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, J. Snoj Tratnik*)
  - 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

18.00 – Participants that will stay in Ljubljana will be invited to Milena garden party with traditional Slovenian foods and wines