Dr Claudine Manach’s group is using a novel metabolomics methodology to support their work identifying new biomarkers of food intake. Here, she discusses this work and her hopes for the future development of the field.

To begin, could you introduce the concept of the food metabolome (FM)?

The FM refers to the whole set of metabolites present in our body that directly derive from the digestion of food. Every day we ingest hundreds of chemicals found in food. These comprise nutrients but also natural non-nutrients such as plant food bioactives and manmade non-nutrients including contaminants, additives or compounds produced during food processing. Once absorbed, these compounds are transformed by our liver enzymes and gut microbiota into a large array of metabolites. The size of the FM is not known because many of the ingested compounds and their metabolites remain unidentified.

In this context, what were the main aims and objectives of the PhenoMeNEp project?

PhenoMeNEp’s objective was to identify new biomarkers of plant food intake for nutritional epidemiology using a metabolomics approach. Low and high consumers of 20 different plant foods were selected on the basis of their reported dietary intakes over 15 years. Their urine and plasma metabolic profiles were analysed by mass spectrometry-based metabolomics and compared in order to identify markers that were only present, or markedly increased, after consumption of the target food. PhenoMeNEp also had important methodological objectives to develop new methods and tools for the analysis and annotation of FM profiles.

How did you become involved in this work and where does your expertise lie?

Our group began developing metabolomics to holistically study the biological effects of nutritional interventions with plant food or extracts. In our initial studies, we identified polyphenol metabolites as major features characterising the urine metabolome after plant food consumption. We realised that the urine metabolome may not only reflect the impact of food on the host physiology, but also contain markers of recent food intakes.

My expertise lies in phytochemicals, especially polyphenols, their dietary sources and metabolism, as well as their analysis in human biofluids. As phytochemical metabolites represent a large part of the FM, this knowledge helps to identify the discriminant metabolites that increase in biofluids after plant food intake.

Who are PhenoMeNEp’s key partners and how has this collaborative approach contributed to the project’s achievements?

The Unit for Research on Nutritional Epidemiology (UREN) has conducted some of the most important cohort studies on nutrition and health in France. Their contribution was essential both as future end users of the discovered biomarkers and as providers of precious samples. They selected subjects and shared high quality data, including dietary intake information, collected over 15 years in the Supplementation en Vitamines et Minéraux Antioxydants (SU. VI. MAX) cohort.

The other major partner was the Metabolism Exploration Platform (PFEM), a mass spectrometry platform dedicated to metabolism studies and founding member of MetaboHUB – the French national infrastructure for metabolomics and fluxomics. PFEM is a national and international leader in metabolomics applied to nutrition. They did an excellent job developing new methods and tools for the analysis and annotation of the FM profiles.

Why is the role of online databases and data sharing important in metabolomics research?

Databases make current knowledge on a large variety of compounds available to everyone, including data useful for metabolomics studies, such as the exact masses of compounds. These databases act as dictionaries with which we decrypt the metabolomes. Sharing of expertise is vital so that we can collectively write these dictionaries as quickly as possible.

Data repositories with standardised formats are also required to compare the discriminating features found in different nutrition studies. For example, if we find a candidate biomarker for apple, we will check that it is associated with apple consumption and not with other foods in the available studies. This will allow prioritisation of the most important markers to identify and validate.

How do you envisage metabolomics developing as a field?

There is now a large community in metabolomics, and annual meetings of the Metabolomics Society gather more than 700 participants. Collective initiatives are underway, for example to standardise methods and implement databases and data repositories.

Regarding the FM as a field, the first stone was laid in July 2013 in Glasgow with the inaugural international workshop, attended by 50 experts, on ‘The food metabolome and biomarkers for dietary exposure’ that we co-organised with Dr Augustin Scalbert from the International Agency for Research on Cancer and Dr Lorraine Brennan from University College, Dublin. We wrote a position paper with the most active scientists in the field to summarise the workshop discussions and identify research gaps and technological needs. This new network is being structured and we hope to organise other meetings and data sharing activities soon.
Delving into diets

To help elucidate the health effects of plant foods, the PhenoMeNEp research project is analysing the food metabolome and building up a comprehensive database on dietary phytochemicals to support future research.

THERE ARE STILL many unresolved questions and inconsistencies in the study of nutrition, including the full nutritional effects of consuming fruit and vegetables, which are largely considered to be healthy foods. Plant foods contain hundreds of phytochemicals, which after ingestion are absorbed and metabolised by the intestine, liver and gut microbiota into a variety of metabolites. Much key information remains unknown in relation to the plant food components that affect health and the interindividual variation in response to their consumption.

Cohort studies are vital to carrying out research into nutrition, but the methods currently available to assess an individual’s diet, such as questionnaires or the identification of a few biomarkers, are imprecise and do not include all the relevant dietary factors. Unlike drugs, which have a focused effect, nutritional compounds from plants act more diffusely, effecting mild changes in a broad spectrum of molecular and physiological targets. Recent technological advances in the fields of mass spectrometry and bioinformatics have opened up a range of exciting possibilities in the analysis of biological samples. These developments have enabled the simultaneous detection of hundreds of host and food-derived metabolites in urine or plasma, and statistical comparisons of complex profiles – this approach is known as metabolomics.

METABOLOMICS

At the French National Institute for Agricultural Research (INRA), one research group is developing this approach to improve understanding of the health effects of plant foods by analysing the food metabolome (the set of metabolites present in human biofluids and tissues that directly originate from the digestion of foods) and the variations in the endogenous (host) metabolome. Because metabolomics permits researchers to analyse the complexity of phytochemical exposures beyond the most studied compounds, this approach could mean that new bioactives and synergies are discovered.

Dr Claudine Manach, Project Coordinator of the PhenoMeNEp project, explains: “Metabolomics, as a holistic exploratory approach, is well suited to study both the variety of phytochemical metabolites our bodies are exposed to and the pleiotropic effects of plant foods on metabolic pathways in an integrative manner. Furthermore it is a data-driven approach likely to generate new hypotheses where knowledge is lacking.”

Although metabolomics is expanding possibilities for researchers, there are limitations to current methods. Manach’s team compare the food metabolomes (FMs) of individuals using multivariate statistics to assess the discrimination between the experimental groups and locate features that contribute most to the discrimination. Next, they identify the discriminating features by comparing their experimental data against the databases. “The main bottleneck is that databases are incomplete, especially regarding compounds of the FM. It’s like reading a book in a foreign language without knowing enough vocabulary to understand what it says,” Manach explains.

NEW BIOMARKERS

PhenoMeNEp aims to develop a novel metabolomics approach in order to discover new biomarkers of intake for a range of plant foods. The study used biological samples from a large French cohort (Supplementation en Vitamines et Minéraux Antioxydants –
The group found clear differences in profiles of low and high consumers for most plant foods.

SU.VI.MAX) and was based on the premise that specific signatures of the recent or usual intake of target foods or dietary patterns could be produced by the analysis of the FM. PhenoMeNEp was the first attempt ever to use this approach and compared the metabolomic profiles of plasma and urine samples taken from individuals who consumed either low or high quantities of fruits and vegetables. They focused on 20 plant foods, based on their nutritional interest or their high frequency consumption within the French population.

The group found clear differences in profiles of low and high consumers for most plant foods – particularly for commonly consumed items that are rich in phytochemicals such as coffee, tea, apple, citrus and tomato. They also identified specific biomarkers of citrus and coffee intake, proline betaine, and diterpenes or alkaloid metabolites respectively. Manach’s next step is to identify biomarkers for other foods and dietary patterns. This will enable mapping of the fingerprint of nutritional exposures to a full picture of dietary behaviours.

**PHYTOHUB**

To help address the data limitations that currently exist, Manach, with the bioinformaticians Frank Giacomoni and Craig Knox, is working on the development of PhytoHub, an online, open-source database to facilitate the identification of phytochemical components of the food metabolome. The database will include approximately 1,000 dietary phytochemicals representing polyphenols, terpenoids, alkaloids and other plant secondary metabolites. For each phytochemical, PhytoHub will provide data on its main dietary sources, known human metabolites, in silico predicted metabolites, and physico-chemical and spectral data.

Users will be able to browse PhytoHub or search by food source, phytochemical name, molecular formula, mass, etc. For example, a list of matching phytochemicals and metabolites, including details about their dietary origin, can be created by querying a specific mass. This allows researchers to quickly and easily pull up possible matches for unknown features of a metabolomic profile. The team hopes to launch PhytoHub in the next few months; and to build the database quickly into a comprehensive source, they will need to cooperate with other laboratories with relevant expertise.

**IMPROVING HEALTH**

The implications of PhenoMeNEp are far reaching, as the innovative approach can be applied to a broad range of areas. One area where FM analysis could be beneficial is in the monitoring of the impact of public health policies and nutritional recommendations at both the individual and population level. This will allow better assessment of adherence to the recommendations and provide more knowledge for making evidence-based recommendations. Improvements in the food processing industry leading to the production of healthier foods could also be furthered by monitoring the impact of food processing on consumers’ nutritional exposures.

Furthermore, the approach could lead to the development of new research areas: “FM analysis gives access to the individual’s nutritional exposures, which opens tremendous research possibilities,” Manach enthuses. She is particularly interested in the determinants (age, genotype, microbiota, etc.) of the interindividual variation in absorption and metabolism of plant food bioactives. However, in order to fully benefit from all the information available in the food metabolome profiles, researchers must continue to improve their tools and methodologies, from study design to data treatment and interpretation. In the future, FM profiles could be related to health outcomes through FM wide association studies, and this could lead to a better understanding of the complex relationships between genes, nutrition and health.