Dr Tim Nawrot of Hasselt University in Belgium provides some insights on the importance of epidemiologic research, and how his latest research efforts will impact the field.

**Impact Objectives**

- Establish prospective epidemiological evidence for molecular mechanisms or early biomarkers, which may underlie the origins or reflect the risk of age-related diseases
- Understand the association of age-related diseases with other processes and the influence of environmental factors

**Shaping the future of epidemiologic research**

Ageing mechanisms and their correlation with environmental exposure are the central topics of your research. Why is it important to address these matters currently?

Overall ageing is not disease-specific but an integrated phenotype. Many environmental risk factors are involved in ageing, causing premature diseases and major health problems in adult populations. Therefore, it is important to determine the internal drivers (e.g. intrauterine environment, maternal factors, genetic variability and inflammation) and the external drivers (e.g. lifestyle, nutrition, environmental pollutants, particulate matter and endocrine disruptors) of unhealthy development or ageing, and study the interaction between them. In this context we expect to implement cost-effective prevention strategies, based on a better understanding of the mechanisms underlying unhealthy development and ageing; promote targeted preventive public healthcare; and contribute to a healthier living environment for pregnant mothers and their children.

**How do you think your current project, ENVIRONAGE (ENVIRONMENTAL INFLUENCE ON early AGEing), will impact the healthcare field in the upcoming years?**

So far, studies on the health effects of air pollution exposure during the most vulnerable stages in life, including the in utero period, are still scarce, and ENVIRONAGE, which was initiated in 2010, was designed with that purpose in mind. Furthermore, there is a need for research in populations, as we are dealing with complex phenotypes, and there is still no generally accepted metric to differentiate normal from dysfunctional development or healthy from unhealthy ageing. We are confident that detailed repeated physiological, and clinical measurements integrated with omic markers, will contribute to understanding early life exposures, and how they might impact future disease development. Some of the results obtained within the framework of the ENVIRONAGE birth cohort study have already been published in international peer-reviewed journals already, and recent findings were presented at international meetings such as the International Meeting of Environmental Epidemiology.

Can you talk about how your partnerships with the Epigenetic Placenta consortium and the EXPOsOMICS consortium contributed to the study’s success?

The ENVIRONAGE birth cohort is involved in diverse networks that aim to provide a collaborative environment by pooling omics data from different studies. Our collaboration with the Epigenetic Placenta consortium helped us to move forward in the field of epigenetics, by forming a resource for researchers who have collected placental tissue from a birth cohort, and are planning to do epigenetic research on the placenta. By combining data and epigenome-wide findings, the statistical power of future studies will increase and yield more robust and consistent results.

With the EXPOsOMICS study, we aim to characterise the external and the internal components of the exposome. EXPOsOMICS is an EU-funded project that uses a holistic approach to study biochemical and molecular changes in the human body in relation to the external exposome by combining epigenetics, transcriptomics, metabolomics and proteomics. We also joined forces with four other European mother-child cohorts to deepen the investigation into the in utero air pollution exposome based on associations with child health and development including birth weight, growth and asthma, among other outcomes.
Lifelong study into ageing

A population cohort study called ENVIRONAGE is set to deepen our understanding of ageing mechanisms and how environmental exposure influences the ageing process throughout life.

Ageing is a biological mechanism universal to every living being, from simple cells to more complex species, which is both irreversible and inevitable. Not only universal and unavoidable, ageing is also a complex mechanism and sensitive to both internal and external factors, leading to a decrease in cellular function and genetic instability.

Telomeres play a pivotal role in ageing and age-related diseases such as cardiovascular diseases, type 2 diabetes, and atherosclerosis. Telomeres are small structures that stabilise the chromosomes and protect them from degradation. When a cell divides, telomeres become progressively shorter, until they wear off and the cell’s chromosomes deteriorate, leading to cell death. Telomere length indicates how many times a cell has divided and is considered a hallmark of ageing.

Similarly, mitochondria – the cell’s powerhouse – are involved in many cellular functions, especially those related to cellular senescence and death. Disruptions in these functions were linked to reductions in cellular lifespan and the onset of endocrine, cardiovascular, and oncologic diseases. Together with telomeres, they form a core aspect of ageing mechanisms.

RETURN TO THE ORIGINS
Dr Tim Nawrot, Professor of Environmental Epidemiology at the Centre of Environmental Sciences at Hasselt University and part-time Associate Professor in the Unit of Environmental Health at Leuven University in Belgium, is the Principal Investigator for a project that seeks to unravel the connection between prenatal environmental exposure and disruption of the core axis of ageing. ENVIRONAGE (ENVironmental influence ON early AGing) is a long-term cohort study that was initiated in 2010 and is funded by the European Research Council and the Flemish Scientific Research Council.

The focus is on understanding the outcomes of prenatal exposure to lifestyle or environmental factors over molecular targets in ageing, such as telomere length and mitochondrial function. ‘Both mitochondrial DNA and telomeres are extremely vulnerable to external damage, and conservation of their function is an important mechanism in extending lifespan,’ explains Nawrot.

ENVIRONAGE was established to further investigate the hypothesis that the great majority of diseases in later life are programmed by environmental factors exposure during gestation, as postulated by previous research. Nawrot’s team speculates that the placenta holds the key for molecular modifications occurring in utero. A dysfunction in placental mitochondria associated with environmental factors leads to excessive oxidative stress and telomere degradation, which in turn translates into a greater susceptibility for age-related diseases. Therefore, the team is working on obtaining evidence that reflects this link in order to elucidate the onset of ageing processes in early life.

A LIFETIME GOAL
In order to evaluate mitochondrial DNA content and telomere length in newborns, the team collected cord blood and placental tissue from newborn-mother pairings recruited at East Limburg Hospital in the Belgian city of Genk right after delivery. DNA extracted from the collected tissues was analysed using real-time polymerase chain reaction (PCR) to estimate relative mitochondrial DNA and telomere length. Measuring these molecular parameters, and studying their correlation with important environmental and lifestyle factors collected via medical records and questionnaires of the mothers participating in the cohort, is vital to strengthen ENVIRONAGE’s primary research concept.

After the initial evaluation at birth, Nawrot’s team followed the participating children in different life stages, starting with a follow-up of four to six years at Hasselt University. ‘At the first follow-up we are able to assess potential outcomes such as cardiovascular outcomes and cognitive function,’ says Nawrot. During follow-up, the mother-child pairs go through a detailed evaluation of their lifestyle data (pertaining to their diets, exercise routines, education), medical data, and most importantly, clinical and neurological development measurements. ‘Next, we investigate whether these early life health outcomes may be associated with environmental factors or altered molecular pathways at the follow-up age.’

ONE OF A KIND
The uniqueness of the ENVIRONAGE consortium lies in several aspects, from performing measurements at the different ‘omics’ levels in relevant biological tissues, to promoting novel preventive public healthcare strategies. ‘The ENVIRONAGE birth cohort is designed with a strong focus on early molecular mechanisms to bridge the gap between exposure and disease,’ says Nawrot. ‘Our study combines both upstream (metabolomics) as well as downstream omic signatures including epigenomics and transcriptomics,’ he adds, which can open up opportunities for the discovery of new biomarkers.

‘We are particularly interested in studying particulate matter (PM) and its potential influence in the ageing pathways,’ reveals Nawrot. In the study, PM exposure is estimated based on the maternal residential address, combining pollution data collected through monitoring networks and land cover data obtained from satellite images. By analysing these parameters, the ENVIRONAGE consortium aims to contribute to a safer living environment for pregnant mothers and their children.

TIMELESS KNOWLEDGE
ENVIRONAGE is an ambitious population study as it encompasses results from a wide cross-generational sample, which can be later generalised to the remaining population. ‘Studying two different segments of the population allows us to explore common mechanisms of ageing influenced by environmental factors throughout life, and will give us a better insight in the potentially higher susceptibility in children and newborns,’ explains Nawrot. ‘An in-depth integrated analysis on how these mechanisms lead to well-defined clinical outcomes gives us the possibility of developing new biomarkers relevant in age-related diseases.’

Above all, a great part of ENVIRONAGE’s potential is drawn from the highly collaborative efforts to establish the lead in environmental health and ageing.
The future of molecular epidemiology depends on these innovative strategies. In this way, we can ensure that ENVIRONAGE and subsequent research activities will continue to contribute to the field of molecular epidemiology, and provide future standards.

Currently, the participation rate on the study is at 61 per cent. As our research is preventive and preclinical we are only able to recruit at time of birth, and we need to convince mothers and their children to participate, not directly for their benefit but for the society in general.

Nevertheless, ENVIRONAGE is gaining momentum as the investigators focus on other influential factors on foetal programming, such as pre-pregnancy BMI, to endorse the study’s hypothesis. If the team succeed in delivering their goals, the scientific community will be one step closer to fully understanding ageing mechanisms.

At four years of age the ENVIRONAGE children are re-invited to participate in the follow-up part of the study, including cardiovascular measurements of the micro- and macrocirculation, cognitive function and bone density.

STEP BY STEP

Although most of the findings from the cohort are limited to the first phase of the study (at birth), the team has already succeeded in showing that air pollution exposure during pregnancy produces significant modifications on placental tissue at a molecular, cellular, and hormonal level; and that these modifications impact mitochondrial function and telomeric length in foetuses.

However, these results must be interpreted with a grain of salt, as Nawrot observes: