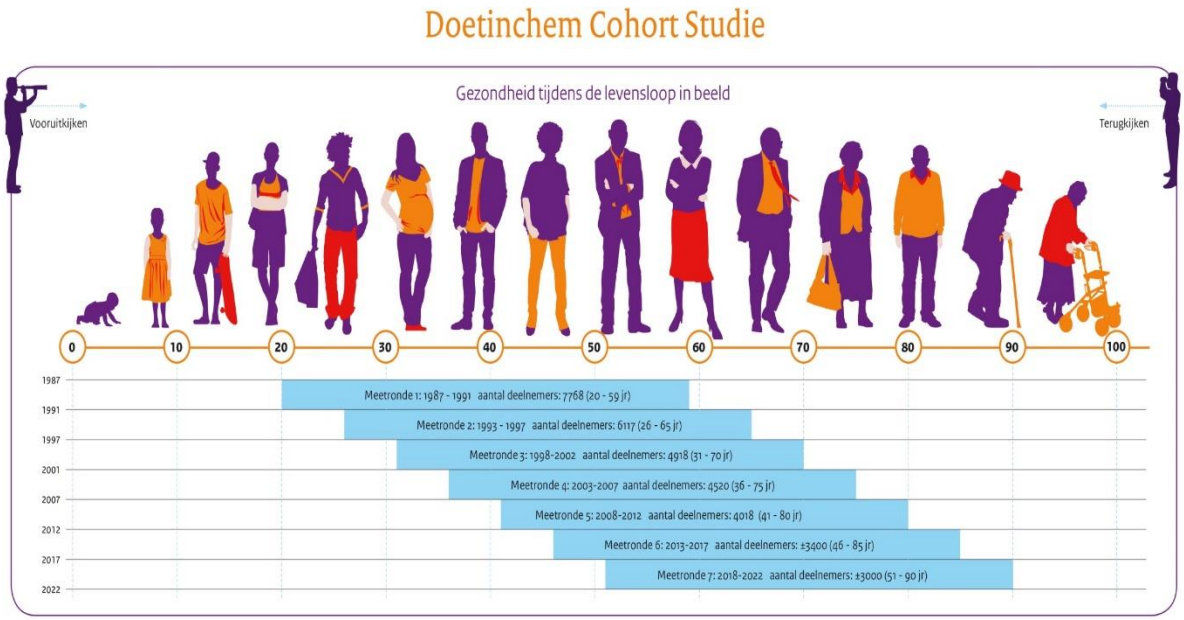
NEWSLETTER VOILA – July 2021

Metabolome determinations in the doetinchem cohort study – RL1

In the autumn of 2020, samples from the Doetinchem Cohort Study were transported to Finland for metabolomics determinations at Nightingale. With the budget of the VOILA-project and the RIVM, all samples from measurement round 4 (N=4527) and measurement round 6 (N=3351) could be measured. From 3199 participants form round 6 we also have a measurement for round 4, from 1328 we only have a measurement on round 4 and from 152 participants only for round 6.



These measurements allow us to look at different types of questions. For round 4 we can relate the metabolome data to health outcomes in later rounds, for round six we can also if we have sufficient follow-up time. We can also look at changes in the metabolome over the ten years between 4 and 6, and how this correlates with changes in lifestyle, other metabolic factors and health outcomes over that period. And the cohort can now also be used for replication of subsequent cohorts and pooling of metabolome data.

NEUROMET

In NEUROMET we are developing novel methods to identify the mechanisms underlying neuroinflammation in *in vitro* models and in humans. Our goal is to study the effect of blood-based metabolic or immune biomarkers on the brain in the context of neurodegenerative diseases and in older adults. We want to study the effect of these markers by perfusing older adults or patient's blood through the blood vessel of a human neurovascular *in vitro* model (NVU) and measure the effect on neurons and astrocytes. To this end, we use metabolomics and tracer-based metabolomics combined with a computational reconstructed metabolic network model of neurons and astrocytes to understand neuroinflammation and to identify the effect of these circulatory markers.

Development of *in vitro* model for neuroinflammation

In order to study neuroinflammation *in vitro*, we are developing a co-culture method containing the essential components for this process. This model will be based on an existing neuro-vascular unit (NVU) model (containing a blood vessel, astrocytes and pericytes), to which we add neurons and microglia.

Neuronal differentiation

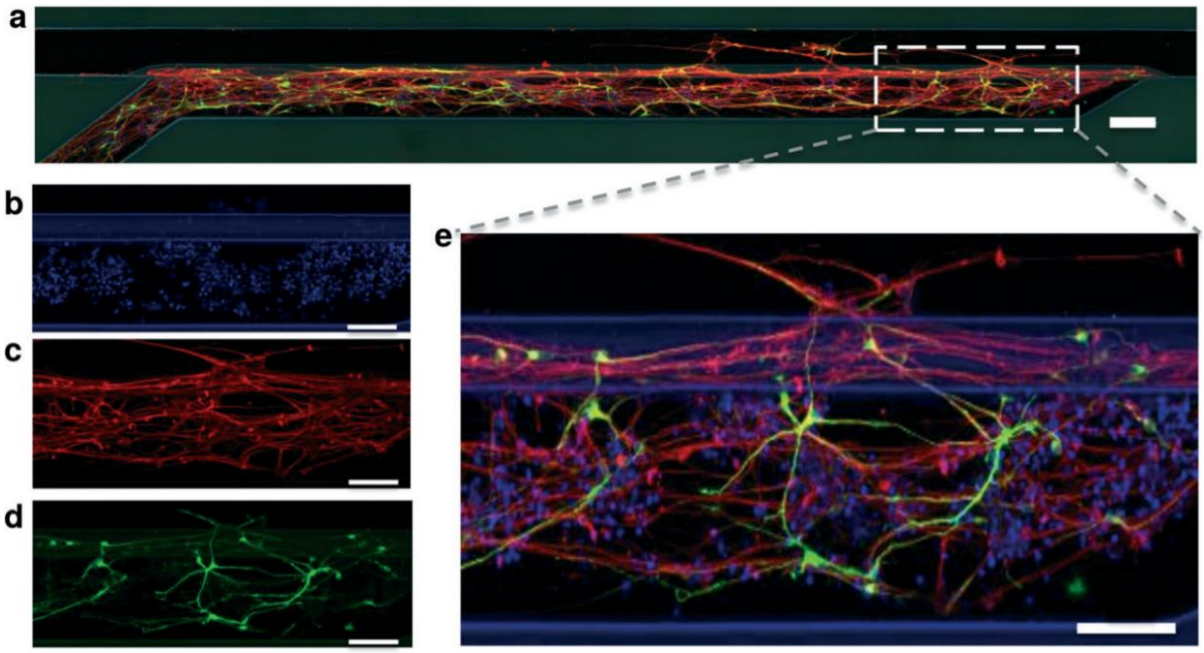


Figure 1. Differentiation of hNESCs into neurons in a 2-lane microfluidic bioreactor of the OrganoPlate. (a) Immunostaining of differentiated neurons in the 2-lane microfluidic chamber after 1 month of differentiation; scale bar 200 µm. Section of the microfluidic chamber stained for: (b) nuclei with Hoechst in blue, (c) TUBβIII in red, and (d) TH in green; scale bar 50 µm. (e) Merged nuclei, TUBβIII and TH stains; scale bar 100 µm [from Moreno, E. L. et al. 2015].

In an earlier project (SysMedPD), we differentiated human neuroepithelial stem cells (hNESCs) to produce dopaminergic neurons with a Parkinson's disease genetic background. This differentiation protocol provides with us a mixture of neurons, with up to 30 percent dopaminergic neurons. As shown in the provided image, this differentiation protocol has been adapted to a microfluidic culture setup, and is available for use as part of the neuroinflammation model.

Astrocyte differentiation

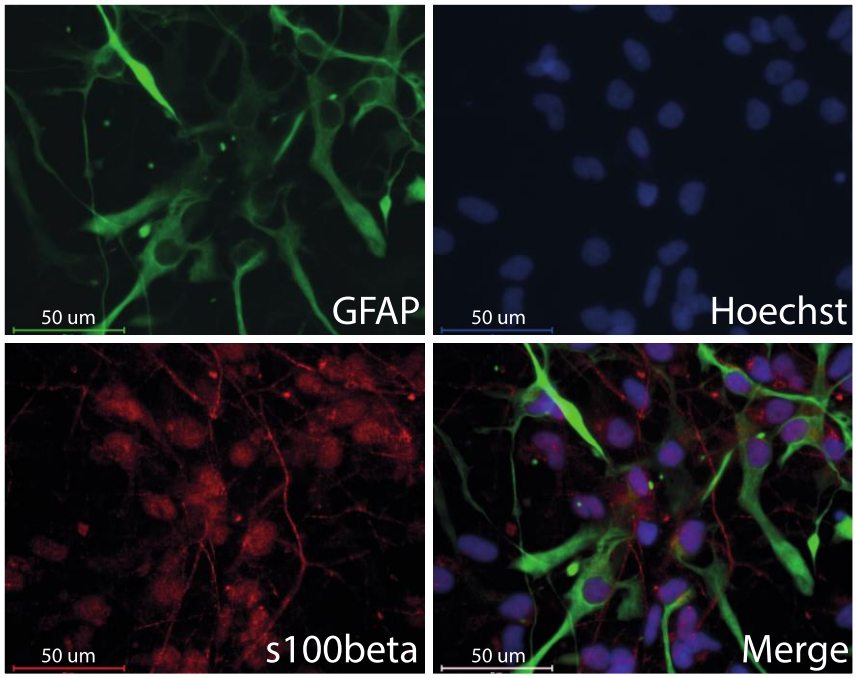


Figure 2. Astrocytes differentiated from hNESCs. Hoechst (blue) shows cell nuclei, GFAP (green) and s100beta (red) mark mature astrocytes.

To get a source of astrocytes with relevant genetic background, we compared different published astrocyte differentiation protocols using the hNESCs available to us. Using these protocols, we were able to differentiate to $GFAP^+$/$s100b^+$ astrocytes, with further characterization ongoing.

Microglia differentiation

For microglial differentiation, induced pluripotent stem cells are required, since these cells are not from the neuroepithelial lineage we have used for the neurons and astrocytes. We have recently acquired these iPSCs, and are currently running our first microglial differentiation protocols.

Development of tracer-based metabolomics to study brain function

An analytical method based on hydrophilic interaction liquid chromatography coupled with tandem mass spectrometry technique was developed, covering a total of 77 metabolites involved in energy metabolism, amino acid metabolism, purine and pyrimidine metabolism, glutathione synthesis, and cofactor de novo synthesis. By using this method, we were able to identify more underlying metabolic regulations by tracing stable isotope-labeled carbon and nitrogen atoms through the metabolic network. Together we further developed an in-house automated tracer-based metabolomics data processing workflow. A preliminary study in healthy dopaminergic neurons was carried out, the metabolic flux results showed us a dynamic metabolic activity within the neuron. For the next step, we will perform a perturbation study using rotenone exposure on neurons, together with PINK 1 gene mutation, and explore the metabolic regulations of neuron dysfunction.

An update on RL3

The protocol for the intervention study was submitted to the METC in May. Currently we are addressing the METC comments and are preparing all the necessary arrangements to start after the summer. The first week of July we have organized a training week generously hosted by Lex and Ale in Maastricht. The three PhD candidates, Ale, Jordi and Charlotte are busy running through the protocols, practicing techniques and are taking part in team building activities. Toward the end of the week they'll be joined by Eline, Lisette, Pol and Lex to discuss outstanding issues and see each other face-to-face for the first time since the formation of the complete team.

Ale, Jordi, Charlotte

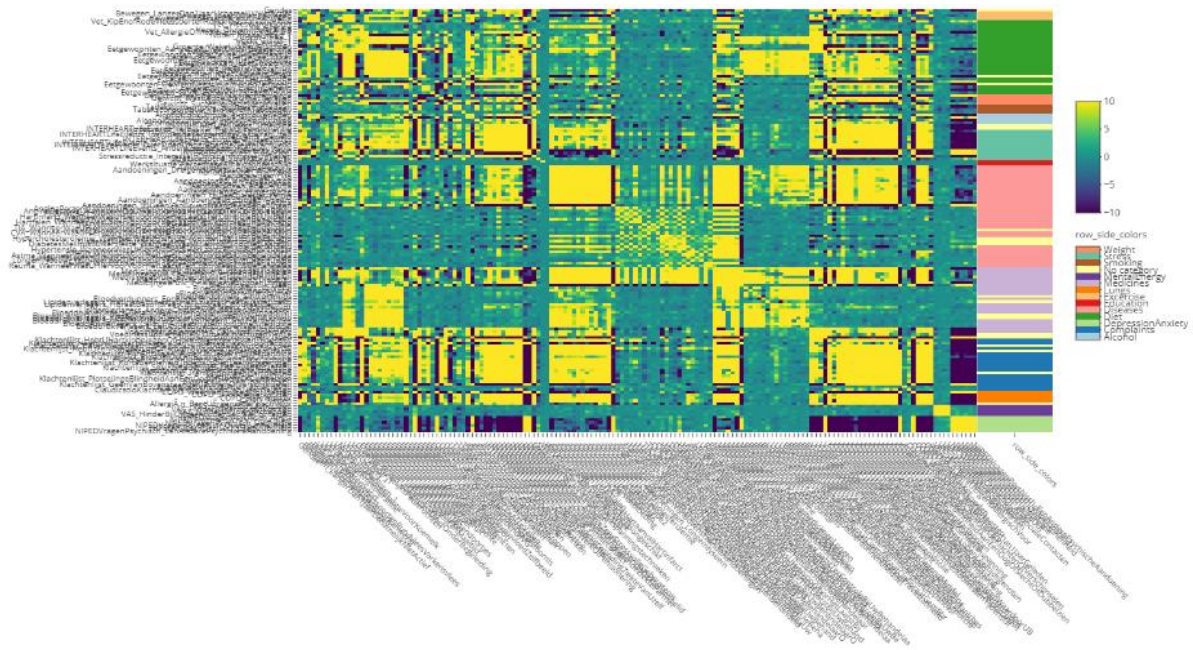
Predicting cardio-metabolic risk from large bodies of questionnaire data – RL4

A student (Thimo Waanders) from the Leiden University Medical Center (LUMC) just started a project to predict cardio-metabolic risk from questionnaire data, as collected by &niped. This project is under joint supervision of Erik van den Akker (LUMC) and Hester van Donkersgoed (&niped).

Cardiometabolic problems can often be slowed, or even reverted, when detected in its early stages by making adjustments in lifestyle. Unfortunately, this opportunity for a fully reversible treatment is often forfeited, as individuals developing cardio-metabolic problems are often unaware of the impending risks, and moreover, these individuals generally go unnoticed in our current health system. This sad notion is illustrated by the fact that we currently even miss out on a large proportion of individuals with overt, often irreversible, cardio-metabolic diseases, i.e. those who really should receive treatment from a physician to prevent further damage from happening. For instance, it is estimated that over 250,000 Dutch citizens have T2D, yet have never been diagnosed, and thus do not receive any medication.

The dataset is provided by &niped (www.niped.nl) and collected via the Personal Health Check. This is a health screening on the basis of several diagnostic measurements in blood in conjunction with an extensive questionnaire on socio-economic factors including lifestyle, diet, and work-related aspects. In collaboration with Dutch foundations for Diabetes (Diabetes Fonds) and Heart disease (Hartstichting), indications have been set when to refer participants for further examination to their respective practitioners. The limitations of the current screening protocols are that they ignore most of the questionnaire data and utilize almost solely the blood-borne diagnostic measurements.

Aim of the project is to develop approaches that leverage the rich questionnaire data that has been collected, in addition to the blood measurements, in over 100,000 participants. By including socio-economic factors including lifestyle, diet, and work-related aspects, we hope to create a second independent line of evidence to identify individuals at risk of cardio-metabolic problems. In addition, any disagreement between predictions made on basis of blood measurements on the one hand, or the questionnaire on the other would be of interest, as it is known that blood measurements can be poor indicators of health, in particular, in the elderly.



Heatmap showing the correlation structure within the questionnaire data. Each cell represents the correlation between two binairy questionairies and ranges from positive (yellow) to negative (blue). The heatmap is symmetric along the diagnonal. Questionaire categories, indicated by colors on the right, typically show high positive correlations, while correlations between categories may vary. Figure by Thimo Waanders, intern on data science and/or modern art.

RL6

The main purpose of research line 6 (Translation, communication and implementation) is to communicate the results of the various VOILA studies to the 70+ population, the general public and to professionals (policy makers, researchers etc.). However, inspired by the challenging times for the 70+ population to stay vital during the current pandemic we are also working on communicating the latest general insights on preventive lifestyle measures for the 70+ population. Guidelines on exercise and diet are mostly focused on a middle-aged target group, while there is ample evidence for specific measures for the 70+ population. In journal club sessions led by dr. Frans van der Ouderaa, we are now establishing core messages for the 70+ population, also taking in account the heterogeneity of this population. An important next step is to link the core messages to the ongoing VOILA studies. For

this we will contact relevant researchers and experts within the VOILA consortium. Also, we want to ask you to reach out to us if there are interesting developments or findings that could be connected to specific preventive lifestyle measures for the 70+ population. We will transform these core messages into attractive microlearnings that will finally be disseminated in collaboration with the communication experts of the Leyden Academy on Vitality and Ageing.