

13th Nordic EMBL Partnership Meeting, Oslo, 17th -19th September 2024

NCMM is delighted to welcome attendees, including group leaders, postdoctoral and senior researchers, PhD students and coordinators, from all of the Nordic EMBL nodes, HCEMM, as well as representatives from EMBL.

This year, we have teamed up with the [Michael Sars Centre](#) at the University of Bergen, established in 1997 and EMBL partner since 2003, to propose a stimulating scientific programme together with all the Nordic EMBL Partnership nodes.

The Organising, Scientific and Young Investigators Meeting Committees are wishing a fruitful meeting!

Code of conduct

Thank you for attending the 13th Nordic EMBL Partnership Meeting. The organisers are committed to providing a harassment-free conference experience for everyone, regardless of gender, gender identity and expression, age, sexual orientation, disability, physical appearance, body size, race, ethnicity, religion (or lack thereof), or technology choices. We expect all participants to behave in a courteous and professional manner, and to treat each other with respect, dignity, and consideration in the spirit of valuing diversity and inclusion of views and opinions.

We do not tolerate harassment of conference participants in any form. Sexual language and imagery is not appropriate for any conference venue, including talks, workshops, parties, and social media. Meeting participants violating these rules may be sanctioned or expelled from the conference *without a refund* at the discretion of the conference organisers.

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Tuesday 17th September

18:30 Registration

19:00 Kenynote lecture by James Hackett (EMBL): Intergenerational Inheritance: *Why your father's microbes matter* – Chair Janna Saarela



Jamie Hackett is a group leader at the European Molecular Biology Laboratory (EMBL), within the Epigenetics and Neurobiology unit in Rome, Italy. He obtained his PhD at the University of Edinburgh, and completed postdoctoral training at the University of Cambridge, UK, under Prof. Azim Surani. Jamie's research group has two overarching scientific themes. (i) To understand the regulatory impact of chromatin changes in health and disease, by engineering precision epigenome editing tools for (pre-)clinical applications. (ii) To investigate the potential for altered epigenetic states to be transmitted through mitosis or meiosis and influence disease risk.

Wednesday 18th September

08:30: Coffee, Registration and Poster hanging

SESSION I. Chairs: Janna Saarela (NCMM) and Charlotte Bocarra (NCMM)

09:00 - 09:15 Welcome and Nordic EMBL Partnership Update: Oliver Billker

09:15 - 09:30 Update from EMBL by EMBL Director General Edith Heard

09:35 - 09:55 Lionel Christiaen (Director - Sars Centre): The Michael Sars Centre: Toward Marine Systems Life Sciences

09:55 - 10:15 New group leader: Anna Mathia Klawonn (DANDRITE): [Immune-to-Brain Mechanisms of Affective State and Parkinson's Disease](#)

10:15 - 10:25 Post-doc talk: Pau Puigdevall (FIMM): [Modelling neurodevelopment using cell villages of iPSC](#)

10:25 - 10:35 Post-doc talk: Hjalte Gram (DANDRITE): [Patient-derived alpha-synuclein aggregate strains as a tool in models of synucleinopathy](#)

10:35 - 11:05 *Coffee & Snacks + Posters on display*

11:05 - 11:25 Group leader: Marios Chatzigeorgiou (Sars Centre): [The molecular and cellular basis of sensory perception in the ocean: A chordate perspective](#)

11:25 - 12:15 Keynote speaker: Rosa Cossart (INMED, France): [Developmental constraints on memory circuits](#)



Rosa Cossart is a distinguished researcher at the French National Institute of Health and Medical Research (INSERM). Specializing in neurobiology, she has dedicated her career to understanding the intricacies of neural circuitry and brain function. After completing her doctoral studies in cellular and molecular neuroscience, Rosa joined INSERM, where she quickly established herself as a leader in the field. Her groundbreaking research has illuminated the synaptic mechanisms underlying various neurological disorders, earning her numerous accolades and research grants. Rosa is also a committed mentor, guiding the next generation of scientists through her work at INSERM and her collaborative projects with leading universities. Outside the lab, she actively participates in science communication, striving to make complex scientific concepts accessible to the broader public.

12:15 - 14:00 *Lunch, Posters and Networking*

SESSION II. Chairs: Barbara Sixt (MIMS) and Marios Chatzigeorgiou (Sars Centre)

14:00 - 14:20 New group leader Iker Aramburu (MIMS): [Illuminating the dark MicroProteome in Innate Immunity](#)

14:20 - 14:30 Post-doc talk Emily Claereboudt (Sars Centre): [Binding modes of Agonists in neuropeptide-gated ion channels](#)

14:30 - 14:40 Post-doc talk Solomiia Korchynska (NCMM): [Sleep architecture dynamics across development](#)

14:40 - 14:50 PhD talk Bianca Korse (MIMS): [Membrane interaction mechanism of the tick-borne encephalitis virus capsid protein](#)

14:50 - 15:10 Selection of flash talks by Meri Kausito (FIMM), Pu Chen (NCMM), Gabin Drouard (FIMM), Jiahao Guo (NCMM), Simon Henriët (Sars Centre)

15:10 - 15:40 *Coffee & Snacks + Posters on display*

15:40 - 16:00 New group leader Ryo Morimoto (MIMS): [Evolutionary trajectory of somatic assembly of antigen receptors based on host-genome editing](#)

16:00 - 16:10 Post-doc talk Himanshu Sharma (MIMS): [Ultrastructure remodeling of the host invasion apparatus of microsporidia parasites](#)

16:10 - 16:20 Post-doc talk Eudald Pascual-Carreras (Sars Centre): [Nutritional control of stem cell cycle dynamics of the sea anemone *Nematostella*](#)

16:20 - 16:30 PhD talk Chiara Castelli (Sars Centre): [System eco-devo: towards using high-content image based morphometrics to quantify the impacts of changing temperatures on developmental dynamics at single cell resolution](#)

16:30 - 16:50 Selection of flash talks by Sandra Holmberg (MIMS), Mikaela Hukkanen (FIMM), Karri Lamsa (HCEMM), Roza Lemma (NCMM), Max Maken (Sars Centre), Birgit Kriener (NCMM)

18:45 - onwards *Cultural event and dinner at Oslo Opera House*

Thursday 19th September

08:30 - 09:00 Arrival & Coffee

SESSION III. Chairs: Esa Pitkänen (FIMM) and Anna Mathia Klawonn (DANDRITE)

09:00 - 09:20 New group leader: Simone Rubinacci (FIMM): [Accurate Haplotype Phasing in UK Biobank Detects Phenotype-Influencing Structural Variants](#)

09:20 - 09:30 PhD talk Oline Eriksen Rio (NCMM): [CRISPR-Cas9 corrected T-cells for personalized primary immunodeficiency disease \(PIDD\) therapy](#)

09:30 - 09:40 Post-doc talk Pekka Vartiainen (FIMM): [Which babies should get RSV immunisation? Using large Nordic registry data to identify children at high risk of severe respiratory syncytial virus infection](#)

09:40 - 09:50 Post-doc Martin Wohlwend (NCMM/MIT): [Noncoding exome genomics identifies ncRNA targets in disease](#)

09:50 - 10:00 PhD talk Rajlkashmi Sawale (DANDRITE): [Role of the gut microbiome in the functional development of the enteric nervous system](#)

10:00 - 10:20 Selection of flash talks by Francisca Pinheiro (DANDRITE), Elham Shojaeinia (NCMM), Aakriti singh (MIMS), Max Tamlander (FIMM), Stefanie Willekens (MIMS).

10:30 - 11:45 *Coffee & Snacks + Posters on display*

SESSION IV. Chairs: Gilles Vanwalleghem (DANDRITE) and Janna Saarela (NCMM)

11:45 - 12:05 New group leader Arafath (Rafa) Najumudeen (FIMM): Spatially resolving metabolism using intestinal cancer as the paradigm

12:05 - 12:15 Post-doc talk Ladislav Hovan (NCMM): [Spatially resolved gene regulatory networks](#)

12:15 - 12:25 Post-doc talk Silvia Turchetto (DANDRITE): [Local regulation of synaptic ubiquitin signaling](#)

12:25 - 12:35 PhD talk Celina Wiik (NCMM): [Unveiling the Epigenetic Alterations in Endometriosis](#)

12:35 - 13:25 Keynote speaker: Renée Beekman (CRG, Spain): [Charting early lymphoma formation from the epigenomics perspective](#)

Dr. R. Beekman holds a Master of Science Degree in Molecular Medicine (2005), a Medical Degree (2007, non-practicing) and a PhD Degree in Hematology (2013), all obtained at the



Erasmus University, Rotterdam, the Netherlands. She is experienced in Epigenetics, Chromatin, Three-Dimensional Chromatin Structure, Genetics, Bioinformatics, Cancer, Hematology, Acute Myeloid Leukemia and Mature Lymphoid Malignancies. She obtained her PhD Degree in the group of Prof. Dr. I.P. Touw, focusing on genetics in acute myeloid leukemia. From 2013-2019 she was a postdoctoral fellow in the groups of Prof. Dr. E. Campo and Dr. J.I. Martin-Subero in the IDIBAPS, Barcelona, Spain.

During her postdoc she focused on the epigenetic characterisation of lymphoid leukemias and lymphomas. In 2018 she received the La Caixa junior leader fellowship to start her own research line to study the role of epigenetic heterogeneity and stochastic gene expression events in development and disease. In January 2020 she started as group leader in the CRG, Barcelona, Spain where she is heading the Single Cell Epigenomics and Cancer Development lab. In her current research she focuses on understanding the accumulation of epigenetic events in normal and malignant B cells using single-cell technologies as well as on using genome-editing strategies to generate and characterise lymphoma-associated pre-malignant in vitro models.

13:25 - 13:40 Prizes, thank you and closing statements

13:45 - 14:30 Lunch, Posters and Networking, followed by departure.

Abstracts from the presentations on Wednesday 18th September

SESSION I

Immune-to-Brain Mechanisms of Affective State and Parkinson's Disease

Klawonn, A.M.

Danish Research Institute of Translational Neuroscience - DANDRITE, Aarhus University, Aarhus, Denmark

How does the immune system influence our mood? Why are people with inflammatory conditions or neurodegenerative diseases more prone to develop depression? How does the gut microbiota signal to the brain and alter our mood? These are some of the questions we are trying to answer, in the hope to uncover better treatments of affective disorders and neurodegenerative disease.

In the present talk, I will give examples from three main project-lines in my laboratory which focus on immune-to-brain signaling in affective homeostasis (sickness and resilience) and relevant affective neurocircuits, such as the Lateral Septum, as well as the role of microglia in early Parkinson's related alpha-Synuclein pathology.

Modelling neurodevelopment using cell villages of iPSC

Pau Puigdevall¹, Benjamin Potter¹, Emma Ryhänen², Adithi Sundaresh¹, Kavya Kalpana Ganesh¹, Riina Lampela¹, Timo Otonkoski^{2,3}, Diego Balboa², Helena Kilpinen^{1,4}

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⁴Faculty of Biological and Environmental Sciences, University of Helsinki, Finland

Neurodevelopmental disorders are a group of conditions caused by the abnormal development of the brain, leading to impairments in cognition, communication and motor skills. Many early-onset developmental disorders result from dysfunctions in chromatin machinery that severely impact gene expression. Induced pluripotent stem cell (iPSC) models combined with single-cell transcriptomics enable the study of these aberrant transcriptional signatures caused by the disease alleles during development. However, scalability of iPSC-based experiments remains a challenge, as large sample sizes are needed to detect the effects of pathogenic variants, often resulting in underpowered studies. To address this, pooled cell culture or 'cell villages' have emerged as a key strategy for large-scale studies. Still, maintaining balanced cell line representation within these villages is difficult due to varying growth dynamics of iPSC lines and the non-cell autonomous effects (NCA).

To understand the factors causing donor imbalance in cell villages, we systematically evaluated the effects of various experimental and cell-line factors on the composition of 8 cell villages, each comprising 5-18 donors, from 14 independent iPSC differentiations into cortical neurons. The villages included both wild type and mutation-carrying cell lines. We used natural genetic variation to deconvolute these villages, employing either single-cell RNA-seq reads (demuxlet) or low-coverage whole genome sequencing (Census-seq).

We observed that specific cell lines consistently become dominant at the neuronal precursor stage, representing 50.4% representation of total cells. This dominance occurred across independent neuronal inductions and was independent of village size. Also, proliferation outliers were more likely to be female-derived lines ($p=0.018$, Fisher's test). To assess the influence of NCA effects on differentiation, we compared the transcriptomic profiles of a subset of lines that were differentiated both individually and as part of a pool. Preliminary

results indicate that the transcriptomic identity of donors in neuronal precursors pools is preserved, with higher expression correlation between matched donors ($R=0.86$, Pearson's) than unmatched ones ($R=0.70$). Similarly, cell type composition correlation was higher in matched ($R=0.84$) versus unmatched donors ($R=0.79$). Finally, to test whether pool (im)balance is retained across different cellular lineages, we differentiated a subset of the same pools to pancreatic islet-like cells. Initial results suggest remarkable shifts in cell line representation between the two lineages, which manifest shortly after induction rather than slowly over time. We are currently exploring the molecular drivers of these lineage-specific effects as well as strategies to minimise the observed pool imbalanced to better capture cellular phenotypes of disease in cell villages.

Patient-derived alpha-synuclein aggregate strains as a tool in models of synucleinopathy

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Synucleinopathies are a group of neurodegenerative diseases characterized by the pathological cellular accumulation of α -synuclein (α -syn) aggregates. α -syn is a small pre-synaptic protein with an unfortunate propensity for forming toxic aggregates. α -syn aggregates are prion-like particles that can spread from neuron to neuron, causing cellular dysfunction and leading to a progressive accumulation throughout the brain.

Variations in the quaternary structure of α -Syn aggregates give rise to aggregate strains that are known to influence the cellular impact of α -Syn aggregates, and are hypothesized to form the basis for the heterogeneity within synucleinopathies. The two synucleinopathies, Parkinson's Disease (PD) and Multiple system atrophy (MSA) present different symptoms and neuropathology, and contain different α -Syn strains.

From the cerebrospinal fluid of PD and MSA patients, we amplified α -syn aggregates using seed amplification assay and investigated the structure and cellular impact of the two disease strains. We investigated cellular inclusion formation in different cell-type models of α -syn aggregation: α -syn overexpressing oligodendrocytes and induced pluripotent stem cell-derived neurons. We demonstrate that aggregates from PD and MSA are structurally and functionally different, with a cell-type-specific inclusion pathology.

This underscores the importance of considering and characterizing the α -Syn aggregate strains used when modeling synucleinopathy, and allows for modeling disease- and ultimately patient-specific signaling pathways in synucleinopathies.

The molecular and cellular basis of sensory perception in the ocean: A chordate perspective

Marios Chatzigeorgiou

Michael Sars Centre, University of Bergen, Norway

The Earth's oceans brim with an incredible diversity of microscopic lifeforms, including motile planktonic larvae, whose evolutionary fitness critically depends on effective dispersal in the water column and subsequent exploration of the seafloor to identify a suitable settlement site to undergo the transformative process of metamorphosis, giving rise to benthic sessile adults.

How their streamlined nervous systems mediate navigation and sensory perception in complex marine environments is a fascinating yet largely unexplored question.

Here we will introduce the genetically tractable marine non-vertebrate chordate *Ciona intestinalis* which has allowed us to gain exceptional insight on the molecular and cellular bases of zooplanktonic navigation and sensory transduction. We will examine how *Ciona* is a sensory multitasker, leveraging polymodal sensory neurons to transduce diverse chemical and mechanical cues, which promote or impede the ethologically relevant settlement behavior. Combining whole-brain imaging and chemogenetics, we will demonstrate that stimuli encoded at the periphery are sufficient to drive global brain-state changes to promote or impede larval settlement behavior. Then we will talk about an ongoing study where we investigate the behavior of swimming *Ciona* larvae under naturalistic conditions, specifically focusing on their response to water flow and grouping context. By employing deep learning tools for pose estimation, in conjunction with microfluidics and calcium imaging techniques, we scrutinized the rheotactic behavior of larvae and their sensory processing. Our findings reveal pronounced positive rheotaxis in *Ciona* larvae, with notable behavioural alterations observed in response to changes in group density. We suggest that early chordates possessed cellular and behavioral correlates of rheotaxis like those found in vertebrates and propose a hypothesis regarding the neural circuitry that potentially underlies rheotaxis.

Developmental constraints on memory circuits

Rosa Cossart

The hippocampus, a brain region traditionally associated with navigation and episodic memory, can be understood as a hub for two distinct forms of navigation: one that integrates external signals to guide movement through physical space, and another that enables self-referenced traversal of our inner mental landscapes. This duality hints at a more fundamental role for the hippocampus in distinguishing between self and non-self.

Central to this process is the hippocampus' ability to differentiate between internally-generated and externally-driven inputs, a function mediated by distinct circuits orchestrated by local inhibitory interneurons. We posit that these dedicated circuits emerge through a developmental process in which the hippocampus learns to segregate self and non-self-signals via its interactions with the body and the environment. Disruptions to this developmental trajectory, we argue, may give rise to disorders characterized by distorted internal models of the world, such as autism and schizophrenia. This presentation aims to unravel the emergence of functional hippocampal circuits over the course of development. Through a synthesis of published and unpublished data, we highlight the dual influence of early developmental programming and ongoing interaction with sensory signals.

SESSION II

Illuminating the Dark MicroProteome in Innate Immunity

Aramburu IV

Laboratory for Molecular Infection Medicine Sweden (MIMS), Department of Molecular Biology, Umeå University, Sweden.

The human genome contains thousands of unannotated short open reading frame (sORF) encoded microproteins or sORF-encoded peptides (sPEP), also known as the dark proteome. Microproteins are peptides smaller than 100 amino acids. In recent years, advances in ribosome profiling, bioinformatic and proteomic approaches have led to the discovery of hundreds of thousands of putative microproteins originated from coding and non-coding genomic regions that provide a pool of unexplored functional genes. A growing number of microproteins have been shown to play key regulatory roles in diverse cellular functions. Nevertheless, the majority remain uncharacterized (reviewed in Schlesinger and Elsässer, 2022). I aim to identify and functionally characterize microproteins present in native and activated innate immune cells combining computational, synthetic biology, protein and genetic engineering approaches.

Schlesinger, D. & Elsässer, S.J. FEBS J. 289, 53-74 (2022)

Flipped binding modes for the same agonist in closely related neuropeptide-gated ion channels

Claereboudt E. J. S., Dandamudi M. and Lynagh T

Michael Sars Centre, University of Bergen, Norway

Neuropeptides are inter-cellular signaling molecules that occur throughout animals and are divergent in sequence, structure, and targets. Most neuropeptides bind and activate G-protein coupled receptors, but some also activate ionotropic receptors (or “ligand-gated ion channels”). This is exemplified by the tetra-peptide H-Phe-Met-Arg-Phe-NH₂ (FMRFa), which activates mollusk and annelid worm FMRFa-gated sodium channels (FaNaCs) from the trimeric degenerin/epithelial sodium channel superfamily. Here, we explored the structure-activity relationships determining FMRFa potency at mollusk and annelid FaNaCs in the light of emerging structural data, using synthetic neuropeptide analogues, heterologous expression, and two-electrode voltage clamp. Substitutions of the FMRFa N-terminal phenylalanine residue (F1) and methionine residue (M2) decreased or abolished FMRFa potency at mollusk *Aplysia kurodai* FaNaC but had little effect at annelid *Malacoceros fuliginosus* FaNaC1. Conversely, F4 substitutions had little effect on FMRFa potency at *Aplysia* FaNaC but either abolished, strongly decreased, or increased potency at *M. fuliginosus* FaNaC1. Accordingly, recently published high-resolution FaNaC structures show that F1 and F4 residues orient deep into the neuropeptide binding pockets of *A. kurodai* FaNaC and *M. fuliginosus* FaNaC1, respectively. We also use non-canonical amino acid substitutions in *A. kurodai* FaNaC to describe the physico-chemical determinants of FMRFa F1 binding to *A. kurodai* FaNaC aromatic side chains. Our results show that the “deeper” of the two FMRFa phenylalanine residues in the binding pocket is crucial for FMRFa potency and outline a remarkable case of the same ligand orienting very differently into similar binding sites of two closely related receptors.

Sleep architecture and sleep oscillation dynamics across development

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Sleep quantity and quality change dramatically across the lifespan of altricial mammals born with immature brains such as humans and rodents. Despite a crucial role for healthy development, we still know quite little about developmental sleep – especially when it comes to architecture and oscillatory dynamics, or to the emergence of global inter-regional brain cross-communication. Existing methods to analyse immature sleep fail to provide the resolution needed to characterise its longitudinal microstructure. We designed light-weighted chronic devices adapted to freely-behaving rats and rat pups to simultaneously record muscle activity (EMG), field activity from prefrontal, parietal and hippocampal cortices, as well as multiunit activity from CA1. We then developed a semi- automated scoring algorithm to analyse the longitudinal sleep microstructure inferred from electrophysiological signals. This allowed us to define sleep substages both during REM and non-REM sleep in developing rodents, while mapping global communications between brain regions. We specifically characterize how sleep architecture exhibits temporal segmentation across the development – hinting at different specific sleep needs across developmental stages. Our results demonstrate that global cross-region oscillations emerge between the second and the third postnatal week, potentially supporting sensory and memory integrative processes.

In summary, with new chronic recording and analytic methods, we determine how sleep micro- architecture changes in rodents across development, revealing the emergence of sleep substages and their predominance during specific developmental windows.

Membrane interaction mechanism of the tick-borne encephalitis virus capsid protein

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Tick-borne encephalitis virus (TBEV, *Orthoflavivirus encephalitidis*) is an enveloped positive-sense single-stranded RNA virus. It is a dangerous human pathogen that causes tick-borne encephalitis (TBE), a severe disease that can result in lethal neurological complications. The TBEV assembly occurs at the endoplasmic reticulum (ER) membrane and involves coordination between the viral genome and three viral structural proteins. During the assembly, multiple capsid (C) protein copies bind to the ER membrane and recruit the viral RNA genome to form a nucleocapsid (NC). However, the molecular mechanisms underlying NC assembly remain poorly understood. To understand the membrane interaction mechanism of TBEV C protein on a molecular level, we combined biochemical and integrative structural biology approaches. Using reconstituted artificial membrane systems and purified C protein, we study the protein-membrane interaction. Our result shows that the C protein binds electrostatically to negatively charged lipid headgroups, followed by hydrophobic membrane insertion. However, the membrane interaction site remains uncharacterized. To address this, we performed hydrogen-deuterium exchange mass spectrometry (HDX-MS) on soluble and membrane-bound C protein. The HDX-MS data indicate a membrane-dependent effect on the deuterium exchange pattern in two regions of the C protein, implying possible protein-membrane or protein-protein interaction sites. These findings are being further investigated using a mutagenesis approach.

Evolutionary trajectory of somatic assembly of antigen receptors based on host-genome editing

Ryo Morimoto

Laboratory for Molecular Infection Medicine Sweden (MIMS), Department of Molecular Biology, Umeå University, Sweden.

Human self-defense against pathogens relies on two crucial arms: innate and adaptive immunity. The intricate synergy between pattern recognition by innate antigen receptors and the anticipatory nature of adaptive antigen receptors, expressed by lymphocytes, is pivotal for our well-orchestrated self-defense mechanisms. It's noteworthy that adaptive immunity is an exclusive feature observed in vertebrate species, setting them apart from invertebrates, which primarily depend on innate immune responses. Here, I will delve into the evolutionary origins and trajectory of our "modern" immune system by introducing an alternative adaptive immunity observed in jawless vertebrates (Hagfishes and Lampreys), a sister group that diverged from jawed vertebrates (ranging from sharks to humans) approximately 500 million years ago. The alternative adaptive immunity in jawless vertebrates encompasses both humoral and cellular arms, with receptors based on leucine-rich repeat modules rather than immunoglobulin modules. The receptor gene loci assembly involves a series of gene conversion steps facilitated by cytidine deaminase (CDA) function, in stark contrast to our V(D)J recombination mediated by RAG1/2 activity. Despite these insights, a considerable knowledge gap persists regarding the intricate systems that paved the way for vertebrate adaptive immunity, often likened to a "Big Bang" development. To address this gap, I recruit non-conventional invertebrate models, such as a water snail species lacking adaptive immunity but equipped with somatically diversified immune effector molecules. The diversification process in these snails mirrors the action of CDA in host-genome editing. The overarching goal of the new projects at MIMS is to unravel the central role of CDAs in the evolutionary emergence of host genome editing for immune function. This research represents the pre-dawn phase of vertebrate adaptive immunity and will shed light on the early stages of our immune system's development.

Ultrastructure remodeling of the host invasion apparatus of microsporidia parasites.

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Microsporidia are a group of obligate intracellular parasites that infect hosts across the animal kingdom. During infection, microsporidian spores translocate their entire cytoplasmic content through a thin, hollow superstructure known as the polar tube. For this, the polar tube transitions from a compact spring-like state to a long needle-like tube capable of long-range sporoplasm delivery. The unique mechanical properties of the building blocks of the polar tube allow for an explosive transition from compact to extended state and support the rapid cargo translocation process. The molecular and structural factors enabling this ultrafast process and the structural changes during cargo delivery are unknown. Here, we employ light microscopy

and in situ cryo-electron tomography to visualize multiple ultrastructural states of the polar tube, allowing us to evaluate the kinetics of its germination and characterize the underlying morphological transitions. We describe a cargo-filled state with a unique ordered arrangement of hibernating microsporidian ribosomes, which cluster along the thin tube wall, and an empty post-translocation state with a reduced diameter but a thicker wall. Together with a proteomic and computational analysis of affinity-purified polar tube components, our work provides comprehensive data on the infection apparatus and its building blocks that aid infection.

Nutritional control of stem cell cycle dynamics of the sea anemone *Nematostella vectensis*

Pascual-Carreras E. and Steinmetz P.R.H.

Michael Sars Centre, University of Bergen, Bergen, Norway

Most bilaterian research organisms (e.g. mammals, *Drosophila*) show nutrient-dependent whole-body growth, mainly carried out by cell proliferation, until reaching a final body size. Many marine invertebrates (e.g. cnidarians, sponges), however, exhibit lifelong growth and cell proliferation plasticity depending on food availability. How the nutritional regulation of cell proliferation underlies systemic growth in animals is therefore poorly understood. The cnidarian *Nematostella vectensis* juvenile polyp shows nutrient-dependent whole-body growth and cell proliferation plasticity. Starvation leads to body shrinkage and reduced cell proliferation, while refeeding triggers explosive growth and cell proliferation resumption. Previous research identified proliferative multipotent *piwi/vasa* stem-like cells and their transient-amplifying progeny populations. However, it is unclear if *piwi/vasa* stem-like cell proliferation is feeding dependent. Here we show that nutrition and Tor signalling control cell proliferation in *piwi/vasa* stem-like cells. During starvation, stem-like cells are preserved, with a small fraction slowly proliferating. Short-term starved *piwi/vasa* stem-like cells paused in G2 /M phase, while long-term starved entered quiescence in G1 phase. Refeeding triggered cell cycle re-entry regardless of their G2 /M-paused or G1-quiescent state. Tor signaling was gradually activated in a portion of stem-like cells after feeding. Furthermore, we demonstrated that Tor signalling was necessary for stem-like cell cycle progression and cell growth during *Nematostella* juvenile growth. Overall, the animal's nutritional state regulates *piwi/vasa* stem-like cell cycle dynamics, with the potential to either pause in G2/M or enter quiescence in G1 phase. The *piwi/vasa* stem cell-like population could be a case study to identify new mechanisms regulating quiescence in animal stem cells.

Systems eco-devo: towards using high-content image-based morphometrics to quantify the impacts of changing temperatures on developmental dynamics at single cell resolution.

Chiara Castelletti, Jonas Maurice Brandenburg, Lionel Christiaen

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Embryonic development is canalized to produce a narrow range of phenotypes despite environmental or genetic variation. Recent increases in the amplitudes of perturbations, owing to climate change, are exposing organisms to new and unpredictable environments. Marine invertebrates are directly exposed to fluctuating temperatures, which can result in significant phenotypic variation. Understanding the complexity of temperature tolerance and stress response will enable us to predict climate change's impact on developing organisms. In this study, we aim to model the correlation between temperature and developmental

dynamics and provide a quantitative estimation of the impact of climate change on early life stages. Because of its highly stereotypical development, the tunicate *Ciona intestinalis* provides an appealing model for evaluating how environmental cues shape development. Here, we tested early embryonic development of *Ciona* at different temperatures. Hatching rates were first used to measure fitness and determine the lower and higher temperature bounds and optimum temperature range, as well as scaling of developmental tempo within the permissive range. Temperature dependent morphological variations were assessed with single cell resolution from neurula stage onward.

From each cell we extracted quantitative parameters that describe shape, size and location within the embryo. By integrating this information, we aim to create a high-resolution phenotypic map of thermal tolerance based on a quantitative description of cellular phenotypes. We hope the map will become a standard tool to track temperature dependent developmental dynamics and to identify asynchrony and susceptibility to challenging temperatures at tissue/cell resolution.

Abstracts from the presentations on Thursday 19th September

SESSION III

Accurate Haplotype Phasing in UK Biobank Detects Phenotype-Influencing Structural Variants

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Genotype imputation has become an essential technique in large-scale genomic studies, providing a cost-effective method to enhance variant density in SNP arrays used in genome-wide association studies (GWAS). This approach is particularly advantageous with the advent of large sequencing biobanks. Recent advancements include significant reductions in sequencing costs for new individuals through low-coverage whole genome sequencing and effective haplotype phasing of rare variants.

I will also introduce a new haplotype-informed method for detecting structural variants (SVs), generating cohort-wide SV genotype dosage estimates for association with phenotypes. By applying the method to the UK Biobank and 1000 Genomes Project datasets, we achieve high genotyping agreement with state-of-the-art pipelines and discover new SV associations, demonstrating the potential of haplotype-informed methods for SV analysis in GWAS.

CRISPR-Cas9 corrected T cells for personalized primary immunodeficiency disease (PID) therapy

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Primary immunodeficiency disorders (PIDs) are multisystemic diseases where a hereditary gene defect causes white blood cells to malfunction. Depending on the type of cells that is most affected, the patients present with a constellation of symptoms, which include susceptibility to viral, bacterial or fungal infections, autoimmune disease, and early cancer. PIDs are caused by mutations in more than 300 genes. The individual mutations are very rare, and new disease genes and mutations are discovered monthly. We hypothesize that PID patients with defective T cell function can be stabilized by correcting the mutation in the T-cells and infusing the cells back as an autologous transplant. CRISPR-Cas9 editing requires the design of guide RNAs (gRNA) and repair templates to allow for efficient and correct DNA repair, without errors and off-target effects. We have investigated the impact of various factors in tool selection and aim to develop a personalized T-cell editing platform for PID therapy. Ultimately, this will form the basis for developing a T-cell specific prediction model.

Which babies should get RSV immunisation? Using large Nordic registry data to identify children at high risk of severe respiratory syncytial virus infection.

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Respiratory syncytial virus (RSV) can cause severe infections especially in the youngest infants, and it is among the most common reasons for hospitalization in young children. Novel immunisation methods have just been approved against RSV and they can transform the disease burden of RSV, but we don't know which children need them the most - our knowledge of risk factors is insufficient for optimal targeting of RSV immunisation.

Our aims were to identify predictors for RSV hospital admission from nationwide registry-based data and to develop and validate a clinical prediction model to guide RSV immunoprophylaxis for infants younger than 1 year.

We studied altogether 2.7 million infants born in Finland and Sweden, along with the data for their parents and siblings. The outcome was hospital admission due to RSV bronchiolitis during the first year of life. From the Finnish data, we searched for the key risk factors among 1511 variables from the infant and family members. After defining 16 key predictors, we trained a clinical prediction model, which was validated in Finnish hold-out data and Swedish data.

In addition to known predictors such as severe congenital heart defects (adjusted odds ratio 2.89, 95% CI 2.28–3.65), we confirmed some less established predictors for RSV hospital admission, most notably oesophageal malformations (3.11, 1.86–5.19) and lower complexity congenital heart defects (1.43, 1.25–1.63). The prediction model's C-statistic was 0.766 (95% CI 0.742–0.789) in Finnish data and 0.737 (0.710–0.762) in Swedish validation data. The infants in the highest decile of predicted RSV hospital admission probability had 4.5 times higher observed risk compared with others. The model's performance was similar to a machine learning (XGboost) model using all 1511 candidate predictors. The C-statistic was similar across different strata of parental income.

In conclusion, the identified predictors and the prediction model can be used in guiding RSV immunisation in infants, or as a basis for further immunisation targeting tools.

Functional genomics of noncoding exons identifies RNA targets

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Recent advances in RNA targeting agents and their delivery provide a unique opportunity to therapeutically target a myriad (>20k) of noncoding RNAs (ncRNAs) implicated in disease. However, impact of ncRNAs on human health is poorly understood and function of most ncRNAs is unknown. Here, we set out to rank ncRNAs according to their genetic impact on human health. We explore the human noncoding exome using genetic finemapping datasets and find trait-associated ncRNA exons under purifying selection, accelerated evolution, mammalian conservation and human constraint. In a series of functional assays including arrayed and pooled (saturation) base editor mutagenesis screens, we validate effects of noncoding exons variants on ncRNA stability and structure, and identify putative functional domains within ncRNAs that can be targeted using repurposed allosteric inhibitors. Furthermore, CRISPR/Cas9 mediated correction of disease-associated variants in ncRNA exons attenuates disease-pathology in human iPSC derived cell models. Overall, our study employs genomic tools to identify noncoding RNA targets implicated in disease. In the era of RNA medicine, we propose targeting noncoding RNA transcripts, or their functional domains as a novel therapeutic modality to combat disease.

Role of the gut microbiome in the functional development of the enteric nervous system and its link to autism spectrum disorders

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The gut-brain axis is the dynamic bidirectional interplay between the gut microbiome, the enteric nervous system (ENS) and the central nervous system. The ENS controls various functions of the gastrointestinal tract such as motility, homeostasis, and nutrient uptake and communicates with the brain in multiple ways, through endocrine and immune signaling, and direct neuronal signaling via the vagus nerve. While several correlative studies have implicated the gut-brain axis in mental health, it also appears that gastrointestinal disorders often accompany neurological disorders, such as autism spectrum disorders (ASD) and Parkinson's disease, among others. However, the mechanistic links underlying this association remain elusive. We hypothesize that the ENS, which is an important relay center in this axis, is also affected in neurological disorders, potentially explaining the concurrent gastrointestinal disorders that frequently accompany such disorders. To investigate this, we are using the zebrafish animal models to study the gut-brain axis in vivo and manipulate it in-situ. Our study focuses on the early development of the ENS and how it is modulated by the gut microbiome in wildtype zebrafish larvae and zebrafish models of autism, using light sheet

microscopy. In conventionalized larvae, the ENS activity intensifies through development from 3 to 5 days post fertilization (dpf) before dropping at 7 dpf. In germ-free animals, the activity is perturbed and seems to develop faster, while the gut motility is less periodic compared to conventionalized or fed animals. This study thus explores the delicate relationship between

the gut microbiome and the development of the ENS, and how its dysregulation affects development and disease.

SESSION IV

Spatial gene regulatory networks

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Gene regulatory networks convey the relationship between regulators such as transcription factors and the expression of individual genes. They are useful for understanding changes in cell phenotype, particularly for complex diseases such as cancer.

Although methods to generate gene regulatory networks from bulk gene expression data are common, it is more challenging to deal with single cell and spatial transcriptomics data due to their sparsity. Here we present an approach to generate spatially resolved gene regulatory networks from spatial transcriptomics data called STOAT (Spatial TranscriptOmics to Assess Transcriptional regulation), building on the methods previously developed in the lab (PANDA, LIONESS) and also a new tool that helps with the generation of prior networks (SPONGE). STOAT adapts the LIONESS approach of generating sample-specific networks to the spatial transcriptomic reality of very sparse gene expression through the use of gene expression averaging. We show supporting evidence for the validity of this method and also its application to a publically available breast cancer dataset as well as melanoma samples provided by our collaborators, highlighting the differences in the classification of individual spots using gene regulatory networks and gene expression data. We determine the limitations of our current approaches in dealing with sparse data and discuss ways to overcome them.

Local Regulation of Synaptic Ubiquitin Signaling

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Regulators of the ubiquitin signaling form a high-risk gene network in major brain disorders. In humans, this network consists of ~600 E3 ligases and ~100 deubiquitylases (DUBs) as potent regulators of proteostasis. To date, identifying which E3 ligases and DUBs are present at synapses, or which synaptic proteins are ubiquitylated, is still beyond our knowledge. To elucidate the synaptic toolbox of E3 ligases and DUBs, I have used DNA PAINT to map and quantify candidate protein copies identified by existing proteomics data. To determine which synaptic proteins are targets of ubiquitin signaling, my ongoing work will enrich synaptic ubiquitylated proteins for ubiquitylomics. This study will shed new light on the synaptic ubiquitin system, providing new potential therapeutical targets for synaptic pathologies.

Unveiling the Epigenetic Alterations in Endometriosis

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This study investigates the epigenetic landscape of endometriosis, focusing on the molecular mechanisms that contribute to its development and progression into endometriosis-associated ovarian cancer (EAOC). By employing techniques such as ChIP-seq for chromatin state analysis and RNA-seq for gene expression profiling, we aim to identify key transcriptional and epigenetic changes that facilitate the transition from benign to malignant states. Our findings will provide new insights into the molecular pathways involved in both the persistence of endometriosis and its potential progression to cancer.

Charting early lymphoma formation from the epigenomics perspective

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Aiming to understand lymphoma formation in its very early stages, we study the presence of lymphoma-specific epigenetic – especially DNA methylation – events in healthy tissues. To do so, we employ our newly developed single-cell DNA methylation method scTAM-seq. Preliminary data suggests that we have identified unknown, rare subpopulations that could represent the cell of origin of specific lymphomas. In addition we aim to understand how lymphoma-specific translocations affect 3D chromatin structure, to better understand the role of these translocations in early tumor formation. Using patient samples and in vitro models, we observe global translocation-related changes in 3D chromatin structure at the inter- and intrachromosomal level, with a potential effect on gene expression. Altogether, our research sheds new lights onto the early events driving lymphomagenesis.

Poster & Flash Talk Abstracts *(by alphabetical order of name of 1st Author)*

Associations of Telephone-Administered and Computerized Cognitive Tests with In-Person Cognitive Testing in a Population-Based Sample of 65-85-year-olds

, A.¹, Saari, T.¹, Urjansson, M.¹, Palviainen, T.¹, Paajanen, T.², Runz, H.¹, Palotie, A.^{1,3,4}, Kaprio, J.¹, Julkunen, V.^{5,6}, Vuoksima, E.¹ & FinnGen

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We investigated the associations of remote cognitive assessment tools with in-person cognitive instruments. We used a population-based sample (n=202) of 65-85-year-olds who participated in TWINGEN biobank recall study excluding those with cognition-affecting diseases. Remote measures were telephone assessment for dementia (TELE), Telephone Interview for Cognitive Status (TICS), its remodified version (TICS-m3) including three-trial word list task, and cCOG-battery, where the total score included modified Trail Making Tests and a three-trial word list task. In-person assessment included the Consortium to Establish a Registry for Alzheimer's Disease neuropsychological battery (CERAD-nb) with verbal and visual memory tasks, fluency, visuconstruction and naming constituting the total score, and Mini-Mental State Examination (MMSE). We analyzed the construct validity using correlation analysis. Additionally, we will analyze the classification abilities of the remote instruments using AUC analysis and evaluate their value in predicting cognition using linear mixed-effects models, controlling for demographic factors.

MMSE correlated 0.31 with TELE, 0.53 with TICS, 0.44 with TICS-m3, and 0.28 with cCOG total scores (p-values < 0.01). For CERAD-nb total score, the correlations were 0.39 (TELE), 0.48 (TICS), and 0.54 (TICS-m3), and 0.61 with cCOG (p-values < 0.01).

In our population-based sample, correlations were moderate and weaker than typically observed in case-control studies including those with Alzheimer's disease. Our study underscores the importance of examining the validity of remote cognitive screening instruments in non-demented populations and points out that remote measures including multi-trial word list task have higher correlations with gold-standard in-person measures than general screening instruments that correlate higher with MMSE.

Long-Term Impact of Stressful Life Events on Breast Cancer Risk: A 36-Year Genetically Informed Prospective Study in the Finnish Twin Cohort

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To investigate potential interactions between stress, genetic predisposition, and breast cancer (BC), we employed a multifaceted approach, analysing polygenic risk scores (PRS), DNA methylation (DNAm) patterns, stressful life events (SLE), and BC risk. Finnish twin cohort data (10,342 women born pre-1958, 719 BC cases 1982-2018) was used. SLE data was gathered from a 1981 questionnaire. Cancer and mortality data were pulled from Finnish Cancer Registry, Finnish Population Register and Statistics Finland. A sub-cohort (3,906 women, 321 BC cases) was used to analyse genetic BC risk using a PRS derived from 313 SNPs. DNAm data from 85 discordant twin pairs explored the association between 212 CpG sites previously linked to BC and SLE exposure. A hazard ratio (HR) of 1.05 (1.02,1.08) was observed for BC per one-event increase in total SLEs. Women exposed to over 10 SLEs exhibited an increased risk of BC incidence (HR 1.93, 1.19, 3.14). Stratifying by birth decade revealed an increase in HR for those born between 1940-1949 (HR 1.07, 1.01,1.12) compared to those born after 1950 (HR 1.01, 0.96,1.06). Genetic analysis linked a 3% increase in BC risk per SD increase in BC PRS (0.92, 1.15). MZ twin pair analysis yielded an HR of 1.14 (1.01, 1.29). DNAm analysis replicated associations with BC-related SLE exposure in 17 of the 212 sites.

This genetically informed study with 36 years of follow-up emphasises the long-term association between SLE and BC risk, while pointing towards the limited role of genetic risk as assessed by PRS. Instead, these results suggest that DNAm could play a role in mediating the effects of SLE on BC incidence.

Cis-regulatory signatures for improved identification and stratification of breast cancer subtypes

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Breast cancer is diagnosed and treated based on the molecular drivers of the individuals cancer. Molecular alterations at cis-regulatory elements, like promoters (cis-regulatory regions surrounding Transcription start site, TSS) and enhancers (cis-regulatory regions distal from TSS) have been shown to alter the regulatory network of the cells, conferring oncogenic behavior. For instance, aberrant DNA methylation pattern have been observed in promoters and enhancers and associated with clinical and histopathological features of breast cancer. We want to investigate the regulatory space of breast cancer further and Identify active RNA TSS and active enhancers in breast cancer that can lead to a better understanding of the regulatory landscape of the subtypes of the disease than we already have. With the presented project we are using the method Super-Low Input Carrier - Cap Analysis of Gene Expression (SLIC-CAGE) to map TSSs of genes and enhancers in over 200 patient samples plus cell lines will use the information to identify refined breast cancer subtypes and extract cis-regulatory signatures driving these subtypes.

A CRISPR homing screen finds a chloroquine resistance transporter-like protein of the *Plasmodium* oocyst essential for mosquito transmission of malaria.

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Summary

Genetic screens with barcoded *Plasmo*GEM vectors have identified thousands of *Plasmodium* gene functions in haploid blood stages, gametocytes, and liver stages. However, the formation of diploid cells by fertilisation has hindered the use of genetic screens to investigate vector-parasite interactions during the mosquito stages of the parasite. In this study, we developed a scalable genetic system that uses barcoded gene targeting vectors equipped with a CRISPR-mediated gene drive to generate homozygous loss-of-function mutants, revealing gene functions in the diploid life cycle stages. In this system, a modified knockout vector containing a *gRNA* integrated into one of the parental alleles serves as a selfish genetic element. This element is designed to direct Cas9 to the intact allele after fertilisation, leading to its cleavage, followed by homology-mediated repair that uses the disrupted allele as a template. We find that this homing strategy is 90% effective in the oocyst, resulting in the generation of homozygous knockouts. We validated the scalability of this approach through a pilot screen of 20 genes and discovered a new food vacuole-like organelle in the *Plasmodium* oocyst, that depends on a homolog of the *Plasmodium* chloroquine resistance transporter, *crt*. The new screening approach now provides a method to study many loss-of-function mutants simultaneously in diploid parasite stages to study oocyst biology systematically. Such investigations will be crucial to understand parasite-vector interactions, the transmission of drug resistance through mosquitoes and to identify new strategies to block transmission for malaria elimination.

Identifying key transcriptional regulators of *PDL1* and their association with survival in cancer through large-scale network analysis.

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In this study, we performed an extensive genome-wide analysis of pathway regulatory heterogeneity in cancer and identified regulatory heterogeneity in the PD1 signaling pathway across various cancer types using data from The Cancer Genome Atlas (TCGA). Our approach involved constructing large-scale gene regulatory networks to map the regulatory interactions between transcription factors (TFs) and their target genes for all TCGA patients. Interestingly, we found that inter-patient heterogeneity of the PD1 signaling pathway correlated with survival outcomes in 7 out of 33 cancer types. To pinpoint the key regulatory interactions linked to survival, we applied LASSO penalized COX regression analysis to the networks edge weights associated with transcriptional regulation of the *PDL1* gene in each cancer type. This analysis revealed several unique and shared transcription factors. Among the shared transcription factors were well-known *PDL1* regulators such as those from the FOS, JUN, and STAT families, as well as potentially novel regulators of *PDL1*, including RFX7, ZNF143, and IRF5. Further understanding of the TFs involved in *PDL1* regulation could potentially lead to the development of targeted therapies, as inhibitors or modulators of these TFs can be designed to interfere with the PD1/PDL1 signaling pathway, potentially enhancing the efficacy of existing immunotherapies.

Genome regulatory landscape of primary breast tumor and metastasis for individual patients

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Interactions between transcription factors and genes ensure healthy cell functioning to maintain a healthy tissue. When this gene regulation is disrupted, cells may start to grow uncontrollably which results in cancer after which further changes may lead to metastases. We are interested in what gene regulatory mechanisms are behind metastasis formation in breast cancer.

To investigate gene regulatory differences between the primary tumor tissue and metastasis of an individual patient, we modeled gene regulatory networks (GRNs) from bulk RNA-seq data of paired primary tumors and metastases. LIONESS, a tool developed in our lab that can model networks for individual samples, is used together with PANDA message passing algorithm to model the GRNs.

In preliminary analysis of these networks, cytokine signaling and PD1 signaling were among the enriched pathways associated to metastatic tissue, which were previously shown to be associated with lower cancer survival. Among other genes, transcription factor regulation of MXI1 was downregulated in metastasis, which inhibits MYC oncogene transcription. By further investigating these pathways, we hope to find upstream regulators of metastasis-specific pathways that could be a potential drug target.

Previous transcriptomic analyses showed that the breast cancer subtype switched between primary tumors and metastasis within individuals. In this work in progress, we are anticipating finding these so-called subtype switches based on our networks. Changes from the primary tumor to metastasis per subtype switch will give insights into gene regulatory patterns underlying metastasis formation per subtype, which could identify potential therapeutic targets for precision medicine.

Single-chain SorCS2 impairs cerebellar morphology and excitability: implications for cognitive and motor function.

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**Shared corresponding authorship*

The cerebellum is involved in higher-order cognitive functioning including spatial learning, attention, and memory, through extensive interconnections between the deep cerebellar nuclei and several limbic structures. Furthermore, aberrant cerebellar functionality is implicated in several neurodevelopmental diseases (NDDs) such as autism spectrum disorder as well as neuropsychiatric diseases. We focus on SorCS2, a member of the sortilin receptor family, that is abundantly expressed in the cerebellar cortex. The concerted action of SorCS2 which forms a tripartite complex with several neurotrophic factors and their receptors is important for neuronal maturation and synaptic plasticity, as well as apoptosis and growth cone retraction. SorCS2 is involved in various types of memory and is a risk factor for several NDDs, including autism. The receptor undergoes proteolytic cleavage resulting in a single-chain receptor which can be further processed into a double-chain isoform, each with distinct expression patterns and biological functions. Whilst both isoforms are highly expressed at early cerebellar postnatal stages, the expression shifts to predominantly the double-chain form from postnatal day 3 and onwards. By utilizing a transgenic mouse model which solely expresses the single-chain isoform, we show that the cellular morphology, dendritic spine density and synaptic composition of Purkinje neurons is impaired compared to wild-type and SorCS2 knockout mice. We propose that correct expression of the different isoforms in a spatiotemporal manner is critical for proper Purkinje cell morphogenesis and synaptogenesis. And that increased single-chain expression contributes to cognitive and motor deficits.

Investigating the interplay between poor sleep and metabolic disorders: A mouse chemogenetic model of inducible and reversible adolescent sleep deprivation

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Our project's overarching goal is to reveal how adolescent sleep deprivation can contribute to the emergence of metabolic dysfunctions later in life. To that end, we need to engineer new rodent models of inducible and reversible adolescent sleep deprivation. We aim to disrupt sleep by stimulating brain nuclei regulating wakefulness using a chemogenetic approach in mouse pups. Specifically, we aim to adapt in adolescent mice, published adult models of sleep deprivation targeting the ventral pallidum, the ventral tegmental area, or the nucleus accumbens. In our proposed models, sleep will be modulated through the activation of a specific DREADD (designer receptors activated only by designer drugs) that can be switched on/off after clozapine N-oxide (CNO) administration. We will introduce the DREADD by microinjecting a viral vector [AAV9-*hSyn-DIO-hM3D(Gq)-mCherry*] in pups at postnatal day 12 from a *Vgat-Cre* mouse line. The *Vgat* (vesicular GABA transporter)-*Cre* genetic model will allow us to restrict the introduction of DREADD to GABAergic neurons. We have refined the coordinates for these brain areas and confirmed that we could successfully and accurately infect these targets with our AAV. Next, we will verify the sleep phenotype of these animal models by recording electroencephalography (EEG) and electromyography (EMG) signals before and after activation of the DREADD during the adolescence period (P21 – P40).

Ultimately, our goal is to use these animal models to score sleep, alongside measurements of food intake, water intake, and energy expenditure – to understand the impact of sleep disruption on the emergence of metabolic disorders.

Unraveling the role of transcriptional networks in pancreatic cancer cell states using machine learning prediction algorithms

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancer types often diagnosed late with little information about the early stages of cancer initiation. Cancer heterogeneity and chemotherapeutic resistance further compound the problem, causing a major obstacle in improving the survival of PDAC patients. The mechanisms of drug resistance in PDAC are not clear and are partially attributed to epithelial-mesenchymal transitions (EMT) and cancer cell plasticity in a complex tumor micro-environment (TME). The state and identity of a cell is controlled by lineage-specific transcription factors (TF). Hence, it is important to study these fundamental mechanisms in PDAC development.

In this project, we utilized state-of-the-art machine learning methods to model robust relations between TFs and gene regulatory networks (GRNs) from treatment-naïve and neoadjuvant-treated PDAC patients. In our analysis, we can recover well-known and new TFs related to pancreatic cell identity and PDAC progression. These TFs were further perturbed in-silico and the cells were examined for their expression profile and role in controlling cell state. The predictions reported a strong activity from both well-known and newly predicted TFs regarding the cell state transition and their regulatory role. This is critical for further validation and in establishing their role in delineating PDAC disease progression and therapy resistance. The findings from our analysis will be presented and discussed in this meeting.

Title: Homozygous loss-of-function mutation in *SIT* leads to combined immunodeficiency due to dysregulated TCR signaling

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Keywords: SIT1, T cells, Immunodeficiency, Hodgkin lymphoma, TCR signaling

Abstract (250 words)

Background: Dysregulation of T cell receptor (TCR) signaling is involved in the pathogenesis of many IEIs. We detected a homozygous splice-site mutation in *SIT1* in a patient presenting with recalcitrant warts, atypical pneumonia, and repetitive Hodgkin's lymphoma. SIT1 encodes for signaling threshold regulating transmembrane adaptor (SIT1) which is a negative regulator of TCR signaling.

Aim: This study aims to characterize the phenotype and disease mechanisms associated with the loss of SIT1.

Methods: Whole-exome sequencing (WES) was used to identify a genetic diagnosis. Immunophenotyping and functional studies of patient's PBMCs and/or CRISPR-Cas9 knockout of healthy donors' T cells were conducted by immunoblotting, flow cytometry, live cell imaging and RNA sequencing.

Results: WES identified a homozygous splicing donor change in *SIT1*, causing loss of SIT1 protein in the patient. Immunophenotyping of patient lymphocytes showed a reduced proportion of naïve T cells and recent thymic emigrants CD4+ T cells. The patient also exhibited low vaccine responses to diphtheria and pneumococcus, coupled with a reduction in memory B cells.

The patient's and SIT1 knockout T cells were hyperresponsive to TCR-mediated activation. Patient's T cells further showed increased phosphorylation of ZAP70 and enhanced proliferation in response to anti-CD3 stimulation. Transcriptomics analysis of SIT1 KO confirmed dysregulated TCR response with upregulated STAT and NFkB signaling. However, the patient CD8⁺ T cells presented a defective cytotoxicity in the killing assay.

Conclusion: We described the loss of SIT1 as a cause of combined immunodeficiency. Our results suggest that SIT1 is a critical regulator of T cell homeostasis, activation, and function.

MULTI-OMIC ASSOCIATIONS OF EPIGENETIC AGE ACCELERATION ARE HETEROGENEOUSLY SHAPED BY GENETIC AND ENVIRONMENTAL INFLUENCES

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Abstract:

Introduction: Connections between the multiome and epigenetic age acceleration (EAA), and especially whether these are influenced by genetic or environmental factors, remain underexplored. We therefore quantified associations between the multiome comprising four layers – the proteome, metabolome, external exposome, and lifestyle – with six different EAA estimates.

Materials and Methods: Two twin cohorts were used in a discovery-replication scheme, comprising young (N=642; mean age= 22.3) and older (N=354; mean age=62.3) twins from FinnTwin12 and the Older Finnish Twin Cohort (OldFTC), respectively. First, associations between EAA estimates and multi-omic factors were assessed in the FinnTwin12 sample using linear mixed-effects models. Then, we used within-pair twin designs to assess genetic and environmental effects on associations. Replication of the associations with metabolites and proteins was performed in the OldFTC sample.

Results: We identified 40 multi-omic factors that were associated with EAA, 28 of which were proteins, after adjusting for sex, smoking, and body mass index. Within-pair analyses showed that genetic confounding heterogeneously affected these associations. Six multi-omic factors remained significantly associated with EAA independent of genetic factors: matrix metalloproteinase 9 (*MMP9*), complement component C6 (*C6*), histidine, lactate, glycoprotein acetyls, and the proportion of individuals aged >90 in the neighborhood. Replication of the results obtained in FinnTwin12 in older adults of OldFTC validated some of the identified associations with EAA.

Conclusions: Our study identifies a large number of multi-omic factors, including plasma molecules and exposures, associated with accelerated epigenetic aging. The twin designs suggest that genetics confounds these associations heterogeneously, with some associations remaining significant after consideration of all genetic and shared environmental effects.

Delayed weaning induces body recomposition in male mice only

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The adipose tissue is an endocrine organ distributed over multiple depots and categorised into two main subtypes: white adipose tissue which stores energy as lipids and brown adipose tissue which dissipates energy as heat. Adipocytes from the white depots can switch from a white to a brown-like phenotype called beiging.

Weaning, defined as the switch from milk to solid diet is a crucial developmental period. Dietary alterations or overfeeding of lactating mother have long-term effects on the metabolism of offspring in both rodents and humans.

To understand how weaning affects the adipose tissue in mice, we have analysed the kinetics of adipose tissue remodelling between weaning (P21) and P35. We next used a model a weaning retardation by separating pups at P21 (SW) or P28 (DW).

From 4 to 8 weeks of age, SW mice kept a body fat percentage rather stable, however male mice maintained with their mother between the 3rd and 4th week showed a decrease in their fat percentage and an increase in their lean mass while DW female mice had a similar body composition as SW. This happens independently of total body mass, energy expenditure and food intake. At 4 weeks of age, male mice harbour a denser adipose tissue innervation and higher cutaneous temperature that indicates beiging of adipose tissue.

These results highlight the importance of the weaning period for the development of adipose tissue. Sexually dimorphic phenotypes and body recomposition following delayed weaning suggest an involvement of hormones that will be further investigated.

Mendelian Randomization elucidating the causality between Narcolepsy and Schizophrenia

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Abstract

Background: Narcolepsy is a severe sleep disorder with characteristics of fatigue, fragmented sleep, cataplexy and hypnagogic hallucinations. Earlier clinical studies have reported the onset of schizophrenia after narcolepsy but the causality behind narcolepsy and schizophrenia is unknown.

Aims: Our goal was to understand the causality between narcolepsy and schizophrenia.

Methods: To estimate the number of comorbidity of both narcolepsy and schizophrenia, we employed data from the FinRegistry that contains data for the total population of Finland. We then used Mendelian randomization and previously published genome-wide association data to test the causality between narcolepsy and schizophrenia.

Results: We observed a robust causal association from narcolepsy to schizophrenia using the HLA-independent lead variants ($P\text{-value} = 6.0 \times 10^{-4}$) and even a stronger association when including the lead variant from the HLA locus ($P\text{-value} = 4.48 \times 10^{-7}$). Schizophrenia did not increase the risk for narcolepsy ($P\text{-value} = 0.225$ for FinnGen Release 12 and $P\text{-value} = 0.061$ for Ollila *et al.* narcolepsy cohort), and there was no evidence of pleiotropy.

Conclusions: Our findings indicate a causal relationship where narcolepsy increases the risk for schizophrenia. Additionally, our results clarify the psychiatric burden influenced by narcolepsy diagnosis.

Unravelling biological mechanisms by looking at single molecules in real-time

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Imagine you could directly see the location and dynamics of individual proteins binding to a piece of single DNA. What if you could assemble your biological complex step by step and see it in action in real-time? What if you could manipulate the structure of your biomolecule and quickly change buffer conditions to test your experimental hypotheses?

Essential biological processes performed by proteins interacting with DNA or cytoskeletal protofilaments are key to cell metabolism and life. In order to understand the molecular basis of life, as well as the pathological conditions that develop when processes go wrong, it is critical to get detailed insights into these processes at the molecular level. Not only at the highest resolution, but also in real time. The C-Trap makes that a reality by combining force spectroscopy with fluorescence microscopy.

Here, we will take you through an exciting journey of different research topics in which dynamic single molecule studies revealed remarkable insights. In each study, the C-Trap system was used; our easy-to-use platform that combines optical tweezers with fluorescence microscopy. We will explain how the ability to control, visualize and manipulate single molecules in real time, changes the way we answer tough scientific questions in the field of DNA processing, single-protein dynamics, liquid-liquid phase separation (LLPS) and beyond.

These experiments show that technological advances in hybrid single-molecule methods can be turned into an easy-to-use and stable instrument that can be easily shared by many research groups to advance scientific output.

Single-cell transcriptome analysis reveals the role of myofibroblastic cancer-associated fibroblasts in chemotherapy-resistance in pancreatic cancer

Abstract

Pancreatic cancer remains one of the most lethal malignancies and understanding the tumor microenvironment (TME) is crucial for developing effective treatments. In this project, we performed in silico analysis of single-cell RNA sequencing data from more than 200,000 cells derived from pancreatic cancer patients with and without neoadjuvant treatment. We identified 12 distinct cell types and focused our analysis on fibroblast cell population due to their important role in TME. Fibroblasts were classified into three major subtypes: regular cancer-associated fibroblasts (CAF), inflammatory CAF (iCAF), and myofibroblastic CAF (myCAF). Our findings reveal that samples from patients with poor treatment response as well as from untreated patients exhibited a higher proportion of myCAFs compared to treatment-responsive patients. Using specific markers derived from each fibroblast subtype, we applied these signatures to the PAAD cohort of TCGA and found a significant association between the myCAF signature and worse prognosis as well as advanced TNM staging. Functional analyses further demonstrated elevated levels of Wnt/ β -Catenin and Notch signaling pathways in the untreated and poor response groups. We are now further characterizing the cell states and associated transcriptional programs within the distinct TME subtypes. The findings and results from our analysis will be presented in the meeting.

Twin pair analysis uncovers novel links between DNA methylation, mitochondrial DNA quantity and obesity

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Alterations in mitochondrial metabolism in obesity may indicate disrupted communication between mitochondria and nucleus, crucial for adapting to changing metabolic demands. Epigenetic modifications, particularly DNA methylation, may influence this intricate interplay, though the specifics remain poorly understood. Leveraging data from the subcohort of the Finnish Twin Cohort (n=173; 86 full twin pairs) that includes comprehensive measurements of obesity-related outcomes, mitochondrial DNA quantity (mtDNAq) and nuclear DNA methylation levels in adipose and muscle tissue, we identified one locus at *SH3BP4* (cg19998400) significantly associated with mtDNAq in adipose tissue (FDR<0.05). *SH3BP4* methylation correlated with its gene expression. Additionally, 14 out of the 35 obesity-related traits displayed significant associations with both *SH3BP4* methylation and mtDNAq in adipose tissue. Using the method that infers causality from examination of familial confounding (ICE FALCON) our data suggests that mtDNAq, insulin sensitivity and certain body fat measures are causal to *SH3BP4* methylation. The examination of mtDNAq and obesity-related traits suggested causation from mtDNAq to obesity which could not, however, be distinguished from potential unmeasured within-individual confounding. In conclusion, our findings underscore the impact of mtDNAq on DNA methylation and expression of the *SH3BP4* gene within adipose tissue, with potential implications for obesity.

Exploring new frontiers of spliceosome function with fast-evolving marine animal genomes

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In eukaryotic genes, almost all introns begin with a GT and end with an AG. The contribution of these sequences to pre-mRNA splicing has been well documented, but the constraints that account for their conservation remain debated.

Whole-genome sequencing of *Fritillaria borealis* revealed that most of the ancient introns have been lost and replaced by new introns with exceptionally diverse splice sites. Canonical GT/AG introns represent less than 10% of all introns and they co-exist with non-canonical introns in the same genes. Using a combination of approaches, we could confirm that non-canonical introns are indeed removed by the spliceosome, which has conserved all the components of its core subunits in *F. borealis*. The radically different splicing landscape therefore evolved without changing significantly the key molecules known to process introns.

We used long-read sequencing techniques to produce a draft annotation of the *F. borealis* genome that includes a larger repertoire of introns and mRNA isoforms. For many non-canonical introns, terminal nucleotides are predicted to establish interactions that are analogous to those formed by canonical introns within the catalytic site of the spliceosome. This observation provides a rationale for how the spliceosome could process non-GT/AG intron ends. Other results indicate that sub functionalization of splicing factor genes, together with the loss of some proofreading molecules, could have supported the recognition of new splice sites. Intronization, made necessary for accommodating the rapid accumulation of a variety of gene-breaking transposons, is possibly a major driving force behind this evolutionary trajectory.

The gut commensal *Blautia* maintains colonic mucus function under low-fiber consumption through secretion of short-chain fatty acids

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Beneficial gut bacteria are indispensable for developing colonic mucus and fully establishing its protective function against intestinal microorganisms. Low-fiber diet consumption alters the gut bacterial configuration and disturbs this microbe-mucus interaction, but the specific bacteria and microbial metabolites responsible for maintaining mucus function remain poorly understood. By using human-to-mouse microbiota transplantation and *ex vivo* analysis of colonic mucus function, we here show as a proof-of-concept that individuals who increase their daily dietary fiber intake can improve the capacity of their gut microbiota to prevent diet-mediated mucus defects. Mucus growth, a critical feature of intact colonic mucus, correlated with the abundance of the gut commensal *Blautia*, and supplementation of *Blautia coccoides* to mice confirmed its mucus-stimulating capacity. Mechanistically, *B. coccoides* stimulated mucus growth through the production of the short-chain fatty acids propionate and acetate via activation of the short-chain fatty acid receptor Ffar2, which could serve as a new target to restore mucus growth during mucus-associated lifestyle diseases.

Does pregnancy accelerate aging and increase mortality risk?

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Life history theory predicts that organisms face trade-offs between resources invested in reproduction versus somatic maintenance. In this context, aging is the price of natural selection for reproductive success. Here, we use a twin study design to revisit the most famous hypothesis of evolutionary biology: the trade-off between reproduction and lifespan. Participants of the Finnish Twin Cohort, born between 1916-1958, have been longitudinally phenotyped with five questionnaires and civil registry data (N=10,783). Reproductive investment is assessed by identifying latent classes of distinct reproductive behaviors based on lifelong pregnancy history. Aging rate is determined utilizing recent advances in epigenetics — DNA methylation-derived epigenetic clocks, such as PhenoAge and PCA-based algorithms. Blood DNA methylation is quantified using Illumina 450k, EPICv1 and EPICv2 platforms. The effects of pregnancy on epigenetic aging and mortality are assessed using multilevel regression and survival models.

We found that pregnancy increased mortality risk - but only when the number of children was over 4. On the contrary to life history theory, the mothers without children had the highest mortality risk. We expect to find lifelong reproductive investment to incur costs on one's aging rate that reflect the history of cellular stress and maintenance, which are stored as epigenetic patterns. Studying twins allows us to minimize the genetic and environmental confounders shared by co-twins, allowing us to elucidate how reproduction underlies aging and lifespan through the prism of evolutionary theories, ultimately deepening our understanding of the determinants of healthy aging.

Transcriptomic analysis of metastatic colorectal cancer identifies enrichment of cancer-associated fibroblasts and up-regulated TNF α -signaling in CMS4 peritoneal metastasis

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Metastatic colorectal cancer (mCRC) is the main cause of CRC mortality, with limited treatment options. Although immunotherapy has benefited some cancer patients, mCRC typically lacks the molecular features that respond to this treatment. However, recent studies indicate that the immune microenvironment of mCRC may be modified to enhance the effect of immune checkpoint inhibitors. This study aimed to explore the metastatic immune microenvironment by comparing immune cell populations in colorectal liver (CLM), lung (mLu) and peritoneal (PM) metastases.

RNA isolated from 20 CLM, 15 mLu, and 35 PM samples was subjected to mRNA sequencing and explored through immune cell deconvolution tools, consensus molecular subtyping (CMS), differential gene expression and gene set enrichment analysis, with respect to the metastatic sites. Clinical data and KRAS/BRAF hotspot mutation status was also obtained for all the cases.

The immune cell fractions were relatively similar between the metastatic sites, except for cancer associated fibroblasts (CAFs), B cells, endothelial cells and CD4⁺ T cells. Notably, PM showed enrichment for CAFs and endothelial cells, consistent with distinct pathways associated with metastatic growth and progression in the peritoneal cavity. PM with the mesenchymal subtype, CMS4, had increased CAFs, endothelial cells and macrophages, along with upregulated genes related to TNF α -signaling via NF- κ B, EMT, and angiogenesis.

Tumor samples from different metastatic sites exhibited a broadly similar tumor microenvironment in terms of immune cell composition, with some intriguing differences. Targeting CAFs-associated pathways, macrophages and TNF α signaling through NR4A could represent potential novel therapeutic approaches in CMS4 PM.

Cell-free DNA methylation signatures for diffuse large B-cell lymphoma subtyping with whole-genome enzymatic methyl sequencing

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Accurate cancer subtyping remains a significant challenge in precision cancer medicine. Liquid biopsy (LB) approaches represent a promising non-invasive method for cancer diagnosis and profiling. Central to LB are cell-free DNA (cfDNA) fragments found in the bloodstream, which originate from normal and tumor cells undergoing cell death and carry cell type-specific genomic and epigenetic signatures. Although methylation signatures in cfDNA have been shown to accurately predict multiple cancers, the methylation profiles of specific cancers and their subtypes, such as lymphomas, are not well understood.

In this study, we analyze cfDNA and tumor samples from ten diffuse large B-cell lymphoma (DLBCL) patients treated in Nordic Lymphoma Group (NLG) Phase II trials LBC-05 and LBC-06. The study includes five germinal center B-cell (GCB) and five non-GCB cases collected at diagnosis, along with cfDNA from three healthy controls. These samples were sequenced using whole-genome enzymatic methyl sequencing (EM-seq) to identify methylated CpG sites. The primary analysis of the raw sequencing data will be performed using the DRAGEN platform's methylation pipeline to extract methylation calls. To identify tissue-specific and cancer-specific markers, we will detect and analyze differential methylation patterns, followed by the classification and profiling of cancer subtypes using machine learning methods. The findings from this study are expected to facilitate the detection of clinically relevant cancer signatures through methylation profiling of cfDNA, advancing precision medicine and targeted therapy interventions.

Heterozygous loss of *MAP4K1*/HPK1 causes a novel Inborn Error of Immunity-disorder characterized by immune dysregulation

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MAP4K1 encodes for Hematopoietic Progenitor Kinase 1 (HPK1), a negative regulator of T cell receptor (TCR) signaling. While there are previous associations between altered *MAP4K1* expression and certain immune-mediated diseases, disease caused by germline *MAP4K1* loss-of-function (LOF) variation had not been described. By exome sequencing and linkage analysis, we identified heterozygous *MAP4K1* LOF variants in two pedigrees with dominantly inherited autoinflammatory symptoms of variable severity. Pedigree 1 included 9 affected patients presenting most commonly with inflammatory arthritis and recurrent fevers, but blood vessel inflammation, rash and EBV-induced HLH were also detected. The single patient from pedigree 2 presented with steroid-responsive nephrotic syndrome and antibody deficiency following rituximab-treatment. We also identified family members who carried the *MAP4K1* variant but did not present with clinical symptoms, suggesting reduced penetrance of the disease phenotype.

Protein analyses confirmed that the variants caused loss of protein production from the variant allele. Lymphocyte immunophenotyping indicated that *MAP4K1*/HPK1-deficiency resulted in increased CD8 T cell effector maturation and perturbations in B memory cell generation. Functional tests on patient and healthy control-derived cells showed that lymphocyte cytotoxicity and regulatory T cell function were intact. *In vitro* TCR-stimulations indicated increased T cell activation and proliferation both in patient cells and in CRISPR-Cas9 generated *MAP4K1*-knockout T cells. Moreover, upon stimulation, patient and *MAP4K1*-KO T cells produced increased amounts of the pro-inflammatory cytokines IFN γ and TNF. Elevated cytokine levels were also detected in patient plasma samples. Western blotting and 3'RNA-sequencing approaches implicated decreased SLP-76 downregulation and increased NF- κ B-mediated TNF α -signaling in disease pathogenesis. Additionally, macrophage stimulations indicated enhanced IFIT2 expression and inflammatory responses in symptomatic patients.

In conclusion, heterozygous loss of *MAP4K1*/HPK1 causes a novel Inborn Error of Immunity characterized by immune dysregulation and inflammation due to increased T cell activation and inflammatory cytokine production.

The role of X chromosomal genetic variation in autoimmune disease risk

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Autoimmune diseases (AIDs) are disorders caused by the immune system's inability to recognize self-antigens from pathogens, leading to misdirected attacks on healthy cells. These diseases affect over 5% of the global population and include over 80 known conditions. A common feature in many AIDs is the predominance of females, with around 80% of patients being women in general. The causes of this sex difference remain unclear, but factors such as hormones, fetal microchimerism, X-chromosome inactivation (XCI), XCI escape, and X-chromosome abnormalities have been proposed as factors underlying the phenomenon.

This research focuses on investigating the potential influence of the X-chromosome in the development of autoimmune diseases, particularly exploring why these disorders often exhibit a female bias. We are analyzing genetic associations across autoimmune diseases using the FinnGen resource ($N \approx 500\,000$), to identify X-chromosomal loci contributing to autoimmune disease risk and to examine the contribution of the X chromosome relative to autosomes in these conditions. We are also evaluating the genetic effects and genetic correlations between the sexes to determine how the genetic influences for the X chromosome differ between males and females. For this study, thirteen autoimmune disease endpoints, e.g., Hyperthyroidism and Grave's disease, were selected based on clear autoimmune etiology, previous evidence of autosomal heritability, and a sufficient number of cases ($N > 1000$) in FinnGen. So far, our study has identified associated loci ($p < 1e-6$) in the X-chromosome in eight of the thirteen endpoints, highlighting the role of X-chromosomal variation in these conditions. Further analyses will be conducted during this summer.

Single-cell history tracing of epigenetic and transcriptional profiles in postmitotic neurons

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Cell identity and states are dictated by gene expression programs, influenced by a range of epigenetic modalities. In the neuronal system, these mechanisms underlie various functions, such as maturation and memory formation, and their deficits are linked to various neurological disorders. Recent advancements in single-cell sequencing technologies, including scRNA-seq, have accelerated our understanding of cellular diversity concerning molecular profiles. However, existing high-throughput sequencing methods have an inherent limitation that they can primarily provide a static 'snapshot' of molecular states due to the need to lyse cells for nucleic acid extraction, posing a barrier in analyzing cell fate-decision dynamics. Thus, a novel technology for whole-genome history tracing, namely multi-timepoints observation, represents a significant innovation.

Here, we report a novel 'time-machine'-like technology for retrospectively tracing the history of epigenetic and transcriptional changes at specific past time points in postmitotic neurons, a concept for which we have recently established a proof of principle. This will enable the analysis of multimodal epigenetic and transcriptional dynamics of over 10,000 genes, as well as more than 100,000 regulatory elements during neuronal fate decisions. We are applying this technology to reveal how heterogeneous subpopulations of memory-encoding engram cells emerge in the mouse brain, with a special focus on their excitability in memory encoding and recall events. Through this approach, we will address the molecular mechanisms of how a subpopulation of neurons becomes functionally relevant in memory formation.

Deep learning-based prediction of clinically actionable genomic alterations from histopathological images

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Precision medicine in neuro-oncology requires detailed molecular characterization of tumor cells for diagnosis due to the large number of CNS tumor entities. Optimal diagnostics is based on a combination of routine histopathology and deep molecular profiling of tumor samples. However, many patients are currently not benefiting from modern therapies due to a lack of sufficient tissue material for molecular profiling, associated costs, or lack of access to novel technologies. Recently, computer vision and deep learning methods demonstrated the feasibility to predict molecular information directly from digital whole slide images. Digital neuro-pathology pipelines can thus potentially aid treatment decisions for a larger patient population and reduce diagnostic costs based on targeted and approved molecular assays.

Our digital pathology pipeline allows us to predict clinically-relevant molecular phenotypes in adult-type diffuse glioma. We developed our models with digitized H&E-stained whole slide images and genomic profiles from the cancer genome atlas (TCGA). We inferred all three WHO2021-defined molecular subgroups of adult-type diffuse glioma (*IDH*-wildtype glioblastoma; *IDH*-mutant astrocytoma; *IDH*-mutant and 1p/19q-codeleted oligodendroglioma) with high performance (AUROC 0.85-0.95). Our approach is explainable and generalizes without loss of performance to external cohorts (The Digital Brain Tumor Atlas). We predict with similar performance somatic alterations in glioma driver genes (eg, *IDH1/2*, *EGFR*, *ATRX*, *TP53*) and diagnostic somatic copy number alterations (eg, -1p, -19q, +7, -10). Moreover, we show that our *TP53* model, trained on adult-type diffuse glioma slides, transferred with similar performance to pediatric-type diffuse high-grade gliomas.

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High throughput screening services at FIMM-HTB laboratory

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The High-Throughput Biomedicine Laboratory (HTB), a part of the FIMM Technology Center, enhances research capabilities through advanced automation and scaling technologies for both local and external scientists. Our facility is equipped with a wide range of liquid handling instruments, including state-of-the-art pipetting robots and ultrasound-mediated acoustic dispensers capable of precise transfers from as little as 2.5 nL. Additionally, our high throughput flow cytometry, automated microscopes, and versatile plate readers facilitate comprehensive assay outcome measurements. The HTB boasts an extensive collection of over 220,000 drug and small molecule compounds, encompassing the diverse EU-OpenScreen library with 100,000 compounds, the FIMM Oncology library with 526 compounds, and the Prestwick FDA-approved drug library with 1,520 compounds among others. These resources are crucial for drug discovery and repurposing, and drug sensitivity testing. We have also developed an online software package BREEZE tailored for data analysis and quality control. Over the past decade, HTB has significantly contributed to numerous drug discovery projects, several clinical trials, and commercial research collaborations with pharmaceutical companies. We invite researchers seeking to broaden their project scopes to consider partnering with HTB.

Aging-associated weakening of the action potential in fast-spiking interneurons in the human neocortex

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ABSTRACT

Aging is associated with the slowdown of neuronal processing and cognitive performance in the brain; however, the exact cellular mechanisms behind this deterioration in humans are poorly known. Recordings in human acute brain slices prepared from tissue resected during brain surgery, enable the investigation of neuronal changes with age. Neocortical fast-spiking interneurons are widely implicated in brain activities underlying cognitive processes, but their high metabolic demand makes them vulnerable to neurodegeneration. Herein, we analyzed the electrical properties of 166 fast-spiking cells in neocortex samples from individuals aged 11–84 years. By studying electrophysiological features of action potentials and neuron intrinsic excitability, we report that action potential overshoot significantly decreases and spike half-width increases with age. Moreover, the action potential maximum-rise speed (but not the repolarization speed or the afterhyperpolarization amplitude) significantly changed with age, suggesting a particular weakening of the sodium channel current generated in cell soma. Cell intrinsic excitability, which is measured as the input resistance, membrane time constant, and cell capacitance remained unaffected by senescence. Thus, we conclude that the action potential in fast-spiking interneurons shows a significant weakening in the human neocortex with age. This may contribute to the deterioration of cortical functions with age.

POSTER ABSTRACT**The ChemBio Platform – the Core Facility for Chemical Biology and High Throughput Screening at NCMM**Johannes Landskron

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The ChemBio Platform (CBP) at NCMM is a core facility for the University of Oslo (UiO) and the Oslo University Hospitals (OUS). It performs services in the field of chemical biology high throughput screening, compound management and bioinformatics, namely data analysis and development of specific analysis tools. It uses libraries of small molecules to target biological functions utilizing state of the art instrumentation for automated screening of several thousand compounds in a variety of biological and biochemical assays. These assays measure read-outs for receptor binding, protein-protein interactions, enzyme activity, as well as whole-cell measurements using high throughput flow cytometry and cellular viability or toxicity. The CBP has ~70,000 chemical substances in libraries that can probe a wide range of chemical space (diversity libraries) or specific targets (e.g., approved drugs in general and cancer drugs, kinase inhibitors, bioactives...). In addition, it serves as the national compound management facility for NOR-Openscreen and EU-OPENSREEN ERIC, which designed the European Compound Library (ECL) of 100,000 compounds of which 5000 compounds serve as a pilot library. The facility also offers screening assay development and gives access to instrumentation such as plate readers and liquid handling robots.

The facility serves both academic and industry users. Most users are local coming from OUS or UiO, but since the platform is part of national and European research infrastructures (NOR-Openscreen and EU-OPENSREEN ERIC, respectively) it also serves users on the national and international level.

Genome-wide screening of noncoding elements associated with breast and prostate carcinogenesis

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Optimal gene expression regulation is crucial for development and homeostasis, with transcriptional regulation being a key layer. Transcription factors control this process by binding to cis-regulatory elements (CREs). Disruptions in CREs are associated with altered gene expression in diseases like cancer, but distinguishing cancer driver- from passenger mutations in CREs is challenging. Our recent investigation showed that somatic mutations in CREs affect regulatory functions, leading to dysregulated gene expression in multiple cancers. While these results are promising, functional studies are needed to confirm that these alterations provide a proliferative advantage to cells.

The advent of CRISPR-based technologies provides a versatile system for genome editing, facilitating the identification of essential elements. Previous studies have been conducted to identify CREs using large-scale CRISPR-Cas9 and CRISPRi screenings. However, these have been conducted in cancer cell lines, which do not account for the effect of perturbation obtained if the CREs are disrupted in normal cells.

We performed large-scale CRISPRi screening using dCas9-KRAB by designing single guide RNAs (sgRNAs) with FlashFry, following the tiling gRNA design. The sgRNAs guide dCas9 to potential CREs in normal breast and prostate cell lines (HMEC and RWPE-1) identified as open chromatin regions. Sequencing data following the screening will allow us to assess the impact of the CRE alterations in providing a selective advantage to the perturbed cells.

Title: Study the evolutionary biology of cardiovascular system in olfactores

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Tunicates have been widely recognized as an important clade for to study the evolutionary origins of vertebrate organ systems, as well as fundamental principles of cell and developmental biology. Tunicates combine a unique phylogenetic position, and a simple and stereotyped embryogenesis, which ends with the hatching of a tadpole-like larvae typical of the chordate phylum. However, several important organ systems do not fully form and become functional before the completion of metamorphosis. These include the conserved and essential digestive, reproductive and circulatory systems. The heart, for example, acquires its blood pumping shape and ability, with contractile cardiomyocytes, at juvenile stages; and the cellular details underpinning the formation of the vasculature, its connection with the heart and the onset of circulation remain elusive, highlighting the need for further investigation. To gain comprehensive insights into the cellular and molecular underpinnings of post-embryonic development in *Ciona intestinalis*, we use animals collected from the Norwegian coast to obtain an extensive time series of whole animal single cell RNA-seq (scRNA-seq) covering 20 time points between the newly hatched larval stage until the maturation of young adults 50 days later. We obtained >160,000 single cell transcriptomes covering the main tissues and cell types composing essential organ systems, identifying known as well as novel marker genes. Focusing on the circulatory system, we pursue an in-depth characterization of the cellular lineages and gene regulatory networks underlying hematopoiesis, vasculo- and angiogenesis, as well as heart organogenesis. This study paves the way to further our understanding of post-embryonic development in *Ciona*, and the evolutionary origins of mesendodermal organ systems in chordates.

Functional Divergence in the DEG/ENaC/ASIC Protein Family and the Quest for Proton-Sensing Determinants

All ion channels in the DEG/ENaC/ASIC protein family have a common quaternary structure. Despite this, DEG/ENaC/ASIC family members have a variety of channel activation modes: some are constitutively active, others open in response to mechanical forces, others in response to external ligands, and some are activated by extracellular acidification. This family's functional divergence offers a great chance to study the molecular mechanisms behind the emergence and diversification of protein functions. Here, we focused on the proton sensitivity of a subgroup of DEG/ENaC/ASIC channels, which are either activated (as in acid-sensing ion channels, ASICs) or inhibited (as in Hydra sodium channels, HyNaCs, and bile acid-sensing ion channels, BASICs) by extracellular acidification. We examined the conservation of potential proton-sensing amino acid residues across different HyNaC/BASIC/ASIC members and species to identify positions that determine sensitivity to acidification. Then, using electrophysiological measurements of heterologously expressed channels, we quantified the effect of mutations of the candidate residues on the proton sensitivity of certain channels. We found important residues for proton-sensing in various members of HyNaC, BASIC, and ASIC, but these residues were not shared across the different channels. This implies two possible scenarios: either proton-sensing evolved in a common ancestor of all HyNaC/BASIC/ASIC channels, with the functionally important residues becoming redundant and dispensable in different extant channels, or proton-sensing evolved independently multiple times, using different residues each time.

A forward genetic screen uncovered a *Chlamydia trachomatis* mutant impaired in its ability to evade xenophagy

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Chlamydia trachomatis is a prevalent obligate intracellular bacterial pathogen causing ocular and sexually transmitted infections. While human cells have intrinsic defense mechanisms that can inhibit the proliferation of such disease agents, successful intracellular pathogens often evolved to evade the defenses. An enhanced understanding of the pathogenic evasion mechanisms may reveal opportunities for therapeutic exploitation of our cells' intrinsic defenses. Therefore, we studied how distinct defense mechanisms limit *C. trachomatis* growth. Specifically, we determined the protective potential of (a) the STING-dependent type-I interferon (IFN) response, *i.e.*, a defense program that can block pathogen growth by starvation, (b) xenophagy, *i.e.*, a specialized form of autophagy that can degrade invading pathogens, and (c) necroptosis, *i.e.*, a form of regulated host cell death that can deplete the pathogen of its growth niche. Genetic disruption of these defense programs did not boost *Chlamydia* growth, emphasizing the pathogen's ability to cope with the defenses. To explore the latent pathogen-suppressed protective capabilities of the defense programs, we further screened a library of *C. trachomatis* mutants and thereby discovered a mutant that displayed reduced growth in wild-type compared to xenophagy-deficient host cells. This suggests a loss of a xenophagy-evading virulence mechanism in the mutant, which further highlights the capacity of xenophagy to restrict *Chlamydia* infection. The mutant bacterial strain can now serve as a tool to identify the xenophagy-suppressing virulence factor(s). Collectively, insights from this research will advance our understanding of how *Chlamydia* modulates host cell biology and could enable the development of new treatments against *Chlamydia* infection.

AI-driven phenotypic image based interaction profiling for functional cancer precision medicine

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Microscopic imaging of cancer tissues offers rich architectural data, yet its potential remains underutilized due to limited analysis tools for such intricate information. There's a pressing need for artificial intelligence (AI)/machine learning (ML)-driven methods to perform in-depth spatial analyses of these cells, aiding biomarker discovery for oncological therapies. The purpose is to develop a deep learning 3D image analysis pipeline that allows an integrated and functional analysis e.g. of complex organoids at single cell level in a spatial context. Specifically, we quantify the architecture and drug vulnerabilities of co-cultured spheroids and immuno-organoids of urological cancers. The methods developed and the findings can both advance biological knowledge and lead to the discovery of new biomarkers for optimizing patient-specific treatments in cancer precision medicine.

Spatial transcriptomics in prostate cancer using multimodal foundation models

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Adequate diagnostics and prognostication of prostate cancer remains challenging due to the multifaceted heterogeneity of the disease. In many solid malignancies, tumor microenvironment (TME) has been shown to play an important role, but for prostate cancer, its importance largely remains to be elucidated. Emerging spatial transcriptomics (ST) methods can shed light on TME. However, ST data is still too limited to fully leverage advanced deep learning (DL). Here, we will utilize existing DL foundation models for digital histopathology and scRNA-seq data to jointly analyze prostate cancer ST and histopathology imaging data. We have collected ST data from 27 samples from 25 prostate cancer patients that were generated using the 10x Genomics Xenium platform. At the current stage, we have performed quality control of the 27 samples and run initial clustering to identify cell types. We will reveal spatially resolved transcriptomic patterns underlying previously reported bulk transcriptomic signatures, investigate the clinical implications of integrated transcriptomic and histological characteristics of prostate cancer and validate our findings in the iCAN Digital Precision Cancer Medicine Flagship project. Our results will contribute to improved clinical diagnostic and prognostic tools for prostate cancer.

Title: Identification of novel breast cancer subtypes through the implementation of single sample network analysis

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Abstract

Understanding the complex regulatory landscape of gene expression that drives cancer progression is a critical, yet challenging prospect. Breast cancer proves to be particularly difficult to understand due to the underlying heterogeneity of patients¹⁻³. The most common method of classifying breast cancer patients into subtypes is through the expression of 50 genes⁴, collectively known as the PAM50 (Prediction Analysis of Microarray 50) gene signature. Use of this gene signature stratifies patients into five distinct breast cancer subtypes: Luminal A, Luminal B, Normal-like Basal-like, and Her2-enriched. While this provides broad-level classification, the methodology behind assigning patients to subtypes has its shortcomings⁵ and not all patients categorized into these subtypes respond equally to treatment. Due to the heterogeneity of breast cancer, further stratification of patients into more distinct subtypes may prove beneficial in the development of new therapies and the subsequent clinical treatment of patients. Through this work, we reconstruct single sample gene regulatory networks⁶ and interrogate these networks to first identify novel breast cancer subtypes, then compare these single sample networks to identify targets for future therapies for breast cancer patients.

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Inhibition of CDK4/6 augments ATRA-induced differentiation across acute myeloid leukaemia subtypes

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Acute myeloid leukaemia (AML) is characterised by a differentiation blockade, preventing cellular maturation and normal haematopoiesis. All trans retinoic acid (ATRA) is a successful differentiation-inducing therapy used to treat acute promyelocytic leukaemia (APL). However, ATRA lacks efficacy in other AML subtypes, highlighting a need for novel differentiation-inducing therapies in the treatment of AML. We aim to identify if ATRA, in combination with CDK4/6 inhibitor palbociclib, induces myeloid differentiation and cell death in a range of AML models. We conducted flow cytometry-based drug sensitivity testing using 7 AML cell lines (HL-60, NB-4, THP-1, OCI-AML-2, Kasumi-1, MV4-11, MOLM-13). The cells were treated with ATRA and palbociclib, as single agents and in combination. Cell differentiation and cell viability were measured using a CD11b antibody and Annexin V/7-AAD, respectively. We observed that palbociclib induced cell cycle arrest in all AML cell lines tested. Interestingly, in HL-60, NB-4, OCI-AML2, and MOLM-13 cells, palbociclib alone induced cell differentiation. Furthermore, palbociclib in combination with ATRA induced increased cell differentiation followed by cell death. The differentiation was confirmed at the morphological level using May-Grünwald Giemsa (MGG) staining. To validate the cell line results, primary AML patient cells are currently being tested. In conclusion, we demonstrate that in AML, CDK4/6 inhibition in combination with ATRA induces cell differentiation and cell death, highlighting the potential of palbociclib as a novel differentiating therapy for use in AML. The ongoing experimentation on AML patient samples will identify the clinical efficacy of palbociclib in combination with ATRA.

AI-Driven Analysis of Stage-Specific Genetic Profiles in Malaria Parasites

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Plasmodium berghei, a rodent malaria parasite, serves as an excellent model for investigating host-parasite interactions due to the accessibility of the parasite, mammalian host, and insect vector. To understand gene function and phenotypes, we employed machine learning models to identify essential and dispensable genes at various stages of the malaria parasite lifecycle. By comparing simulation results with experimental high-throughput genetic screens, we achieved a 70% accuracy rate across stages. Our machine learning predictive models, trained using growth rates of ***Plasmodium berghei*** knockout mutants and gene/protein-based features, aim to elucidate gene functions and aid in drug discovery by identifying essential genes on a genome-wide scale. These efforts showed moderate success, achieving a 70% accuracy rate in predicting gene essentiality based on input features.

TP53 and KAP1 mediated derepression of retrotransposons in cancer cells upon inhibition of chromatin modifiers

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Transposable elements (TE) are widespread in human genome and are enriched in transcription factor binding sites thus can act as gene regulatory elements. In normal cells, TEs are epigenetically repressed by numerous mechanisms that include binding of tumor suppressor p53 and KRAB-associated protein 1 (KAP1). Epigenetic therapy has been shown to induce derepression of TEs resulting in activation of immune response against cancer cells. However, the molecular mechanism of TE regulation by distinct chromatin modifier enzymes (CME) in the context of p53 and KAP1 is still elusive. Here, we used FDA-approved epigenetic drugs to systematically inhibit distinct CMEs in p53 wild-type, p53-mutant, and KAP1 heterozygous colorectal, esophageal and prostate cancer cells. We show that inhibition of distinct CMEs results in derepression of TE subfamilies both in a common and cell type-specific manner. Our results indicate that co-inhibition of DNMT and HDAC synergistically and robustly derepresses individual TE loci in a cancer cell-specific manner. KAP1, a known repressor of TEs, activates TE expression upon co-inhibition of DNMT and HDAC. In addition, loss of functional p53 results in stronger TE activation and TE-chimeric transcript expression. This robust immune response elicited by co-inhibition of DNMT and HDAC is due to induced inverted repeat Alu expression that is commensurate with reduced ADAR1-mediated Alu RNA editing. However, SETDB1 inhibition activates ADAR1 expression that is associated with increased Alu RNA editing. Collectively, our systematic analyses provide insights for rational use of epigenetic therapies in distinct cancer types.

Clear cell renal cell carcinoma (ccRCC) is characterized by a unique tumor microenvironment (TME) with significant vascularization and immune infiltration, making it a target for anti-angiogenesis and immune checkpoint blockade (ICB) therapies. In our study, we performed high-throughput 33-marker immunofluorescence imaging on 437 surgically treated localized ccRCCs, focusing on CD45^{high} tumors, potentially responsive to immunotherapy. By analyzing tumor center, edge, and adjacent benign areas, we revealed complex spatial interactions among tumor, vascular, stromal, and immune cells. In CD45^{high} tumors, loss of epithelial markers was associated with higher expression of PD-L1 and fibroblast activation protein (FAP), indicative of epithelial-mesenchymal transition (EMT). These EMT^{high} tumors, enriched with M2-like macrophages, exhausted T-cells, and FAP⁺ CAFs, were associated with poor outcome in the overall, early stage (pT1-2), and CD45^{high} patient groups. Moreover, this phenotype correlated with reduced response to sunitinib treatment. These findings suggest that patients with this tumor subtype, even in early stages, could potentially benefit more from immuno-oncology therapies. Our findings highlight a dynamic interplay between immune infiltration, stromal biology, and EMT in localized ccRCC, suggesting new avenues for therapeutic strategies.

NUP98 is important in flavivirus infection through binding of viral RNA

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The nuclear pore complex (NPC) is composed of multiple nucleoporins (NUPs) and mainly enables the exchange of RNA and proteins between the nucleus and cytoplasm. NUP98 is one of the major components of the NPC, being involved in several import and export pathways by interacting with several transport factors and mRNA directly. Previous studies have suggested both proviral and antiviral functions of NUP98 in viral infection, yet little is known about its function in flavivirus infection. Flaviviruses are small enveloped positive strand RNA viruses, such as tick-borne encephalitis virus and West Nile virus, that cause major global health problems, with millions of human infections annually and no antiviral treatments currently available.

In this study we show that NUP98 is recruited from the nuclear envelope to the cytoplasm where it colocalizes with dsRNA at the replication vesicles during infection with different flaviviruses. Furthermore, using surface plasmon resonance, cross-link immunoprecipitation (CLIP) and CLIP-sequencing we observe that NUP98 directly interacts with a conserved site of the viral RNA. Blocking of the interaction site using peptides and also knock-down NUP98 using CRISPR results in reduced infection of flaviviruses, indicating it to be a proviral factor in infection. Taken together our results suggest a mechanism in which NUP98 is recruited to the replication vesicles during flavivirus infection to modulate infection by binding to the viral RNA.

Unravelling personalized drug vulnerabilities in pediatric solid tumors

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Pediatric cancer patients with high-risk primary, refractory, or relapsed tumors still lack effective treatment strategies and suffer frequently from both acute and delayed (long-term) adverse drug effects, including secondary cancers. These tumors are generally characterized by very few druggable molecular alterations, diminishing the advantage of genetics in defining patient-specific treatments.

To accelerate the development of innovative therapies for children with cancer, we aim to obtain deeper biological insights on potential druggable pathways of pediatric tumors and empower the pediatric preclinical studies. Here, we describe our recent functional precision medicine studies of pediatric solid tumors, including exome sequencing and transcriptomics analysis for fusion genes, as well as *ex vivo* drug testing of patient-derived cancer cells (PDCs) in a clinical real time. We also present the “fast-track” functional precision medicine concept, incorporated as a part of biobank operations to return the clinically relevant findings back to the treating clinicians.

As a conclusion, our findings show that the functional precision medicine approach can i) add to the care of pediatric cancer patients already at primary diagnosis with therapeutic as well as diagnostic impact, and ii) increase our biological understanding of drug vulnerabilities of pediatric cancers.

Targeting peptide drugs based on the activity state of synapses

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The overactivity of excitatory neurotransmission has been implicated in the pathogenesis of several brain diseases, turning the components of glutamatergic synapses into attractive drug targets. Yet, the development of drugs that reduce glutamatergic transmission has been largely hindered by the need to preserve normal brain function. In this project, we will engineer a coiled coil that undergoes phosphorylation-dependent dimerization in the presence of activated CAMKII, a biochemical feature of strong excitatory synapses. Thus far, we have redesigned the homodimeric antiparallel coiled coil APH6 to incorporate the recognition motif of one of the most abundant CAMKII brain isoforms, CAMKII α . Preliminary results suggest that the redesigned coiled coil has a lower tendency to form dimers, as intended, which we hypothesize will be recovered upon phosphorylation. In addition, we have created a FRET construct that allows to assess antiparallel coiled coil formation in a timely and effective manner, facilitating the screening of different sequences. Importantly, once we have a peptide that assembles into an antiparallel coiled coil upon CAMKII phosphorylation, we will address whether this occurs in primary neurons after long term potentiation. The creation of CAMKII dimerization modules will open a new avenue for studying the pathogenic mechanisms underlying neurologic disorders and, especially, for the development of drugs that target specific activity states of synapses.

Integrating multi-omics data with gene regulatory networks in cancers

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Cancer is a complex and heterogeneous disease caused by disruptions in normal cellular functions. The emergence of high-throughput 'omics technologies has resulted in their wide application to cancer studies, greatly increasing our understanding of the disruptions taking place at the different 'omic levels. The role of gene regulation as a core driver of biological processes and its role in the development and progression of cancer has been well established and gene regulation can be represented as a network of interactions between regulators and their targets. These networks are known as gene regulatory networks (GRNs).

Many tools and methods for integration of multi-modal data have become available in the last few years, however there are not yet established best practices regarding their use. Results can vary depending on the specific dataset or tool in use, as well as on the specific analysis being performed.

In this study, we integrated sample specific GRNs with multi-omics data in ten cancer datasets from The Cancer Genome Atlas (TCGA), using joint dimensionality reduction (JDR) approaches. We compared JDR models where we included GRNs with models where we did not, to determine the usefulness of GRNs in these integrative contexts. We also benchmarked the performance of several JDR tools when using PCA on the data first and used a "leave-one-out" approach to try to better understand the contribution of individual omics to the JDR models.

Intestinal α -defensins play a minor role in modulating the small intestinal microbiota composition as compared to diet

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The intestinal microbiota is at the interface between the host and its environment and thus under constant exposure to host-derived and external modulators. While diet is considered to be an important external factor modulating microbiota composition, intestinal defensins, one of the major classes of antimicrobial peptides, have been described as key host effectors that shape the gut microbial community. However, since dietary compounds can affect defensin expression, thereby indirectly modulating the intestinal microbiota, their individual contribution to shaping gut microbiota composition remains to be defined. To disentangle the complex interaction among diet, defensins, and small-intestinal microbiota, we fed wild-type (WT) mice and mice lacking functionally active α -defensins (*Mmp7*^{-/-} mice) either a control diet or a Western-style diet (WSD) that is rich in saturated fat and simple carbohydrates but low in dietary fiber. 16S rDNA sequencing and robust statistical analyses identified that bacterial composition was strongly affected by diet while defensins had only a minor impact. These findings were independent of sample location, with consistent results between the lumen and mucosa of the jejunum and ileum, in both mouse genotypes. However, distinct microbial taxa were also modulated by α -defensins, which was supported by differential antimicrobial activity of ileal protein extracts. As the combination of WSD and defensin deficiency exacerbated glucose metabolism, we conclude that defensins only have a fine-tuning role in shaping the small-intestinal bacterial composition and might instead be important in protecting the host against the development of diet-induced metabolic dysfunction.

Abstract title

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Tick-borne encephalitis virus (TBEV, *Orthoflavivirus encephalitidis*) is a positive-strand RNA virus, and a dangerous neuropathogen. During virus infection, the host cell's endoplasmic reticulum (ER) membrane is extensively remodeled to facilitate virus production. As the viral genomic RNA is replicated via a highly immunogenic double-stranded intermediate, the viral RNA synthesis machinery is sequestered into ER-derived replication vesicles. The vesicles have a high membrane curvature, and the mechanistic details of their formation are not known.

We have investigated the TBEV non-structural 1 (NS1) protein, a key factor in replication vesicle formation. We have used reconstituted artificial lipid membrane systems combined with purified recombinant NS1 to study the protein-membrane interactions facilitating the replication vesicle formation. Our results show that NS1 binds to negatively-charged lipid headgroups and inserts into the membrane. Additionally, we have identified key residues for these interactions, and by combining these data with integrative structural biology approaches, we can start to mechanistically understand the function of the TBEV NS1.

Using pangenomes to identify interspecies boundaries within *Bacillus cereus sensu lato*

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Bacillus cereus sensu lato or the “*B. cereus* group” is a complex of Gram-positive, spore forming bacteria. Members of the *B. cereus* group include, among others: *B. anthracis* - the causative agent of anthrax; *B. cereus sensu stricto* - a foodborne pathogen; and *B. thuringiensis* - a biocontrol agent. Species-level taxonomic classification of *B. cereus* group strains is essential from both a public health and industrial perspective, as different strains are subject to varying forms of treatment and/or regulation. However, species identification in the *B. cereus* group is currently a challenge, as there is no universally accepted taxonomy.

Biological and environmental barriers can influence gene gain/loss events and selection for specific genes within a species. Typically in bacteria, each species unit has a distinct gene content “signature”, which makes it distinguishable from other species units. In this study, we sought to examine the pangenome of the *B. cereus* group and use differences in gene content to identify potential interspecies boundaries. Here, we began with 5,976 *B. cereus* group genomes from the BTypperDB database (www.btyper.app/) and estimated the core and accessory genomes of a nonredundant subset. Using dimensionality reduction methods and various other bioinformatic tools, we investigated the relationship between gene content and *B. cereus* group lineage. We also attempted to identify genetic markers fixed in specific *B. cereus* group lineages, which could aid in epidemiological studies and outbreak investigations. Overall, this work attempts to provide a deeper understanding of the genetic diversity within the *B. cereus* group.

BTyperDB: a community-curated, global atlas of *Bacillus cereus sensu lato* genomes and metadata for epidemiological surveillance

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Existing public data portals linking whole-genome sequencing (WGS) data to epidemiologically relevant metadata can lead to dangerous pathogen misidentifications when applied to *Bacillus cereus sensu lato* (*s.l.*). Current taxonomic assignments are inadequate for evaluating *B. cereus s.l.* pathogenic potential at the strain level, as many important virulence factors can be gained, lost, and variably present within and across species boundaries. Moreover, incomplete metadata in general-purpose pathogen databases also makes epidemiological surveillance challenging.

To combat these issues, we developed BTyperDB, an atlas of *B. cereus s.l.* genomes with standardized, community-curated metadata. Briefly, all genomes submitted to the National Center for Biotechnology Information (NCBI) Genbank database as a *B. cereus s.l.* species were downloaded ($n=3,428$ genomes; accessed 8 August 2022). An additional 3,473 previously unassembled genomes were assembled using Shovill v1.1.0/SKESA v2.4.0 or Unicycler v0.5.0. All 6,901 genomes underwent quality control (via QUAST v5.0.2 and CheckM v1.1.3), taxonomic assignment (via BTyper3 v3.3.3 and the Genome Taxonomy Database Toolkit v2.1.0), and *in silico* typing (via BTyper3). Low-quality and misidentified genomes were removed, resulting in 5,976 *B. cereus s.l.* genomes.

Genomes within BTyperDB can be queried via an interactive web application (www.btyper.app). Users can identify and download strains isolated from various sources and geographic regions and query genomes based on the presence or absence of important virulence factors. To showcase its utility for pathogen surveillance, we used BTyperDB to identify emerging anthrax toxin- and capsule-harboring lineages.

In summary, BTyperDB can help improve *B. cereus s.l.* surveillance, source tracking, outbreak detection and response efforts.

Predictors of Response and Rational Combinations for the Novel MCL-1 Inhibitor MIK665 in Acute Myeloid Leukemia

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Preclinical investigations of MCL-1 inhibitors in acute myeloid leukemia (AML) have yielded promising results and led to clinical studies. However, results from clinical trials have shown diverse responses, most likely attributable to AML patient heterogeneity. To better stratify patients with AML for MCL-1 inhibition, we evaluated the ex vivo sensitivity of 42 primary AML samples to the selective MCL-1 inhibitor MIK665 (S64315) in the aim of identifying indicators of response as well as efficacious combination partners. By contrasting the clinical and molecular profiles of the samples, we observed that sensitive samples to MIK665 had a more differentiated phenotype. On the other hand, resistant samples displayed higher levels of ABCB1 (also known as multi-drug resistance protein MDR1) and of the anti-apoptotic protein BCL-XL, thus unveiling novel potential biomarkers of primary resistance to MCL-1 inhibitors. To induce sensitivity, we treated MIK665-resistant AML samples with a combination of MIK665 and FDA-approved BCL-2 inhibitor venetoclax. The combination was more effective at eliminating AML cells than either of the agents alone. Additionally, the combination restored sensitivity of AML samples with primary venetoclax resistance. Overall, this study indicates that elevated expression of ABCB1 or BCL-XL is a potential predictor of resistance to MCL-1 inhibition in AML. Moreover, this work demonstrates that a combination of MIK665 with venetoclax has the potential to restore sensitivity in patient populations resistant to either of the single agents, a finding warranting further clinical evaluation.

Zebrafish embryos with reduced deoxyhypusine synthase exhibit dysmorphology, epileptiform activity, and reduced GABAergic neuronal arborization

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The >7000 rare diseases affect >300 million patients worldwide, of which neurodevelopmental disorders (NDDs) are a major category. An ultra-rare NDD, with only 5 patients identified to date, is caused by mutations in *DHPS*, which encodes the highly conserved enzyme deoxyhypusine synthase. DHPS is essential to synthesize hypusine, a rare amino acid that only occurs as a post-translational modification of eukaryotic initiation factor 5A (eIF5A). DHPS deficiency syndrome causes epileptiform activity and seizures, cognitive impairment, and mild facial dysmorphology. To further elucidate DHPS' role during early brain development, we generated a zebrafish loss-of-function model by partial knockdown of *dhps* expression using an antisense morpholino oligomer (MO) targeting the exon 2/intron 2 (E2I2) splice site of the *dhps* pre-mRNA. *dhps* knockdown embryos exhibited dose-dependent dysmorphology characterized by microcephaly, axis truncation, and body curvature, as well as developmental delay. These results were confirmed with a second MO targeting the start codon of *dhps* mRNA, and could be partially rescued by co-injection with *in vitro* transcribed *dhps* mRNA. Ongoing experiments will evaluate the potential activity of anti-seizure drugs and drug candidates in this model.

Development of a Narrow-Spectrum Therapy Against *Chlamydia trachomatis*

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The obligate intracellular bacterial pathogen *Chlamydia trachomatis*, being a primary cause of bacterial sexually transmitted infections and the most common infectious cause of blindness, imposes a substantial public health burden globally. Current treatment strategies rely on broad-spectrum antibiotics, which do not always prove adequate in eradicating the infections and can disturb the delicate balance of the microbiome. We recently identified novel antichlamydial molecules, two of which demonstrated exceptional potency and selectivity *in vitro*. Our current research aims to assess their performance in an *in vivo* model of infection and to identify their molecular modes of action. Owing to the significant advancements offered by nanotechnology in *in vivo* testing, we have developed potential oral delivery systems using lipid-based nanocarriers. We have further characterized the nanoformulations *in vitro* with respect to their physiochemical properties, cellular uptake, biocompatibility, and antichlamydial efficacy, which overall revealed favorable characteristics. Now, we will move forward to conduct *in vivo* pharmacokinetic profiling and efficacy testing in established mouse models. To identify the molecular targets of the compounds, we applied thermal proteome profiling and identified mutations that can confer drug resistance. These approaches revealed potential targets in chlamydial fatty acid synthesis. Ongoing studies focus on validating these targets using genetic and biochemical approaches.

Overall, we anticipate our research to contribute to developing an effective narrow-spectrum therapy against chlamydial infections and to enhancing our understanding of *Chlamydia* biology at the molecular level. This knowledge will help advance public health outcomes and improve patient care globally.

Systematic assessment of protective polygenic effects in susceptibility gene variant carriers

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Recent studies demonstrate that polygenic risk scores (PRS) modify disease risk in individuals carrying moderate- and high-risk variants in known disease susceptibility genes. For instance, breast cancer risk is considerably impacted by polygenic background in *BRCA1/2*, *PALB2*, or *CHEK2* variant carriers. However, the focus has mainly been on assessing the impact of a high PRS in variant carriers, with less understanding of the impact of a low PRS. Moreover, prior studies on single variants indicate that the impact of low PRS might be smaller in high- than moderate-risk variants, possibly due to detrimental impact on disease-relevant biological pathways.

We systematically investigated the effect of low PRS in variant carriers for 9 common diseases (cancers, cardiometabolic, and autoimmune diseases) in FinnGen (N=520,210). We first analysed coding-variant associations for each disease. In variant carriers, we estimated effect sizes of low and high PRS (<40th decile, >60th decile, reference: 40-60th). We then compared the ratio of the effect sizes of low and high PRS against the coding-variant effect size (OR 1.03-5.15, min. N carriers 352). We did not observe any systematic differences in effect sizes of PRS tails as a function of the coding-variant effect size (trend p=0.08). Results were similar by variant function (missense p=0.26; frameshift p=0.93). We are currently performing similar analyses in UK Biobank.

In conclusion, the effect of low PRS is similar across different susceptibility genes, indicating that even in high-risk variant carriers, a low PRS may compensate for the effect of variant carriership.

Synaptic Regulators of Calcium homeostasis

Ca²⁺, as an important signaling ion for synaptic transmission and plasticity, is regulated by local Ca²⁺ channels and pumps that act as synaptic 'sources' and 'sinks'. Our current knowledge of these sources and sinks is largely based on biochemical information averaged across many synapses. We do not even know whether these averaged models depict the true capacity for Ca²⁺ handling at individual synapses, let alone define a disease state based on them. Using DNA PAINT, we mapped and quantified the major intracellular Ca²⁺ pump bound to the ER membrane (i.e. SERCA) at individual synapses. We observed a surprising clustering of SERCA copies in the spine apparatus, a mysterious extension of the ER into the postsynaptic compartment, as well as a huge variability of local SERCA copy-number between individual synapses.

Data-driven subgrouping of individuals on Alzheimer's continuum based on amyloid- β brain imaging

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Amyloid positron emission tomography (PET) scans are used to define amyloid- β (A β) accumulation which is a biomarker of Alzheimer's disease (AD). Typically, individuals are classified as either A β negative or A β positive, with the latter indicating AD-related pathology. However, early detection of AD requires identifying distinct patient subgroups based on the distribution of A β in the brain. This study aims to develop a deep learning model to define A β subgroups using A β PET scans and tracer information.

We aimed to develop a Conditional Convolutional Variational Autoencoder (CCVAE) to define subgroups based on amyloid PET scans, conditioned on amyloid tracer type. Our dataset comprised 3,127 PET scans from the Alzheimer's Disease Neuroimaging Initiative and 1,788 scans from the Anti-Amyloid Treatment in Asymptomatic Alzheimer's study. Scans were pre-registered to the MNI152 template and normalized to the whole cerebellum before training. To visualize and define A β subgroups, we extracted latent features from the CCVAE bottleneck and clustered them using UMAP.

The anticipated outcomes of this study include the identification of distinct A β distribution patterns that could lead to a better understanding of AD progression. Biologically, this could reveal variations in disease mechanisms, while clinically, it could enhance early diagnosis, improve monitoring of disease progression and treatment response, leading to more effective interventions for AD.

Genome-wide polygenic risk scores for colorectal cancer have implications for risk-based screening

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Hereditary factors, including single genetic variants and family history, can be used for targeting colorectal cancer (CRC) screening, but limited data exist on the impact of polygenic risk scores (PRS) on risk-based CRC screening. Using longitudinal health and genomics data on 453,733 Finnish individuals including 8801 CRC cases, we estimated the impact of a genome-wide CRC PRS on CRC screening initiation age through population-calibrated incidence estimation over the life course in men and women. Compared to the cumulative incidence of CRC at age 60 in Finland (the current age for starting screening in Finland), a comparable cumulative incidence was reached 5 and 11 years earlier in persons with high PRS (80–99% and >99%, respectively), while those with a low PRS (< 20%) reached comparable incidence 7 years later. The PRS was associated with increased risk of post-colonoscopy CRC after negative colonoscopy (hazard ratio 1.76 per PRS SD, 95% CI 1.54–2.01). Moreover, the PRS predicted colorectal adenoma incidence and improved incident CRC risk prediction over non-genetic risk factors. Our findings demonstrate that a CRC PRS can be used for risk stratification of CRC, with further research needed to optimally integrate the PRS into risk-based screening.

Dissecting the structural and functional elements of human super-enhancers

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Super-enhancers (SE) are genomic elements that consists of multiple enhancers acting in a synergistic way to regulate gene expression. SEs are characterized by enrichment of active histone mark H3K27ac along with binding of many transcription factors (TF). Functionally SEs have been implicated to be important for regulation of genes related to cell identity and cancer. Furthermore, it has been shown that biochemical condensates formed by a process of phase separation are important for establishing efficient SE–promoter interactions. Phase separation is facilitated by proteins such as TFs, and especially their intrinsically disordered regions (IDR) have been closely linked to the formation of biochemical condensates. Here, we have used massively parallel enhancer assay STARR-seq along with various genomics datasets to detect classical enhancers (STARR-seq positive) and chromatin-dependent enhancers (STARR-seq negative) within SEs in human cancer cell lines. We identified a specific TF binding exclusively to classical enhancers, which we also show being able to drive phase separation *in vitro* and in nuclear extracts. Deletion of this TF resulted in changes in chromatin structure, gene expression and epigenetic marks. Collectively, our results suggests that classical enhancers have an important role in the regulating the structure and function of super-enhancers as well as genome organization.

Tail Watching: Uncovering the Roles of the SorCS1-3 C-terminal Domains in Protein Trafficking

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Synaptic plasticity, the alteration of synaptic strength in response to novel information, is widely considered the molecular mechanism underlying memory formation. Changes to the arrangement and structure of the post-synaptic density drive this process. Uncovering the mechanisms by which proteins traffic molecules to and from the post-synaptic density is therefore a necessary step in advancing our understanding of memory.

SorCS1-3, members of the mammalian VPS10p-D receptor family, are crucial to the proper functioning and survival of neurons. They are highly prevalent in the central nervous system, have known functions in protein trafficking, and are associated with various neurological disorders, including Alzheimer's disease. While SorCS1-3 share structural elements, they harbour unique cytoplasmic tail regions. Studies have shown that the tail region of the related yeast protein VPS10p is indispensable for its protein transportation function. Furthermore, the tails of other mammalian VPS10p-D family members are instrumental in driving Golgi-endosomal transport. Collectively, these findings support roles for the SorCS1-3 tails in protein trafficking.

This project aims to characterise the interactomes of the SorCS1-3 tails and to uncover the contributions of these regions to the protein trafficking functions of SorCS1-3. To do this, we will combine proteomics and functional assays in primary neuronal cultures.

Modelling epilepsy-causing STX1B mutations in Zebrafish

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Approximately 25-30% of people suffering from epileptic seizures do not respond to currently available antiseizure medication (ASMs). Additionally, ASMs tolerated well by adults may cause severe side effects in children. Thus, there is a high need for models to test novel therapeutic options. Especially rare forms of epilepsy, where evaluation of intervention may be challenging due to the limited number of subjects, will benefit from animal models suited for rapid drug screening.

A large number of non-responders suffer from seizure phenotypes caused by mutations in genes encoding SNARE-complex proteins or one of its co-factors. The SNARE-complex is required for the fusion of neurotransmitter-containing synaptic vesicles with the cell membrane, to achieve synaptic signal transmission.

Mutations in genes of the SNARE complex give rise to a broad array of clinical outcomes, such as seizures, intellectual disability and movement disorders. To develop effective therapeutics for individuals with SNAREopathies, knowledge about the molecular consequences of specific genetic alterations is essential. In this study, we investigate two epilepsy-causing mutations in the SNARE-motive of Syntaxin-1B, E210K and L221P. We describe changes in complex stability and binding affinity in vitro, as well as the effects on epileptiform activity in vivo.

Midlife and old-age cardiovascular risk factors, educational attainment, and cognition at 90-years

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We examined the associations of midlife and old-age cardiovascular (CV) risk factors and education with 90-year cognition.

The study participants were 96 nonagenarians from population-based twin cohort study. CV risk factors included blood pressure (BP), body mass index (BMI), and physical activity (PA), and cholesterol assessed via questionnaires in 1975, 1981, 1990, and 2021-2023. The Cardiovascular Risk Factors, Aging and Dementia (CAIDE) and an educational-occupational score were used as midlife dementia risk scores. Telephone-based cognitive assessments included semantic fluency and immediate and delayed recall of a 10-word list (three trials). We used regression analyses to predict 90-year cognition with CV risk factors, dementia risk scores, age, sex, education, follow-up time and Apolipoprotein E genotype.

Those with 7-11 years and ≥ 12 years of education had better immediate recall than those with ≤ 6 years of education ($p=0.003$ & $p<0.001$). Those with ≥ 12 years of education scored higher than those with ≤ 6 years of education in semantic fluency and delayed recall ($p<0.001$). Those with high middle-age BP scored higher in semantic fluency ($p=0.005$), immediate recall ($p=0.023$) and delayed recall ($p=0.003$). Those with high old-age BP scored lower in semantic fluency ($P=0.010$). Total CAIDE score was not associated with any cognitive measures. Higher educational-occupational scores associated with better semantic fluency ($p=0.003$), immediate recall ($p=0.001$) and delayed recall ($p<0.001$).

Higher educational and occupational attainment was associated with better cognitive function in late old age. Elevated BP during middle age was associated with better old age cognitive function; however, this association reversed in old age.

Genome-wide association study on ACE-inhibitor switching identifies missense variants in *NTSR1* and *CACNA1H*

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Persistent dry cough is the most common side effect of angiotensin-converting enzyme inhibitors (ACEi). Treatment guidelines advise resolving it by switching to an angiotensin receptor blocker (ARB). We aimed to identify genetic predictors for ACEi-associated cough.

We conducted a genome-wide association study (GWAS) within 84,759 first-time ACEi users (mean age 58.1 years, 44.7% female) from the FinnGen study, which includes medication purchase register follow-up since 1995. We defined cases (N=15,145) as ACEi users who switched to an ARB within 1 year of treatment initiation, and the rest (N=69,614) as controls. After GWAS, we performed fine-mapping with SuSiE to identify causal variants.

We identified 11 genome-wide significant loci in *KCNA2*, *NRXN1*, *OSBPL10*, *KCNIP4*, *PREP*, *MAPKAP1*, *HELLPAR*, *CACNA1H*, *L3MBTL4*, *SLCO4A1/NTSR1*, and *NTSR1*. Fine-mapping pointed to 17 credible sets (CS). Lead CS variants included two missense variants: rs148569146-G-C (p.G301R, *NTSR1*, OR=0.39, AF=1.4%) and rs3751664-C-T (p.R788C, *CACNA1H*, OR=0.88, AF=11%). Lead CS variants were not phenome-wide associated with any other traits FinnGen. The relative risk for a cough diagnosis (ICD-10 or ICPC-2) during follow-up was 3.6 in cases compared to the GWAS controls (P<0.001).

We expand earlier work by identifying four new loci (*NRXN1*, *OSBPL10*, *HELLPAR*, and *CACNA1H*) and two new protein-coding variants associated with ACEi-to-ARB switching. Of particular interest is the 28-fold Finnish-enriched missense variant in *NTSR1*, which, to our knowledge, has not been reported before. Our results provide new evidence for the neurological hypothesis of ACEi-associated cough. The protective missense variants in *NTSR1* and *CACNA1H* could potentially guide the development of cough-suppressing therapies.

A genetic screen for factors involved in translation reactivation of *Plasmodium* maternal mRNAs.

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Plasmodium parasites cause malaria, a deadly disease that remains a global health concern due to lack of effective vaccines and the rise of drug resistance. Developing the much-needed therapies relies on understanding the parasite's biology. *Plasmodium* undergoes different developmental stages in both vertebrate and mosquito hosts. These stages are characterized by distinct transcript and protein expression patterns crucial for survival and transitioning from one developmental stage to another. In the female sexual forms known as female gametocytes, some mRNAs are translationally silenced in ribonucleoprotein granules under the regulation of the DOZI protein complex. These mRNAs are translated after fertilization and the resulting proteins are essential for zygote development. However, the mechanisms of translation reactivation following fertilization are unknown. In our study, we are performing a targeted screen to find proteins involved in de-repressing translation in *Plasmodium berghei*. Reporter cell lines with repressible transcripts for a fluorescent protein will be used to track DOZI mediated translation repression. A pool of barcoded mutants from the *PlasmoGEM* resource will then be generated in such reporter lines and mutants that result in defects in reporter expression will be identified after activation of female gametocytes. The phenotype will be assigned to the causal gene mutant by barcode sequencing. Top hits will be validated by individually knocking out the genes to confirm the phenotype and assess transcriptome profiles. We postulate this screen will uncover mechanisms underpinning sexual developmental transformations that are essential for the mosquito transmission of malaria parasites.

Galectin 3: a promising target for 3D optical imaging of virus-induced neuroinflammation

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Viral brain infections induce neuroinflammation. Although this is a natural defense mechanism, it can shift towards a neurotoxic state. In viral encephalitis, neuroinflammation is believed to be a disease escalating factor and a potential cause of extensive neuronal loss underlying neurological consequences. Visualization of virus-induced neuroinflammation in the whole brain would allow identification of its spatio-temporal pattern and contribute to a better understanding of neurological complications. Here, we aimed to identify targets to visualize virus-induced neuroinflammation by 3D optical imaging.

IPS^{-/-} mice were intracranially inoculated with Langat virus and at endpoint, brains were harvested and processed for immunofluorescence and whole brain 3D optical imaging. After whole mount immunofluorescent labeling, light sheet fluorescent microscopy (LSFM) images were acquired. To assess the influence of intracranial injection on inflammation, Western Blot was performed on PBS-injected mice.

Gal3 was most favorable for 3D optical imaging of virus-induced neuroinflammation with high expression in infected brains while nearly absent in non-infected controls. Gal3 was expressed in microglia/macrophages, while astrocytes and neurons were devoid of Gal3. Gal3 coincided with CD45 but not with TMEM 119, indicating visualization of infiltrating macrophages. Gal3 could not be detected on Western Blot in PBS infected brains, meaning that none of the Gal3 signal can be attributed to intracranial injection. LSFM allowed simultaneous visualization of infection and Gal3 and, Gal3 positive regions on LSFM corresponded to Gal3 positive regions on confocal microscopy. Together, these data confirm that we can visualize virus-induced neuroinflammation on the whole brain level by targeting Gal3.

Dissecting the gene expression readout of distinct elements in human super-enhancer clusters

Super-enhancers (SEs) are genomic elements composed of multiple enhancers that synergistically regulate gene expression, playing crucial roles in cell identity and cancer. We have functionally classified the enhancers within SEs into classical enhancers (STARR-seq positive) and chromatin-dependent enhancers (STARR-seq negative) using massive parallel reporter STARR-seq assay and together with other orthogonal genomics datasets. Our recent results show the role of classical enhancers bound by specific family of TFs in mediating phase separation through formation of biochemical condensates and in controlling 3D genome organization. The goal of my project is to understand the role of classical enhancers in gene expression control using CRISPR-Cas9 based genome editing of non-coding regulatory elements and their functional readout using single cell RNA-seq. We will further dissect the role of these classical enhancers in mediating promoter-enhancer interactions to detect changes in chromatin conformation.