Peter Gordebeke and Cornelia van Duijn introduce CoSTREAM, a project working to understand the causes of stroke and Alzheimer's disease

Partners in crime

The second leading cause of death and one of the leading causes of disability worldwide, stroke is expected to affect 23 million people for the first time in 2030, in no small part due to a rapidly ageing population. Already some 15 million people across the globe experience a stroke each year, with one in four of them developing some form of dementia, most commonly Alzheimer's disease (AD), within ten years. The frequency of this co-occurrence is such that there is now an urgent need to better understand the common cause of these two diseases.

It is here that CoSTREAM comes in, a five-year project funded by Horizon 2020 which will combine novel analytical strategies and emerging technologies in the fields of genetics, metabolomics, brain imaging and clinical prediction to identify the common mechanisms and pathways in stroke and AD and determine possible targets for future therapeutic intervention.

Here, project manager Peter Gordebeke, of the European Institute for Biomedical Imaging Research (EIBIR), tells Portal what the project has achieved so far, how it will elucidate our understanding of the link between stroke and AD, and how he hopes it will overcome barriers toward the development of new, evidence-based treatments for these two most devastating diseases.

Stroke and Alzheimer's disease have long been considered 'partners in crime' – what has held back prior attempts to explain their link, and how is CoSTREAM working to change this?

There are major gaps in our understanding of the pathophysiology underlying the relationship between vascular risk factors, including stroke, and AD. Current model systems are focused on each disease separately, and the interplay between these two diseases has remained unexplored.

Basically, we do not know the cause and consequence in the cascade, or what the genetic drivers, or intermediate steps, or independent effects on different tissues are in different tissues. We also need a better understanding of how the molecular signatures, genetic or metabolic, relate to lesions in the brain, and to discover the earliest changes in the brain in stroke and AD using magnetic resonance imaging (MRI) or positron emission tomography (PET).

From a radiological perspective, we need to know how changes in brain connectivity, micro- and macro-vascular, contribute to cognitive impairment in these diseases and whether there is a relationship between genetic, metabolic and molecular features with downstream effects in stroke and AD.

Recent developments in imaging research combining MRI and PET may yield a marked improvement in understanding the pathological pathways and predicting the risk of AD in stroke patients. There is a major gap in our knowledge of how the pathology – as seen at MRI level – can be prevented from progressing by compensatory mechanisms involving genes, metabolism, and environmental or psychosocial factors.

To understand the pathways underlying stroke and AD, and to develop innovative treatment and prevention, there is an urgent need for an accurate, high-throughput experimental model of the neurovascular unit to study these pathways and test potential interventions.

The current state of stroke management is to deal with stroke as a clinical event, managed in isolation. There is a clear gap in neurology between the care of vascular and neurodegenerative diseases such as AD. As a consequence, there is a missed window of opportunity to prevent AD as comorbidity by intervening in vascular pathways.

CoSTREAM will use a wide approach to investigate the co-occurrence of stroke and AD. The project aims to:

- Identify joint genetic and environmental pathways in stroke and AD;
- Discover metabolic pathways that explain the occurrence of AD in stroke patients;
- Understand how genetic, environmental and metabolic pathways relate to structural and functional alterations as seen through MRI and PET;
- Discover compensatory mechanisms protecting against both stroke and AD;
- Determine predictive accuracy of metabolic and imaging markers for stroke and AD;

- Develop new directions for therapeutic interventions based on the metabolic and imaging markers;
- Develop a neurovascular 'organ-on-a-chip' model for high-throughput research; and
- Use genetic data to evaluate potential therapeutic approaches which target shared processes between AD and stroke.

Much has been made of the importance of maintaining healthy and active lifestyles to lower your risk of stroke or Alzheimer's disease. How will CoSTREAM elucidate our understanding of such preventative and compensatory measures?

There has been major success in the prevention of both stroke and Alzheimer's disease, yet these disorders remain the major causes of morbidity and mortality in the elderly. To better understand preventative and compensatory measures for stroke and AD, CoSTREAM will make use of data from long-term follow-up studies and complement these with new findings to discover compensatory mechanisms and validate prediction models. CoSTREAM will implement the findings of genetic, metabolic and imaging research to discover and understand compensatory mechanisms that may counteract pathology driven by genetic and environmental risk factors.

Ageing and AD are closely related processes, and both are the result of a lifelong accumulation of biological alterations and organ damage. On the basis of the current evidence, we hypothesise that brain reserve-related factors can reduce the risk of stroke and AD, as well as the adverse effects of brain lesions on accelerated cognitive decline. High brain reserve is typically produced by a long-term exposure to positive psychosocial factors, active lifestyles and psychiatric wellbeing. These factors may also act as compensatory factors that modify the vulnerability due to vascular risk factors and brain lesions.

CoSTREAM has access to a combined dataset spanning more than 11,500 cases of stroke, nearly 20,000 cases of Alzheimer's disease (including mild cognitive impairment) and over 74,000 healthy controls with up to 25 years of follow-up, including multiple repeat assessments. It includes two large, ongoing follow-up studies.

In particular, we will take advantage of two databases that have already been built: the Swedish National study on Aging and Care in Kungsholmen database (SNAC-K) at the Karolinska Institute, and the Rotterdam Study at Erasmus MC, the Netherlands. Using information from these studies on vascular risk factors, lifelong psychosocial factors and lifestyle, and the availability of neuroimaging techniques gives us the opportunity to verify our working hypothesis *in vivo.*

In the long-term follow-up cohorts we'll determine how much of the variation in stroke and AD risk can be explained by metabolites and imaging markers, and how much of the unexplained variation is due to genetic background. We will examine whether the risk of cognitive impairment and AD varies among persons with the same brain lesion load and how much of the variation in risk can be explained by vascular and neurodegenerative lesions as determined by imaging.

CoSTREAM will also determine which genetic and contextual factors are associated with vascular and degenerative lesions, and how these effects differ over the life course. It will also determine which are the compensatory mechanisms that lower the risk of cognitive decline in people with vascular and neurodegenerative brain lesions.

There is a lack of understanding as to what extent psychosocial factors modulate the risk of cognitive decline and AD. CoSTREAM will further determine whether there are specific time windows for optimal protective effects, and what the possible psychosocial factors are.

Additionally, we will exploit the follow-up studies to translate the findings to clinical practice and develop toolboxes for combining metabolomic measurements, imaging and compensating mechanisms into prediction models for epidemiology, clinics and public health.

CoSTREAM will explore whether the risk of stroke and cognitive dysfunction (AD, dementia and cognitive decline) varies among persons with similar vascular burdens. Here it is important to find out if compensatory factors modify the association between vascular risk factors and stroke, and if these factors also modify the association of vascular risk factors with cognitive dysfunction.

To study the compensatory mechanisms, the following factors will be taken into account:

- Brain reserve-related compensatory factors, such as education, work complexity, social network, leisure activities, perception and amount of stress;
- Data from cognitive assessments;
- Data from stroke assessments;
- Vascular burden, including risk factors such as smoking, alcohol consumption, physical inactivity, unhealthy diet, hypertension, diabetes, high total cholesterol, obesity, heart diseases; and
- Gender.

Information on these factors is available from different life periods and will be combined and compared to investigate and identify compensatory mechanisms.

Can you expand on the organ-on-a-chip *in vitro* model CoSTREAM aims to create? How do you hope this will advance drug development in ways that previous attempts have failed?

A major barrier in the development of new, evidence-based treatments for stroke and AD is the lack of animal and cellular models for research. To overcome this obstacle, MIMETAS will develop an organ-on-a-chip *in vitro* model of the neurovascular unit, with neurons, glial cells and endothelial cells of the blood-brain barrier, as part of the CoSTREAM project.

Studies aimed at understanding vascular and neuronal pathways, their interaction, and how to intervene to prevent irreversible neurodegeneration have so far always been limited due to a lack of models and/or tissue at early stages of stroke or AD. The organ-on-a-chip model provides opportunities to rapidly and extensively study the neurovascular unit even in the early stages of disease.

Limited *in vitro* cellular and animal models have played a key role in translating the discovery of rare mutations (in *APP, PSEN1* and *PSEN2*) into the amyloid cascade hypothesis, which has not only changed our understanding of AD but has also formed the basis of biomarkers (*Aß40/42, Tau* and PET tracers) that are widely used in the clinical diagnosis of AD today. In addition, genome-wide association studies (GWAS) have been tremendously successful in uncovering genes involved in the aetiology of stroke and AD. The major challenge we're facing is to translate the information on low risk genes identified by GWAS. These low risk genes are most likely working in concert with non-genetic vascular factors.

Classical animal and cellular studies investigating single genes are expensive and not time-efficient. The organ-on-a-chip model, using MIMETAS' OrganoPlate technology, enables high-throughput screening of new therapeutic interventions in a highly relevant *in vitro* model. This makes the organ-on-a-chip model cost- and time-efficient. Additionally, the availability of such a platform is a prerequisite for a systems medicine approach in the development of new therapeutics and validation of drug targets.

The rapid pace of the development of induced pluripotent stem cells (iPSCs) research offers unique opportunities and provides unfettered possibilities to study neuronal metabolism in iPSCs derived from patients that developed AD due to a combination of low risk genes.

MIMETAS, in collaboration with Leiden University, the Netherlands, is able to differentiate iPSCs in the OrganoPlates used for the organ-on-a-chip model, and can now generate neurons, astrocytes and glia cells from skin and urine. Studies of AD iPSCs in 3D cultures have already been shown to closely resemble *in vivo* neuronal organisation, as well as accurately model the biochemical alterations and histopathological findings observed in post-mortem brain tissue from AD patients.

The development of the organ-on-a-chip platform will allow scientists to integrate large-scale genetic, clinical and epidemiologic research with focused metabolomic research involving iPSCs. Within CoSTREAM the model will be used for research aiming to understand the metabolic profiles that are underlying the occurrence of AD in stroke patients. Beyond the scope of the project, it provides a model to develop and study the effects of novel therapeutic interventions.

CoSTREAM will use reprogrammed iPSCs derived from somatic cells available in the public domain, but will in parallel develop models of patients with stroke and AD from our cohorts that are known to carry a combination of genetic risk factors associated to specific vascular pathways. This will allow us to study the effect of the combination of the known risk genetic variants with small effects on the molecular level in the neurovascular unit. Not only will the organ-on-a-chip be crucial in unravelling the pathophysiological processes within the neurovascular unit, but these models can also be used to efficiently explore therapeutic intervention: experiments in cell cultures are easier to model, control and interpret, are less expensive and do not require complex ethical regulations.

A unique feature of our iPSCs and organ-on-a-chip work is that we use a closed system in which the iPSCs and organ-on-a-chip models are differentiated in 96 well plates. Furthermore, we have succeeded in miniaturising and automating our metabolic measurements of samples collected as medium or after cell lysis.

Ultimately, the organ-on-a-chip models will be brought to the market as cost- and time-efficient screening instruments for existing and novel medication.

What early progress has been made since your launch in December, and what are your immediate priorities heading towards your second year of the project?

Since the start of CoSTREAM research has mainly focused on identifying shared genetic and metabolomics factors of stroke and Alzheimer's disease, as part of Work Package 1 and Work Package 2 respectively.

Shared genetic factors will then be used to identify common mechanisms and pathways and then link these with metabolic, imaging and clinical findings. We are basing ourselves on the

data of large genetic consortia that we are leaders in aiming to understand the genetics of AD and stroke. Our goal has been a targeted one: is there a genetic overlap between stroke and AD, as well as their subtypes? To what extent is there a genetic correlation between the two diseases so that the genes leading to one disease inevitably also evoke the other disease?

In the first period, CoSTREAM has also been exploring a wide range of metabolites to discover novel pathways underlying the co-occurrence of stroke and AD, with the ultimate goal of identifying persons with a potentially modifiable metabolic aetiology. This is achieved by applying untargeted metabolomics (i.e. to cover fully the physicochemical and biochemical metabolite space) to genes that relate to both stroke and AD. We are finding metabolites implicated in metabolites associated with ageing, diet (fish) and fat metabolism in both diseases.

In a complementary approach, we will use targeted biology-driven metabolomics addressing metabolites of selected genes and their role in the pathway. Metabolites identified through these two approaches can then be correlated to imaging data, including imaging data acquired within CoSTREAM over the coming months. Further down the line, identified genetic and metabolic factors will be used in a Mendelian randomisation setting to test their causal association with stroke and AD.

In the immediate future we will start with two clinical studies, performed by the Karolinska Institute and the University of Geneva, Switzerland, as part of Work Packages 3 and 4.

We will use novel PET ligands and ultra-high-field MRI to study pathophysiological mechanisms common to both stroke and AD. We want to image the earliest molecular brain changes in stroke and AD, study changes in brain connectivity and perfusion as contributors to cognitive impairment, and investigate the relationship of genetic, metabolic and molecular features with downstream effects in stroke and AD.

Additionally, we will explore the compensatory effect of healthy behaviour and lifestyles against the harmful effects of the pathological mechanisms and pathways of stroke and AD. Using the imaging markers from and metabolites used and identified by CoSTREAM, we will investigate to what extent they explain the variation in occurrence of stroke and AD and how much of the unexplained variation is due to putative compensatory mechanisms. In this way, we aim to find potential compensatory mechanisms that lower the risk of the co-occurrence of stroke and AD.

The CoSTREAM project proposal was awarded the highest evaluation score possible by Horizon 2020 reviewers – what advice would you give to other applicants when preparing their own proposals?

For proposal writing, the CoSTREAM consortium relied heavily on the expertise of the EIBIR, the project's partner for management and dissemination.

EIBIR has extensive experience in supporting European researchers with proposal writing and project applications, particularly for research projects under the Horizon 2020 framework.

All administrative tasks for the application were handled by EIBIR, which was especially welcomed by consortium members who were not familiar with Horizon 2020 or were first-time applicants.

By having a separate, dedicated consortium member manage the entire application process, communication between partners and administrative matters, we were able to save time and redirect attention from figuring out templates and guidelines to the scientific content of the excellence, impact and implementation sections of the proposal. The most important aspect is of course addressing all aspects of the call throughout your proposal.

Towards the end of the proposal preparation phase we identified some weaknesses in the proposal internally and with help from our national contact point. EIBIR worked closely with each responsible consortium member to strengthen that particular section. In this regard, it was particularly helpful to have EIBIR on-board, with over a decade of experience in the preparation of proposals for European Commission research frameworks.

The biggest challenges in proposal writing are addressing everything correctly and paying attention to detail. Generally speaking, the easiest part to write is the science, whereas the impact, management structure and project management are areas that most scientists are not familiar with and where experience can greatly benefit the quality of your proposal.

HORIZON 2020

Peter Gordebeke
Project Manager
CoSTREAM

www.costream.eu