

# **IMPACT OF CARCINOGENIC POLYCYCLIC AROMATIC HYDROCARBON EXPOSURE TO CHILDREN RESPIRATORY MORBIDITY**



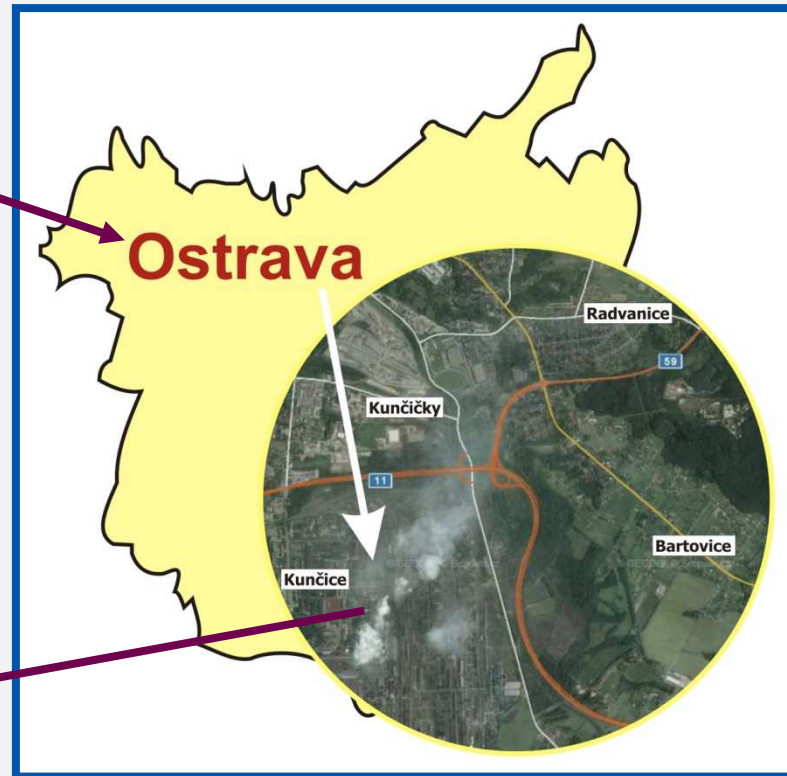
**Radim J. Sram, M. Dostal, H. Libalova, P. Rossner, Jr, E. Schallerova,  
H. Gmuender**

**Institute of Experimental Medicine AS CR, v.v.i., Prague, Czech Republic  
Genedata AG , Basel, Switzerland**



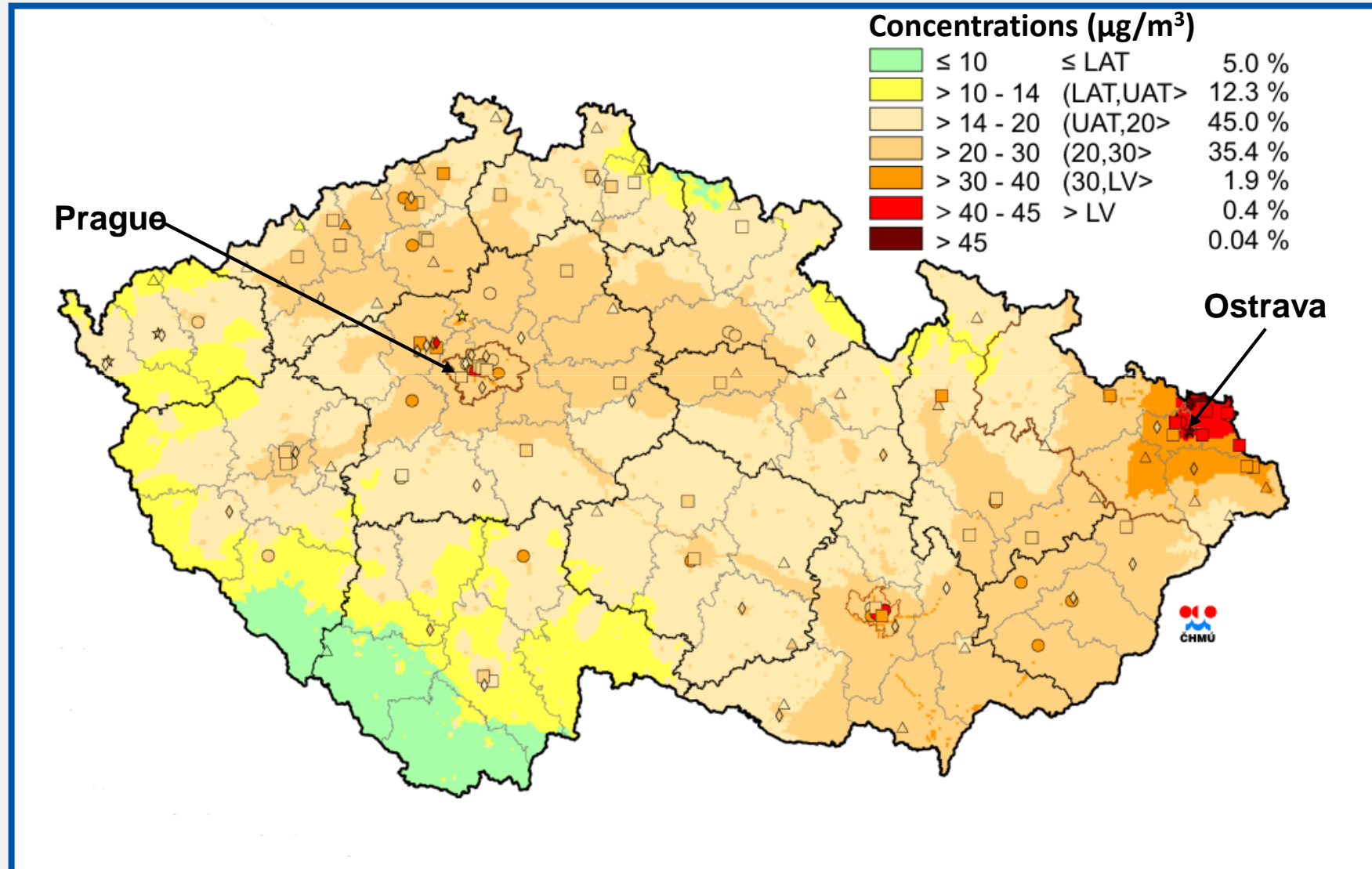
**Conference ISES-ISEE 2010  
Seoul, Korea, September 1, 2010**





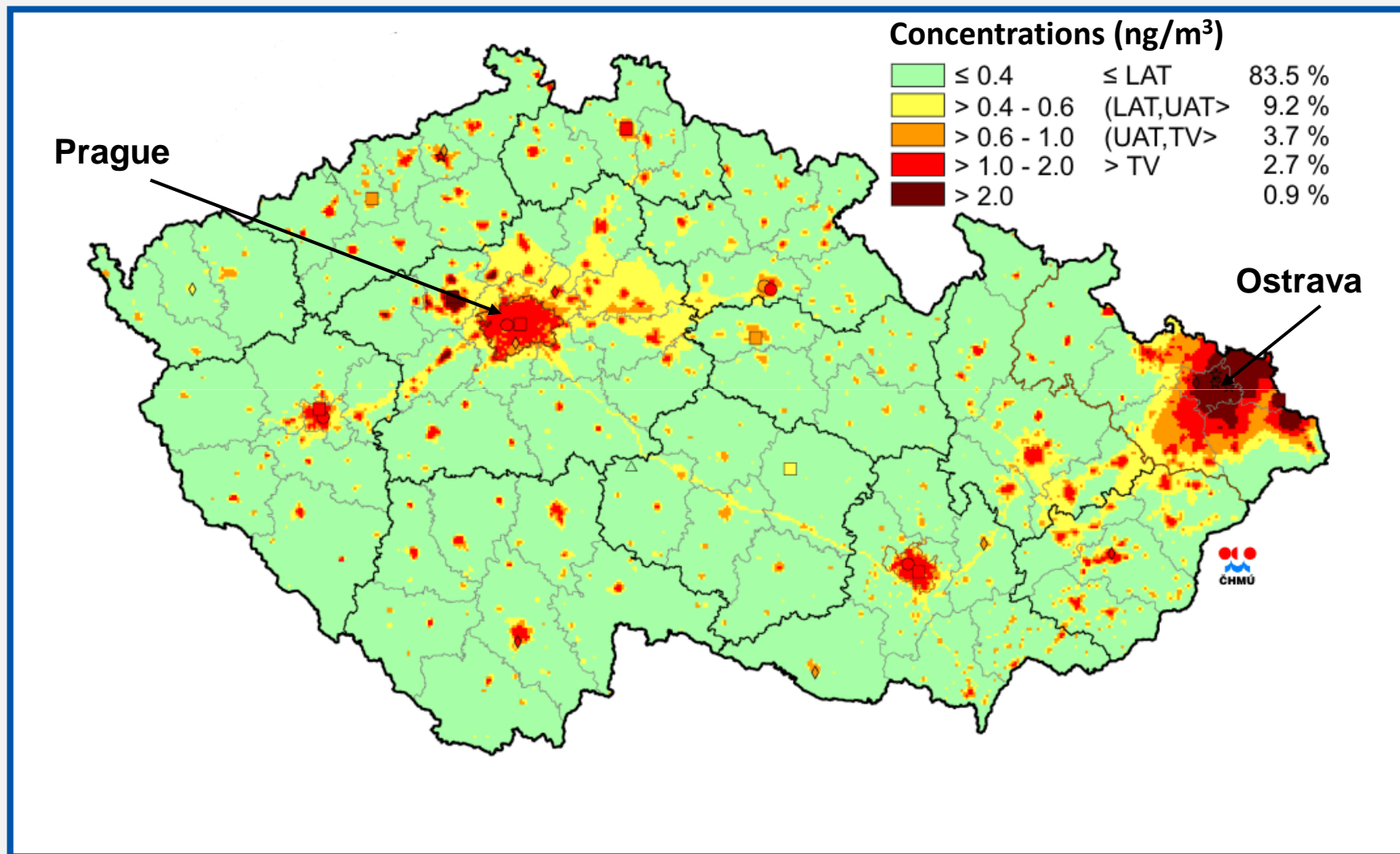


# PM10 - 2008



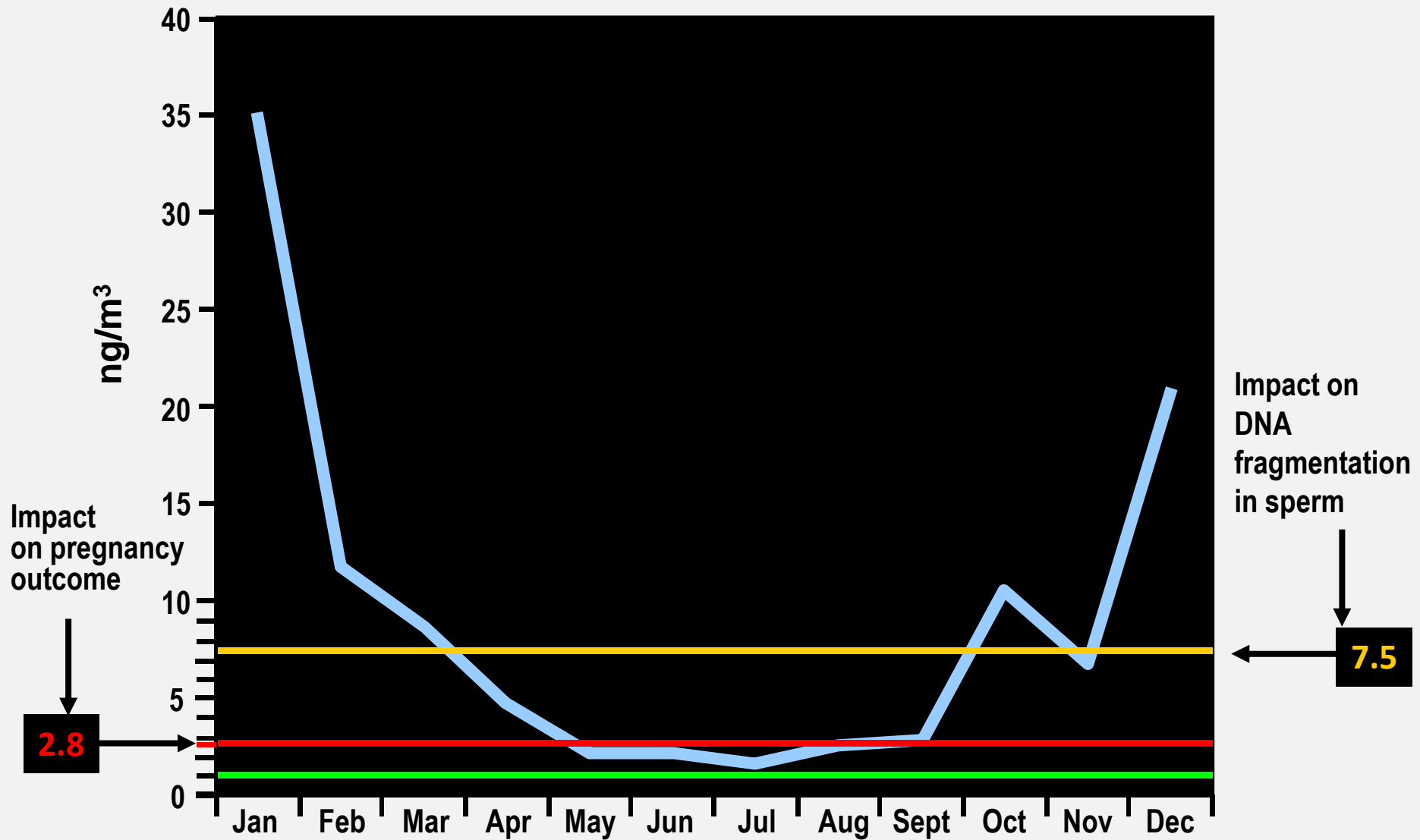


# B[a]P - 2008



# Monthly concentrations of benzo[a]pyrene

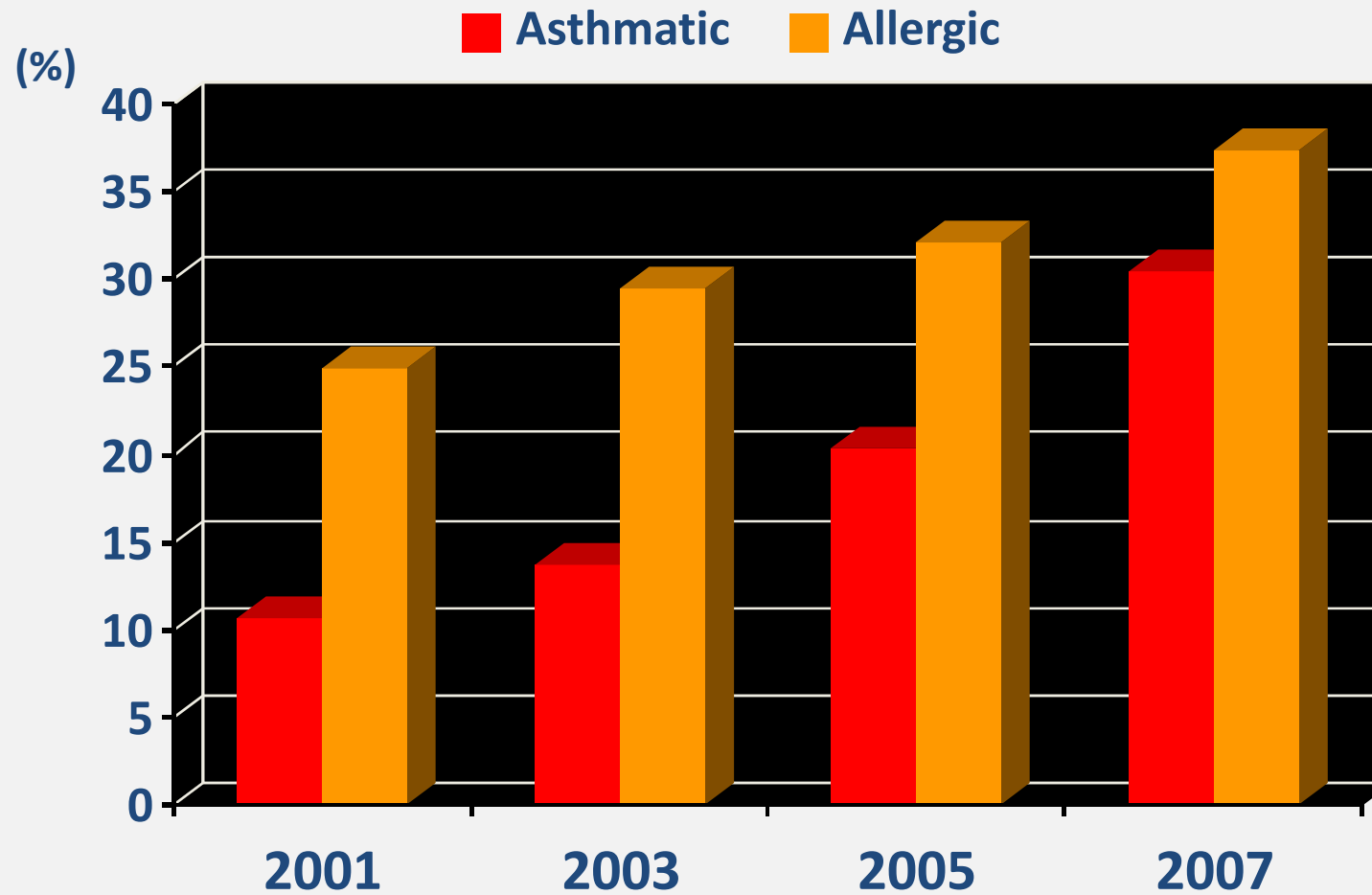
Bartovice (Ostrava) 2009





# ASTHMATIC AND ALLERGIC CHILDREN

## OSTRAVA – BARTOVICE 2001 - 2007



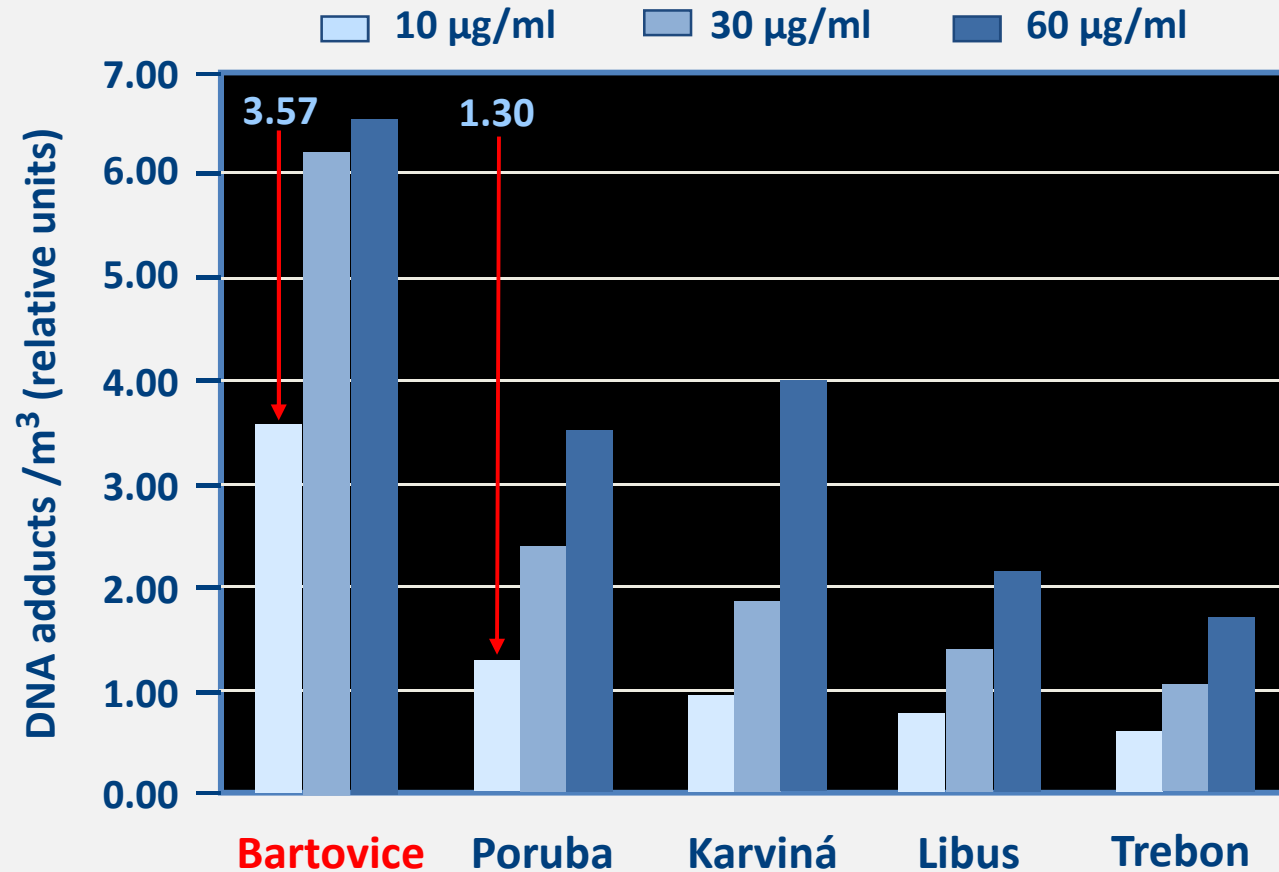


# AIR POLLUTION 2009 (CHMI)

Locality	PM10 $\mu\text{g}/\text{m}^3$	PM2.5 $\mu\text{g}/\text{m}^3$	B[a]P ng/ $\text{m}^3$
Ostrava-Poruba	34.0 $\pm$ 28.1	27.6 $\pm$ 21.1	3.3 $\pm$ 1.0
Ostrava-Bartovice	47.6 $\pm$ 42.1	35.1 $\pm$ 29.	9.2 $\pm$ 4.2
Karvina	44.7 $\pm$ 36.8	X	7.4 $\pm$ 2.0
Havirov	43.9 $\pm$ 35.9	X	X
Prague-Smichov	30.5 $\pm$ 25.1	22.1 $\pm$ 18.4	0.9 $\pm$ 0.4
Prague-Libus	25.4 $\pm$ 21.9	18.7 $\pm$ 15.9	0.8 $\pm$ 0.4
Ceske Budejovice	23.0 $\pm$ 19.7	17.4 $\pm$ 14.5	1.4 $\pm$ 0.5
Prachatice	31.8 $\pm$ 17.2	28.1 $\pm$ 9.2	1.4 $\pm$ 2.1

x - not measured

# Genotoxicity - DNA adducts (by $^{32}\text{P}$ -postlabeling)



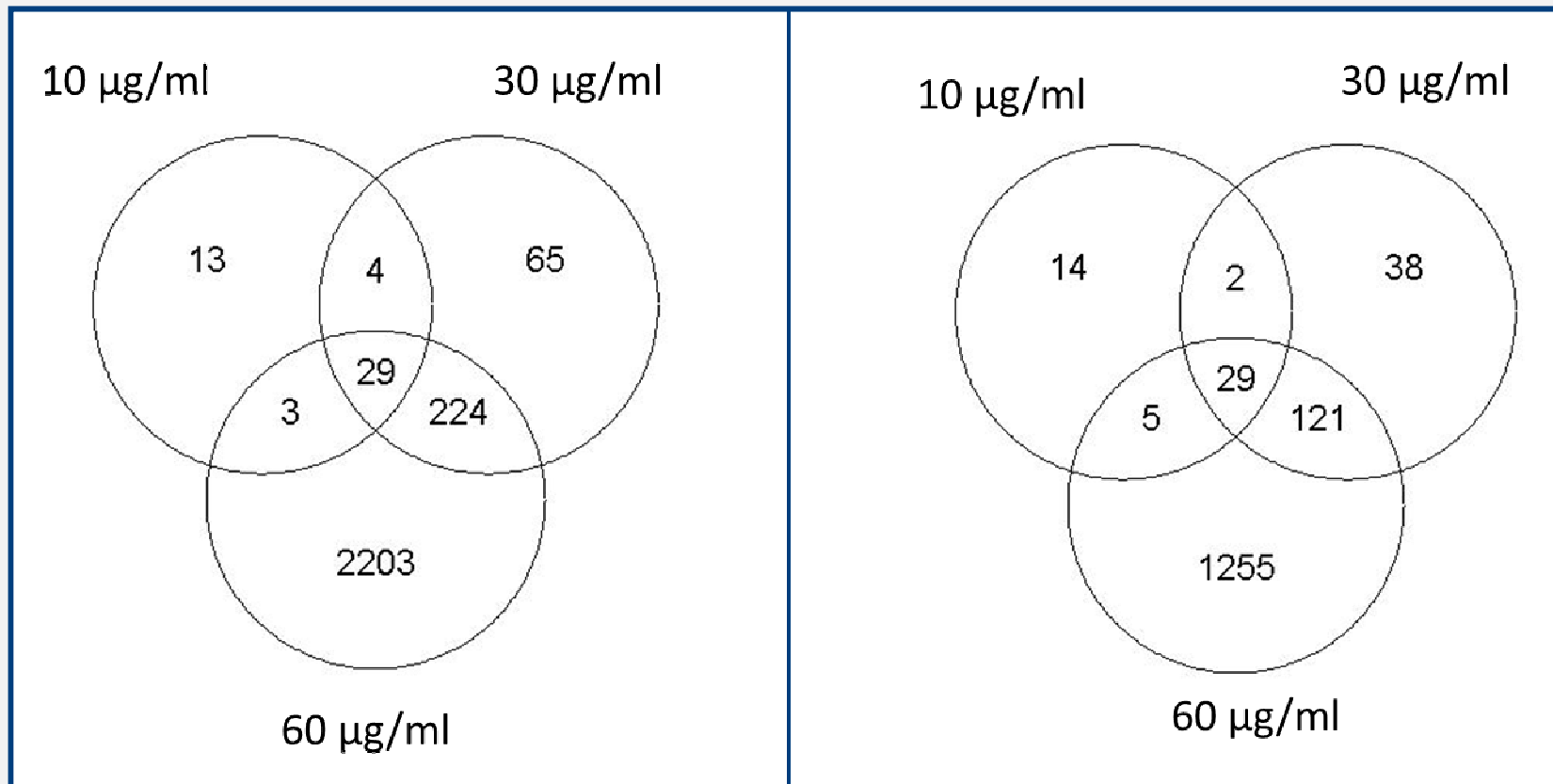
# Deregulated genes

Bartovice vs. Poruba

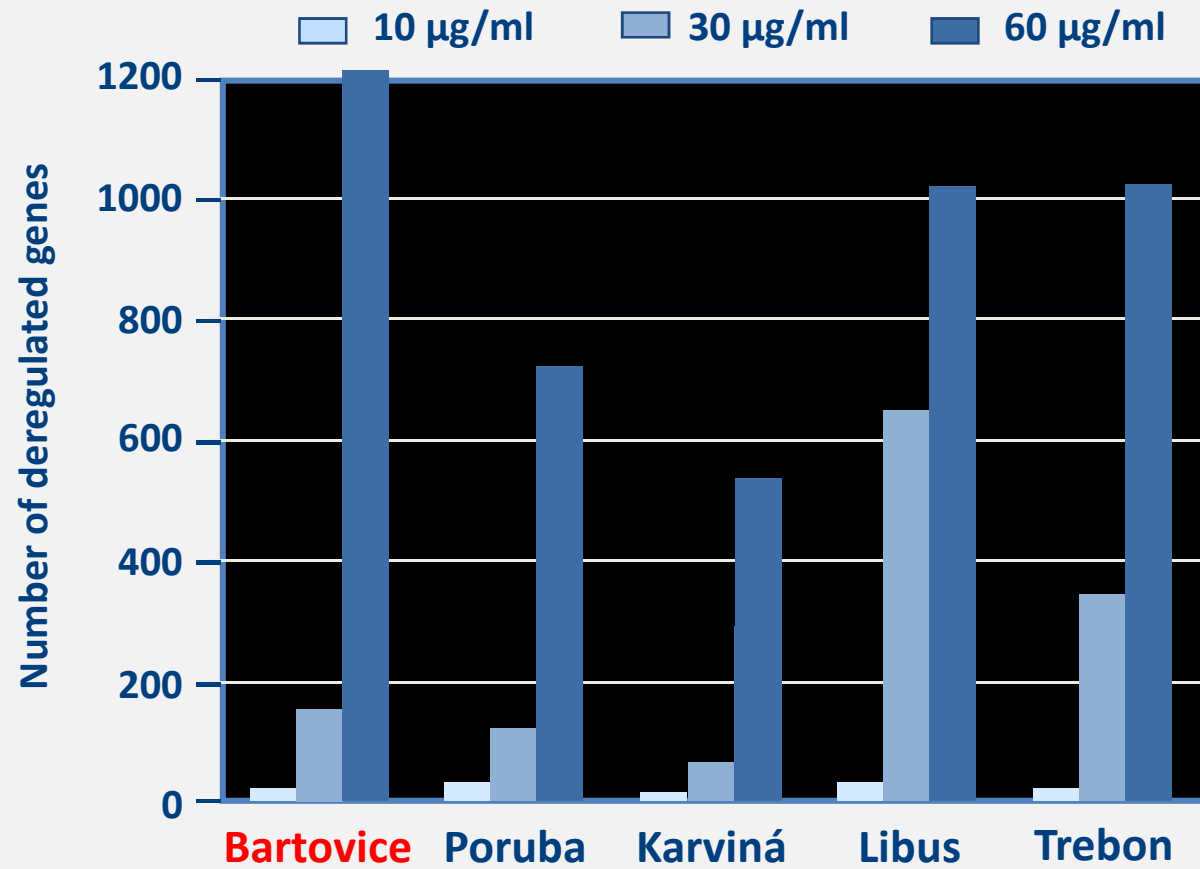
HEL cells

**Bartovice**

**Poruba**



# Deregulated genes



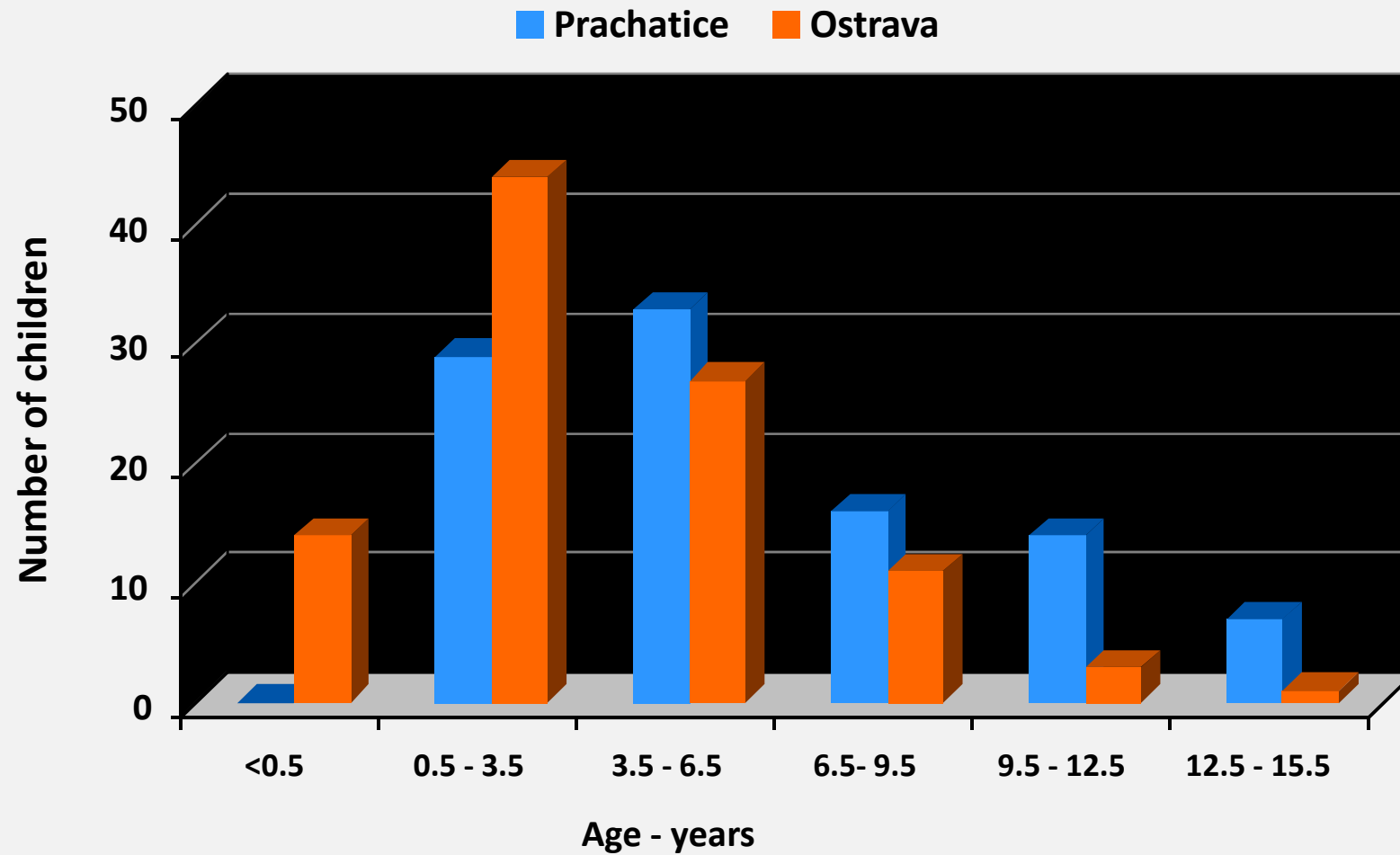


# OSTRAVA PROGRAM

## ASTHMA BRONCHIALE AND BIOMARKERS

- In the most polluted part (Ostrava-Bartovice) were selected 100 asthma bronchiale diagnosed children and 100 healthy children, aged 6-15 years.
- As matching controls were selected 100 asthma bronchiale diagnosed children and 100 healthy children, aged 6-15 years in the district of Prachatice.
- Genetic polymorphisms (768 genes-metabolism, DNA repair, immunology by Illumina chips)
- Gene expression (whole genome by Illumina chips)
- Oxidative damage: 8-oxodG, 15-F2T-isoP, proteins
- PAU-DNA adducts from saliva
- Vitamins A, C, E
- Cotinine

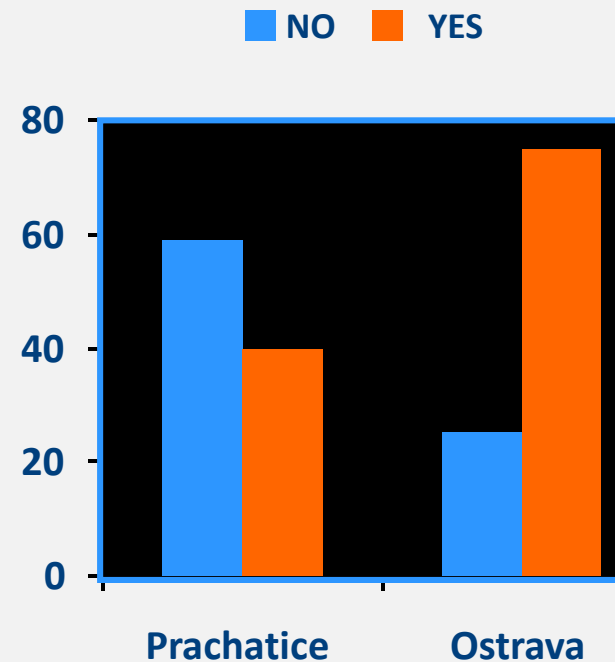
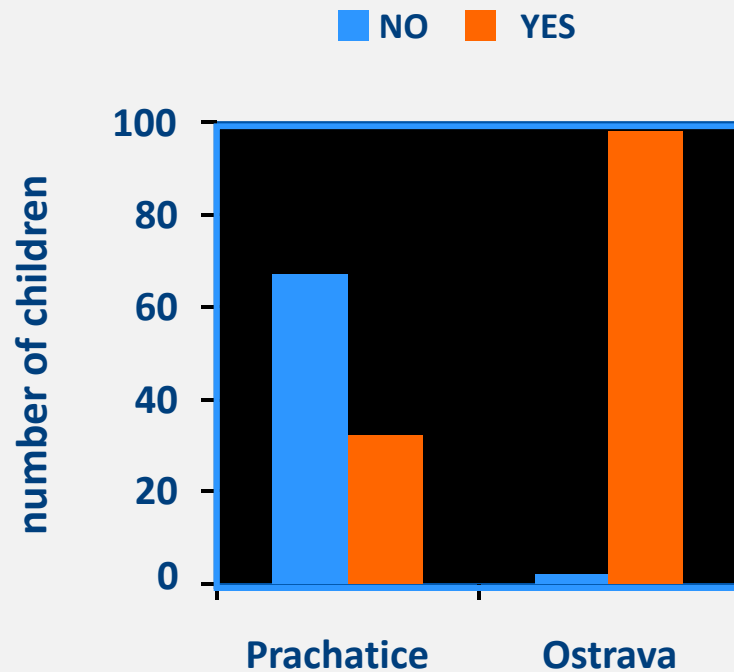
# First signs of asthma bronchiale - relationship to age



# Children with AB

Respiratory diseases  
>5x/year, age 2-5 years

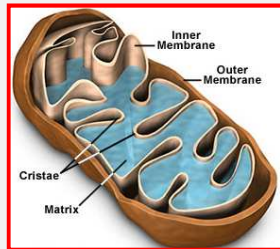
Asthma bronchiale active  
during the last 12 months



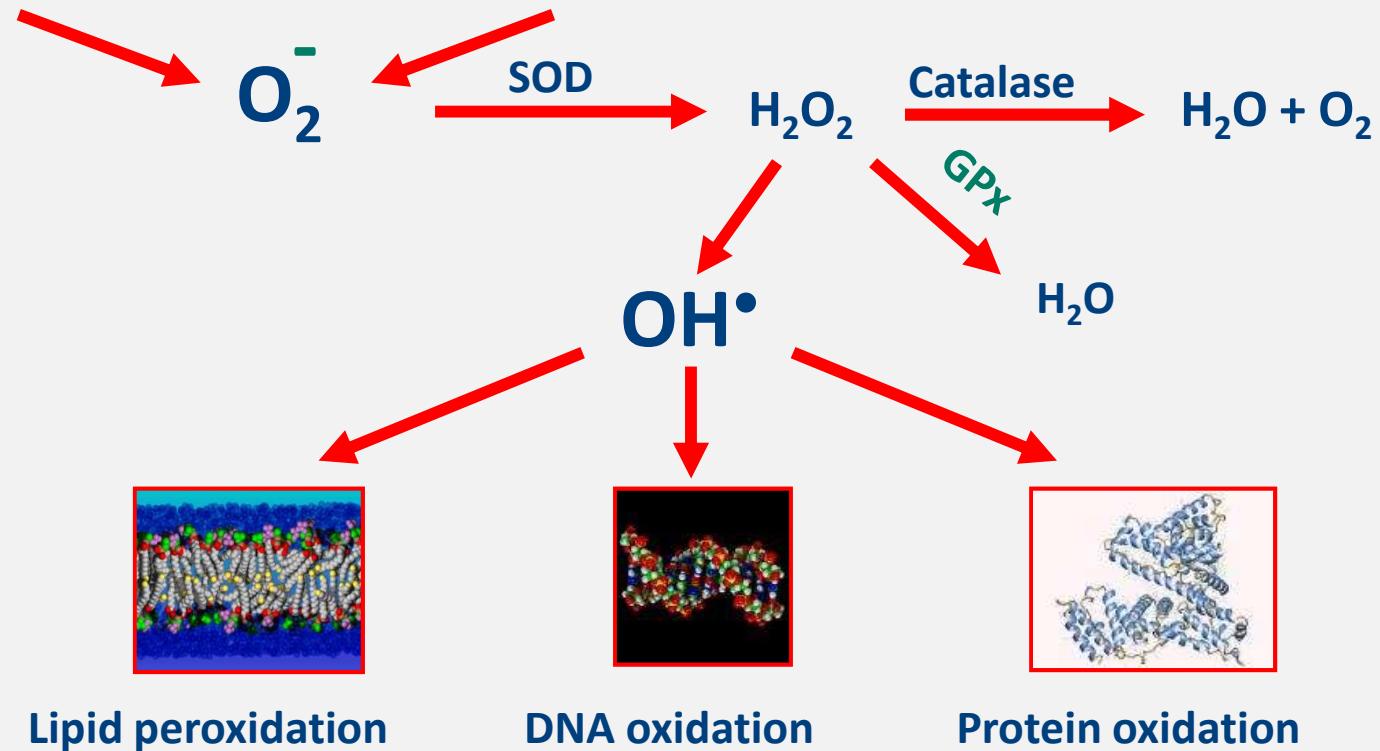


# Reactive oxygen species

## Endogenous sources

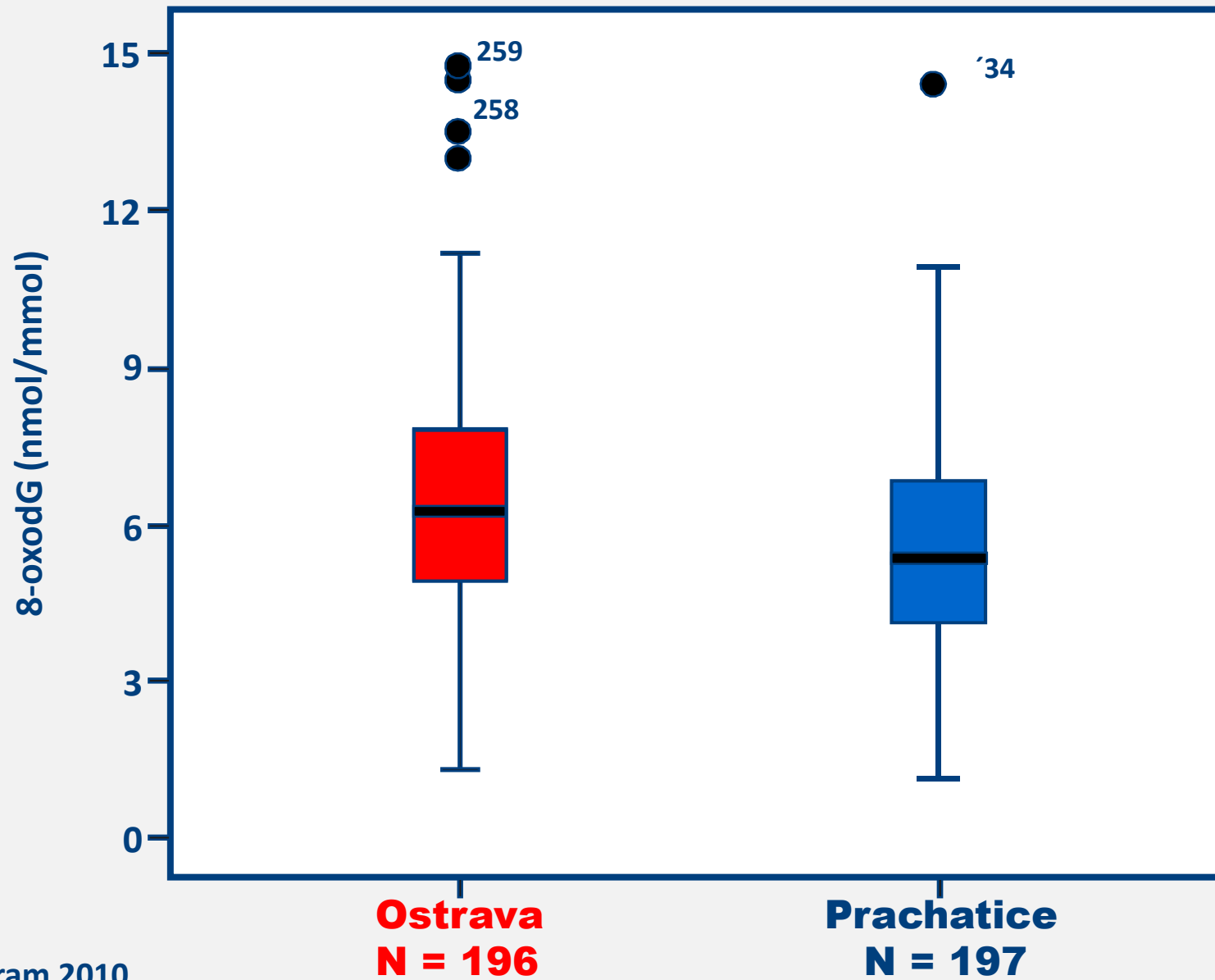


## Exogenous sources



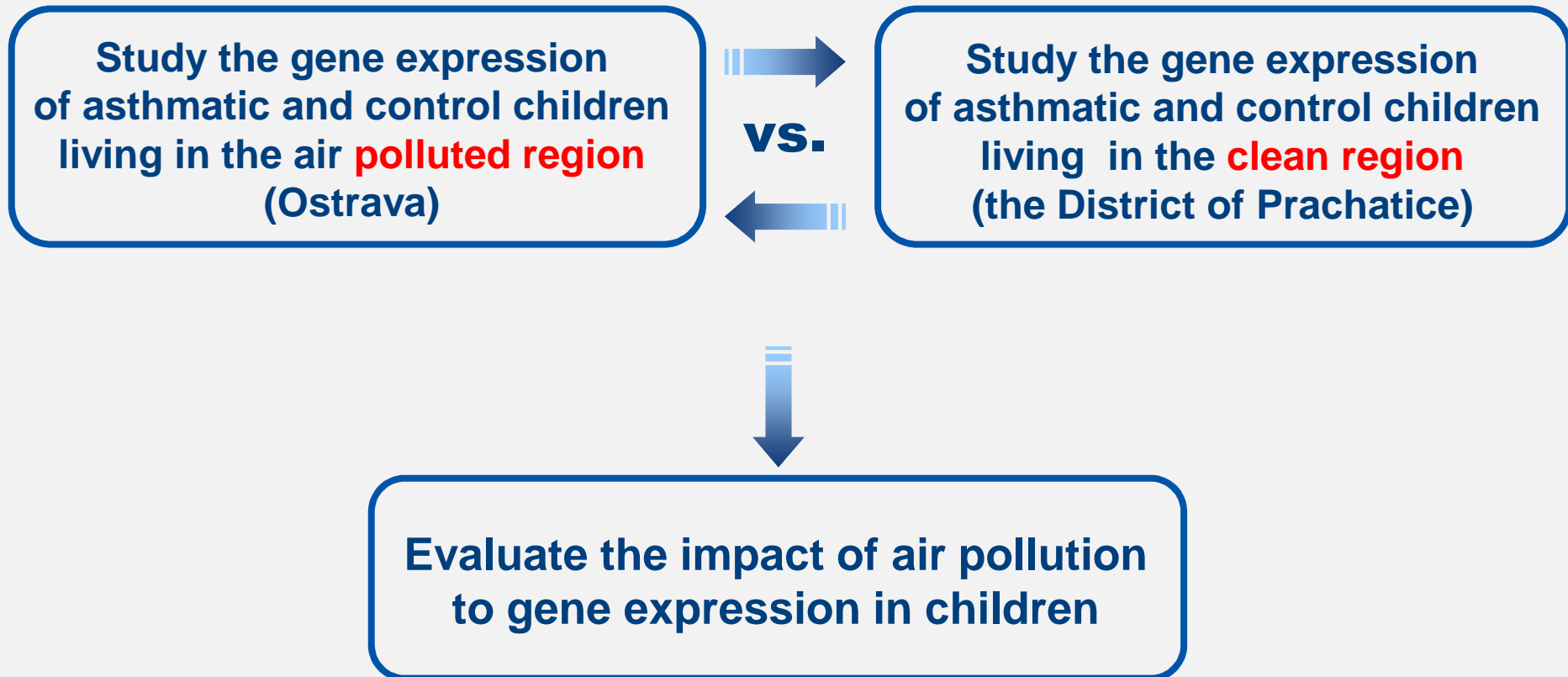
# 8-oxodG in urine

$p < 0.001$

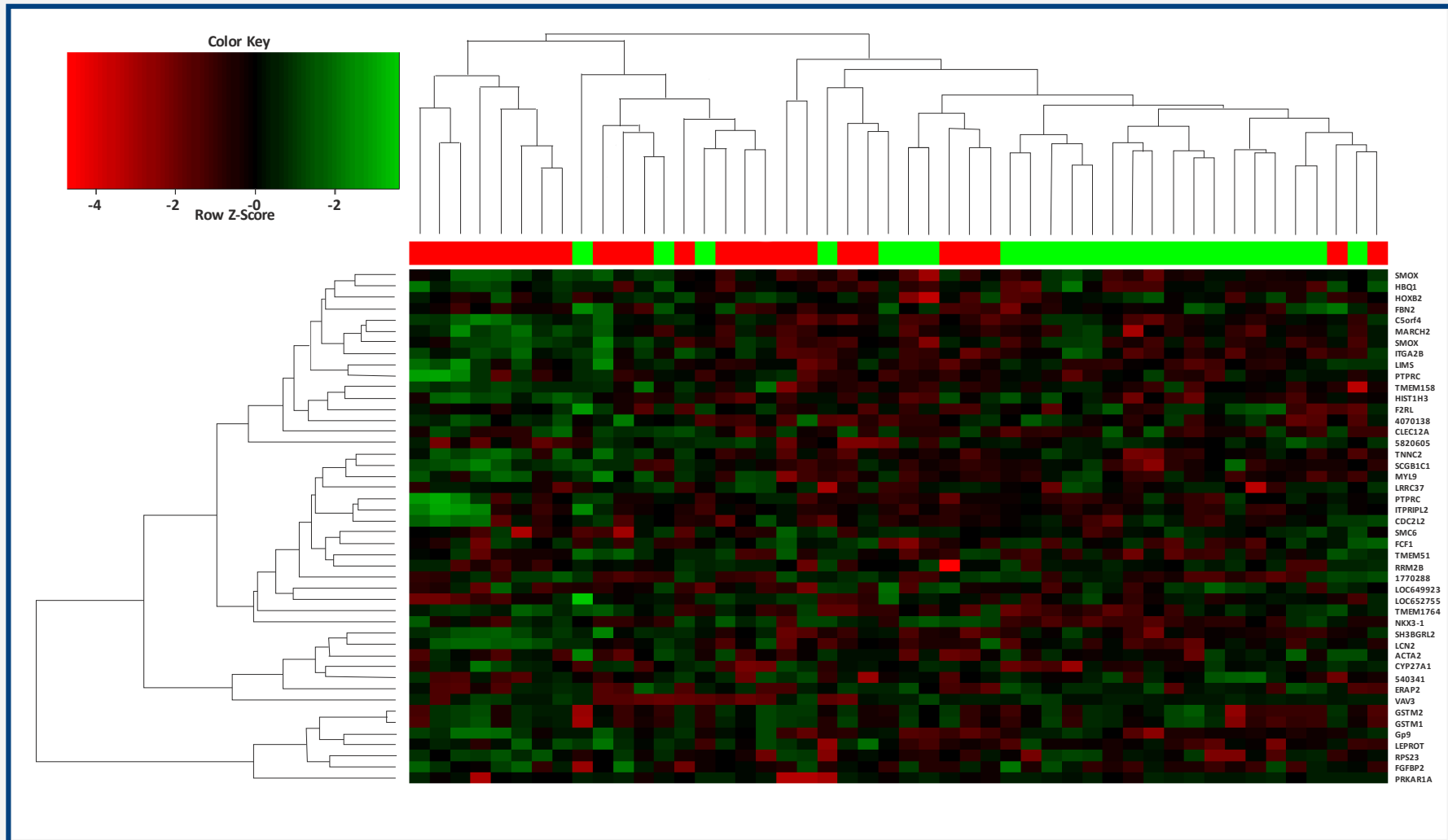




# GENE EXPRESSION



# HIEARARCHICAL CLUSTERING OF DIFFERENTIALLY EXPRESSED GENES IN ASTHMATIC (red) AND HEALTHY (green) CHILDREN SAMPLES



# Analysis of transcriptomics data from children with asthma

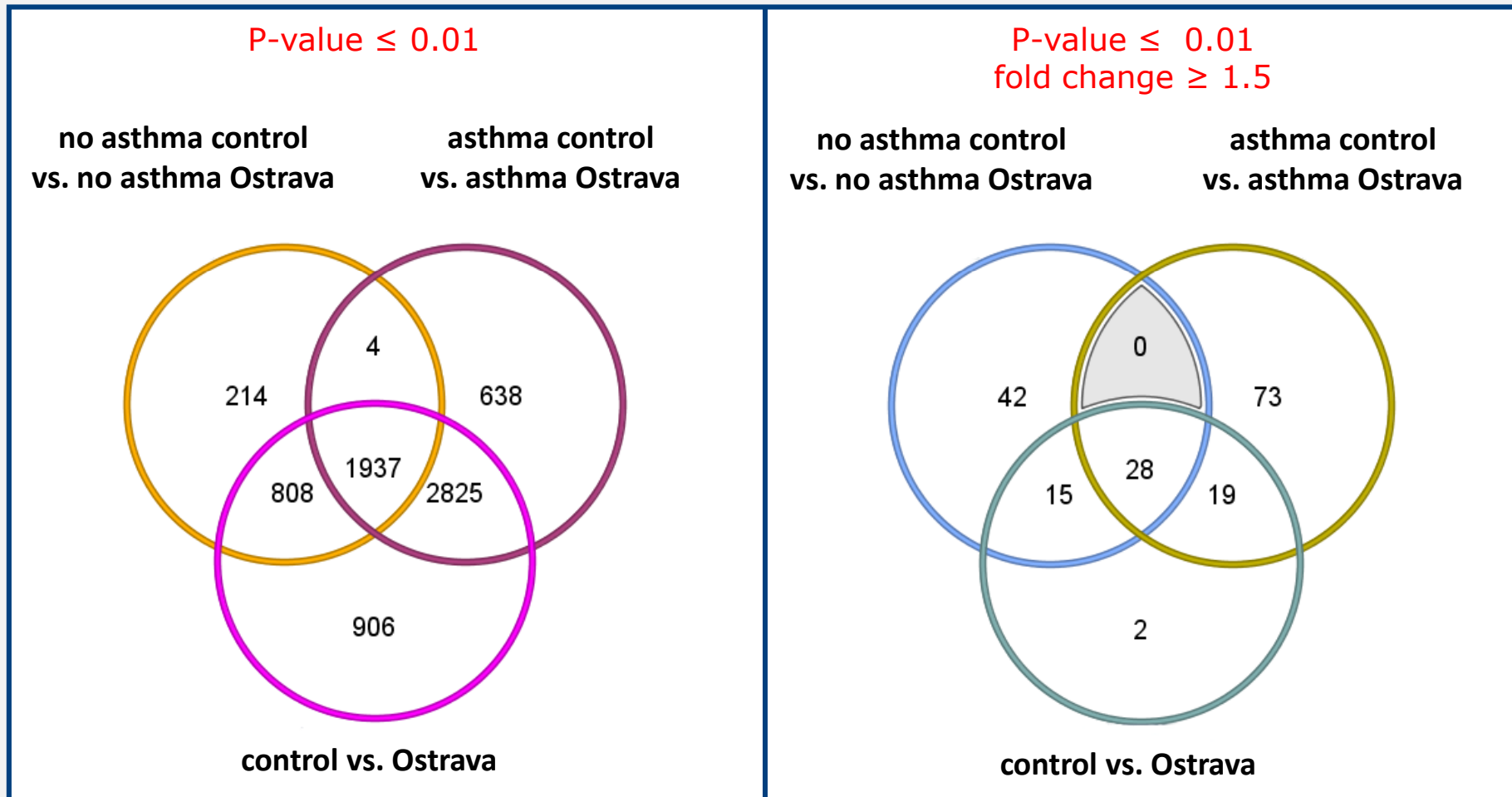
2<sup>nd</sup> report

Dr. Hans Gmuender, Scientific Consultant  
Genedata AG, Basel  
July, 2010

# Comparison of 'no asthma' or 'asthma' results from the two regions

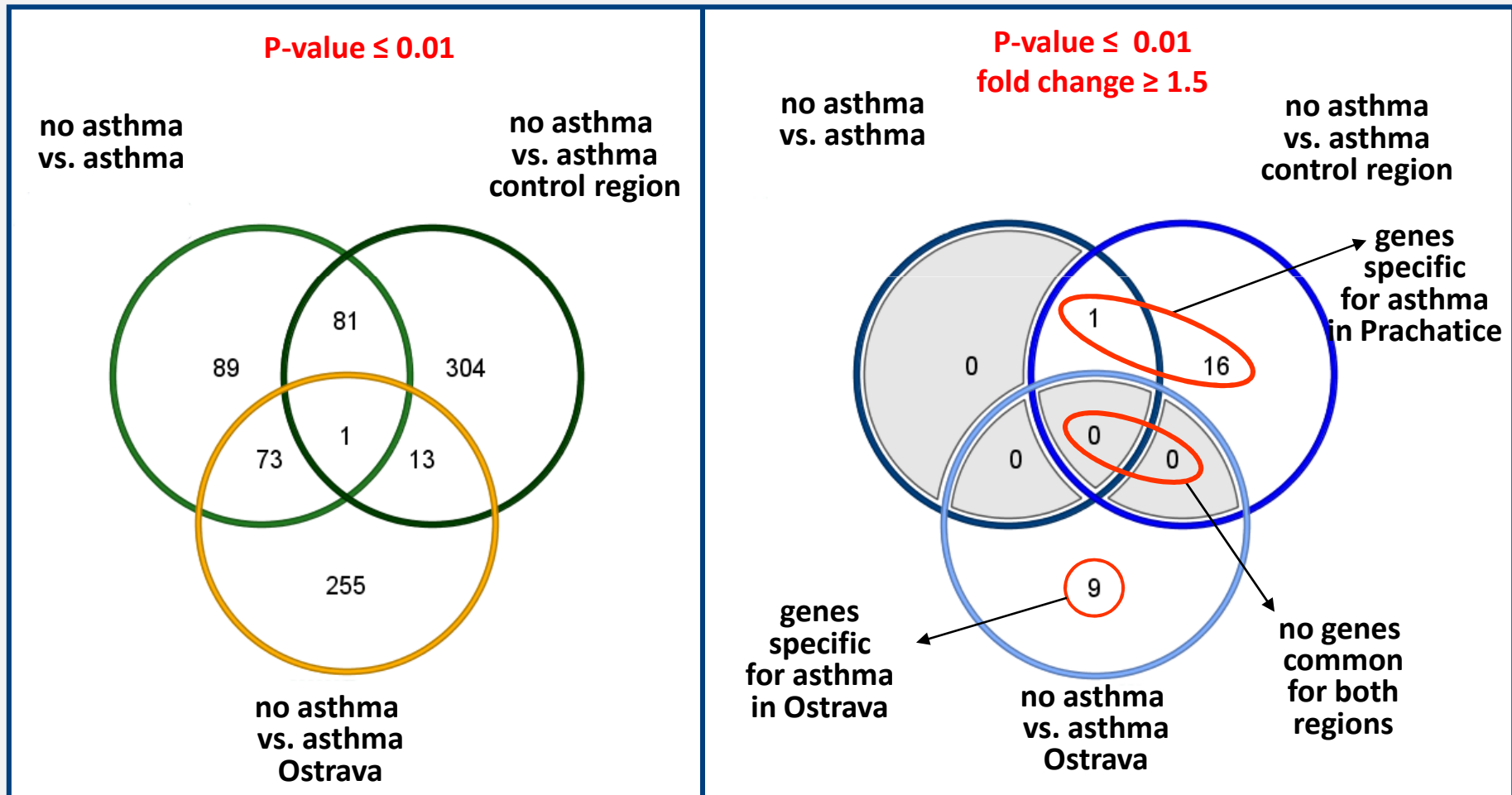
There is a significant amount of differentially expressed genes comparing the 2 'no asthma' or the 2 'asthma' groups, respectively, indicating a strong region effect

The Venn diagrams below show the number of significant transcripts obtained with these two comparisons and with the disease-independent comparison 'control' vs. 'Ostrava'



# Comparison of 'no asthma' vs. 'asthma' t-test results

In the Venn diagrams shown below, the t-test results obtained using all experiments either with a p-value cutoff of 0.01 or a p-value cutoff of 0.01 and at least a 1.5 fold change are compared



# Results of t - tests

- 368 experiments with the best RIN – determination of diferencial gene expression (t–test)
- 48 803 transcripts detected
- p-value 0.01
- Corrected for 100 repeats – Benjamini-Hochberg False Discovery Rate (FDR)
- relationship of medians between experiments (fold changes = FC) – threshold for the significant change – 1.5 times for up-regulated and 0.67 times for down-regulated genes

t-test	# transcripts p-value < 0.01	BHq-value	# transcripts p-value < 0.01 and FC>1.5
asthma vs. control	244	0.642	1
asthma Prachatice vs. control Prachatice	399	0.398	17
asthma Ostrava vs. control Ostrava	342	0.451	9
ANOVA asthma + region	5708	0.028	251
Prachatice vs. Ostrava	6476	0.024	64
control Prachatice vs. control Ostrava	2963	0.052	85
asthma Prachatice vs. asthma Ostrava	5404	0.029	120

# Control region no asthma vs. control region asthma

NAME	SYMBOL	DEFINITION	P-Value	Ratio of Medians
670010	LOC650298	PREDICTED: similar to 40S ribosomal protein S26	5.03E-03	2.14
5910424	SLC29A1	solute carrier family 29 (nucleotide transporters) member 1, nuclear gene encoding mitochondrial protein, transcript variant 4	1.13E-03	1.72
780369	SIGLEC8	sialic acid binding Ig-like lectin 8	5.28E-05	1.64
1980598	OLIG2	oligodendrocyte lineage transcription factor 2	2.38E-06	1.63
6110343	CCL23	shemokine (C-C motif) ligand 23, transcript variant CKbeta8	2.20E-04	1.61
2030142	CLC	Charcot-leyden crystal protein	5.22E-05	1.61
7570484	TFF3	trefoil factor 3 (intestinal)	1.98E-04	1.60
2470364	PRSS33	protease, serine, 33	4.34E-03	1.60
5360706	GPR82	G protein-coupled receptor 82	5.79E-04	1.58
7560138	PMP22	peripheral myelin protein 22 (PMP22), transcript variant 2, mRNA	7.43E-04	1.54
2940767	CEBPE	CCAAT/enhancer binding protein, epsilon	5.53E-04	1.53
4050678	EMR4	egf-like module containing, mucin-like, hormone receptor-like 4	8.56E-05	1.52
5080180	CACNG6	calcium channel, voltage-dependent, gamma subunit 6, transcript variant	1.23E-03	1.51
5690400	GSTTP2	glutathione S- transferase theta pseudogene 2 on chromosome 22 XM_941198 XM_945014 XM_945016	3.48E-04	0.65
6220202	ZADH1	zinc binding alcohol dehydrogenase, domain containing 1	1.34E-03	0.64
840309	LEP	leptin (obesity homolog, mouse)	7.02E-03	0.64
6040670	CYCSLI	cytochrome c, somatic-like 1 on chromosome 6	1.95E-03	0.63

## Ostrava region no asthma vs. Ostrava region asthma

NAME	SYMBOL	DEFINITION	P-Value	Ratio of Medians
2450070	LOC440345	PREDICTED: hypotheticalprotein LOC 440345, transcript variant 3	5.81E-06	3.10
1450349	LOC653158	PREDICTED: similar to hypothetical protein MGC40405, transcript variant 1	1.13E-04	2.39
1770288			2.81E-02	2.10
3060358			8.32E-04	1.89
6280138	PPM1B	protein phosphatase 1B (formerly 2C), magnesium-dependent, beta isoform, transcript variant 4	5.88E-04	1.87
4010040	HBG2	hemoglobin, gamma G	2.29E-01	1.73
2630373			2.30E-03	1.66
2350274	AHSP	alpha hemoglobin stabilizing protein	1.01E-05	1.65
6550164	DEFA4	Defensin, alpha 4, corticostatin	1.42E-02	1.63
3310484	C7orf54	chromosome 7 open reading frame 54	7.81E-04	1.60
430605	TMEM137	PREDICTED: transmembrane protein 137	1.88E-04	1.53
6510170	IFIT3	Interferon-induced protein with tetratricopeptide repeats	2.72E-03	0.60

The 3 transcripts detected in addition using linear models are highlighted yellow

# Linear models

For all potential confounding or interacting factors a two-factor model was applied:

-y (expression of a single gene) ~ disease + confounding factor

Only the transcripts showing at least 50 % valid values per group, with p-values below 0.01, and at least 1.5 fold changes between 'no asthma' and 'asthma' were considered to be significant

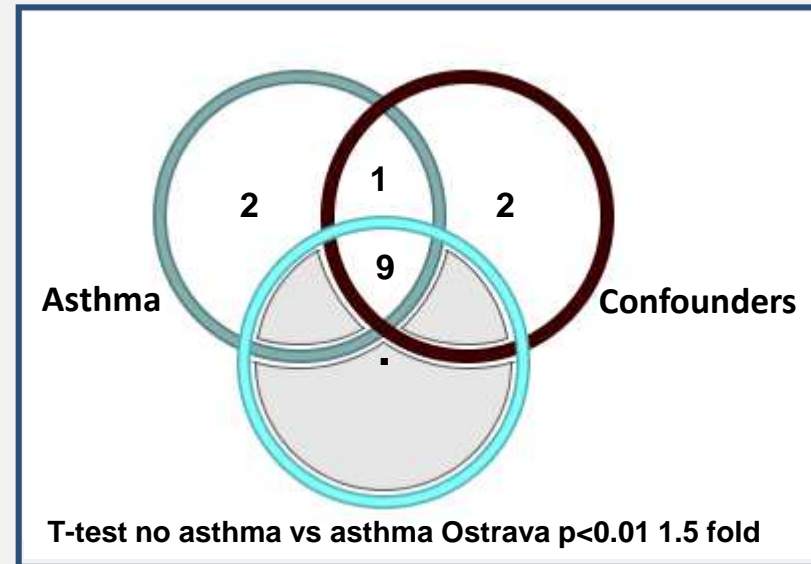
# Effect of confounding factors on comparisons 'no asthma' vs. 'asthma' in the two regions

- ❑ gender
- ❑ smoking father, smoking mother
- ❑ antibiotics, corticoids
- ❑ allergic rhinitis, atopic dermatitis, asthma
- ❑ biomarkers: 8-oxodG, 15-F2t-IsoP,  
carbonyl, micronuclei, cotinine

**NO EFFECT WAS OBSERVED**

# Summary of linear model results

- ❑ For the Ostrava region 3 additional transcripts (DEFA4, HBG2, ID 1770288) were found to be significant for asthma and 3 (HLA-DRB6, RPS4Y2, ID 1770288) for the confounding factors; the overlap between these 3 significant transcripts is 1 (ID 1770288).
- ❑ For the control region all significant transcripts overlap with the significant transcripts from the t-test, even the transcripts found to be significant for the confounding or interacting factors.
- ❑ No additional disease-specific transcript was detected.
- ❑ There is still no overlap between the 17 transcripts specific for the control region and the 12 transcripts specific for Ostrava.



# Fisher's Exact Test

Fisher's Exact Test was performed with the groups:

- ❑ No asthma vs. asthma control region p-value < 0.01;  
399 transcripts
- ❑ No asthma vs. asthma control region p-value < 0.01  
and at least 1.5 fold changes; 17 transcripts
- ❑ No asthma vs. asthma Ostrava p-value < 0.01;  
342 transcripts
- ❑ No asthma vs. asthma Ostrava p-value < 0.01;  
12 transcripts
- ❑ Intersection of common genes p-value < 0.01;  
14 transcripts

# Affected pathways

- ⇒ Control region **p=0.01** 1.5 fold  
Cytokine-cytokine receptor interaction
- ⇒ Control region **p=0.01**  
Inositol phosphate metabolism  
Phosphatidylinositol signaling system  
Lipoic acid metabolism
- ⇒ Ostrava **p=0.01** 1.5 fold  
MAPK signaling pathway
- ⇒ Ostrava **p=0.01**  
Pathogenic Escherichia coli infection  
Parkinson's disease  
Lipoic acid metabolism

# Summary

- Results indicate disease specific and region-dependent gene expression profiles.
- Gene expression profiles for asthma children are different between the polluted and control regions.
- In each region were observed specific transcripts comparing asthma vs. no asthma, 17 transcripts in the control region, 12 transcripts in the polluted region.
- Comparing “no asthma” or “asthma” groups, a strong region effect was observed (57 and 92 transcripts with > 1.5 fold changes).

## **Summary (contd)**

- **Asthma bronchiale in Prachatice – the allergic type of asthma response to allergens.**
- **Asthma bronchiale in Ostrava - the nonallergic type of asthma induced by irritants as air pollution, ETS, viruses.**

# **ACKNOWLEDGEMENT**

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