Oscillating Wildly?

Characterising Metabolic Phenotypic Variability in the Human Exposome

Toby Athersuch  Imperial College London 19th April 2017

Yale School of Public Health :: Environmental Health Sciences :: Lifetime Exposures and Human Health :: The Exposome

Declaration of Interest:
The author declares that the research for and communication of this independent body of work does not constitute any financial or other conflict of interest.
Figure 2. Steps that define the Systems Toxicology paradigm, from biological network models to dynamic adverse outcome pathway (AOP) models. The development of dynamic AOP models enabling the simulation of the population-level effects of an exposure is the ultimate goal of Systems Toxicology. This development follows three broad steps of maturity from top to bottom. The first level consists of the development of causal computable biological network models that link the system’s interaction of a toxicant with the organ-level responses. Such models can be used to quantify the biological impact of an exposure in the context of quantifiable end points such as histology or physiological measurements. In a second step, as more mechanistic knowledge derived from quantitative measurements accumulates, dynamic models linking the exposure with the organ-level responses can be developed. Ultimately, the third level of maturity is reached when the link between the exposure and the population outcome can be represented by mathematical models that enable the simulation of population-level effects of an exposure. Blue arrows denote causal links, which are mainly derived from correlative studies. Artwork by Samantha J. Elmhurst (www.livingart.org.uk).

Genomic analyses identify molecular subtypes of pancreatic cancer

The consensus molecular subtypes of colorectal cancer

Molecular subtyping for clinically defined breast cancer subgroups

Molecular subtyping and improved treatment of neurodevelopmental disease
"In the past decades, huge efforts have been devoted to a gene-based approach, identifying the specific genetic defects that... predispose an individual to disease. However, it is increasingly understood that such an approach, although enormously successful, is far from sufficient, especially because most cellular components exert their function through intricate networks of regulatory, metabolic, and protein interactions.

Therefore, the impact of different (and often disease-causing) genetic and epigenetic variations are not restricted but may spread in the intracellular network, affecting the activity and/or function of gene products that otherwise carry no defects.

Because of these complex interdependencies among a cell’s molecular components, the possibility of deep functional and causal relationships between apparently distinct disease phenotypes is apparent.
"

Multimorbidity – two or more concurrent chronic diseases

“…internal (biological) and external (social/environmental) perturbations of the genomic, proteomic and metabolomic networks … leading to different phenotypic expressions of the same underlying disease processes.

…Theoretically, this leads to the conclusion that much observable ‘multimorbidity’ results from a common underlying physiological disease process

…These processes affect the whole person across molecular, personal and social domains and physiologically leads to a new state of objective and subjective homeokinetic adaptation.

…While the emergence of the new stable physiological state may entail characteristics that enable better adaptation, it also can lead to a variety of ‘diagnosable diseases’.

“

Allostasis and Resilience

Reproduced from: Ghini et al. 2015. Allostasis and resilience of the human individual metabolic phenotype. J. Proteome Res. 14 (7) 2951-2962. Copyright 2015. American Chemical Society
Probabilistic "Pachinko" Metabolism

Systematic prospective and longitudinal collections in large-scale epidemiological studies open up new research opportunities:

- In adult studies, to explore responses to exposures and develop predictive models of chronic disease;
- In **mother-child cohorts**, to understand and discover the impact of **pre-natal and post-natal exposures** on the onset of child and subsequent adult physiological conditions;
- Availability of blood and urine samples for exposure and omics analysis

Previous metabolomics studies have investigated the impact of various **anthropometric factors** in an attempt to understand the human metabolome and inter-individual differences:

- Age
- Sex
- BMI / obesity

- Mainly focused on adults
- **Similar studies on children and adolescents are less common**
Metabolic profiles exhibit high temporal variability:

- Nicholson (2011) showed 60% (plasma) and 47% (urine) of biological variation in $^1$H NMR-detectable metabolite concentrations was stable and representative of **familial** and **individual-environmental** factors.

- Child studies / populations require specific metabolic variability assessment due to its different vulnerability to environmental factors.
  - Childhood represents a **critical physiological window** (pubertal)
  - Exposures at this time may be responsible for several metabolic deregulations.

- Data on short-term temporal variability of metabolomics markers in repeated urine samples are lacking, particularly in child studies.

Assessment of metabolic phenotypic variability in children’s urine using $^1$H NMR spectroscopy

**Aim:** To determine the most appropriate urine sample collection period / timepoint for large-scale epidemiology studies children (morning or nighttime urine, or a pool of both).

20 children (8-9 years) selected from the of the larger, multi-centre Human Early-Life Exposome (HELIX) project cohort.
- Spanish panel study, INMA (INfancia y Medio Ambiente; Spain).

Daily urine collections each day for one week:
- Spot urine (morning)
- Spot urine (night-time)

A pooled sample was also prepared, combining morning and nighttime collections for each individual, each day.

The Human Early-Life Exposome (HELIX): Project Rationale and Design

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FP7 HELIX – Mother-Child Cohorts

MoBa
KANC
BiB
EDEN
INMA
RHEA
## FP7 HELIX – Mother-Child Cohorts

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Enrollment Years</th>
<th>Participants</th>
<th>Age in 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>BiB</td>
<td>2007-2010</td>
<td>14,000</td>
<td>4-7</td>
</tr>
<tr>
<td>EDEN</td>
<td>2003-2006</td>
<td>2,000</td>
<td>8-11</td>
</tr>
<tr>
<td>INMA</td>
<td>2004-2006</td>
<td>2,500</td>
<td>8-10</td>
</tr>
<tr>
<td>KANC</td>
<td>2007-2009</td>
<td>4,000</td>
<td>5-7</td>
</tr>
<tr>
<td>MoBa (Oslo region only)</td>
<td>2004-2008</td>
<td>8,000</td>
<td>6-10</td>
</tr>
<tr>
<td>RHEA</td>
<td>2007-2008</td>
<td>1,500</td>
<td>6-7</td>
</tr>
</tbody>
</table>
FP7 HELIX – Study Design

1. Entire cohorts
   - $n = 32,000$ mother–child pairs from the 6 cohorts
   - Existing data:
     - Exposures
     - Phenotypes
     - Social factors, diet, etc.
   - New spatial models for outdoor exposures
   - Outdoor exposome

2. HELIX subcohort
   - $n = 1,200$ mother–child pairs from the 6 cohorts
   - Exposure biomarkers
   - Exposure questionnaires and models
   - Omics
   - Behavior, diet, social factors, etc.
   - Harmonized phenotypes
   - Total exposome

3a. Child Panel Study
   - $n = 150$ children from the 6 cohorts
   - Repeat biomarkers
   - Omics
   - Smartphones
   - Exposure sensors
   - Diaries (diet, habits)

3b. Pregnancy Panel Study
   - $n = 150$ volunteer women
   - Repeat biomarkers
   - Smartphones
   - Exposure sensors
   - Diaries (diet, habits)

4. Health impact assessment
   - Europe-wide data from birth cohorts ($> 300,000$ mother–child pairs) and surveys
     - Burden of disease estimates
     - Complex benefit–harm scenarios
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<tr>
<th>Exposure group</th>
<th>Entire cohorts (n = 32,000)</th>
<th>HELIX subcohort (n = 1,200)</th>
<th>Child Panel Study (1 week in 2 seasons) (n = 150)</th>
<th>Pregnancy Panel Study (1 week in 2 seasons) (n = 150)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCB-153, DDE, HCB, PBDE-47</td>
<td>—</td>
<td>Biomarkers: in stored pregnancy blood samples&lt;sup&gt;a&lt;/sup&gt; and in newly collected child blood samples.</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PFAS (PFOS, PFOA, PFBS, PFHxS, PFNA)</td>
<td>—</td>
<td>Biomarkers: in stored pregnancy blood samples&lt;sup&gt;a&lt;/sup&gt; and in newly collected child blood samples. PBPK models for pregnancy and childhood.</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Metals (Hg, Pb, and TMS)</td>
<td>—</td>
<td>Biomarkers: in stored pregnancy samples&lt;sup&gt;b&lt;/sup&gt; and in newly collected child samples: blood (Pb), urine (TMS), and hair (Hg).</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Phthalates (13 metabolites)</td>
<td>—</td>
<td>Biomarkers: in stored pregnancy urine samples&lt;sup&gt;b&lt;/sup&gt; and in newly collected child urine samples (last night and first morning void).</td>
<td>Biomarkers: in daily repeat urine samples. Daily data on diet, cosmetics. PBPK model for DEHP.</td>
<td>Biomarkers: in daily repeat urine samples. Daily data on diet, cosmetics. PBPK model for DEHP.</td>
</tr>
<tr>
<td>Phenols (BPA, parabens, TCS, BP3)</td>
<td>—</td>
<td>Biomarkers: in stored pregnancy urine samples&lt;sup&gt;b&lt;/sup&gt; and in newly collected child urine samples (last night and first morning void).</td>
<td>Biomarkers: in daily repeat urine samples. Daily data on diet, cosmetics.</td>
<td>Biomarkers: in daily repeat urine samples over whole week. Daily data on diet, cosmetics.</td>
</tr>
<tr>
<td>OP pesticides</td>
<td>—</td>
<td>Biomarkers: in stored pregnancy urine samples&lt;sup&gt;b&lt;/sup&gt; and in newly collected child urine samples (last night and first morning void).</td>
<td>Biomarkers: in daily repeat urine samples in two seasons. Daily data on diet and repellent use.</td>
<td>Biomarkers: in daily repeat urine samples in two seasons. Daily data on diet and repellent use.</td>
</tr>
<tr>
<td>Water DBPs</td>
<td>Estimates available from previous HiWATE project during and after pregnancy.</td>
<td>New questionnaire in children on water consumption and swimming combined with water company data.</td>
<td>Water consumption diaries.</td>
<td>Water consumption diaries.</td>
</tr>
<tr>
<td>Indoor air: BTEX, NO&lt;sub&gt;2&lt;/sub&gt;, PM&lt;sub&gt;2.5&lt;/sub&gt;</td>
<td>Existing questionnaire data on indoor sources during and after pregnancy.</td>
<td>New questionnaire in children on cooking, heating, cleaning, and ventilation.</td>
<td>Passive BTEX and NO&lt;sub&gt;2&lt;/sub&gt; sampling in the home. Active PM&lt;sub&gt;2.5&lt;/sub&gt; sampling. Questionnaire on cooking, heating, cleaning, and ventilation.</td>
<td>Passive BTEX and NO&lt;sub&gt;2&lt;/sub&gt; sampling in the home. Active PM&lt;sub&gt;2.5&lt;/sub&gt; sampling. Questionnaire on cooking, heating, cleaning, and ventilation.</td>
</tr>
<tr>
<td>ETS</td>
<td>Existing questionnaire and cotinine data during and after pregnancy.</td>
<td>New questionnaire in children. Biomarkers: cotinine measurement in newly collected child urine and/or hair samples.</td>
<td>Questionnaire on ETS.</td>
<td>Questionnaire on ETS.</td>
</tr>
</tbody>
</table>

**Abbreviations:** BP3, benzophenone-3; BPA, bisphenol A; BTEX, benzene, toluene, ethylbenzene, xylene; DBPs, disinfection by-products; DDE, dichlorodiphenylchloroethylene; DEHP, di(2-ethylhexyl) phthalate; ETS, environmental tobacco smoke; HCB, hexachlorobenzene; Hg, mercury; NO<sub>2</sub>, nitrogen dioxide; OP, organophosphate pesticides; Pb, lead; PBDE-47, polybrominated diphenyl ether-47; PCB-153, polychlorinated biphenyl-153; PFAS, perfluoroalkyl substances; PFBS, perfluorobutanesulfonic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; TCS, triclosan; TMS, total metal spectrum.

<sup>a</sup>Where measurements are available from previous studies, these will be used. "Pool"ing of ≥ 2 urine samples when available.
<table>
<thead>
<tr>
<th>Exposure group</th>
<th>Entire cohort ((n = 32,000)), for pre- and postnatal exposure periods</th>
<th>Subcohort ((n = 1,200))</th>
<th>Child Panel Study (1 week in 2 seasons) ((n = 150))</th>
<th>Pregnancy Panel Study (1 week in 2 seasons) ((n = 150))</th>
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<tr>
<td>Ambient air pollutants</td>
<td>LUR model for (NO_2), PM(<em>{2.5}), PM(</em>{10}), PM(<em>{\text{coarse}}), PM(</em>{2.5}) absorbance, PM (_{\text{elemental analyses}}). Routine monitoring and OMI satellite data for temporal variability.</td>
<td>LUR model for (NO_2), PM(<em>{2.5}), PM(</em>{10}), PM(<em>{\text{coarse}}), PM(</em>{2.5}) absorbance, PM (_{\text{elemental analyses}}). Routine monitoring and OMI satellite data for temporal variability.</td>
<td>Inhalation rates and mobility (GPS) data from smartphones. Personal monitoring (24 hr) of PM(_{2.5}) (and black carbon).</td>
<td>Inhalation rates and mobility (GPS) data from smartphones. Personal monitoring (24 hr) of PM(_{2.5}) and black carbon.</td>
</tr>
<tr>
<td>Noise</td>
<td>Existing municipal noise maps to obtain spatial estimates. Address-based modeling of noise at the most and least exposed facade.</td>
<td>New questionnaires in children on bedroom position, noise perception, etc. Noise estimates based on maps and questions.</td>
<td>Time–activity and mobility (GPS) data from smartphones.</td>
<td>Time–activity and mobility (GPS) data from smartphones.</td>
</tr>
</tbody>
</table>

Abbreviations: GIS, geographic information system; GPS, global positioning system; LUR, land use regression; \(NO_2\), nitrogen dioxide; \(NO_x\), nitrous oxides; OMI, ozone monitoring instrument; PM\(_{2.5}\), particles ≤ 2.5 μm in size; PM\(_{2.5}\) absorbance, measurement of the blackness of PM\(_{2.5}\) filters—a proxy for elemental carbon, which is the dominant light-absorbing substance; PM\(_{\text{coarse}}\), particles between 2.5 and 10 μm in size; PM\(_{10}\), particles ≤ 10 μm in size.
## Omics Measurements

<table>
<thead>
<tr>
<th>Omics technique</th>
<th>Entire cohort (n = 32,000)</th>
<th>Subcohort (n = 1,200 mother–child pairs)</th>
<th>Child Panel Study (1 week in 2 seasons) (n = 150)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolomics</td>
<td>—</td>
<td>Untargeted $^1$H NMR spectroscopy and semitargeted UPLC-MS analysis in urine; targeted analysis in serum (using Bioclates Absolute IDO p180 Kit) in newly collected child samples.</td>
<td>Further analysis of daily urine samples and single serum sample at the end of each week (in winter and summer seasons) to evaluate sources of variation and short-term exposure–omics associations.</td>
</tr>
<tr>
<td>Proteomics</td>
<td>—</td>
<td>Targeted analysis in newly collected child plasma samples depending on results of analysis in the Child Panel Study.</td>
<td>Initial iTRAQ and MRM (or similar) analyses in plasma samples collected at end of each week (in winter and summer seasons) to evaluate sources of variation and short-term exposure–omics associations.</td>
</tr>
<tr>
<td>Transcriptomics</td>
<td>—</td>
<td>Next-generation sequencing (Illumina HiSeq2000) or microarray analysis of both mRNAs and miRNAs in newly collected child whole blood samples. In addition, plasma will be collected to analyze miRNAs in the future.</td>
<td>Analysis of blood samples at the end of each week (in winter and summer seasons) to evaluate sources of variation and short-term exposure–omics associations. In addition, plasma will be collected to analyze miRNAs in the future.</td>
</tr>
<tr>
<td>DNA methylation</td>
<td>—</td>
<td>Infinium Human Methylation 450 BeadChip for genome-wide methylation analysis of DNA extracted from newly collected child whole blood samples.</td>
<td>Analysis of blood samples at the end of each week (in winter and summer seasons) to evaluate sources of variation and short-term exposure–omics associations.</td>
</tr>
</tbody>
</table>

Abbreviations: $^1$H NMR, proton nuclear magnetic resonance; iTRAQ, isobaric tags for relative and absolute quantitation; MRM, mass spectrometry–based multiple reaction monitoring; miRNA, microRNA; mRNA, messengerRNA; UPLC-MS, ultra performance liquid chromatography–mass spectrometry.

*Details of the techniques are described in Supplemental Material, Detailed description of omics techniques to be used in HELIX, pp. 4–6. *The Pregnancy Panel Study will collect biological samples similar to those of the Child Panel Study. Omics analyses are currently not foreseen in the pregnant women, but samples will be stored for future analysis, e.g., to evaluate whether specific omics findings from the children are replicated in the pregnant women.*
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   \( n = 32,000 \) mother–child pairs from the 6 cohorts
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4. Health impact assessment
   Europe-wide data from birth cohorts (> 300,000 mother–child pairs) and surveys
   - Burden of disease estimates
   - Complex benefit–harm scenarios

Uncertainties
- Temporal variability
- Behaviors
- Validation
- Internal dose

Exposure estimates
Exposure–response estimates
Precision High-Throughput Proton NMR Spectroscopy of Human Urine, Serum, and Plasma for Large-Scale Metabolic Phenotyping

Anthony C. Dona,†,§ Beatriz Jiménez,†,‖ Hartmut Schäfer,‡ Eberhard Humpfer,‡ Manfred Spraul,‡ Matthew R. Lewis,†,§ Jake T. M. Pearce,†,§ Elaine Holmes,† John C. Lindon,† and Jeremy K. Nicholson*,†,§,‖

Analysis

- 1D $^1$H NMR spectra acquired at 14.1 T using standard protocols for urinalysis with water suppression (Dona et al. 2014)

- 300 K / 128 transients / recycle delay 4s / spectral width 12000 HZ

- Spectra processed using recursive segment-wise peak alignment

- 44 metabolites quantified by integration of representative resonance
- Signal-to-noise calculated for each integral

- Signal intensities corrected where possible using compensation factor for incomplete $T_1$ relaxation based on inversion recovery data

- Spectral assignment using in-house database / HMDB / 2D NMR / STOCSY / spike-in

Analytical variability was assessed:

- Coefficient of variation (CV%) was calculated for NMR signals representing individual metabolites across repeat analysis of the same representative pool sample:
  - 7.2% (mean)
  - 7.7% (median)

- Metabolites with a low signal to noise ratio presented a higher CV% (typically >10%)
  - N$^\prime$-methylnicotinamide
  - N-methylnicotinic acid
  - scyllo-inositol.
Intra-class correlation coefficients (ICC) were calculated to assess the stability of metabolites over the sampling period:

- ICC is a measure of the reliability of repeated measures over time, defined as the ratio of between-subject variance to the total variance (between-subject plus within-subject).
- In this context: higher ICC for metabolite = more stable over time

\[
ICC = \frac{\sigma_{0\&}}{\sigma_{0\&} + \sigma_{1}}
\]

\(\sigma_{0\&}\) - variance between group
\(\sigma_{1}\) - variance within group

ICC values for the **pooled samples** indicated the highest stability of the metabolite profiles over the sampling period.
Decomposition of biological variation for each annotated metabolite using a mixed model analysis of variance.
- Metabolites are ordered by estimated inter-individual variance

Estimates for the proportion of biological variance explained by each of three components –
- Collection (diurnal)
- Child (individual)
- Combined between-day and technical variability
Summary

- Good analytical reproducibility and precision was demonstrated for the $^1$H NMR spectroscopic platform (median CV for all metabolites 7.7%)

- **Pooled samples** captured the best *inter-individual variability* with 19 out of 44 metabolites with ICC values above 0.5 whereas only 11 and 8 metabolites were above ICC 0.5 respectively in morning and night-time samples.

- Trimethylamine, *N*-acetyl neuraminic acid, 3-hydroxyisobutyrate, 3-hydroxybutyrate/3-aminoisobutyrate, tyrosine, valine, 3-hydroxyisovalerate were the metabolites that showed the greatest *inter-individual variability* with ICCs in pooled samples over 0.7.

- TMAO, proline betaine, acetate, *N*-methylpicolinic acid were the least stable with ICCs under 0.2.

- We *suggest* future studies should collect urine day pool samples to capture best the *inter-individual variability*. 
Development and Application of Ultra-Performance Liquid Chromatography-TOF MS for Precision Large Scale Urinary Metabolic Phenotyping

Matthew R. Lewis,†,‡ Jake T. M. Pearce,†,‡ Konstantina Spagou,‡ Martin Green,§ Anthony C. Dona,†,‡,¶ Ada H. Y. Yuen,† Mark David,† David J. Berry,† Katie Chappell,‡ Verena Horneffer-van der Sluis,† Rachel Shaw,†,‡ Simon Lovestone,¶ Paul Elliott,¶ John Shockcor,‡ John C. Lindon,‡ Olivier Cloarec,¶ Zoltan Takats,‡ Elaine Holmes,‡ and Jeremy K. Nicholson*†,‡

Jointly funded for 5 years by the
• National Institute for Health Research
• Medical Research Council
• Director: Nicholson (ICL)

In partnership with
• Waters Corporation
• Bruker
• King’s College London

**Nuclear Magnetic Resonance**  **Mass Spectrometry**  **Informatics and Chemometrics**
Phenome Centre
UPLC & MS Resources

15x Acquity UPLC
10x Xevo G2-S Q-ToF
5x Xevo TQ-S

Centre R&D / Training Resources

2x Waters Synapt G2-S HDMS
2x Waters Xevo TQ-S
3x Acquity UPLC
1x NanoAcquity UPLC
1x Acquity UPC²
10x UPLC-MS
Xevo G2-S Q-ToFs

- Molecular profiling for biomarker discovery
- Broad metabolite coverage from a limited number of complimentary techniques

5x UPLC-MS
Xevo TQ-S

Targeted analysis for biomarker validation, pathway probing, and profiling augmentation

Sample capacity: 25,000 to 100,000 samples analysed per year
Assays per instrument

Courtesy of M. Lewis
RPC

HILIC

Assays per instrument

Courtesy of M. Lewis
Neutrulation

**HILIC**
- (bases)
- (acids and neutrals)

**RPC**
- (lipids)
- (moderate species)

**Assays per instrument**

Courtesy of M. Lewis
Perspectives

• Systems toxicology is central to deciphering the exposome:
  • **Omics analyses** will help populate biological network models to link exposures and endpoints
  • **AOPs** will help anchor metabolic phenotypic information and provide a format/language for comparing studies

• Dynamism, cumulative effects and time-series are a priority:
  • All exposures and responses have a **unique context**
  • Understanding aspects of **individual variation, allostasis and resilience** will help delineate when substantial perturbations to metabolic disease networks have actually occurred.
  • Understanding **metabolic phenotypic variation** will inform probabilistic network models of metabolic disease networks

• We recently explored the variation in $^1$H NMR urinary spectra obtained from children in the INMA cohort of the **EU FP7 HELIX** project:
  • **A daily pooled urine** is suggested in preference to morning or night-time spot collection on the basis of ICC distribution
• **Longitudinal** studies with multiple high-quality biosample collections needed (high temporal resolution) to deconvolve short and long-term responses, diurnal variation, cyclic processes

• Continued characterisation of **individual metabolic variation** to provide snapshot baselines for future comparisons

• Community deposition of data (funder mandated?) for **open access** and analysis with transparent protocols / metadata

• High-capacity, well-resourced **metabolic phenotyping centres** to reduce barrier to entry for researchers
Acknowledgements

Imperial College London

Jeremy Nicolson
Elaine Holmes
Ian Wilson
John Lindon

Paul Elliott
Paolo Vineis
Ioanna Tsoulaki

Muireann Coen
Hector Keun
Liza Selley
Maitre
ChungHo Lau
Alexandros Siskos
Olly Robinson
Elizabeth Want
+ HELIX collaborators

Matthew Lewis
Jake Pearce
+ NPC researchers
+ many others

Royal Society of Chemistry

Waters

Bruker

European Commission Integrated Translational Medicine Partnership (ITTP)

CSM

BTS

Exposomics

Building the Early Life Exposome (HELIX)

NPC

National Phenome Centre
Thanks for your attention