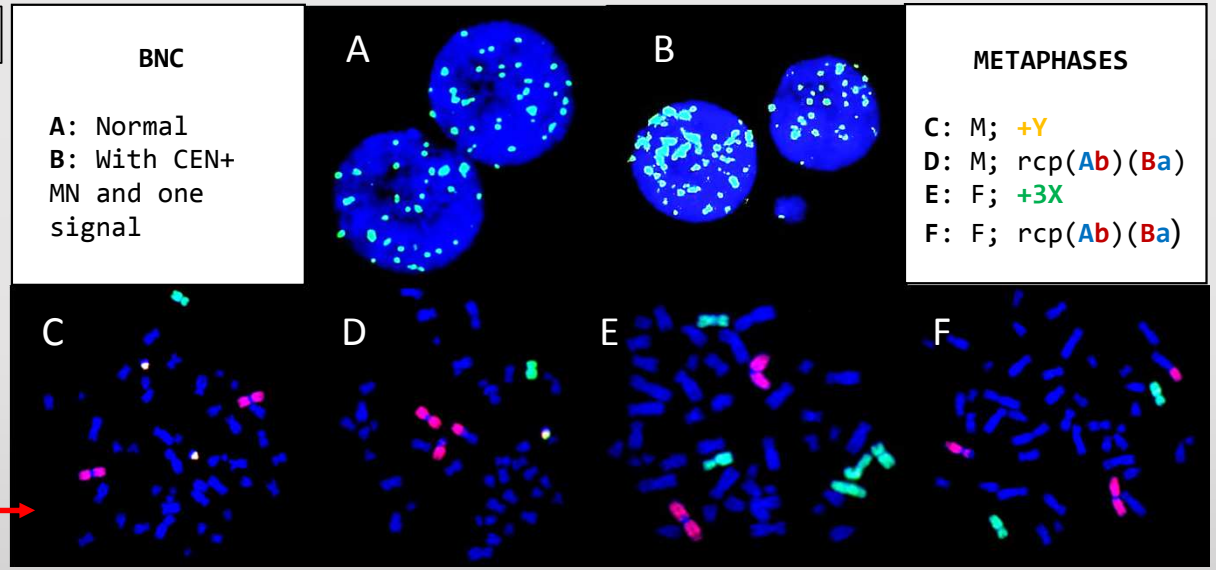
Results, WCP FISH

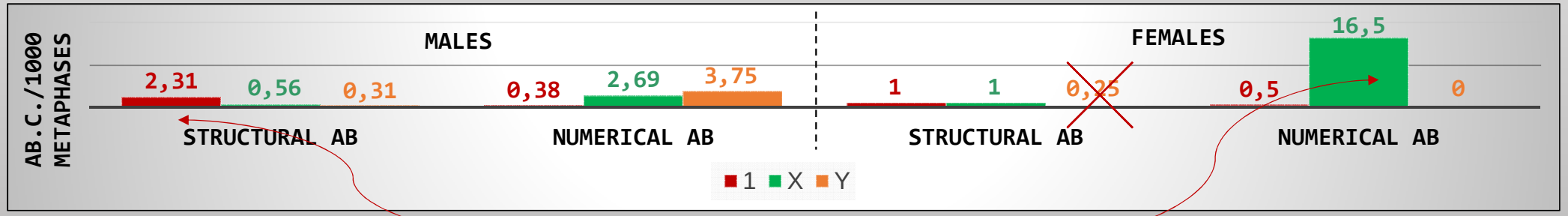
- **Gender**-related DNA damage differences were observed by to MN analysis → detail WCP FISH.

Total, CEN+ and CEN- MN/1000 BNC in males and females

Year / Gender	N	Total	p	CEN+	CEN-
2016					
Males	30	9.70	0.002	5.03	4.66
Females	11	16.09		11.00	5.09
2017					
Males	26	10.15	0.018	6.23	3.92
Females	14	13.57		8.00	5.57

Examples of cytogenetic findings analyzed by MN test with Pan-centromeric FISH (A-B) and by WCP FISH (C-F)



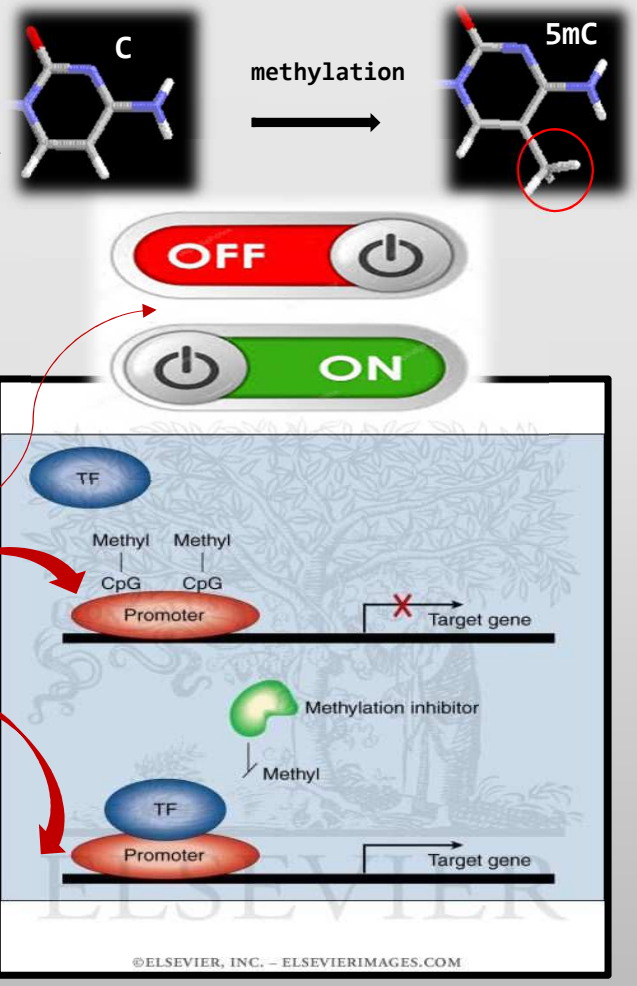


- Structural ab. – dominant in males (autosomes) x numerical ab. – dominant in females (gonosomes).

Part III: Epigenetic part of the study

1. Background and methods – DNA methylation

- **4 basic bases** in DNA (A, T, C, G).
- Crucial role of **cytosine in epigenetics**!
- Cytosines in CpG dinucleotides can be methylated to **form 5-methylcytosines**.
- Roughly **28 million** CpG sites in human genome.
- 40% is located **in promoters** of genes, commonly in clusters (CpG islands).



Methylation of cytosines in CpG sites of DNA is linked to **control of gene functions**

↑ methylation in promoter = ↓ gene expression
↓ methylation in promoter = ↑ gene expression

DNA methylation is an important mechanism in prenatal programing of genes affected by environmental stressors.

GLOBAL DNA METHYLATION (quantitative)

GENE-SPECIFIC DNA METHYLATION (qualitative)

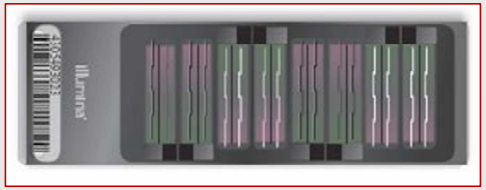
I. RT-qPCR MethyLight or Taqman assay

II. Array – Illumina Infinium Human Methylation BeadChips
- 27K (27 000+ CpG)-0.1%
- 450K (485 000+ CpG)-1.7%
- 850K (850 000+ CpG)-3%

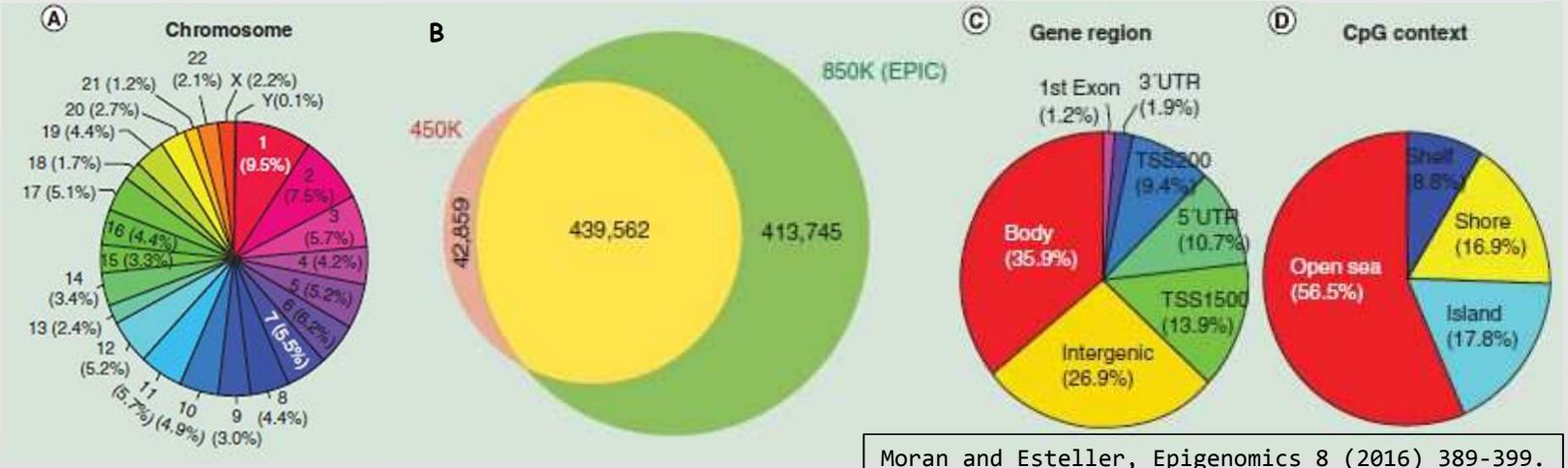
III. Whole-Genome Bisulfite Sequencing

Part III: Epigenetic part of the study

2. iScan system



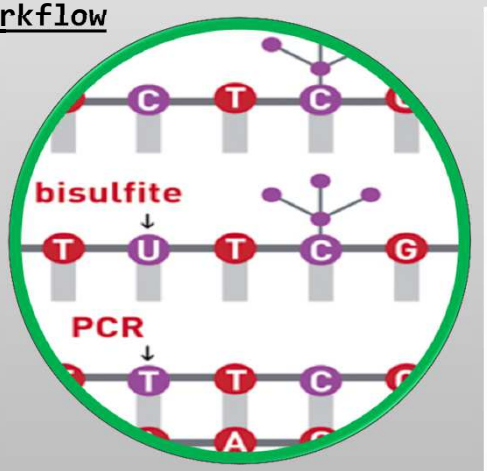
- **850K EPIC arrays**

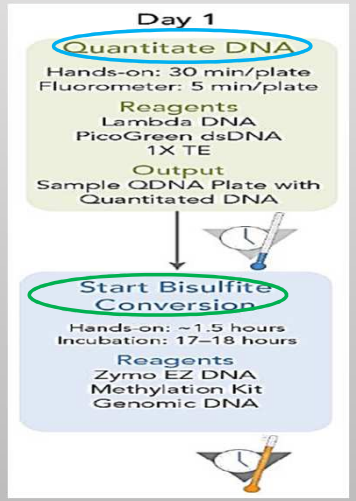


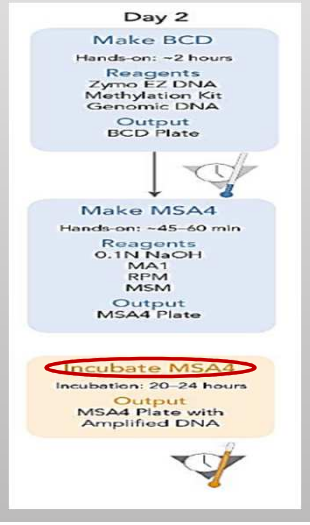
Moran and Esteller, Epigenomics 8 (2016) 389-399.

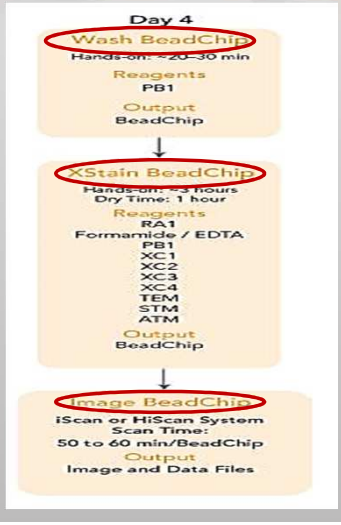
- **4 DAY Illumina workflow**

DNA isolation
Quantification
BS conversion
Fragmentation
Precipitation
Resuspension
Hybridization
Washing
Staining
Scanning
+
(Bioinformatics)







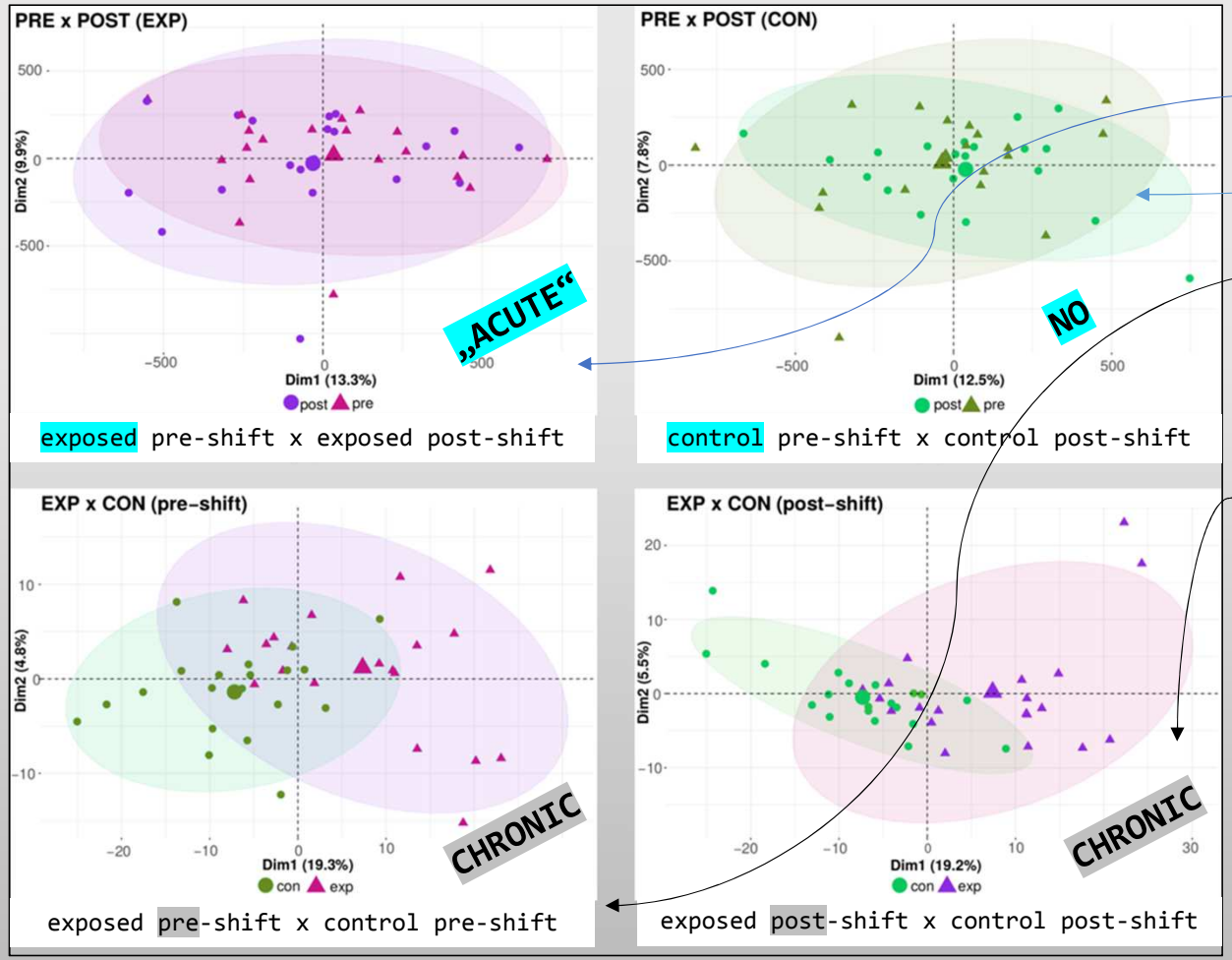


Part III: Epigenetic part of the study

Results 1

Rossnerova, A. & Honkova, K. et al.
IJMS **2020**, 21, 2420.

DNA methylation profiles in **groups** of workers occupationally exposed to nanoparticles and controls (**2018**)



- Acute (short-term, daily) exposure **is not accompanied** by DNA methylation pattern changes as well as in controls.
- Chronic (long-term, years) exposure **is accompanied** by DNA methylation pattern changes in exposed subjects.

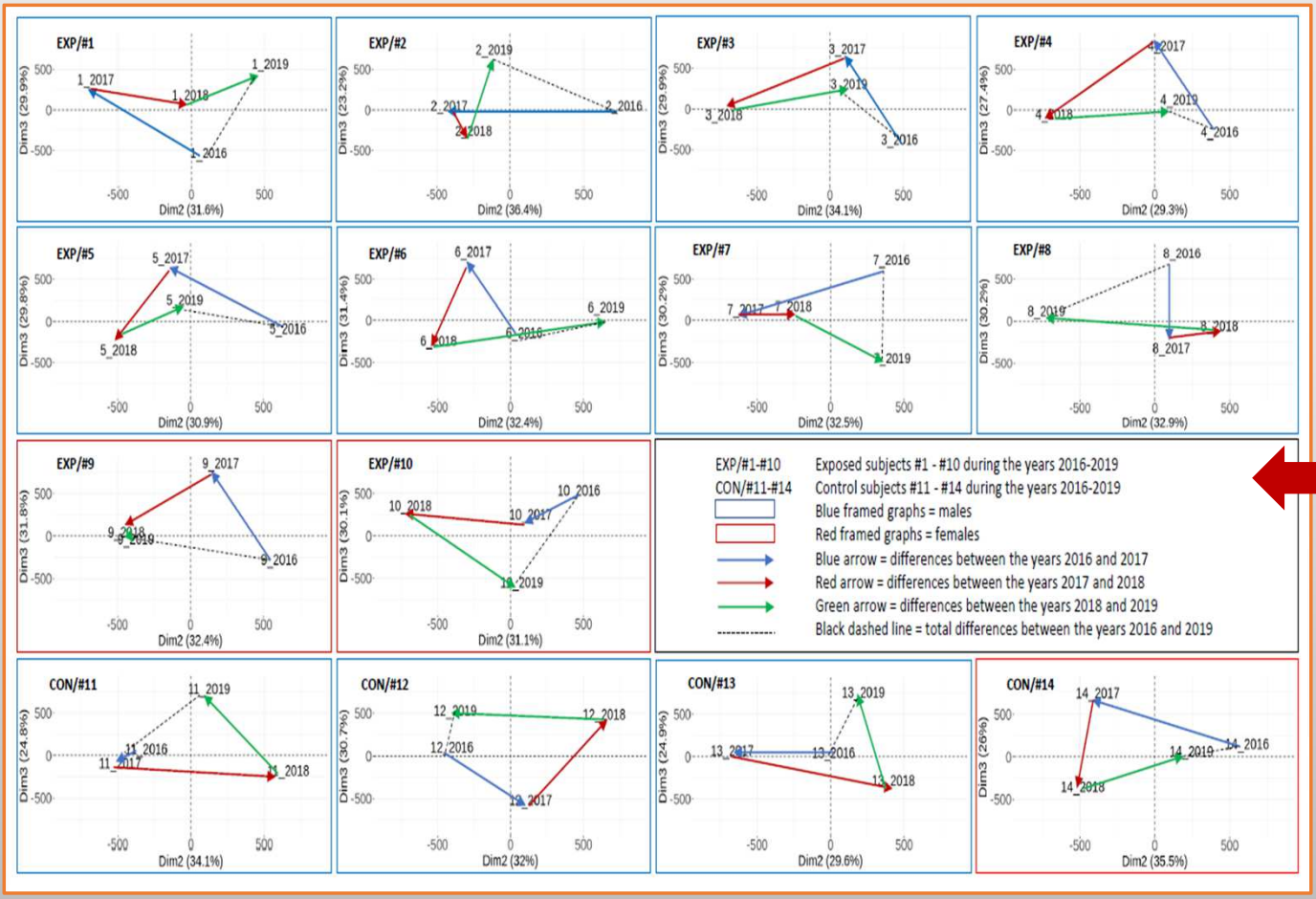
- Significant differences in methylation after long-term exposure included: **341 CpG loci hypomethylated and 364 hypermethylated.**
- 14 most significant CpG differences were detected in genes involved in lipid metabolism, the immune system, lung functions, signaling pathways, cancer development and xenobiotic detoxification.

Part III: Epigenetic part of the study

Results 2

Rossnerova, A. et al. IJMS **2021**, 22, 7834.

Individual DNA methylation profiles in 10 workers exposed to nanoparticles and 4 controls (**2016-2019**)



- The results show the **shift in DNA methylation pattern during the years,** in all the exposed and control subjects.
- The overall **range of differences varied** between the years in individual persons.
- The **differences** between the first and last year of examination (a three-year period) **were 72% greater in the NP exposed** subjects, in comparison with the controls (850K CpG).
- The **differences** between the first and last year of examination (a three-year period) **were 60% greater in the NP exposed** subjects, in comparison with the controls (**705 CpG**).

Part III: Epigenetic part of the study

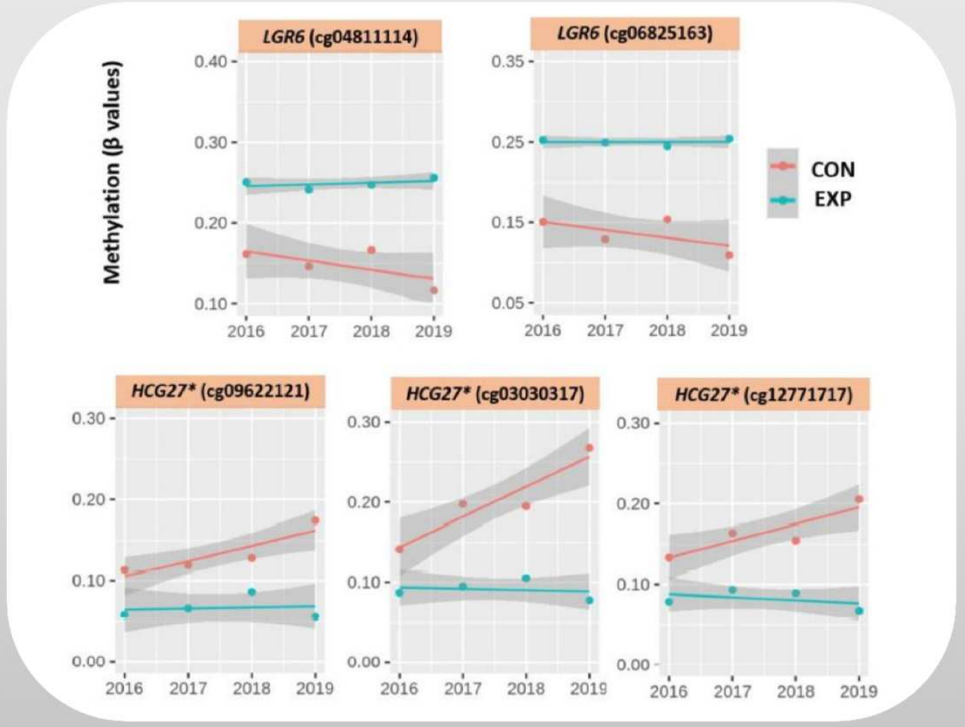
Rossnerova, A. et al. IJMS **2021**, 22, 7834.

Results 2

Individual DNA methylation profiles in 10 workers exposed to nanoparticles and 4 controls (**2016-2019**)

- The selected **14** most differently methylated cg loci **were relatively stable in the exposed** subjects. (The **differences** between the first and last year of examination (a three-year period) **were 16% lower in the NP exposed** subjects, in comparison with the controls (**14 CpG**).

- Specific type of long-term exposure can contribute to the **fixing of relevant epigenetic changes** related to NP inhalation (**adaptation**).



Beta value trajectories of the exposed and control subjects in four consecutive years for 5 CpG loci with significant beta value differences.

Part IV: Results and theory of adaptation

Rossnerova, A. et al. Mutation Research **2017**, 773, 188-203.

Previous data

Theory of adaptation of humans was first time **formuled** based on the data obtained during 10 years **air pollution** biomonitoring research in the Czech Republic.

- Aim: To review the results of cytogenetic and -omics studies with the aim to find the meaningful interpretation of the surprising, sometimes opposite results.

- Results: The reaction of the human body to the short-term and long-term air pollution exposure varied depending on previous exposure history.

Theory suggested the **epigenetic adaptation to long-term chronic exposure** that should protect our DNA (decreased DNA damage levels) and memorize the events by DNA methylation settings in case of future re-exposure.

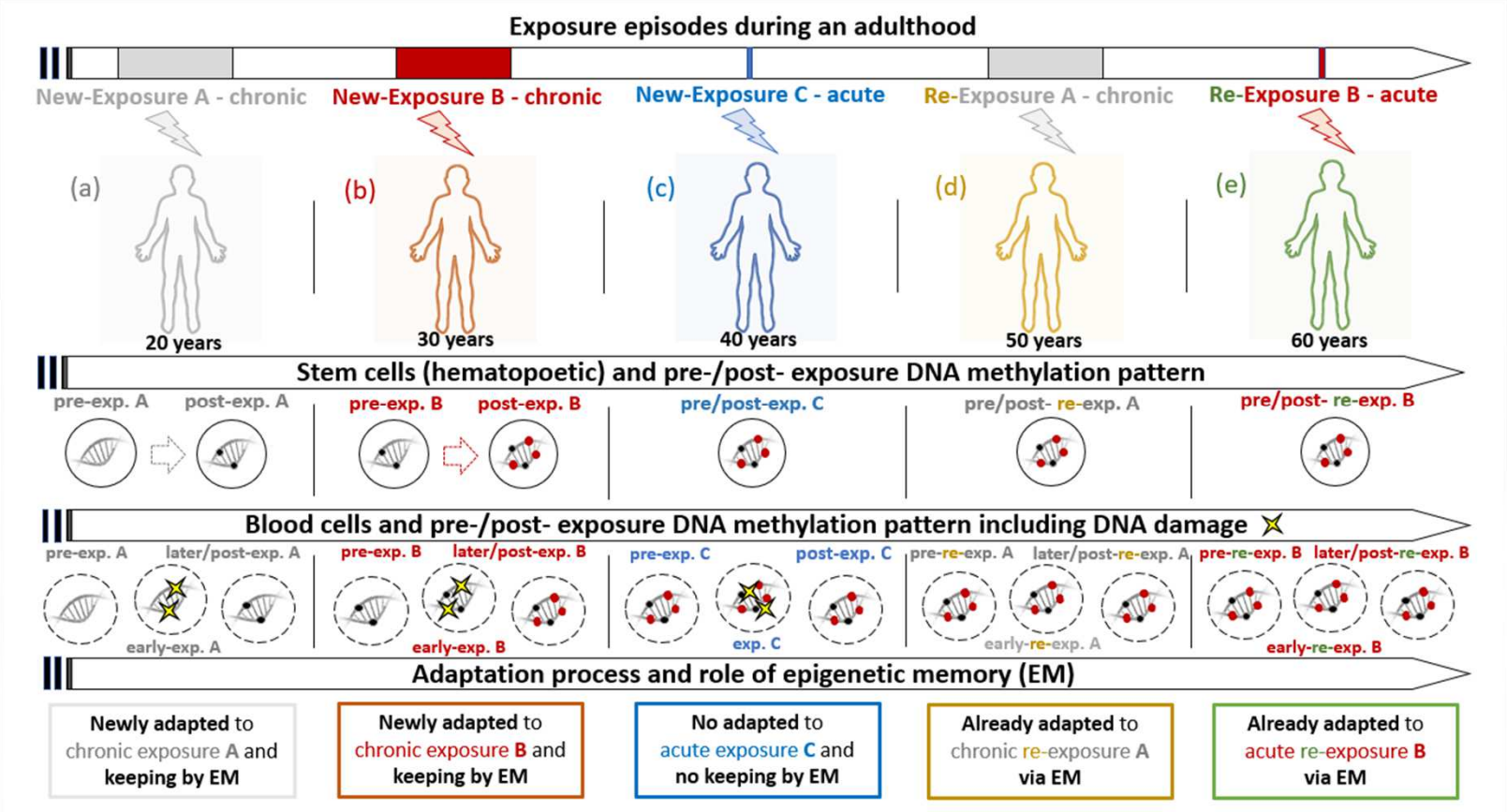
Versatility of the theory for various environmental stressors ?

Part IV: Results and theory of adaptation

New data

Rossnerova, A. et al. IJMS **2020**, 21, 7053.

The model of the process of adaptation to environmental exposure and its "storage" by epigenetic memory (EM) in stem cells.



Different exposure scenarios during the life and their consequences:

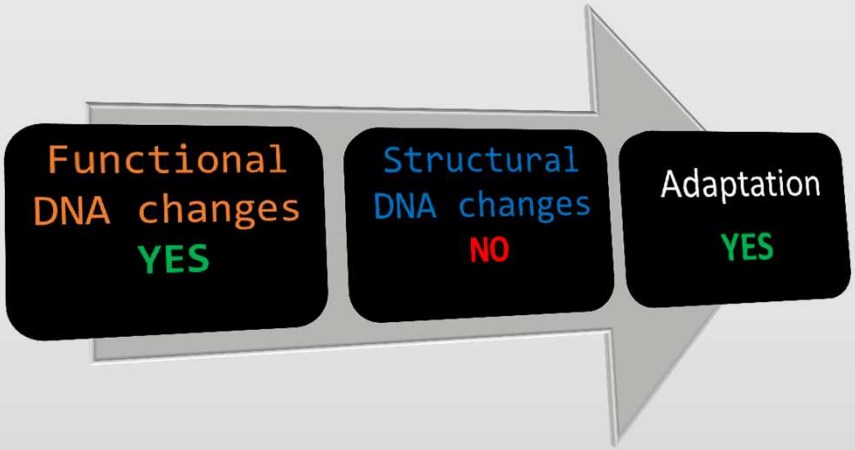
- (a) **New** adaptation to chronic exposure A and preservation by EM.
- (b) **New** adaptation to chronic exposure B and preservation by EM.
- (c) **No** adaptation to acute exposure C and no preservation by EM.
- (d) **Already** adapted to chronic re-exposure A via EM.
- (e) **Already** adapted to acute re-exposure B via EM.

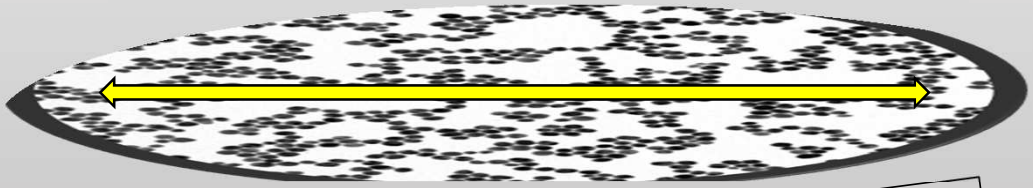
Versatility ? - YES

Part IV: Results and theory of adaptation

General conclusions

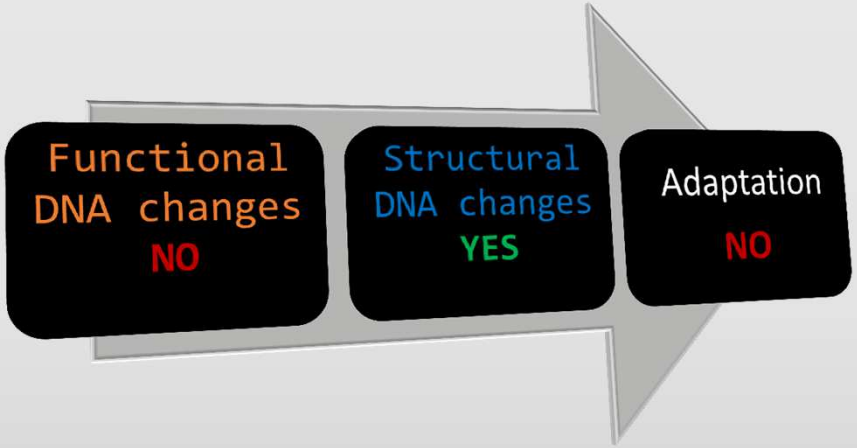
Long-term (chronic) exposure to NPs





- Long-term (chronic) exposure to NPs contributes to epigenetic changes/adaptation followed by reduction of DNA damage.

Short-term (acute) exposure to NPs



- Short-term (acute) exposure to NPs is not associated with a substantial adaptation of DNA methylation pattern and is followed by increase of DNA damage.

Acknowledgements

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Collaborations

Sampling and biomarkers

Monitoring of exposure

Cohorts and workshops

Preparation of samples

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IKEM Institute for Clinical and Experimental Medicine

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