This project has received funding from the European Union's Seventh Programme for research, technological development and demonstration under grant agreement N°603946

1 HEALS Publishable summary

1.1 HEALS context and objectives

We recall here briefly the main features of the HEALS (Health and Environment-wide Associations based on Large population Surveys) project context and objectives that have been presented elsewhere more extensively.

Explaining and predicting chronic health outcomes implies that the individual environmental exposures, biomarkers of exposure and effect, internal body processes and signals, genetic variations and epigenetic marks are reliably measured simultaneously during his/her entire lifespan. This entails the introduction of the notion of the exposome, i.e. the totality of exposures from preconception onwards, simultaneously identifying, characterizing and quantifying the exogenous and endogenous exposures and hence modifiable risk factors that predispose to and predict diseases throughout a person's lifespan. The HEALS project brings together in an innovative approach a comprehensive array of novel technologies, data analysis and modelling tools that support efficiently exposome estimation and exposome-wide association studies to improve health risk assessment of environmental stressors.

The general objective of HEALS is the refinement of an integrated methodology and the application of the corresponding analytical and computational tools to real-life population-based samples for performing environment (or exposome)-wide association studies (EWAS) in support of EU-wide environment and health risk assessments. This constitutes the HEALS paradigm (see figure below).

In the HEALS project, EWAS is applied to three classes of health outcomes - asthma and allergies, metabolic diseases (mainly overweight, obesity and type 2 diabetes), neurodevelopmental and neurodegenerative disorders -, the prevalence of which is elevated in the European population and worldwide, even at early life. Their prevalence is also increasing mostly because of the intervention of environmental factors. The considered outcomes are deemed to be interrelated to each other and to share common risk factors, as well as common toxicity pathway nodes.

More in detail, the HEALS approach intends to harmonize environmental, socio-economic, exposure and effect biomarkers and health data at different levels (from ecological to individual) and to collate processes and computational pipelines that are necessary for comprehensive environmental exposure-health associations explaining the development of the considered health outcomes.

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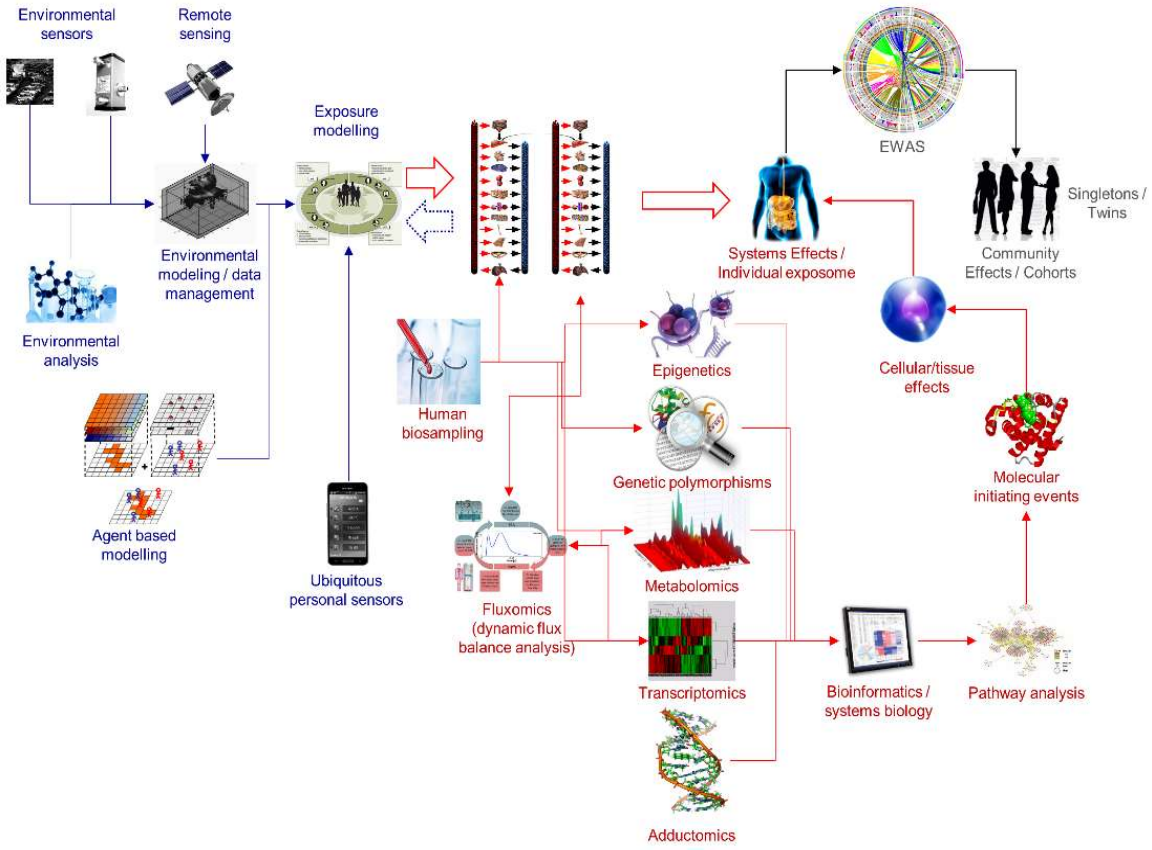

The overall HEALS approach is verified and refined in a series of pre-existing population studies across Europe including singletons and twin cohorts and registries, accounting for different class of ages, levels of environmental exposure, windows of exposure, socio-economics, biomarkers of exposure and effects and epi/genetic variability, with focus on the above mentioned health outcomes. It is successively applied in a pilot environmental EXposure and Health Examination Survey (EXHES) of children including sets of singletons and of twins with matched singletons (each twin's pair having also a matched singleton) and their parents covering ten EU Member States.

To attain its objectives, the HEALS project has been structured in 7 streams and 20 work-packages (WPS). And this work organisation has been effective with most of the objectives and technical goals of the period 19 – 36 months achieved. Six streams are still active.

For the third period of work, the specific objectives comprised:

1) the completion of the internal exposome in the pre-existing data
2) the completion of external exposome in the pre-existing data
3) the conduction of EXHES
4) and as a permanent objective, the continuation of reporting, networking, disseminating and training at the national and international level.

More up-to-date information on HEALS can be found at the project public website http://www.heals-eu.eu/.

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1.2 Work performed and results achieved in the third period

Main results

Major achievements consist in the creation of unique databases of exposures and health at the European level and the identification of algorithms, models, tools for exploring them and thus unravelling the exposome. First results on health determinants have been obtained. The activities of reporting, networking, disseminating, training are continuing in a successful way.

Databases

Health data:

A harmonized database containing datasets on symptoms, diseases, phenotypes from pre-existing studies of singletons and twins is presently available. So far, it contains records for more than 50000 individuals, most followed-up in time, some since birth and even *in utero* life. Almost half are twins, which will allow investigating the proper role of environment and epigenetic marks in monozygotic twins sharing the same genetic background. This dataset is presently expanding to accommodate other health data from other datasets. Because of the heterogeneity of data, harmonization and standardization of the data was performed. New data are being collected in EXHES that has started in 9 countries and is even finished in 1 country (Germany) for a total of almost 2000 mother-child pairs.

Internal exposome:

During the 3rd period, the work has progressed consistently towards the implementation of the HEALS conceptual framework in terms of the internal exposome through the enhancement and development of different interdisciplinary components. This allowed us to bring forward the implementation of the tools developed in the project to assess and estimate the internal and external exposome at the individual level and to obtain the first results.

Biomarkers of exposure and effects were compiled from various datasets. Omics have been employed on samples available from existing exposure/disease outcome studies. Progress was made towards the completion of the major objectives in terms of biokinetic modeling for internal dose and exposure reconstruction, the generic multi-route lifetime PBBK model, which includes among others gestation and breastfeeding and incorporates *in utero* exposure and mixtures interactions and novel bioinformatics for predictive biomarkers discovery. In addition, information from other existing initiatives on omics was collected and links to other omics databases were made accessible.

External exposome:

Two harmonized databases were built: one containing risk factors from pre-existing population-based studies of singletons and twins, and the other the exposure data available at the European level. A considerably large number of environmental data sets including food contamination have been identified, collected and analysed for their quality (assessing their QA/QC procedures). Environmental factors are assessed at various levels ranging from the

ecological to the individual. A probabilistic exposure modelling framework to assess external exposure to chemicals for selected population groups was established. Data on non specific environmental determinants of exposure (social economic, community characteristics, health care system…) were also modelled and collected. Model derived information was recorded. The HEALS Environmental Data Management System (EDMS) was implemented to pull together the totality of the available environmental data. First application of exposure data were conducted.

To sum up, in terms of databases we have concluded:

- The creation of a large harmonized dataset of general population and twin data in view of EWAS. Initiatives have been taken to share existing epidemiological data and biospecimens in view of conducting pooled analyses in addition to study-specific analyses.
- Establishment of the HEALS Environmental Data Management System (EDMS) containing the environmental data necessary for assessing the external exposome
- A database of metabolites and metabolic pathways associated with phenotypes of adverse health outcomes to reconstruct the internal exposome
- The HEALS GeoData platform has been developed containing exposome and health data at the European data
- The EXHES database using e-EXHES to convey in EDMS and the HEALS GeoData platform.

All datasets collected are referenced in space and time and are going to be collated in the HEALS plateform (GeoDatabase), a unique tool for the investigation of exposome that we expected to become a key resource for the exposome and environmental health academic, research and regulatory communities.

Modeling and EWAS

Methodological advancements consisted of:

- Establishment of the methodological framework for EWAS
- Methodologies for linking omics technologies to population studies
- Implementation of generic multi-route lifetime PBBK
- Review and gathering of methods in bioinformatics for descriptive and predictive data mining
- Applications of SES-informed exposure and risk model to population data and Agent Based Modeling (ABM)
- Development of imputation methods and machine learning have been applied to prevent the consequences of the lack of missing data

– Bioinformatics was applied to omics.

As a consequence, revision of the methods to be applied in EWAS has progressed. They have been applied to asthma and to metabolic diseases (overweight, obesity and diabetes 2) respectively. In terms of the internal exposome, suitable data mining techniques for biomarker identification and methods for EWAS were finalized in view of processing mixed data from various sources. Definition of the methodological developments for taking into account of socio-economic status in the exposome approach was also finalized. Using the results from a task force and further literature searches, we were able to create preliminary 'rules' for our Agent Based Modeling (ABM) platform. Omics analysis have been conducted in urine and blood samples. DNA have been used for methylome analysis in 128 samples. The resulting idat output files of intensity have been shared on the HEALS/AUTH server. At the time of this reporting raw data import, conversion of methylated and unmethylated signals to beta values and normalization have been performed from AUTH partner using the R programming language. Pathway analysis is still on going.

Additional results

Among additional major milestones reached during this reporting period the following are key:

- Review of the recent literature about exposure and health association studies, and about biomarkers of exposure and their effects on health outcomes under study in HEALS have been conducted (asthma and allergy, neurodevelopmental and neurodegenerative problems and metabolic disorders, namely childhood obesity and type 2 diabetes).
- A pilot study testing the reliability of personal and remote sensors for monitoring of individual exposure was conducted in 5 countries. It provided recommendations for the EXposure and Health Examination Survey (EXHES) field campaign.
- The CANARIN, a remote sensor able to assess PM1, PM2.5, PM10, T, Hum, pressure and GPS in real time has been validated in France.
- A novel external exposure modelling framework which supports the objectives of HEALS has been developed. The approach makes use of data and models collected within Stream 3 as a whole following a life-course approach of external exposome characterization. For the first time such methodology was applied to a group of ~550 individuals (twins) and showcased the applicability of the methodology to existing studies. This allows to associate and link different life-long multi-stressor exposure profiles to specifics of individuals. Furthermore, this may allow to draw policy-relevant conclusions about population groups of society.
- Regarding the rest of activities, training and dissemination activities: reporting, meeting, networking and disseminating towards scientists but also stakeholders were held.
- In addition, the key training tools are available in the *ad hoc* web-based training portal (HEALS Moodle platform).
- Omics analysis has been performed *epidemiological cohort studies, specifically:*

- Repro-PL (PI Kinga Polanska, NIOM), cord blood SNP profiling and DNA methylome analysis, urinary and cord blood plasma metabolomics (NMR, LC-MS)
- Phime (PI Milena Horvat, JSI): urinary and cord blood plasma metabolomics, SNP profiling
- And the ISS twin cohort (PI Eugenia Doggliotti , ISS) adductomics (8-oxodG), anti-oxidant capacity, metalomics)
- EXHES cohort study has provided:
    - 154 urine and serum samples from Spain (URV) coming from pregnant women from the three different semesters of the pregnancy have been analysed for metabolomics (NMR, LC-MS)
    - 600 urine and serum samples from Germany (UKR), analysis is currently ongoing (NMR, LC-MS)

To the extent of mechanistic understanding for possible omics derived markers of exposure, and/or health status, *in vitro* models were employed, and more specifically:

- HepaRG (UPD) transcriptomics, MS based proteomics and metwbolomics, after treatment with a mixture of TCDD and alpha-endosulfan. Study of the same combination in the human colon cell line Caco2 with an emphasis on the alteration of cell metabolism and mitochondrial function
- HepaRG (UPD) transcriptomics, MS based proteomics, NMR and LC-MS based metabolomics after treatment with a mixture of phthalates and metals, based on results in REPRO PL and PHIME cohorts.
- Lung cells (CERETOX): MS based proteomics

In all the fields, analyses are progressing and results are expected soon. These results will be published in peer-reviewed journals.

As example of investigation of internal exposome. The work was initiated by the collection of existing omics protocols available at the HEALS partners, so to effectively start employing omics on samples available from (1) existing exposure/disease outcome studies; (2) newly developed exposome studies: the HEALS EXHES twin study. This activity has resulted in delivering D5.2, including an extensive overview of protocols and SOPs for omics technologies to be applied to biological samples from HEALS. In addition, some methods have been refined by partner FERA, UM and AUTH, in particular on adductomics and metabolomics. The next step was the application of omics technologies in several models in relation to environmental stressors (a.o. metals, phthalates, dioxins, organophosphates, phthalates) in existing human molecular epidemiological cohort studies:

- Repro-PL (PI Kinga Polanska, NIOM), cord blood SNP profiling and DNA methylome analysis, urinary and cord blood plasma metabolomics (NMR, LC-MS). From NMR metabolomics, 49 and 40 peaks were annotated in case of plasma and urine samples respectively, while from LC-MS/MS urine and plasma metabolomics 380 and 1700 peaks were annotated respectively. All of that resulted in the identification of 274 unique pathways.
- Phime (PI Milena Horvat, JSI): urinary and cord blood plasma metabolomics, SNP profiling. From the NMR analysis, 16 annotated peaks in case of plasma samples from mothers and 17 metabolites in case of children' plasma were annotated. From the LC-MS/MS analysis, 600 peaks in case of plasma samples from mothers were annotated,

while 800 metabolites were annotated from children plasma samples. Pathway analysis resulted in the identification of 93 unique pathways.
Pathway analysis in case of both studies (Repro_PL and Phime) revealed that alterations in urine metabolites are related to the TCA cycle, suggesting impaired mitochondrial respiration; the latter is central to energy metabolism and cellular signaling and plays fundamental roles in synthesis of nucleotides and active transport processes.

To the extent of mechanistic understanding for possible omics derived markers of exposure, and/or health status, *in vitro* models were employed, and more specifically*:*

- HepaRG (UPD) transcriptomics, MS based proteomics and metabolomics, after treatment with a mixture of TCDD and alpha-endosulfan. Study of the same combination in the human colon cell line Caco2 with an emphasis on the alteration of cell metabolism and mitochondrial function. Choline, valine and pyruvate level were increased whereas glutamate and citrate were decreased by the TCDD treatment (25nM), suggesting at least a perturbation of the energetic metabolism.
- HepaRG (UPD) transcriptomics, MS based proteomics, NMR and LC-MS based metabolomics after treatment with a mixture of phthalates and metals, based on results in REPRO PL and PHIME cohorts. Integrated pathway-level analysis of transcriptomics and proteomics data revealed that co-exposure to phthalates and heavy metals leads to the perturbation of the urea cycle. Co-mapping of proteomics and metabolomics data revealed that their common drivers are responsible for the homeostasis of metabolic pathways related to choline, phosphatidylcholine, phospholipases and triacylglycerol metabolism. The identification of the urea, phosphatidylcholine biosynthesis I and phospholipases metabolic pathways is of particular interest since these pathways have been also identified in human samples from the REPRO PL and PHIME cohorts.

Lung cells (CERETOX): MS based proteomics. From the analysis it was identified that several targets deregulated in the transcriptome experiments (UPD) were also deregulated at the protein level (CERETOX) in the three experiments performed using LC/MS technology. The results showed that those proteins are mainly related to detoxification response networks (molecular function: organic cyclic and heterocyclic compound binding) and RNA-translation (up-regulation of ribosomal proteins)

Dissemination

A huge amount of papers has been finalized and accepted or are presently submitted (see list in the WP and at the end of this document). So far, whe have 104 papers published in peer-reviewed journals. Amongst the most important journal: LANCET, EHP, JACI, Journal of Applied Toxicology…

Other papers in preparation and to be submitted to peer-reviewed journals include:

1. The HEALS methodological framework and study design – soon to be submitted in Environmental Health Perspectives.
2. "A critical review of how much of the difference in disease between socio-economic and other social groups can be explained by differences in the "group" exposome".
3. An overview of results from cohorts/population studies relating adverse health outcomes related to overweight, obesity, diabetes and metabolic disorders to environmental exposures of endocrine disruptors.

The HEALS paradigm and related issues were also presented several times in scientific congresses and meetings and to stakeholders in public events.

Last but not least, the HEALS project is quoted in international publications.

1.3 Expected final results and potential impact and use

HEALS findings will be employed to understand the development of the multifactorial diseases that demand both genetic and environmental exposures to occur and assess the respective health risk to the European population. More in general, the lessons learned from HEALS will be translated into scientific advice towards the development of protocols and guidelines for setting up of a larger European environment and health examination survey and enhancing health risk assessment via the operational use of the exposome.

1.4 Relevant contact details and list of partners

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