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HEALS

Health and Environment-wide Associations based on Large population Surveys

FP7-ENV-2013- 603946

http://www.heals-eu.eu/

Deliverables 4.1 and 5.1

Workshop on Internal Exposome Markers in HEALS

WP 4 and 5

Version 1

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Document History

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Foreword

The workshop “Internal Exposome Markers in HEALS” (organised by Stream 2, in particular WP 4 and WP 5) took place in Ljubljana, Slovenia from May 26 to 28, 2014. The meeting consisted of a two days’ workshop (Internal Exposome Markers in HEALS – 26 and 27 May) and one day Technical meeting (28 May) for WP 4 and WP 5.

The meeting was organized in the Conference Center MONS by the local organizer JSI and the WP 5 leader TNO.

The workshop represented an important step forward in the implementation of the EWAS and EXHES protocols in HEALS. Active participation of HEALS participants resolved numerous issues related to the use of existing Human biomonitoring samples (HBM) samples available in cohorts as part of EWAS. Harmonization of approaches for the analysis of exposure and –omics markers has also reached and concrete planning of actions were set up. The technical meeting discussed technical points including guidelines for exposure and –omics markers.

This report consists of the following chapters:

1 Agenda

Workshop on Internal Exposome Markers in HEALS

Ljubljana, 26.-27. May, 2014

Monday, 26. May

8:30 – 9:00 **Registration**

9:00 – 9:15 **Opening (***M. Horvat, R. Stierum***)**

SESSION - 1

9:15 - 9:45 1.1. Setting the stage for HBM and HEALS (*D. Sarigiannis, R. Stierum***)**

- HBM (exposure and - omics) in the context of EWAS – what is needed and who needs to do what?
- How can the existing data and samples support the EWAS?
- Hypothesis: research questions driven vs. agnostic approach?

Outcome of this session: general understanding of what we really need to do in terms of biomarker and - omics research, to contribute to the ultimate construction of the exposome.

9:45 – 10:45 1.2. Biomarkers and -omics in HEALS & the methodologies *(M. Horvat)*

- Exposure, susceptibility and effect biomarkers:
  - Metals, metalloids and other elements (*I. Falnoga*)
  - Organic contaminants and their metabolites (*J. Grimalt, L. Leondiadis*)
  - Other stressors in relation to health impacts: obesity, neurodevelopment and asthma (*G. Calamandrei, G. Viegi, I. Annesi-Maesano*)

10:45 - 11.00 Coffee break

11:00 – 12:45 1.2. Biomarkers and -omics in HEALS & the methodologies *(R. Stierum)*

- "Omics" and biomarkers – technology, concepts, possibilities & challenges, and final suggestion & decision for inclusion in HEALS (*R. Stierum, D. Sarigiannis*), 15 minutes each
  - **Metabolomics**. Metabolomics at Fera and AUTH (*M. Dickinson*) *George Theodoridis and Eleni Gkika* available via Skype for interactions, 25 minutes presentation
  - **Adductomics.** Exposure and susceptibility to endogenous and exogenous alkylating agents (*A. Povey*)
  - **SNP profiling:** SNP genotyping - different platforms for different questions (*W. van Workum*)
  - **DNA methylation:** DNA methylation and epigenetics (*S. Kouidou*)
  - **miRNA profiling:** miRNA profiling technologies (*G. Viegi* presenting on behalf of Agata Giallongo)
  - **Transcriptomics**: Transcriptomics providing the mechanistic basis for causality in EWAS (*D. Sarigiannis*)
  - **Functional assays:** DNA repair functional assays within the HEALS project (*E. Dogliotti*)

Outcome of this session: To have the background for further discussion on pre-selected biomarkers and -omics methodologies, taking into account the state of the art knowledge and recent practices.

12:45-13.30 Lunch

13.30–15.00 1.3 Phenotyping/endotyping in the HEALS paradigm (*I. Annesi-Maesano*)

- Phenotyping/endotyping:
  - Asthma and allergies (*I. Annesi-Maesano*)
  - Diabetes and overweight (*E. Ramos*)
  - Neurodevelopmental troubles (*G. Calamandrei*)
  - Methodology for phenotyping/endotyping (*S. Banerjee*)
- Round table on (*I. Annesi-Maesano, G. Calamandrei; G. Viegi, R. Stierum, D. Sarigiannis*):
  - "Omics" and phenotyping/endotyping: are -omics a necessary step or viceversa
  - HBM and phenotyping/endotyping as an intermediate step in finding causal relationship
  - External exposome input
  - Harmonized approach in phenotyping/endotyping – what is needed?
  - Data missing imputation – Need for standardization and harmonization

Outcome of this session: To have the background for further discussion on phenotyping/endotyping in relation with -omics and biomonitoring in view of EWAS, taking into account the state of the art knowledge and recent practices.

15:00 - 15.15 Coffee break

15:15 – 15:30 Training needs related to Session 1 (*M. Schuhmacher, M. Horvat, R. Stierum***)**

SESSION - 2

15:30 – 17:00 2.1. Existing HEALS cohorts: scientific rationale, available data and samples (*G. Calamandrei, G. Viegi, I. Annesi-Maesano***)**

In this session the three WP leaders should explain which type of samples, analysis and data exist for biomarkers and exposure data. Some most representative studies will be presented. The examples of the whole study protocols and scientific questions will be presented (10 - 15 min each).

- Respiratory allergies and asthma (*G. Viegi*)
- Neurodevelopmental and neurodegenerative disease (*G. Calamandrei*)
- Childhood obesity and diabetes type 2 (*I. Annesi-Maesano*)
- REPRO_PL cohort (*K. Polanska*)
- PHIME (*J. Snoj Tratnik*)
- DEMOCOPHES cohort (*D. Mazej*)
- INMA cohort (*J. Grimalt*)
- The Italian twin study *(L. Nistico)*

Outcome of this session: Scientific rationale of the existing HEALS cohorts, including practicalities of implemented protocols in existing exposome like studies performed so far, including truly available samples/study designs for HEALS from WP14,15,16, storage condition etc. Documents describing

these cohorts should be available prior the meeting.

17:00 -18:00 2.2. HBM in existing HEALS cohorts – round table discussion to address and answer the following questions: (*G. Viegi***)**

Questions A *(facilitator: M. Horvat)*

- Which cohorts are the most comparable in terms of **exposure** markers data?
- Which data are missing in cohorts to make them comparable?
- Can we fill the gaps by additional analysis of exposure markers, if appropriate samples are available (sampling and storage) and do we have sufficient resources to perform the analysis?

Questions B *(facilitator: D. Sarigiannis)*

- Are the existing data of **susceptibility** and **effect** markers useful and comparable between different existing cohorts?
- Can we fill the gap by additional testing on existing samples? Which, and do we have resources to perform testing?
- In case the existing samples are not appropriate or missing, shall we plan additional sampling of study subjects? Resources and feasibility (and the number of study subjects) needs to be discussed!

Questions C *(facilitator: R. Stierum)*

- Which other -omics analysis/technologies are suitable for the existing samples?
- If yes, in which cohorts and to what extend (number of subjects)? (Logistics and the budgetary issues to be addressed)
- Is it meaningful to perform additional sampling from study subjects? (for example non-invasive sampling of saliva, for SNP profiling, etc…).

Outcomes of this session:

- clear overview of existing samples and their suitability in HEALS in general
- identification of missing data and planning of additional analysis, particularly related to omics technology
- detailed plan (who does what and a timetable) for the preparation of the protocol for the implementation of missing analysis

19:00 – Reception in the MONS hotel

Tuesday, 27. May

SESSION – 2 continues ….

8.30 – 9:15 2.2. HBM in existing HEALS cohorts

- Summary conclusions of the Day 1 of the workshop (*M. Horvat, R. Stierum, D. Sarigiannis, G. Viegi*)

9:15 – 9:30 Training needs related to Session 2 (*M. Schuhmacher, M. Horvat, R. Stierum***)**

SESSION – 3

9:30 – 10:30 3.1. HBM (biomarkers and -omics) and the EXHES protocol (*I. Annesi-Maesano*)

- General protocol for EXHES, with a core programme discussed in detail (number of subjects, matrices, analytes, -omics markers, statistical consideration in terms of minimum

requirements from each country, etc...) (*I. Annesi-Maesano*).
- Which SOPs exist and which still need to be prepared (*N. Baiz*)
- Supplementary EXHES samples and protocols (*I. Annesi-Maesano*)
- Detailed work plan and sharing of responsibilities (*A. Moustafa*)

Outcome of this introduction: an overview of the whole complexity of the EXHES protocol and the definition of all the stages that will be addressed at the workshop in Ljubljana. It has to be clear what, who and when we have to do in each step of the whole protocol. Will the HBM protocol also include effect testing, if any? This part may also be used to distribute responsibility for the preparation of the materials/methodology for each part of the protocol.

10:30 – 10:45 Coffee break

10:45 -12:30 3.2. Core protocol of EXHES with SOPs presented (*I. Annesi Maesano***)**
Pre-sampling stage
Sampling design and recruitment strategy (during pregnancy, at delivery, exclusion/inclusion criteria, questionnaire, etc…) (*I. Annesi-Maesano*)
Ethical issues and approval (*I. Annesi-Maesano)*
Communication materials for the participants (*A. Moustafa*)
Informed consent (*S. Maio*)

Sampling and shipment
Sampling SOPs for hair, urine, maternal milk, blood, saliva, cord blood, cord tissue, etc … (including aliquoting of biological samples, when needed and relevant questionnaires during sampling) (*N. Baïz*)
SOPs related questionnaires (*N. Baïz*)
Sample labeling and storage SOPs and related management package (*A. Moustafa*)
Sample shipment SOP (*A. Moustafa*)
Quality control during shipment (any T sensors needed?)

SOPs for metal analysis (*J. Snoj Tratnik*)
SOPs for organic compounds (*L. Leodiadis, J. Grimalt*)
SOPs for omics protocols (*R. Stierum*)
SOPs for other metrics (creatinine, lipid content in milk, hemogram, *the exact list must be compiled*) -
Quality assurance protocols and design for all analytical work (*M. Horvat*)

12.30 – 13.30 Lunch

13.30 – 15.00 Session 3.2. continues

Database format (*S. Nousiainen*)
Data entry from questionnaires (manual or computer assisted) (*S. Cerrai*)
Quality assurance during data entry/treatment (*S. Cerrai*)

Example from DEMOCOPHES to be presented (*J. Snoj Tratnik, D. Mazej*)

Outcome of this session: very detailed and clear overview of all the steps, documentation to be prepared, distribution of responsibilities to finalize the protocols.
15:00 – 15:30 Coffee
15:30 – 16:00 Training needs related to Session 3 (*M. Schuhmacher, M. Horvat, R. Stierum***)**

16:00 – 17.30 3.3. Supplementary EXHES programme (*I. Annesi-Maesano***)**

The same steps as in session 3.1. To be discussed and tasks divided. The scope of this session is to identify which countries will implement supplementary programme and how the HEALS integrate their

work into the overall framework.

- New research questions (*I. Annesi-Maesano, M. Horvat, R. Stierum*)
- Proposed SOPs (*N. Baïz*)
- New aspects of proposed SOPs compared to previous SOPs (*N. Baïz*)

18:15 – Tour of old Ljubljana and the dinner at Ljubljana castle

HEALS Technical meeting for WP 4 and WP 5 participants

Wednesday, 28. May

8:30 – 9:30 **Summary of conclusions of the Internal Exposome Markers Workshop (***R. Stierum and M. Horvat***)**

9:30 – 12.00 - Split into two sessions if necessary (rooms will be identified):

WP4 – HBM – lead JSI (*M. Horvat*)

Partners: UPMC, JSI, LMU, UM, CSIC, OIKON, NCSRD, URV

1. Periodic reporting (1 – 6 M)
2. **Status of the deliverables:**
    a. **D 4.1.** Workshop on the feasibility and extend of sharing biomarker data in Europe and integration of existing data in the HEALS EWAS approach (M8) – lead JSI
    b. **D 4.2.** Guidelines for appropriate "biomarker of exposure" selection for EWAS studies (M12) – lead JSI

WP5 –Omics (*R. Stierum*)

AUTH, UPD, ISS, TNO, FERA, CERETOX, CNR, UKR, SXS

- Final check and confirmation on (bilateral) collaborations emerging from the workshop:
- Optimizing the -omics workflow, samples, technologies, etc.
- e.g. WP14 partner X collaborates with WP5 partner Y on samples xxx from Cohort II, with -omics technology Q); WP5 partner W responsible for -omics analysis in EXHES and so on and forth.
- Final distribution of workload amongst WP5 for EXHES and existing samples from WP14,15,16
- In relation to this budgeting issues

12:00 -14:00 Next actions, road map (*D. Sarigiannis, M. Horvat, R. Stierum, I. Annesi-Maesano)*

14:00 End of the meeting

14:00 Lunch

2 Minutes for each session

2.1 Session 1

9:15-9:45 Setting the stage for HBM and HEALS (Denis Sarigiannis)

Denis Sarigiannis

Coupled EWAS-GWAS approach. Individualized profiles. Biomarkers of exposure, biomarkers of effect. Whole genome targeted. Then some overview on technologies, towards biological pathways. Then overview on databases. SOP specification, exposure biology workflow, identify data transfer protocols, annotation of datasets, characterization of samples, and definition of storage/sample requirements. Bioinformatics/Biocomputing. Existing samples. Phenotypic anchoring!!

Patel (2011) Canonical Correlation of Biomarkers of Environmental Exposures.

Ingrid Falnoga

Overview of biomonitoring for metals, metalloids and other elements. Biochemical markers of effect. Biomarkers of susceptibility. Metals interact with one another, in terms of driving susceptibility to specific metal exposures. Effect modifiers, genetic factors. Then overview on SNPs (e.g. apolipoprotein).

Then slide on multi-biomarker approach by -omics technologies. Discovery of new biomarkers, in relation to one another, also in relation to existing biomarkers. Standardization issues, e.g. metabolomics, low levels of exposure!!! Mainly interest in biomarkers of susceptibility (SNPs), less interest in exposure via e.g. metabolomics to discover **biomarkers of effect**, as a consequence of low levels of exposure. Interest in Selenoproteins!!! Levels of selenium in Europe lower than US.

Question by Joan Grimalt: (1) what analysis on what samples? (2) Lack of selenium in Europe not a problem, but in China?

Milena: you can measure with ICPMS all metals. In addition, -omics may be applied. Selenium might be an issue, in still highly exposed areas.

Leondiadis Leondios

Persistent Organic Polutants. Resistant to degradation, bioaccumulation. Dirty Dozen list Stockholm 2001. PCBs, PFCs etc. dioxins. PFCs: aliphatic hydrocarbonic acids, fully perfluorinated. Bioaccumulations, long transport distances, harmful effects?? Emerging POPs. Do not accumulate in fat tissue, but aggregate with blood proteins, target organ liver. Shows study on PFOS presence in Greece population. Also analysis on food packaging compounds and food.

Joan Grimalt

Joan gives a good overview of possible POPs humans may be exposed to from different sources.

Persistent organic pollutants. PCB, HCB, DTT, HCHs endosulphanes. POP, resistant to degradation.

Interesting, humans/animals do not have mechanisms for handling POPs, for metals and PAH organisms have better mechanisms, as they are exposed in the past. PAH, e.g. measurement of hydroxy metabolites in urine. Is the consensus here that we should more focus on POPs, as for the others (PAH) biomarkers are available, and human metabolisms is capable of handling PAHs.

Nonyphenol, fish sex transformation. New organic pollutants, e.g. pharmaceuticals, insect repellants, drug abuse. Methylterbutylethers (lead replacement in gasoline). MTBE cycle. Neurotoxic pesticides.

Some discussion at the end of talk: Prioritization of compounds. Which POP compounds can be done in combined methods? What is analytically feasible to combine, to analyze most POPs presented. Opinion by Denis, first look into metabolomics profiling, then towards targeted on specific POPs.

Martha: suggestion make a matrix, table. Leave the door open for questions specific to specific partners.

Gemma Calamandrei

Biomarkers of exposure, biomarkers of effect. Existing cohorts. Prenatal, neonatal, infancy.

What markers do we have, including potential associations? What do we need to measure (in terms of new biomarkers), to complement the missing gap? Address new research issues, selecting -omics markers, advance hypothesis. Biomarkers of susceptibility: neurodevelopment. Paraoxonase 1. SNPs for PON1, BDNF, transferrin, progesterone receptors. MAO-A monoamine oxidase A polymorphisms affecting impact of MeHg and PCB.

Biomarkers of effect, e.g. placental miRNA profiles and DNA methylation of specific genes associated with measures of infant neurobehavioral outcomes. Can we use surrogate tissue plus e.g. -omics as proxy to detect markers of effect that occur in brain???

General question: can we use surrogate tissue (e.g. PBMC, plasma) for the effects of exposure and health impact in the target tissue??

Biomarker of pesticides in placenta.

Her suggestion:

- Cytokines and anti-oxidant capacity in serum.
- Store PBMCs in EXHESII
- Steroid hormones
- Select genetic polymorphisms

Giovanni Viegi

Markers related to asthma/allergy. He shows factors contributing to asthma (pollens, molds, air pollution etc.). Asthma outcomes: biomarkers.

Total and allergen specific IgE. Exhaled nitric oxide. Sputum eosinophiles, blood eosinophil, Urine leukotriene. CT scanning, sputum neutrophils, SNPs, susceptibility.

Then overview table with different biomarkers. Induced sputum, exhaled NO, exhaled breath condensate electronic nose.

General question: To which extent do we have access to BAL fluid? What other biomaterials can we obtain from lung?? Sputum???? Yes.

Isabella Annesi-Maesano

Obesogen chemicals and health effects. TBT, bisphenol A, POPs (dioxon, orange agents, PCBs, chlorinated pesticides) Other factors (low calcium, micronutrient intake, short sleep duration, high disinhibition eating behavior trait, exercises, micronutrient intake, body mass etc.

Major issues:

- Biological samples

- Questionnaires
- What is needed for HBM and omics.
    - Crucial: hierarchisation, how can existing data and samples support EWAS. Research question, agnostics, but also driven targeted.
    - Feasibility.

Biomarkers and -omics in HEALS & the methodologies

Mike Dickinson: Metabolomics

Overview of Fera. Current methods, sample methods. Background profiles, fate and behavior of chemicals, biomarkers discovery. Methodologies; non-targeted (agnostic) and semi-targeted.

NMR, LC-MS, GC-MS. NMR: reproducible, high throughput, coverage of compounds, quantitative, non-destructive!! Low sensitivity, skilled operation. MS: relatively fast, sensitive, minimal sample preparation, in particular important for non-targeted agnostic approaches. Downsite: Irreproducible responses, large datasets, will not detect all classes of compounds.

Two technologies combined? Then he continues on SOPs and sample collections. Constant manner.

Consistency in analysis, sample preparation, data processing, and data acquisition protocols QC procedures etc.

Then examples: ethanol exposure in rodents. TSE in ruminants.

General question: Question if sensitivity is an issue with NMR, and exposures are generally low, better not use for environmental exposure assessment. Perhaps NMR better for disease/health impact assessment?? LC MS perhaps better for monitoring actual exposure????

Wilbert van Workum: SNP profiling

ServiceXS DNA analysis. Some background info.

Next gen, single molecule sequencing, SNP discovery, expression analysis, high throughput RT PCR.

Current description of work 48 markers on ~2000 samples using fluidigm technology. Genotyping (undefined)

Few platforms technology,

Fluidigm: 96 markers on 96 samples simultaneously, budget ~10000 samples. Microfluidics mixes samples with assays (picoliter scale), fluidigm website for movie.

Illumina iSelect, Affymetrix axiom

High density genotyping. Affymetrix genotyping Illumina Genotyping.

Axiom biobank array, GWAS, ADME content etc. customizing for heals

- Check on samples:
- Questions focus on methylation or SNPs. Medium cohort (~2000, 30 K SNPs) or large cohorts wit less markers.
- Hypothesis driven vs agnostic
- Which markers?

General question: decisions to be made on larger amount of SNPs in smaller samples vs smaller amount in larger samples.

Sofia Kouidou: DNA methylation

Developmental epigenetics: recent developments, bets, and profits.

Epigenetic profiling, cytosine to 5-methylcytosine conversion. Then overview about genetic versus epigenetic hereditary. Tissue specific! Promotor methylation. CpG islands. Hypermethylation of promotor regions shut down expression, however not that simple.

Binary modification codes. Choice to make: DNA methylation, histone modification???

Promotor methylation-gene silencing (mainly in carcinogenesis), shore methylation differences, internal promotors poly A sites, repeats. Global differences, genetics of epigenetics.

Then about bisulfite conversion sequence based technology, convert 5-mC to U, Re-read (read U as T) requires a lot of sample.

Methylation specific PCR

Methylation susceptible genes CpG SNPs associated with type 2 diabetes and differential methylation in human pancreatic islands.

Smart epigenetic chips.

47 identified

General remark: in terms of prioritizing samples/stressors: should there be a toxicological rationale for looking into methylation changes (e.g. inhibition of 5-methylcytosine methyl transferase) changes of nucleotide pool)???

SNPs of relevance to driving DNA methylation! Level of genes, level of SNPs, level of criteria of selection.

miRNA profiling: G. Viegi, instead of Agata Giallongo

Environmental influences on gene function: miRNAs as biomarkers of exposure.

- Use total RNA extraction.
- 1000 miRNA vs 40000 total transcripts……Agilent Array 8x15K ver 3 miRNA expression.
- Contribution to general external, internal and specific external exposure
- miRNA profiling used to distinct current smokers from non smokers.
- miRNA profiling used to distinct Alzheimer patients.
- Also in lung cancer patients
- Elaborate on second step,
- His opinion: no fishing expedition, but very specific. Confirmatory analysis.

General question, how stable are miRNA over time, in order to reflect past exposure, or future disease progression. Generally stable: Stability of miRNA yes, because some of these are used for markers in cancer biology.

Transcriptomics: Denis Sarigiannis

Pathway based analysis, Denis shows in vitro toxicogenomics in cells exposed to indoor air, PAHs, outdoor air. 5% top up and down regulated genes, pathways and GO category selection. Wikipathways, genespring based pathway analysis.

Cannonical Maps of regulatory networks of genes. Suggestion of secondary mechanisms. Suggestion for confirmatory analysis, using e.g. proteome, metabolome analysis.

General question. Stability of gene expression changes at pathway level, turnover of lymphocytes etc. etc.

General question use of in vitro data to map what the half-life is of chemically induced pathways and compare to in vivo exposure scenarios

Andy Povey: exposure and susceptibility to endogeneous and exogeneous alkylating agents.

Exposure dimethylnitrosoamine, reported as cause of liver cirrhosis. Carcinogenic, mutagenic, toxic etc. affecting S-adenosyl methionine metabolism.

Exposure, direct or altered by co-exposures, life long and variable. 20 known exposures to alkylating agents. Possibly hundreds in intestinal that can be alkylated.

O6-methyl guanine associated with tumor formation. MGMT transgenic models, fit the expectations, knock-out more tumor formation.

N7 exposure associated in man (e.g. smoking). Atl1 protein binds to 06-methylguanine binding, but does not remove it. Any is developing an assay for this.

No MGMT deficiency in the general population. Low MGMT associated with cancer formation.

General question: obvious effect to cancer formation, how about relations with asthma/allergy, endocrine disruption obesity, metals/neurodevelopment (the HEALS issues)?

Eugenia Dogliotti: DNA repair functional assays

8-oxo guanine, 8-oxo deoxyguanosine, NER, endonucleases. No effect of diet on 8-oxodG. Primarily related to DNA repair processes.

Then she shows a table on associations between HEALS priority agents and 8-oxodG.

E.g. PM, VOC, toxic metals.

Disease also, obesity, gastritis, Alzheimer, brain damage

Constant background level of DNA damage I PBMLs 4.2 8-oxodG per 106 guanines

8-oxodG increases with ageing. Also mother /child studies show effects of maternal exposure on 8-0x0dG in mother, but also in child, related to development.

Preterm and newborns show slower DNA repair, oxidative DNA repair as well as BPDE induced NER.

In vitro assays

Preliminary

Phosphorylation of anti-gH2Ax dsb repair

General remark, 8-oxodG/8-oxoG, □H2Ax, specific for exposure or disease, or can it be better used as a general marker of stress, either as a consequence of a variety of chemical exposures, or as a consequence of the development of a variety of diseases.

Her opinion primary exposure.

Phenotyping/endotyping in the HEALS paradigm: Isabella

Phenotype: endpoint outwards manifestation

Endotype: is proposed to be a subtype of a condition defined by a distinct pathophysiological mechanism.

Asthma, possibly multiple diseases, phenotypes etc.asthma phenotype describes clinically observable characteristics of a disease, related to different endotypes.

Gene expression profiling, COPD.

Examples of asthma phenotype: one associated to allergic stimuli Th2 associated asthma:

Early onset allergic asthma; eosinophilic inflammation; favorable response to steroids...

Early-onset allergic asthma vs late onset persistent eosinophilic asthma (sputum eosinophila). Also genetic markers, familiar resemblance. Several TH2 cytokine SNPs, high number of mutations in Th2-related genes (Il4, Il13, and Il4Ra). Cytokines can be used to find Th2 phenotypes. Exhaled FeNO.

Non Th2-associated phenotype.

Several phenotypes need for clustering.

Endotypes: clinical characteristics; biomarkers; lung physiology; treatment response, etc.

Endotypes based upon the same allergic mechanisms. Endotypes: personalized medicine.

Then to obesity. Susceptibility, genetic, epigenetic. T2D loci for susceptibility. 70 genes. Molecular, cellular, physiological level. Adipose tissue remodeling of adipocyte membrane remodeling. Elov16. Then several cell lines. Cross-talk between obesity and asthma. Overview factors with obesity and diabetes.

DIABETES and overweight: Elisabeth Ramos

BMI as classical approach. Causes: environmental and genetics. Lots of loci's identified: FTO; MC4R PPARG; TMEM18, UCP2-866G/A polymorphisms. Obesogenic environment. Food availability, mobility, sleep patterns, bisphenol A etc.

Susceptibility periods

- Perinatal
- 18 first months
- Adiposity rebound
    - Puberty
    - Marriage/divorce
    - New job

Epiteen cohort. BMI trajectories

Obesity-diabetes interactions. T1D, LADA (latent autoimmune diabetes in adults).

Obesity apple vs pear. Also, localisation within the body is a risk factor.

Genetics and environment.

Porto Cohorts

Gemma Calamandrei: Neurodevelopmental troubles

Neurodevelopmental disorders (ND), ADHD, learning disabilities, and autism. Genetic factors only in 5% of ND. Polygenic-based conditions, multifactorial etiology with environmental chemicals…. Then different stages during neurodevelopment. Brain vulnerability to environmental factors. Development of synaptic connection, influence by gut microbioma, chemical exposure. Immune anomalies.

Neurodevelopment: effects of chemicals do not show overt phenotypic changes early on in life, but are only revealed at a later moment in time, however, subtle changes occur early on in life.

Can we find markers for biomarkers of exposure and effects AND behavioral phenotype? Learning, social relationship. Brain anomalies. ADHD and ADHD endophenotypes. Several associations with BPA, Phthalates exposure PAH, PFC, tobacco, alcohol.

Methodology for phenotyping/endotyping: S Banerjee.

Unsupervised classification problem. We have observed several characteristics form individuals

Genotype + environment +genotype*environment interactions → phenotype.

E.g. clustering based classification into phenotype, K-means clustering approach. Ward's method to maximize between and minimize with variability. Average linkage, complete linkage.

K-means clustering no of clusters etc. etc. latent class analysis, Finite mixture models.

2.2 Session 2

Cohorts available, with available samples.

WP14 Asthma allergy: Sara Maio

Twin registry: population based data. Use matrix asked for many information. Clinical assessment, medications, environmental exposure. WP14: biological allergens exposure, particle matter sources. Exposure assessment by objective assessments. Table 1. Overview for existing cohorts. Some problems with exposure data, information about clinical findings. Their problem is to find ways to find ways of characterization of exposure! Missing allergen information, missing PM data.

E.g. retrospective exposure assessment. All available information for 13 pre-existing information.

Also information on available samples.

General question, biomonitoring/omics only for markers for exposure since missing allergen information, missing PM data is you main problem?

WP15. Neurodevelopment and neurodegenerative disease: Calamandrei

Wp15- cohorts EDEN, ReproPL Phime, JSO, HUMIS for total of 2875 children

Exposure assessment include biomarkers of exposure as well as exposure to environmental pollutants.

Biomarkers of effect: see each cohort. Biomarkers of susceptibility: gene polymorphisms, see each cohort. Neurobehavioral maturation: Bailey scale within 3 years of life.

Discussion between Gemma, Denis and Joan: what could be available? What kind of analysis could HEALS contribute to?

HUMIS cohort Norway?

Childhood obesity and diabetes type 2 (Annesi-Maesano)

WP16 Childhood obesity and diabetes: Annesi-Maesano

Table available with study overviews and endpoints, sample info not yet

Kinga Polanska: REPRO-PL

8-12 weeks of pregnancy, questionnaire saliva, blood, urine hair, depending on stage of pregnancy

Delivery: cord blood; questionnaires. GIS data to relate to air pollution, occupational exposure data, stress, APGAR, lifestyle, previous and current pregnancies. Etcetera.

Outcome information of children born: physiological parameters etc. health parameters

Saliva (cotinine) Pb, Hg, PAH, 1 hydroxypyrene, phthalates: maternal urine, children, trace elements etc.

Follow up of children next child examination at 7 years of age. 600 children are followed up for 2 years. Health status estimations, psychomotor development.

Available for HEALS detailed questionnaires, urine samples, buffy coat n=1000 stored at -20 ^{o}C. Urine samples n=1500, stored at –20^{o}C; plasma (HepLi) (1 mL) stored at –70^{o}C (n=700).

Phime: Janja Snoj Tratnik

Large PHIME study. Mixed elemental exposure, low levels of methyl mercury to fish consumption.

Pregnant women recruited from the healthy population, cord blood upon delivery, milk hair, 18 months children tested for neurodevelopment. Now aim for collections of further samples.

Focus on methyl mercury from fish consumption.

Cord blood, plasma, serum. Milk, cord tissue in the table.

Also susceptibility at genetic level, glutathione metabolism, involved role in detoxification. GSTT1, and GSTM1 deletion.

Findings: no effect between bailey scores and mercury. Nevertheless associations between bailey and fish consumption. Total mercury vs methyl mercury. Associations between mercury and selenium in cord blood. Genetic polymorphisms. ABC transporters.

Nice Table giving an overview.

Darja Mazej: Democophes/Cophes all sample

Goal harmonized approach

EU level, Slovenian level

EU level: urine, hair 120 pair/country 3600. Different analytes

Slovenia urine hair blood 120 pairs and fathers →370. Different analytes

Available samples/data Slovenia: 370 hair, 370 urine (-80^{o}C) during sampling period not stored at -80 ^{o}C.

For the EU part, more complicated. National human biomonitoring program in Slovenia. Lactating women and men from the same region. 1200 samples.

COPHES: Different analytes, metals some POP, questionnaire. Urine, blood and milk stored at -30 ^{o}C.

Question by Viegi: should we go back to get informed consent. Democophes: need to go back to participants to get (re)informed consent. Empowerment. Collaborative atmosphere between researchers and subjects, possible reanalysis is possible.

INMA cohort (Grimalt/coworker Merce)

Different regions from Spain, total subjects 3757. Each place has own researcher that is owner of the data. 12 weeks questionnaire, 32 weeks, delivery, 14 mo, 4 y, 7-8y

Air pollution monitoring, passive sampler. Various biomarkers, anthropometric measurements. Resreach INMA cohort serve public health recommendations. Catalan study as well including age effects on POP levels. No samples, but existing data is available,

Question: can you go back for resampling, answer: is difficult. Data is available.

The Italian twin study Nistico

20333 twins enrolled baseline info include zigosity, education job etc.

Aim: estimate contribution of generic and environmental factors of phenotypes investigate biological difference of MZ pairs discordant for exposures or phenotypes. Many parameters, on disease diabetes, autoimmune disease, autistic traits, blood parameters heavy metals. Hand grip, respiratory symptoms.

Sub-study: MUBICOS (multiple birth cohorts. 164 families, 91 families. Saliva, other examples of studies: Euroclot, PIO etc. phenotypes, blood parameters, exposures etc genetic environmental factors etc.

Biobank: DNA from saliva N=2700; Blood DNA 430, plasma from heparine blood 180; platelet free plasma from citrate blood 240 serum 430 buffy coat citrate blood 240 lymphocytes

HBM in existing HEALS cohorts-round table discussion to address and answer the following questions

Now Questions A (facilitator M Horvat)

Statement by Milena: samples and cohorts are available

- Which cohorts are most comparable in terms of exposure markers?
  Chemical stressors analyses in blood, hair
    - Heavy metals/metalloids
        - Incompatibility exist: some were analysed in blood, some in urine, some in maternal milk, and some in hair. Incompatibility exist in biomonitoring
    - POP (PCBs)

        - PCBs available, PPD, PFOS
    - Those contaminants, non-persistent, phthalates, BPA, available in some cohorts but not in all.
- Plan for analysis of exposure markers in the existing cohorts
- Martha: focus on health effects. Only include studies in which an association with health effects are shown. Should we plan additional analysis in existing cohorts, yes only in existing cohorts for which endpoints are included. Neurodevelopment. Look what is available. Toxic metals. PBBs. Chlorinated pesticides.

    - Pisa study. Need for effect biomarker. Suggestions:
        - Metals to be analysed in serum with ICMS; possibly inflammatory marker.
        - Suggestion by Denis: Possibly benzene PBPK modelling (WP5), then including analysis of benzene metabolites (urinary???).
        - Possible nested case control, only looking at the health impact individuals (economics).

Prioritization of studies

See table Isabella, **priority cohorts**: repro-PL (Kinga) **300 samples**, to Wilbert

Question on SNPs, is 300 enough? Perhaps not for phenotypic anchoring, but perhaps for benchmarking snpS to toxicological pathways. Pilot

Resampling on Phime 50 samples on, (Isabella), split, and technical details.

Sampling sending and shipping instructions.

Unique identifier on sample available, needs to be done **shipment agreement with courier!!!! Agree.**

Sampling instructions.

Sample numbers.

    - REPRO_Poland
        - Urine 1500 samples; plasma saliva 1500, hair and whole blood ~500 whole blood??
            - Detailed table, available, very little to be added. Perhaps Phthalates for additional subjects, as only 150 subjects have been measured.

    Who can do phthalates, to be discussed between Leondios and Joan. Perhaps Canada. Perhaps FERA can help out and BPA, organochlorine and phthalate

    - FERMA, maximum amount

    - Portugese samples, epiteen, geracao. Nested case control.

- Scientific analysis and prioritization.

Proposal:

30 min parallel to complement the table by wp14, 15, 16 leaders

Then start addressing A, B, C very systematically through the studies.

Day 3 Wednesday May 28

EXHES protocol presented: -omics in smaller subset

- Eliandra: check for compatibility of possible targeted proteomics in blood cells after transcriptomics.
- Triage of samples for possible proteomics (targeted later on)
- Sending DNA vs sending blood??? Have DNA isolated in one lab??
- PBMC isolation.
- Blood amount for RNA

- EXHES, discuss sample 'economy' within WP5, e.g. sharing sample for DNA and RNA isolation

- Majority of studies exposure data available, endpoint data not always

  Three studies selected

  - Phime, Repro-PL highest priority. Possibly Eden
  - Based upon sample availability and storage conditions
  - Start made with prioritising samples
  - Detail further actions needed
  - Exact number of samples, cost calculations

  SNPs
  - Dedicated?
  - Shortlisted candidates Diabetes, Metals etc.
  - GWAS too little individuals likely
  - Incidence SNPs compared to toxicological/disease pathways
  - Metabolomics
  - Targeted/untargeted
  - Resampling Phime
- Partners doing work identified

Outcome of discussions relating to WP5:

Please find attached the latest version of the spreadsheet we created for samples to start the global piloting from existing cohorts, using omics. Initial activities

REPRO-PL and Phime selected as initial candidate studies for pilots

- Power calculations on REPRO-PL for SNP analysis: Wilbert SXS
- Kinga will look for samples covering complete data sets (exposure, phenotype) availability of sample
- Metabolomics on resampled samples from Phime: contacts between Fera and JSI: Janja, Mike
- WP5 please check (again) EXHES SOPs for compatibility.
- Rob will send link.
- Janja and Ingrid need to look at the HEALS SOPs for sample collection (designed for EXHES, but compatible for other studies as well) available on google drive.
- Establishment of an SNP 'targeted' team to design a tox, disease, ADME array:

  - People involved Ingrid, Isabella, Lorenza, Gemma, Wilbert, Leondias (he will send a list with POP related SNPs).

- DNA methylation, targeted, pending outcome of initial analysis.

Sample tracking and shipment procedures, Denis

3 List of participants

	Name of participant	Institution	Country	HEALS partner number
1.	Aggerbeck Martine	Université Paris Descartes	Paris, France	Participant 6 (UPD)
2.	Annesi Maesano Isabella	Université Pierre et Marie Curie	Paris, France	Participant 1 (UPMC)
3.	Baiz Nour	Université Pierre et Marie Curie	Paris, France	Participant 1 (UPMC)
4.	Banerjee Soutrik	Université Pierre et Marie Curie	Paris, France	Participant 1 (UPMC)
5.	Barouki Robert	Université Paris Descartes	Paris, France	Participant 6 (UPD)
6.	Bignami Margherita	Istituto Superiore di Sanità	Rome, Italy	Participant 8 (ISS)
7.	Böse-O´Reilly Stephan	LMU - University of Munich, KUM – Hospital of the University of Munich, Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine	Munich, Germany	Participant 9 (LMU)
8.	Calamandrei Gemma	Istituto Superiore di Sanità	Rome, Italy	Participant 8 (ISS)
9.	Cerrai Sonia	Institute of Biomedicine and Molecular Immunology of CNR (IBIM-CNR)	Palermo, Italy	Participant 20 (CNR)
10.	Charlton Adrian	Department for Environment, Food and Rural Affairs (DEFRA)	Sand Hutton York, United Kingdom	Participant 14 Food and Environment Research Agency (FERA)
11.	De Lauente Joaquím	Fundació Parc Científic De Barcelona	Barcelona, Spain	Participant 17 (CERETOX)
12.	De Oliveira Eliandre	Fundació Parc Científic De Barcelona	Barcelona, Spain	Participant 17 (CERETOX)
13.	Dickinson Michael	Department for Environment, Food and Rural Affairs (DEFRA)	Sand Hutton York, United Kingdom	Participant 14 Food and Environment Research Agency (FERA)
14.	Doglioti Eugenia	Istituto Superiore di Sanità	Rome, Italy	Participant 8 (ISS)
15.	Falnoga Ingrid	Jožef Stefan Institute	Ljubljana, Slovenia	Participant 5 (JSI)
16.	Gari Merce	Spanish Council for Scientific Research (CSIC). Institute of Environmental Assessment and Water Research (IDÆA)	Barcelona, Spain	Participant 15 (CSIC)

17.	Grimalt O. Joan	Spanish Council for Scientific Research (CSIC). Institute of Environmental Assessment and Water Research (IDÆA)	Barcelona, Spain	Participant 15 (CSIC)
18.	Horvat Milena	Jožef Stefan Institute	Ljubljana, Slovenia	Participant 5 (JSI)
19.	Jurewicz Joanna	Nofer Institute of Occupational Medicine	Lodz. Poland	Participant 10 (NIOM)
20.	Kouidou Andreou Sofia	Aristotle University of Thessaloniki,	Thessaloniki, Greece	Participant 2 (AUTH)
21.	Leondiadis Leondios	National Centre for Scientific Research Demokritos,	Athens, Greece	Participant 22 (NCSRD)
22.	Lindahl-Jacobsen Rune	University of Southern Denmark	Odense, Denmark	Participant 28 (SDU)
23.	Maio Sara	Institute of Clinical Physiology of CNR (IFC-CNR) - Pulmonary Environmental Epidemiology Unit (EPAP),	Pisa, Italy	Participant 20 (CNR)
24.	Mazej Darja	Jožef Stefan Institute	Ljubljana, Slovenia	Participant 5 (JSI)
25.	Meccia Ettore	Istituto Superiore di Sanità,	Rome, Italy	Participant 8 (ISS)
26.	Moustafa Amir	Université Pierre et Marie Curie	Paris, France	Participant 1 (UPMC)
27.	Nistico Lorenza	Istituto Superiore di Sanità,	Rome, Italy	Participant 8 (ISS)
28.	Nousiainen Sami	Technical Research Centre of Finland,	Espoo and Tampere,	Participant 11 (VTT)
29.	Polanska Kinga	Nofer Institute of Occupational Medicine	Lodz. Poland	Participant 10 (NIOM)
30.	Povey Andrew	University of Manchester - Centre for Occupational and Environmental Health and Maternal and Fetal Health Research Group	Manchester, United Kingdom	Participant 12 (UM)
31.	Ramos Elisabete	Medical School Of The University Of Porto	Porto, Portugal	Participant 21 (FMUP)
32.	Sarigiannis Dimosthenis	Aristotle University of Thessaloniki	Thessaloniki, Greece	Participant 2 (AUTH)
33.	Schuhmacher Marta	Universitat Rovira i Virgili (URV), Centre for Environmental, Food and Toxicological Technology	Tarragona, Spain	Participant 23 (URV)
34.	Snoj Tratnik Janja	Jožef Stefan Institute	Ljubljana, Slovenia	Participant 5 (JSI)
35.	Špirić Zdravko	OIKON Ltd. - Institute for applied ecology	Zagreb, Croatia	Participant 19 (OIKON)
36.	Steckling Nadine	LMU - University of Munich, KUM – Hospital of the University of Munich, Institute and Outpatient Clinic for Occupational, Social and	Munich, Germany	Participant 9 (LMU)

		Environmental Medicine		
37.	Stierum Rob	Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek	Zeist, the Netherlands	Participant 13 (TNO)
38.	Van Workum Wilbert	SXS	Leiden, Netherlands	Participant 25
39.	Viegi Giovanni	Institute of Biomedicine and Molecular Immunology of CNR (IBIM-CNR)	Palermo-Italy	Participant 20 (CNR)

HEALS Institut "Jožef Stefan", Ljubljana, Slovenija

Workshop on Internal Exposome Markers in HEALS, Ljubljana, 26.-28. May, 2014

List of Participants

1.	Aggerbeck Martine	
2.	Annesi Maesano Isabella	
3.	Baiz Nour	
4.	Banerjee Soutrik	
5.	Barouki Robert	
6.	Bignami Margherita	
7.	Böse-O´Reilly Stephan	
8.	Calamandrei Gemma	
9.	Cerrai Sonia	
10.	Charlton Adrian	
11.	De Lauente Joaquim	
12.	De Oliveira Eliandre	
13.	Dickinson Michael	
14.	Doglioti Eugenia	
15.	Falnoga Ingrid	
16.	Gari Merce	
17.	Grimalt Joan	
18.	Horvat Milena	
19.	Kouidou Andreou Sofia	
20.	Leondiadis Leondios	
21.	Maio Sara	
22.	Mazej Darja	
23.	Meccia Ettore	
24.	Moustafa Amir	
25.	Nikovski Damjana	
26.	Nistico Lorenza	
27.	Nousiainen Sami	
28.	Odumah Anderson Christiana	

HEALS Institut "Jožef Stefan", Ljubljana, Slovenija

Workshop on Internal Exposome Markers in HEALS, Ljubljana, 26.-28. May, 2014

29.	Pavlin Majda	
30.	Polanska Kinga	
31.	Povey Andrew	
32.	Ramos Elisabete	
33.	Sarigiannis Dimosthenis	
34.	Schumacher Marta	
35.	Snoj Tratnik Janja	
36.	Špirić Zdravko	
37.	Steckling Nadine	
38.	Van Workum Wilbert	
39.	Viegi Giovanni	
40.	RUNE LINDAHL-JACOBSEN	
41.	Rob Stierum	
42.	Joana Jurkiewicz	JOANNA JUREWICZ
43.		

4 Minutes

Workshop on Internal Exposome Markers in HEALS

Ljubljana, 26.-28. May 2014

Organization and hosting of the meeting: Jožef Stefan Institute (JSI), Ljubljana

Start: 26.5.2014 , **End**: 28.5.2014

Foreword:

The workshop represented an important step forward in the implementation of the EWAS and EXHES protocols in HEALS. Active participation of HEALS participants resolved numerous issues related to the use of existing Human bio-monitoring samples (HBM) samples available in cohorts as part of EWAS. Harmonization of approaches for the analysis of exposure and –omics markers has also reached and concrete planning of actions were set up. The technical meeting discussed technical points including guidelines for exposure and –omics markers.

Participants

1-UNIVERSITE PIERRE ET MARIE CURIE	UPMC	Annesi_Maesano Isabella Baiz Nour Moustafa Amir Banerjee Soutrik
2-ARISTOTELIO PANEPISTIMIO THESSALONIKIS	AUTH	Sarigiannis Dimosthenis Kouidou Andreou Sofia
23-UNIVERSITAT ROVIRA I VIRGILI	URV	SCHUHMACHER MARTA
5-Jožef Stefan Institute	JSI	Falnoga Ingrid Horvat Milena Mazej Darja Snoj Tratnik Janja
6-Université Paris Descartes	UPD	Aggerbeck Martine Barouki Robert
8-Istituto Superiore di Sanità	ISS	Bignami Margherita

		Calamandrei Gemma Doglioti Eugenia Meccia Ettore Nistico Lorenza
9-University of Munich, KUM – Hospital of the University of Munich, Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine	LMU	Böse-O´Reilly Stephan Steckling Nadine
10-Nofer Institute of Occupational Medicine	NIOM	Jurewicz Joanna Polanska Kinga
11- Technical Research Centre of Finland	VTT	Nousiainen Sami
12- University of Manchester - Centre for Occupational and Environmental Health and Maternal and Fetal Health Research Group	UM	Povey Andrew
13- Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek	TNO	Stierum Rob
14- Food and Environment Research Agency (FERA)	FERA	Charlton Adrian Dickinson Michael
15- Spanish Council for Scientific Research (CSIC). Institute of Environmental Assessment and Water Research (IDÆA)	CSIC	Gari Merce Grimalt O. Joan
17- Fundació Parc Científic De Barcelona	CERETOX	De Lauente Joaquím De Oliveira Eliandre
19- OIKON Ltd. - Institute for applied ecology	OIKON	Špirić Zdravko
20- Institute of Biomedicine and Molecular Immunology of CNR (IBIM-CNR)	CNR	Cerrai Sonia Maio Sara Viegi Giovanni
21- Medical School Of The University Of Porto	FMUP	Ramos Elisabete
22- National Centre for Scientific Research Demokritos	NCSRD	Leondiadis Leondios
25-SX Services Leiden	SXS	Van Workum Wilbert

28- University of Southern Denmark	SDU	Lindahl-Jacobsen Rune

Agenda:

Workshop on Internal Exposome Markers in HEALS

Ljubljana, 26.-27. May, 2014

Monday, 26. May

8:30 – 9:00 **Registration**

9:00 – 9:15 **Opening (***M. Horvat, R. Stierum***)**

SESSION - 1

9:15 - 9:45 1.1. Setting the stage for HBM and HEALS (*D. Sarigiannis, R. Stierum***)**

- HBM (exposure and - omics) in the context of EWAS – what is needed and who needs to do what?
- How can the existing data and samples support the EWAS?
- Hypothesis: research questions driven vs. agnostic approach?

9:45 – 10:45 1.2. Biomarkers and -omics in HEALS & the methodologies *(M. Horvat)*

- Exposure, susceptibility and effect biomarkers:
  - Metals, metalloids and other elements (*I. Falnoga*)
  - Organic contaminants and their metabolites (*J. Grimalt, L. Leondiadis*)
  - Other stressors in relation to health impacts: obesity, neurodevelopment and asthma (*G. Calamandrei, G. Viegi, I. Annesi-Maesano*)

10:45 - 11.00 Coffee break

11:00 – 12:45 1.2. Biomarkers and -omics in HEALS & the methodologies *(R. Stierum)*

- "Omics" and biomarkers – technology, concepts, possibilities & challenges, and final suggestion & decision for inclusion in HEALS (*R. Stierum, D. Sarigiannis*), 15 minutes each
  - **Metabolomics**. Metabolomics at Fera and AUTH (*M. Dickinson*) *George Theodoridis and Eleni Gkika* available via Skype for interactions, 25 minutes presentation
  - **Adductomics.** Exposure and susceptibility to endogenous and exogenous alkylating agents (*A. Povey*)
  - **SNP profiling:** SNP genotyping - different platforms for different questions (*W. van Workum*)
  - **DNA methylation:** DNA methylation and epigenetics (*S. Kouidou*)
  - **miRNA profiling:** miRNA profiling technologies (*G. Viegi* presenting on behalf of Agata Giallongo)
  - **Transcriptomics**: Transcriptomics providing the mechanistic basis for causality in EWAS (*D. Sarigiannis*)
  - **Functional assays:** DNA repair functional assays within the HEALS project (*E. Dogliotti*)

12:45-13.30 Lunch

13.30–15.00 1.3 Phenotyping/endotyping in the HEALS paradigm (*I. Annesi-Maesano*)

- Phenotyping/endotyping:
  - Asthma and allergies (*I. Annesi-Maesano*)
  - Diabetes and overweight (*E. Ramos*)
  - Neurodevelopmental troubles (*G. Calamandrei*)
  - Methodology for phenotyping/endotyping (*S. Banerjee*)
- Round table on (*I. Annesi-Maesano, G. Calamandrei; G. Viegi, R. Stierum, D. Sarigiannis*):
  - "Omics" and phenotyping/endotyping: are -omics a necessary step or viceversa
  - HBM and phenotyping/endotyping as an intermediate step in finding causal relationship
  - External exposome input
  - Harmonized approach in phenotyping/endotyping – what is needed?
  - Data missing imputation – Need for standardization and harmonization

15:00 - 15.15 Coffee break

15:15 – 15:30 Training needs related to Session 1 (*M. Schuhmacher, M. Horvat, R. Stierum***)**

SESSION - 2

15:30 – 17:00 2.1. Existing HEALS cohorts: scientific rationale, available data and samples (*G. Calamandrei, G. Viegi, I. Annesi-Maesano***)**

- Respiratory allergies and asthma (*G. Viegi*)
- Neurodevelopmental and neurodegenerative disease (*G. Calamandrei*)
- Childhood obesity and diabetes type 2 (*I. Annesi-Maesano*)
- REPRO_PL cohort (*K. Polanska*)
- PHIME (*J. Snoj Tratnik*)
- DEMOCOPHES cohort (*D. Mazej*)
- INMA cohort (*J. Grimalt*)
- The Italian twin study *(L. Nistico)*

17:00 -18:00 2.2. HBM in existing HEALS cohorts – round table discussion to address and answer the following questions: (*G. Viegi***)**

Questions A *(facilitator: M. Horvat)*

- Which cohorts are the most comparable in terms of **exposure** markers data?
- Which data are missing in cohorts to make them comparable?
- Can we fill the gaps by additional analysis of exposure markers, if appropriate samples are available (sampling and storage) and do we have sufficient resources to perform the analysis?

Questions B *(facilitator: D. Sarigiannis)*

- Are the existing data of **susceptibility** and **effect** markers useful and comparable between different existing cohorts?
- Can we fill the gap by additional testing on existing samples? Which, and do we have resources to perform testing?
- In case the existing samples are not appropriate or missing, shall we plan additional sampling of study subjects? Resources and feasibility (and the number of study subjects) needs to be discussed!

Questions C *(facilitator: R. Stierum)*

- Which other -omics analysis/technologies are suitable for the existing samples?
- If yes, in which cohorts and to what extend (number of subjects)? (Logistics and the budgetary issues to be addressed)
- Is it meaningful to perform additional sampling from study subjects? (for example non-invasive sampling of saliva, for SNP profiling, etc…).

19:00 – Reception in the MONS hotel

Tuesday, 27. May

SESSION – 2 continues ….

8.30 – 9:15 2.2. HBM in existing HEALS cohorts

- Summary conclusions of the Day 1 of the workshop (*M. Horvat, R. Stierum, D. Sarigiannis, G. Viegi*)

9:15 – 9:30 Training needs related to Session 2 (*M. Schuhmacher, M. Horvat, R. Stierum***)**

SESSION – 3

9:30 – 10:30 3.1. HBM (biomarkers and -omics) and the EXHES protocol (*I. Annesi-Maesano*)

- General protocol for EXHES, with a core programme discussed in detail (number of subjects, matrices, analytes, -omics markers, statistical consideration in terms of minimum requirements from each country, etc...) (*I. Annesi-Maesano*).
- Which SOPs exist and which still need to be prepared (*N. Baiz*)
- Supplementary EXHES samples and protocols (*I. Annesi-Maesano*)
- Detailed work plan and sharing of responsibilities (*A. Moustafa*)

10:30 – 10:45 Coffee break

10:45 -12:30 3.2. Core protocol of EXHES with SOPs presented (*I. Annesi-Maesano***)**

Pre-sampling stage (I. Annesi-Maesano, A. Moustafa, S. Maio)

Sampling and shipment (N. Baïz, A. Moustafa)

SOPs for analysis and quality assurance (*J. Snoj Tratnik, L. Leodiadis, J. Grimalt, R. Stierum, M. Horvat*)

12.30 – 13.30 Lunch

13.30 – 15.00 Session 3.2. continues

Database format (*S. Nousiainen*)

Data entry from questionnaires (manual or computer assisted) & Quality assurance (*S. Cerrai*)

15:00 – 15:30 Coffee

15:30 – 16:00 Training needs related to Session 3 (*M. Schuhmacher, M. Horvat, R. Stierum***)**

16:00 – 17.30 3.3. Supplementary EXHES programme (*I. Annesi-Maesano***)**

The same steps as in session 3.1.: To be discussed and tasks divided. The scope of this session is to identify which countries will implement supplementary programme and how the HEALS integrate their work into the overall framework (*I. Annesi-Maesano, M. Horvat, R. Stierum*, *N. Baïz*)

18:15 – Tour of old Ljubljana and the dinner at Ljubljana castle

HEALS Technical meeting for WP 4 and WP 5 participants

Wednesday, 28. May

8:30 – 9:30 Summary of conclusions of the Internal Exposome Markers

Workshop (*R. Stierum and M. Horvat***)**

9:30 – 12.00 - Split into two sessions if necessary (rooms will be identified):

WP4 – HBM – lead JSI (*M. Horvat*)

Partners: UPMC, JSI, LMU, UM, CSIC, OIKON, NCSRD, URV

3. Periodic reporting (1 – 6 M)
4. **Status of the deliverables:**
    a. **D 4.1.** Workshop on the feasibility and extend of sharing biomarker data in Europe and integration of existing data in the HEALS EWAS approach (M8) – lead JSI
    b. **D 4.2.** Guidelines for appropriate “biomarker of exposure” selection for EWAS studies (M12) – lead JSI

WP5 –Omics (*R. Stierum*)

AUTH, UPD, ISS, TNO, FERA, CERETOX, CNR, UKR, SXS

- Final check and confirmation on (bilateral) collaborations emerging from the workshop:
- Optimizing the -omics workflow, samples, technologies, etc.
- e.g. WP14 partner X collaborates with WP5 partner Y on samples xxx from Cohort II, with -omics technology Q); WP5 partner W responsible for -omics analysis in EXHES and so on and forth.
- Final distribution of workload amongst WP5 for EXHES and existing samples from WP14,15,16
- In relation to this budgeting issues

12:00 -14:00 Next actions, road map (*D. Sarigiannis, M. Horvat, R. Stierum, I. Annesi-Maesano)*

14:00 End of the meeting

14:00 Lunch

Session 1[1]		**Who**	**Estimated date**	**Status**[2]
I	*General understanding of what we really need to do in terms of biomarkers and -omics research, to contribute to the ultimate construction of the exposome*			
I	*Background for further discussion on pre-selected biomarkers and -omics methodologies, taking into account the state of the art knowledge and recent practices.*			
I	*Background for further discussion on phenotyping/endotyping of the health conditions considered in HEALS (asthma/allergies, overweight/diabetes, neurodevelopment) in relation with -omics and biomonitoring in view of EWAS, taking into account the state of the art knowledge and recent practices.*			
Session 2		**Who**	**Estimated date**	**Status**[2]
I	*Some most representative studies were presented, including the examples of the whole study protocols and scientific questions, to provide scientific rationale of the existing HEALS cohorts, including practicalities of implemented protocols in existing exposome like studies performed so far, including truly available samples/study designs for HEALS from WP14,15,16, storage condition etc.*			
I	*Clear overview of existing samples and their suitability in HEALS in general; identification of missing data and planning of additional analysis, particularly related to -omics technology;* *detailed plan (who does what and which timetable) for the preparation of the protocol for the implementation of missing analysis*			
D	We need to focus on health effects. Only include studies in which an association with health effects is shown. Should we plan additional analysis in existing cohorts, yes only in existing cohorts for which endpoints are included. Neurodevelopment: Look what is available. Toxic metals. PBBs. Chlorinated pesticides.			
A	Table produced containing available cohorts and samples/analysed stressors and other available information (e.g. health outcome, questionnaire data). Chemical stressors analysed in blood, hair: a) Heavy metals/metalloids (heterogeneity and incompatibility exist: some were analysed in blood, some in urine, some in maternal milk, some in hair. Incompatibility exists also in biomonitoring. b) POPs (PCBs available, PPD, PFOS)			

	c) Phthalates, BPA, available in some cohorts but not in all			
D	**Prioritization of studies** - three studies were selected based upon sample availability and storage conditions: - PHIME - Repro-PL (highest priority!) - and possibly Eden (but this requires time). Details on further actions are needed: Exact number of samples, cost calculations needed.			
A	Repro-PL: **300 samples** will be sent to AUTH and servicexs for -omics	NIOM, SXS	JULY 2014	ON-GOING
A	Resampling on PHIME: 10-20 samples, according to the EXHES protocol, technical details needed.	IJS, FERA	SEPT 2014	ON-GOING
A	Definition of Sampling and shipping instructions	UPMC, TNO		ON-GOING
Session 3		**Who**	**Estimated date**	**Status**[2]
I	Overview of database format, data entry from questionnaires (manual or computer assisted)			
I	SOPs for pre-sampling, sampling and shipment in EXHES study	UPMC, TNO		
D	Detailed meeting dedicated only to SOPs needed	PARTNERS INVOLVED IN WP17	29. SEPT 2014	ON-GOING
Technical meeting, WP 4				
I	Status of the deliverables			
A	**D 4.1.** Workshop on the feasibility and sharing of biomarker data in Europe and integration of existing data in the HEALS EWAS approach	JSI	M8	CLOSED
A	**D 4.2.** Guidelines for appropriate "biomarker of exposure" selection for EWAS studies (M12)	JSI, LMU	M12	ON-GOING
A	Preparation of outline of the deliverable D4.2 and time-planning			
Technical meeting, WP 5				
I	Optimizing the -omics workflow, samples, technologies, etc.	TNO		
D	REPRO-PL and PHIME selected as initial candidate studies for pilots			
A	Power calculations on REPRO-PL for SNP analysis	SXS, UPMC		ON-GOING
A	Metabolomics on resampled samples from PHIME: contacts between FERA and JSI	JSI, FERA		ON-GOING
A	Look at the HEALS SOPs for sample collection (designed for EXHES, but compatible for other studies as well) available on google drive; sample tracking and shipment procedures	JSI		ON-GOING
A	Establishment of an SNP 'targeted' team to design a tox, disease, ADME array.	JSI, ISS, UPMC,		ON-GOING

		SXS, NCSRD		
Type 1	**Dissemination**	**WHO**	**ESTIMATED DATE**	**STATUS 2**
I	On-going discussion			