ABSTRACT BOOK

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Metabolomics and lipidomics insights into Barth syndrome cardiomyopathy

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Barth syndrome (BTHS) is a rare X-linked recessively inherited disorder caused by variants in the TAFAZZIN gene. The pathogenic variants lead to impaired conversion of monolysocardiolipin (MLCL) into matured phospholipid cardiolipin (CL). The accumulation of MLCL and mature CL deficiency is a diagnostic marker for BTHS. The clinical spectrum includes cardiomyopathies, skeletal myopathies, neutropenia, and delays in growth and development. In severe BTHS patients, the cardiac phenotype is early onset, heterogeneous and unpredictable. Ultimately, severely affected patients require a cardiac transplantation early in their life. Unfortunately, the pathophysiological mechanisms of BTHS are poorly understood, and treatment options for BTHS remain symptomatic.

In this study, we investigated a unique collection of heart samples from five paediatric male BTHS patients (5 month-15 years old) and 24 non-failing donors (19-71 years old). We performed metabolomics and lipidomics using UPLC-mass spectrometry (LC-MS). The lipidomic profile of BTHS confirmed the findings of MLCL accumulation and CL depletion, corroborating what has been reported in literature. In addition, the acylcarnitine profile showed significantly decreased long-chain acylcarnitines in the BTHS samples, suggesting a possible shift in energy metabolism from fatty acid oxidation to glycolysis. Metabolomics showed significantly decreased metabolites including adenosine triphosphate (ATP), creatine phosphate, acetyl-CoA and carnitines (short, medium and long-chain), indicative for energy deficiency. Increased metabolites included pyruvate, lactate, alanine and serine, which point to a metabolic preference towards glycolysis.

Our analysis comparing heart biopsies from BTHS individuals to non-failing donors, reveal that BTHS have a unique and distinct metabolic and lipidomic profile. Our findings suggest that the BTHS heart can undergo a metabolic switch from fatty acid oxidation to glycolysis when compared to control. The latter switch may represent a compensatory mechanism in response to cardiac energy deficiency.

Factors associated with neurologic deficits in children with cerebral malaria compared to children with other forms of severe malaria

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Introduction: Children with severe malaria may develop transient or lifelong debilitating neurologic deficits. Although these deficits have been identified among children with cerebral malaria (CM), they have not been explicitly identified in other forms of severe malaria. Factors associated with these neurologic deficits among children with CM have been identified, however factors associated with these deficits which may also occur in other forms of severe malaria have not been identified. This study assessed neurologic deficits and factors associated with neurologic deficits in children who had CM compared to those who had other forms of severe malaria. Other forms of severe malaria included: severe malaria anemia (SMA), respiratory distress (RD), malaria with seizures (MS) and prostration (PR).

Methods: This was a secondary analysis of data from the Neurodevelopmental outcomes in children with severe malaria (NDI) study. The NDI study conducted between 2014 and 2018 assessed neurodevelopmental outcomes in five most common types of severe malaria (CM, SMA, RD, MS, PR) followed up to 12 months post hospital discharge. Exposure variables for this study included: age, gender, study site, number of seizures after admission, seizures duration, malnutrition, hypoxia, severe acute kidney injury and duration of coma. The outcome was neurologic deficits at discharge and at one month following hospital discharge. The effect measure was odds ratios. A multivariable logistic regression model was used to assess factors associated with neurologic deficits among children with CM and children with other forms of severe malaria.

Results: A total of 54 children with CM and 495 with other forms of severe malaria were included in the analysis. The proportion with CM with neurologic deficits were 37%, 11.1% and 5.6% while those of with non-CM were 9.5%, 2.2% and 1.8% at discharge, one month and 12 months post-discharge respectively. Among those with CM, duration of coma was associated with neurologic deficits (AOR= 6.49, 95% CI: 1.10-38.46) at discharge. At one-month postdischarge, study site (AOR=15.47, 95% CI: 1.37-174.29) was associated with neurologic deficits. Among those with non-CM, moderate malnutrition (AOR=7.17, 95% CI: 1.64-31.26) and severe acute kidney injury (AOR=6.39, 95% CI: 1.26-3.92) was associated with neurologic deficits at discharge. At one-month post-discharge, seizure duration (AOR=8.62, 95% CI: 1.03-71.79) was associated with neurologic deficits.

Conclusion: Neurologic deficits occur in children with other forms of severe malaria, that is, severe malaria anemia, respiratory distress, malaria with seizures and prostration. Factors associated with neurologic deficits after severe malaria in children differ between those with cerebral malaria and those with other forms of severe malaria. Given this difference, it is important to consider treatment and care specific to these different forms of severe malaria in an effort to control and prevent neurologic deficits among children after severe malaria.

Effectiveness of Tackle your Tics, a brief, intensive group-based exposure therapy programme for children with tic disorders

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Introduction: Behavioural treatment is the first-line treatment for tic disorders, but tic reduction and treatment availability remain relatively low. Patient associations emphasise the need for treatments that additionally improve children's tic-related coping skills and quality of life.

Methods: Results will be presented of a randomised controlled trial (N=106, 2020-2022) studying the efficacy of Tackle your Tics, a four-day intensive group treatment for youth (9-17 years) with Tourette syndrome or chronic motor or vocal tic disorder. Tackle Your Tics offers exposure and response prevention treatment and supporting components, such as coping strategies workshops by experts-by-experience and active parent involvement. Assessments were performed pre- and posttreatment and at 3 and 6 months follow-up, to study the effects on tic severity (as measured with the Yale Global Tic Severity Scale, our primary outcome measure), quality of life, tic-related cognitions, emotional/behavioural functioning, family functioning and treatment satisfaction.
Results: Outcomes directly post-treatment improved in both the treatment group (n=52) and waiting list (n=54), but showed no superior effect of Tackle your Tics compared to the waiting list. Importantly, on longer term this brief four-day group treatment was effective in improving tic-related impairment, quality of life and emotional/behavioural functioning. Moreover, at 6 months after the start of the brief treatment, 39% of the treated children were rated as responders in reduced tic severity.

Conclusion: Tackle your Tics is no ‘quick fix’ to reduce tics, but effective on longer term, in improving the quality of life, tic-related impairment and emotional or behavioural problems in daily life. Therefore, it can be considered a worthwhile innovative format for treating children and adolescents with tic disorders.

Modulate glycosylation to reduce the immunogenicity of Factor VIII in the treatment of Haemophilia A

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Introduction: Researching innovative strategies to tolerate biologicals is an urgent need in the field of pharmaceutical biotechnology. Immunogenicity is the major challenge in the treatment of Haemophilia A, where neutralizing antibodies against Factor VIII (FVIII) make the therapy inefficient for 30% of the patients. Therefore, we are developing glycan based strategies to target FVIII to an inhibitory axis on dendritic cells. Specifically, sialic acid plays a major role in the maintenance of peripheral tolerance. It signals immune suppression through ITIM – carrying receptors (Siglecs) expressed on a variety of immune subsets.

Methods: By using microfluidic, we assembled polylactic/glycolic-acid nanoparticles (NP) encapsulating FVIII immunodominant peptides and displaying sialic acid. Size and molar concentration were measured by dynamic light scattering techniques. Binding to plant lectins and Siglecs was assessed by ELISA, while uptake in monocyte-derived dendritic cells was studied by flow cytometry. Cytokines in the medium of stimulated moDCs and moDC/CD4+Tcells co-cultures were assessed by ELISA. Expression, maturation markers and the glycosylation profiles of the cell lines were measured by flow cytometry. A HEK293T cell line was genetically modified by CRISPR/Cas9 knock-out of GCNT1. The C1 domain of FVIII was produced by transient transfection with PEI and purified by nickel chromatography, exploiting the HisTag. Statistical analyses were performed with GraphPad Prism 9.5.

Results: On the one hand, in a competitive ELISA we confirmed the presence of sialic acid on the surface of the NPs and its binding to Siglec-9. We confirmed that the NPs are efficiently captured by moDCs and are able to modulate cytokine secretion (IL6, IL10, TNFα, IL12p70). We co-cultured moDCs loaded with the NPs with a CD4 Tcell clone isolated from an haemophilic patient to demonstrate that the antigen is correctly processed and presented on MHC-II. On the other hand, we generated a HEK293T GCNT1 knock-out (KO) cell line that overexpresses the ligand for Siglec-7. Next, we produced the C1 domain of FVIII and set up a Siglec7-biotin ELISA to assess its glycosylation profile.

Conclusion: To summarize, we successfully established two approaches that make use of glycans to deliver an immunogenic biological (FVIII) to the inhibitory receptors Siglec-7 and Siglec-9 on moDCs. This resulted in a lower inflammatory response compared to the unmodified

protein, proving promising as a novel strategy to tolerate FVIII and ameliorate the treatment of Haemophilia A.

Antenatal Cardiotocography in Primary Midwife-led Care: Women's Satisfaction

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Introduction: In the Netherlands, antenatal cardiotocography (aCTG), used to assess fetal well-being, is performed in obstetrician-led care. To improve continuity of care, an innovation project was designed wherein primary care midwives perform aCTGs for specific indications. The aim of this study was to examine the satisfaction and experiences of pregnant women who received an aCTG in primary midwife-led care and to explore which factors were associated with high satisfaction.

Methods: Data were collected through a self-administered questionnaire based on the Consumer Quality Index. The primary outcome was general satisfaction on a 10-point scale, with a score above nine indicating participants were "highly satisfied".

Results: In total, 1227 women were included in the analysis. The study showed a mean general satisfaction score of 9.2. Most women were highly satisfied with receiving an aCTG in primary midwife-led care (77.4%). On the Consumer Quality Index, the mean satisfaction level varied from 3.98 (SD± 0.11) for the subscale "client satisfaction" to 3.87 (SD± 0.32) for the subscale "information provision" on a 4-point scale. Women at between 33 and 36 weeks gestation were more likely to be highly satisfied (adjusted OR (aOR)=3.35). Compared to a completely comfortable position during the aCTG, a mostly comfortable or somewhat comfortable level had decreased odds of being associated with a ranking of highly satisfied (aOR 0.24 and 0.19, respectively).

Conclusion: This study shows that pregnant women are satisfied with having an aCTG in midwife-led care. Providing aCTG in midwife-led care can increase access to continuity of care.

Environmental Impact Assessment of Reusable and Disposable Surgical Head Covers

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Importance: Health care delivery is responsible for significant global greenhouse gas emissions, a large part of these emissions originating from the supply chain and waste of surgical equipment, including surgical attire. In order to guide the transition towards sustainable healthcare, appraisal of the environmental impact of reusable and disposable surgical equipment is needed.

Objective: The objective of this study was to compare the environmental impact of reusable polyester surgical head covers with single-use disposable viscose and polypropylene (PP) surgical head covers.

Design: We performed life cycle assessment (LCA), the standard method to quantify the environmental impact of a product within all stages of the life cycle in accordance with ISO 14040:2006 and ISO 14044:2006 guidelines. The functional unit was defined as an annual supply of head covers.

Setting: The study was performed at the Amsterdam University Medical Centres (AUMC, Amsterdam, The Netherlands), a large tertiary hospital using 100,000 head covers annually.

Main Outcomes and Measures: The primary outcome measure was the carbon footprint expressed in carbon dioxide equivalents (CO2-eq). We included 17 environmental impact categories as secondary outcomes, including ozone depletion, fine particulate matter and water ecotoxicity.

Results: Comparative analysis showed that reusable head covers have a 61% smaller carbon footprint than disposable viscose and a 56% smaller footprint than disposable PP head covers. For 16 out of 17 secondary outcomes, reusable head covers had a smaller environmental impact than both types of disposable head covers. Replacing 100,000 disposable head covers with 1000 reusable head covers in a university teaching hospital saves 933 to 1173 kg CO2-eq each year, and reduces waste disposal by 94%.

Conclusions and Relevance: This LCA shows that reusable head covers have a significantly lower environmental impact than disposable viscose and disposable polypropylene head covers, with no difference in clinical utility. These results can provide guidance for clinicians and policy makers to change best practices by taking environmental impact into account, driving positive change in the healthcare sector and, consequently, contributing to the mitigation of climate change and its adverse health effects.

Data Interoperability enabling Precision Medicine in Neurodevelopmental Disorders

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Introduction: Neurodevelopmental disorders (NDDs) are complex, multifactorial conditions and to better understand the dynamic progression of NDD in an individual, we must integrate various data sources, covering clinical, genetical biological and environmental factors. However, this is hindered by fragmented medical record silos, isolated databases, and incompatible systems, making NDD progression difficult to study. Our hypothesis is that by making data interoperable, we can break translational barriers and offer an unbiased analysis of NDD complexity through data integration and visualization.

Methods: The methods include the creation of a NDD data interoperability pipeline leveraging pre-build components covering data curation, exploration, visualization, and knowledge graph overlays. The objective of this project is to demonstrate the potential of data interoperability within various contexts for NDD, such as Dutch consortia, clinical trials, EEG data, and European trials.

Results: The anticipated results involve validating the platform's ability to bridge gaps in NDD data, revealing correlations between pre-clinical and clinical parameters, and gaining insights into the natural history of NDDs. This approach can lead to more precise and tailored treatments, particularly for rare genetic NDDs.

Conclusion: In conclusion, this project strives to combat the fragmentation of NDD data by creating a robust medical data interoperability pipeline. By integrating and visualizing (pre-)clinical data sources, the project aims to enhance our understanding of NDD origin and progression. This has the potential to revolutionize NDD diagnosis and treatment and serve as a model for data interoperability in the broader context of neurodevelopmental disorders.

Connecting the Blood-Brain Barrier to Cerebral Organoids in a membrane-free hiPSCbased organ-on-chip platform

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Introduction: Effective therapies remain an unmet clinical need for numerous brain disorders. This is partly because therapeutic compounds need to cross the blood-brain barrier (BBB), a multi-cellular structure which actively limits drug transport into the brain. The search for effective drugs is further hampered by the lack of predictive validity of animal models in human biology. Hence, there is an urgent need for novel model systems that mimic the human brain complexity to test delivery and efficacy of potential therapeutic compounds for brain diseases. The advent of human induced pluripotent stem cells (hiPSCs) led to the generation of hardly accessible cell types and self-assembled cellular structures that recapitulate the organ complexity, named organoids.

Methods: We have developed a novel method to generate hiPSC-derived brain microvascular endothelial cells (iBMECs). We have applied and optimized protocols to differentiate brain pericytes (iBPCs), astrocytes (iAstros), and cerebral organoids (cOrgs), using autologous hiPSC lines. A custom-made microfluidic chip was designed and produced using proprietary technology, providing the structure for the formation of a perfusable BBB vessel in direct contact with a cOrg.

Results: iBMECs express key BBB markers, such as tight/adherens junction proteins and efflux transporters, present proper transendothelial electrical resistance (TEER) and metabolic activity, and respond to inflammatory stimuli by reducing TEER, upregulating cell adhesion molecules, and forming stress fibres. iBPCs and iAstros present the canonical markers for each cell type and are able to support the formation of a vascular network when co-cultured in 3D with iBMECs. Although Matrigel enhances the development of neural rosettes in cerebral organoids, it is expendable for their formation and long-term maintenance. The cOrg is able to grow in the chip and astrocytes outgrow from the cOrg towards the endothelised BBB compartment.

Conclusion: A novel human autologous 3D in vitro model was developed to overcome the limitations of 2D cultures and animal models. Given its properties, this model is suitable for drug delivery studies and disease modelling, with enhanced physiological relevance. Further studies will focus on validating the astrocyte-BBB connection and BBB properties

Tumor Sialylation: a Master Regulator of Anti-tumor Immunity in Colorectal Cancer

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Introduction: Abnormal levels of tumor-associated glycans correlate with increased invasion and metastasis in many types of cancer, including colorectal cancer (CRC). Sialic acids, a family of 9-carbon monosaccharides, are thought to play key roles in immunosuppression through their binding to sialic acid-binding immunoglobulin-type lectin (Siglec) receptors on immune cells. They are present at the end of carbohydrate chains on glycoproteins and glycolipids and mainly occur in α2,3- or α2,6-linkages. How sialylated glycans modulate immune responses in CRC still remains unclear.

Methods: To study this, we introduced the ST6Gal1 enzyme to upregulate α2-6 linked sialic acids on the murine CRC CT26 cell line, since overexpression of α2-6 sialylation correlates to poor survival in human CRC patients. We further modified CT26 cells also with CRISPR/Cas9 by knocking out either the CMAS gene, to remove all the sialic acids, or ST3Gal5, the enzyme that adds sialic acids specifically on glycolipids. The generated sialovariant cell lines were then injected subcutaneously in the flank of Balb/c mice and the tumor growth was monitored.

Results: Overexpression of ST6Gal1 did not alter tumor growth in vivo, indicating that α2-6 sialic acids do not hamper anti-tumor immunity. In contrast, CT26 lacking cell surface sialic acids displayed a significantly reduced tumor growth in vivo, resulting in increased survival of the mice. This was paired with an increase of antigen-specific CD8+ T cells and a reduction of NK cells. However, depletion of CD8+ T cells slightly increased the tumor growth of CMAS KO tumors, implying an additional role for other immune cell subsets in the anti-tumor immune response. When removing the sialic acids on glycolipids, there was no difference in tumor growth between the ST3Gal5 KO and the control group, demonstrating that only sialic acids on glycoproteins are crucial in tumor progression.

Conclusion: In conclusion, we successfully generated different sialylated glycovariants in a murine CRC cell line and demonstrated that removal of sialic acids enhances anti-tumor immunity. This highlights a key role for sialylated glycans on proteins in controlling immunosuppression in colorectal cancer.

Eliciting preferences of persons with dementia and informal caregivers aging in place in the Netherlands: a discrete choice experiment protocol

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Introduction: Ageing in place (AIP) for persons with dementia is encouraged by European governments and societies. Healthcare packages may need reassessment to account for the preferences of care funders, patients and informal caregivers. By providing insight into people's preferences, discrete choice experiments (DCEs) can help develop consensus between stakeholders. This protocol paper outlines the development of a Dutch national study to co-create a healthcare package design methodology built on DCEs that is person-centered and helps support informal caregivers and persons with dementia to AIP. A subpopulation analysis of persons with dementia with a migration background is planned due to their high risk for dementia and under-representation in research and care.

Methods: The DCE is designed to understand how persons with dementia and informal caregivers choose between different healthcare packages. Qualitative methods are used to identify and prioritise important care components for persons with dementia to AIP. This will provide a list of care components that will be included in the DCE, to quantify the care needs and preferences of persons with dementia and informal caregivers. The DCE will identify individual and joint preferences to AIP. The relative importance of each attribute will be calculated. The DCE data will be analysed with the use of a random parameters logit model.

Results: -

Conclusion: Ethics approval was waived by the Amsterdam University Medical Center (W23_112 #23.137). A study summary will be available on the websites of Alzheimer Nederland, Pharos and Amsterdam Public Health institute. Results are expected to be presented at (inter)national conferences, peer-reviewed papers will be submitted, and a dissemination meeting will be held to bring stakeholders together. The study results will help improve healthcare package design for all stakeholders.

Long-term PTSD prevalence and adverse psychological, function and economic consequences: a 12-15 year follow-up in adults with traumatic injury

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Introduction: There is a large number of longitudinal studies investigating the prevalence and impact of PTSD following civilian trauma. However, most of these studies limit their final assessments to a year following trauma and at most within a decade post-trauma. The goal of the current follow-up study was to examine the long-term prevalence of PTSD, along with adverse psychological, functional and economic outcomes 12-15 years post-trauma in Dutch adults with traumatic injury. We also examined whether there were gender-specific effects present.

Methods: *N* = 194 Dutch adults (34% women) admitted to the emergency department with traumatic injury (Mouthaan et al., 2014; van Zuiden et al., 2022) completed a follow-up assessment at 14.3 years post-trauma (SD = 1, range: 12-15). Participants completed assessments of clinician-rated PTSD symptom severity (CAPS-5), and self-report questionnaires on psychological (anxiety, depression, alcohol use), functional (well-being, general and health-related quality of life) and economic (productivity loss at work, health care consumption) outcomes.

Results: 4.9% of participants (5.8% for men; 3.1% for women) fulfilled DSM-5 diagnostic criteria for PTSD related to their traumatic injury of 12-15 years ago. PTSD symptom severity was associated with more depressive and anxiety symptoms; less well-being and lower (health-related) quality of life; and higher health care consumption. There were no significant interaction effects of PTSD symptom severity and gender on these outcomes.

Conclusion: Almost 5% of Dutch adults fulfilled PTSD diagnostic criteria related to their traumatic injury of over a decade ago. Higher PTSD symptom severity was linked to more adverse psychological, functional and economic outcomes. These findings highlights the importance of early detection and treatment of PTSD in order to reduce its long-term negative impact on individuals and larger society.

Enriching the Evidence Base of Co-creation in Public Health with Methodological Principles of Critical Realism

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Introduction: With the popularity of co-creation approaches in public health research, there is a need for a framework guiding how we can optimally apply co-creation based on meta-theoretical principles. Critical realism seems a good candidate for providing such meta-theoretical principles. Therefore our objectives are to clarify and elaborate on the critical realism methodological principles and then apply such principles to a public health case study.

Methods: We conducted a Google Scholar search for critical realism methodological principles, identifying Wynn and Williams' unique set of five principles as the only one of its kind. Utilizing the literature mapping software "Litmaps", we searched for additional relevant papers, complemented by reference checking. Next, we clarified and elaborated on the five principles through a scrutiny of the included papers. Finally, we explored the value of the principles by applying them to a case study.

Results: We included twenty-two relevant papers to explore the five critical realism methodological principles. These principles can offer added value for co-creation research in public health, for instance, due to the emphasis on plurality and a longer-term empirical research design. By using these principles, research can be made more meaningful in its deeper exploration into alternative explanations which may not have been previously considered, resulting in potentially more effective public health interventions.

Conclusion: The critical realism methodological principles seem well-suited as a meta-theoretical framework for evidence-based co-creation. Hence, we posit that incorporating these principles into co-creation research within public health has the potential to enhance its application and strengthen empirical foundations.

The Ten-Year Association between Change in Speech-in-Noise Recognition and Falls due to Balance Problems: A Longitudinal Cohort Study

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Introduction: Hearing and balance issues increase risk of falls, particularly among older adults, causing injury and disability. Both hearing loss and fall-related injuries accelerate around age 50. While crosssectional studies have found significant associations between hearing impairment and falls, longitudinal studies are limited and show mixed results. The role of dizziness on this association is also unclear, and whether hearing aid (HA) use can reduce fall risk remains uncertain. The present study examines the 10-year longitudinal relationship between hearing ability and falls (Research Question (RQ) 1), the impact of dizziness (RQ2), and the effect of HA use on fall risk (RQ3). Further understanding these associations can augment the comprehensiveness of fall prevention programs and help improve the quality of life of adults at risk for falls.

Methods: Data from the Netherlands Longitudinal Study on Hearing between 2006–2022 was collected via an online survey and hearing test every five years. The hearing test measures auditory speech recognition in noise by determining a participant's signal-to-noise ratio (SNR) and resulting speech reception threshold in noise (SRTn) score. Available data was divided into two 10-year intervals: T0 (baseline) to T2 (10-year follow-up), and T1 (5-years) to T3 (15-years). Eligible participants for all RQs were ≥40 years at baseline, without congenital hearing loss, and non-cochlear implant users (n=592). For RQ3, eligibility required an SRTn ≥-5.5 dB SNR (n=422). Analyses included survey variables on demographics, hearing, dizziness, recurrent/incident falls, chronic conditions, and psychosocial health. Logistic regressions using General Estimating Equations in SPSS assessed all RQs.

Results: Poor SRTn at baseline was significantly associated with a higher odds of falling recurrently (≥2 falls) (OR:2.91, 95% CI [1.21, 7.02]). Among individuals with obesity, those with poor baseline SRTn had a higher odds of falling incidentally (1 fall) 10 years later (OR:14.7, 95% CI [2.12, 103]). A 10-year worsening in SRTn was significantly associated with higher odds

of falling recurrently (OR:2.20, 95% CI [1.03, 4.71]) but not incidentally. No significant interaction was found between dizziness and 10-year change in SRTn. HA use (no use or <2 years of use vs. ≥2 years of use) was not significantly associated with incident nor recurrent falls. Although sex significantly modified this association, the effect of HA use on falls was not statistically significant among men nor women.

Conclusion: A longitudinal association between the deterioration in SRTn and recurrent falls after 10 years was confirmed in this study. This result stresses the importance of identifying declines in hearing earlier and justifies including hearing assessments within current fall risk prevention programs. Further prospective research that collects additional details on the different types of dizziness could provide better insight into the role of vestibular symptoms on the relationship between hearing loss and falls. Mixed results of HA use on fall risk warrant further investigation into the temporality of this association and possible differences between men and women. This comprehensive approach holds the potential to inform health professionals and enable more personalized care to at-risk patients, strengthening overall fall prevention strategies.

The association of tobacco smoking and metabolite levels in the anterior cingulate cortex of first-episode psychosis patients: A 1H-MRS study

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Introduction: Tobacco smoking amongst patients with schizophrenia is 2-3 times higher compared to the general population, and it is associated with worse clinical outcomes such as relapse and readmission. Remarkably, few neuroimaging studies have explored the effect of tobacco on the brain in psychosis. In particular limited studies have assessed the effect of tobacco on the levels of different neurometabolites, whilst neurometabolites such as glutamate and choline have been implicated in psychosis and tobacco smoking separately. The current study therefore aimed to investigate the influence of chronic smoking on neurometabolite levels in the anterior cingulate cortex (ACC) of first-episode psychosis (FEP) patients and controls, as shared ACC abnormalities may contribute to the development or maintenance of smoking in schizophrenia.

Methods: 59 FEP patients and 36 controls were included in the study. Proton magnetic resonance spectroscopy (1H-MRS) was acquired using a Point Resolved Spectroscopy (PRESS) pulse sequence on a 3 Tesla Philips MRI scanner. Associations between smoking status (i.e. smoker yes/no) or average number of cigarettes smoked per day in the past year and Glx (glutamate + glutamine, as proxy for glutamate) and total choline (tCh) levels were assessed at baseline in both groups separately using analysis of covariance (ANCOVA) and linear regressions. For 37 patients, six months follow-up data were acquired to assess longitudinal changes in neurometabolite levels using linear mixed models. We controlled for the use of cannabis in all analyses, considering the strong correlation of cannabis use with tobacco smoking.

Results: No significant differences in Glx levels in the ACC were found between smoking (n=28) and non-smoking (n=31) FEP patients at baseline nor in multi-cross-sectional analyses. Smoking patients showed lower tCh levels compared to non-smoking patients at baseline and in multi-cross-sectional analysis, although not surviving multiple comparisons

correction(pFDR=0.06 and pFDR=0.08 respectively). Negative associations were observed between cigarettes smoked per day, and ACC Glx (pFDR=0.03) and tCh levels (pFDR=0.02) in controls but not in FEP patients.

Conclusion: We found that tobacco smoking is associated with differences in neurometabolite levels in controls but not in FEP patients. The observed alterations in tCh levels, suggesting a reduction in cellular proliferation processes, might result from exposure to the neurotoxic effects of smoking. Additionally, schizophrenia patients already appear to have an aberrant glutamate system. It is hypothesized that schizophrenia-related glutamate signalling abnormalities stem from dysregulated nicotine acetylcholine receptor (nAChR) expression. Nicotine, the primary substance in tobacco, activates these nAChRs. Thus, differences between patients and controls regarding Glx might be explained by pre-existing disease-related glutamate deficits or alterations at the nicotine acetylcholine receptor level, resulting in variations in tobacco-related associations with neurometabolites. Future research could investigate this hypothesis by focusing on nAChR (dys)functioning using PET imaging.

A novel 3D heart on chip system to investigate vasculature-cardiomyocyte interaction in ischemia reperfusion injury

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Acute myocardial infarction (AMI) is a leading cause of morbidity and mortality. Where reperfusion is the current standard, it often results in myocardial ischemia reperfusion injury (MI-R). MI-R is a multifactorial process with an intricate interplay between multiple cell types, where the primary insult in the coronary vessels results in cardiomyocyte dysfunction and death. As currently, no clinically effective cardioprotective agents has not been found to date, it is important to increase our knowledge on how these tissues communicate.

Our aim is to further investigate the molecular mechanisms of MI-R using a newly developed a 3D heart-on chip model. This model comprises of a vascular channel consisting of human coronary endothelial cells (CAECs) with and a layer of human cardiomyocytes (iPSC-CMs) on top. Both channels are separated by a porous membrane, allowing direct cell-cell communication. It can be mechanistically stretched, individually subjected to flow and secretory output can be collected.

Using live-cell imaging, we were able to show a confluent vascular channel as visualized by F-actin, VE-cadherin and Hoechst, and Troponin-T and Hoechst in our matured CM channel. Our model offers the possibility to study EC-CM interactions under (patho)physiological conditions, as no decrease in cell viability (LDH release) decrease in cellular stress (BNP-1 for CM and IL-8 for EC) was observed (> 7days). Functionally, decreased CM beating frequency was observed in this 3D CM-EC chip-model, indicating increased CM maturation due to the protective endothelial effects under quiescent conditions. Currently, this model is used to study the molecular underpinnings of MI-R.

Enhancing Cancer Detection: Machine Learning Insights from Fragmentation Patterns of Plasma Cell-free DNA

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Introduction: Cell-free DNA (cfDNA) consists of DNA fragments released in the bloodstream following cell death. cfDNA analysis presents a non-invasive alternative to conventional invasive tissue biopsy in oncology, particularly for cancer detection. The encoded patterns within cfDNA fragments serve as a valuable resource, providing insights into the genomic make-up of tumors and their distinctive chromatin structure. These encoded patterns can be effectively harnessed through machine learning methods. However, despite the potential for early cancer detection and intervention, existing methods suffer from limited sensitivity and flexibility. We aim to enhance cancer detection sensitivity through machine learning classifiers by gaining deeper insights into the characteristics of cfDNA molecules.

Methods: Our study focuses on developing machine learning classifiers for cancer diagnosis using cfDNA fragment-end patterns. These patterns, derived from the orientation of cfDNA fragment-ends relative to the reference genome, serve as distinctive genomic signatures, distinguishing cancer from control samples. Extracted from open-chromatin regions, cancer and control sample patterns are labeled as 1 and 0, respectively. Combining these patterns and their corresponding labels within identical regions across both cancer and control samples, we construct a dataset. This dataset enables training classification models to predict cancer status of specific genomic regions based on observed fragment-end patterns. The trained model evaluates an unknown sample by generating probabilities for each pattern's likelihood of indicating cancer. Ultimately, sample-level predictions are obtained by applying a threshold to the mean probabilities.

Results: In our initial analysis, we examined a cohort of 28 B-cell lymphoma and seven healthy control samples employing logistic regression, achieving 100% specificity and 85.7% sensitivity. To broaden the scope of our research, we intend to increase both the scale and diversity of our cohort, incorporating a broader range of cancer types, including brain cancers. Additionally, we will explore the utilization of alternative machine learning methods, such as deep learning, to expedite and improve cancer classification.

Conclusion: Our preliminary results underscore the potential of our method for enhancing cancer diagnosis and uncovering cancer-specific DNA regions, which may indicate tumor-derived signals. The development of a robust diagnostic classifier based on cfDNA analysis could have significant implications for early detection of cancer and intervention, leading to improved patient outcomes and reduced healthcare costs.

The role of sex hormones in severe mental illness: a genetic exploration

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Introduction: There are prominent sex differences in severe mental illness (SMI) which have been reported across domains such as prevalence, symptomatology, and treatment response[1]. Increasing evidence suggests that sex hormones might play a pivotal role in these differences[2]. While the existing literature has provided relevant correlational insights into the relationship between hormones and SMI, causal questions remain unanswered. Elucidation of the role of hormones in SMI is crucial to tailor gender-specific prevention and treatment. Here, we aim to investigate the relation of sex hormones with SMI using complementary genetic methods that can estimate genetic overlap and test whether associations observed in previous observational research reflect causal effects.

Methods: In this study, the main focus was the relationship of oestrogen and testosterone with SMI (depressive disorder, bipolar disorder and schizophrenia), as these are the sex hormones most strongly associated with SMI. The analyses were performed stratified by sex, where possible, and also in both sexes together, using publicly available summary-level data from genome-wide association studies (GWASs).

First, to estimate genetic overlap, we computed genetic correlations (using linkage disequilibrium score regression) of oestrogen, testosterone, and related reproductive traits with depressive disorder, bipolar disorder, and schizophrenia. Second, we performed Mendelian randomization (MR) to examine the causal effects of oestrogen and testosterone on SMI, as well as the reverse. To ensure the reliability of the results, a broad range of sensitivity methods was applied.

Results: The results showed a widespread pattern of both positive and negative genetic correlations of sex hormone traits with depressive disorder and schizophrenia. In total 20 significant genetic correlations were observed, of these 14 remained significant after FDR correction. We found that the genetic correlations of depressive disorder differed depending on menopause status, so that a negative correlation between depressive disorder and oestrogen levels occurred only post-menopause. Evidence for causal effects was largely lacking, however there was weak but consistent evidence for a positive effect of testosterone in schizophrenia. We observed that susceptibility to higher testosterone levels increased the risk of schizophrenia

in men. In the other direction, susceptibility to schizophrenia increased sex hormone levels in both sexes.

Conclusion: This study presents evidence for the involvement of the endocrine system in the susceptibility to severe mental illness, offering new insights into the complex aetiology of depressive disorder, bipolar disorder and schizophrenia. Our findings emphasize the need to integrate the influence of sex hormones into the multifactorial model of mental illness aetiology. However, the complexity of this model, and the limited scope of this study prevented us from dissecting the precise role of each sex hormone and, therefore, pinpoint prevention targets or formulate clinical implications. Future research should build upon these findings using larger and more precisely phenotyped samples, and focus on the identification of individuals particularly vulnerable to hormonal disturbances. These steps will facilitate the development of tailored treatment strategies.

References

1. Gobinath, A. R., Choleris, E. & Galea, L. A. Sex, hormones, and genotype interact to influence psychiatric disease, treatment, and behavioral research. J Neurosci Res 95, 50-64 (2017). https://doi.org/10.1002/jnr.23872
2. Gogos, A., Ney, L. J., Seymour, N., Van Rheenen, T. E. & Felmingham, K. L. Sex differences in schizophrenia, bipolar disorder, and post-traumatic stress disorder: Are gonadal hormones the link? Br J Pharmacol 176, 4119-4135 (2019). https://doi.org/10.1111/bph.14584

ADJUST: A stiffness adjustable ankle-foot-orthosis for rapid human-in-the-loop orthosis selection

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Introduction: Spring-like ankle-foot-orthoses (AFOs) can augment walking for individuals with calf muscle weakness by improving the gait pattern and reducing energy cost of walking[1]. To maximize the effectiveness of spring-like AFOs regarding reduction in walking energy cost, the AFO stiffness, e.g. how easy the AFO bends, needs to be individually optimized[2]. However, implementation of individual AFO stiffness optimization is hindered by time-consuming procedures due to the necessity to manually change the stiffness. Therefore our goal is to develop an untethered wearable AFO capable of changing stiffness during walking, enabling fast and feasible methods to introduce individual AFO optimization within the clinic.

Methods: An integrated design approach was used wherein perspectives of patients, orthotists, rehabilitation specialists and technicians were involved to develop a stiffness-adjustable AFO. Adjusting the AFO's stiffness was achieved by changing the resistance around the ankle joint with leaf springs and a mechanical transmission. To enable clinical use, a custom calf case was developed that is semi-flexible and fits a large range of patients. Furthermore, the AFO shoe can be changed to accommodate different sizes of feet. AFO stiffness range was measured using the BRUCE stiffness tester[3].

Results: A variable stiffness AFO, the ADJUST-AFO, was successfully build (weight 2 kg, without shoes) and can be controlled wirelessly with a laptop over WiFi. Through a variable-stiffness-mechanism located dorsally on the AFO, the stiffness can be changed within a clinical relevant range of 1-7 Nm/deg. The stiffness can be changed during the swing phase, when the load on the system is minimal. Bench testing and walk tests with five healthy participants showed good functioning and safety of the device. Validation of the optimization procedure with the ADJUST-AFO in patients is underway.

Conclusion: The ADJUST AFO was successfully developed and fulfilled its main function, which was wirelessly altering the stiffness during gait in a clinical relevant range. Therefore it holds the promise to significantly improve the stiffness optimization process, although improvement regarding the weight are warranted.

[1]Brehm MA, Nollet F, Harlaar J. Energy demands of walking in persons with postpoliomyelitis syndrome: relationship with muscle strength and reproducibility. Arch Phys Med Rehabil. 2006 Jan;87(1):136-40. doi: 10.1016/j.apmr.2005.08.123. PMID: 16401452.

[2] N.F.J. Waterval et al., "Stiffness-Optimized Ankle-Foot Orthoses Improve Walking Energy Cost Compared to Conventional Orthoses in Neuromuscular Disorders: A Prospective Uncontrolled Intervention Study," in IEEE Transactions on Neural Systems and Rehabilitation Engineering, vol. 28, no. 10, pp. 2296-2304, Oct. 2020, doi: 10.1109/TNSRE.2020.3018786.
[3] D.J.J. Bregman, A. Rozumalski, D. Koops, V. de Groot, M. Schwartz, J. Harlaar, A new method for evaluating ankle foot orthosis characteristics: BRUCE, Gait & Posture, Volume 30, Issue 2, 2009, Pages 144-149, ISSN 0966-6362, https://doi.org/10.1016/j.gaitpost.2009.05.012

Understanding B-cell repertoire sequencing data through a multiscale computational model of the germinal center

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Introduction: The adaptive immune response provides an important line of defense against antigens present in our body. It involves B-cells, memory B-cells (MBCs), and plasma cells (PCs) and is responsible for immunological memory and antibody response. The germinal center plays an important role in the adaptive immune response. Sequencing of B-cell immune receptor repertoires helps us to understand said response. However, it only provides information about the clonotypes and their frequencies. This is useful to identify dominant clones of high frequency but, generally, additional experiments are required to further characterize the clones by measuring, for example, their affinity or function. Since such experiments are not always performed, are time consuming, and/or expensive we aimed to develop a computational model to gain general insight in B-cell repertoires.

Methods: We present a multiscale model of the germinal center (GC) to establish the relationship between clonal abundance and affinity, and to establish the extent that PCs with high B-cell receptor (BcR) mRNA content may disturb the identification of dominant clones. Since we simulate B-cell repertoires generated from a single GC we also compared the extent that these repertoires deviate from experimental repertoires established from blood or tissue.

Results: Our simulations show that there is a limited correlation between clonal abundance and affinity and, in addition, there is large affinity variability among subclones that descended from the unmutated common ancestor. Furthermore, we show that the presence of PCs does not significantly affect the number of dominant clones. Finally, as expected, immune repertoires generated from our single GC model deviate in several aspects from experimental repertoires. At the same time, these simulations show some general characteristics of BcR repertoires that might be important to considered during data interpretation and the design of follow-up experiments.

Conclusion: We consider our model as a first step towards the simulation of repertoires through the simulation of a GC reaction. Insights from these simulations -limited correlation between (sub)clonal abundance and affinity, large affinity variability among same-ancestor subclones and no effect of PC presence in the number and identification of dominant clones in single GCs by BcR-RNA sequencing- guide data interpretation and the design of follow-up experiments to facilitate the development of strategies to select (sub)clones for further characterization or development to therapeutic antibodies, vaccines and a better understanding of (auto)immune responses.

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Myeloperoxidase causes arrhythmogenic remodeling in cardiac slices

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Introduction: In atrial fibrillation (AF) and postischemic ventricular arrhythmia, neutrophils excessively release myeloperoxidase (MPO) into cardiac tissue, increasing its susceptibility to arrhythmia. Increased MPO precedes AF onset and predicts treatment failure. We hypothesized that MPO contributes to structural and electrophysiological remodeling.

Methods: Viable 380 µm-thick slices were generated using a vibrating microtome from healthy pig ventricles (with a homogenous architecture) and human left atrial appendages from AF patients undergoing thoracoscopic ablation (as a disease-specific model). The slices were then incubated with 1 µg/mL MPO or its vehicle (milliQ, control) for 24 hours (ventricular slices: n=15/group, n=2 pigs; atrial slices: n=18 MPO, n=14 control, n=6 patients). The slices were then electrically mapped using an 8x8 multielectrode array, and action potentials (APs) were recorded using a microelectrode during pacing. Staining and qPCR were used to assess fibrosis and extracellular matrix (ECM) gene expression, respectively.

Results: Compared to controls, MPO-treated ventricular slices exhibited increased tissue anisotropy and slower longitudinal and transverse conductions (26.2 [5.6] vs. 34.3 [6.3] cm/s, p=0.006; 11 [2.2] vs. 16 [3.2] cm/s, p=3*10^-4, respectively). Conduction was more heterogeneous in MPO-treated ventricular slices, containing more collagen and fibroblasts than control. Microelectrode recordings revealed more depolarized resting membranes in ventricular cardiomyocytes exposed to MPO vs. control (-66.6 [9.8] vs. -77.7 [6.1] mV, p=0.01). Relative to controls, MPO-treated atrial slices also exhibited increased tissue anisotropy, with slower longitudinal and transverse conductions (18.9 [6.2] vs. 35.7 [10.3] cm/s, p=0.004; 9 [3.3] vs. 21.7 [3.5] cm/s, p=7*10^-7, respectively) and more heterogenous collagen comprised of long fibrotic strands. MPO depolarized atrial cardiomyocyte resting membranes compared to controls (-47.7 [2.8] vs. -71.7 [8.7] mV, p=0.0002).

Conclusion: MPO causes functional and structural remodeling in healthy or diseased atrial and ventricular myocardium. This leads to arrhythmogenic heterogeneous conduction slowing.

These findings suggest that antagonizing MPO is a potential clinical target for preventing or managing reentrant arrhythmias.

Evaluation of novel mid-region and C-terminal-specific CSF β-synuclein ELISAs for Alzheimer's disease diagnosis

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Background: Elevated cerebrospinal fluid (CSF) β-synuclein levels have emerged as a potential specific biomarker for Alzheimer's disease (AD) using an N-terminal-targeted ELISA [1, 2]. The N-terminus is more conserved across synuclein variants, potentially leading to assay cross-reactivity [3]. This suggests potential improved diagnostic efficacy of assays targeting the more β-synuclein-specific mid and C-terminal regions.

Methods: We developed two novel CSF ELISAs targeting mid and C-terminal β-synuclein, and set-up the N-terminal assay previously described [2]. We validated the precision, sensitivity and selectivity of the ELISAs. Next, we validated the diagnostic performance of the ELISAs by analyzing 44 routine CSF samples (22 AD and 22 control with CSF Aβ42 and p-tau181 profiles.

Results: All three assays detected β-synuclein in all clinical samples with mean intra-assay precisions below 10%CV (Figure 1). The β-specific-synuclein capture-antibody used for both the mid-region and C-terminal assays did not react with recombinant α-synuclein. In contrast, the capture antibody of the N-terminus assay did show reactivity with α-synuclein protein. CSF β-synuclein levels measured with all three ELISAs significantly discriminated AD from controls (Figure 2, all: $p<0.05$). Fold-change differences in median values were 2.3 (N-terminus), 1.6 (mid-region), and 2.2 (C-terminus). Age and sex-corrected ROC analyses showed comparable diagnostic performance across the assays (AUCs: N-terminus: 0.75 (95%CI: 0.60-0.90), mid-region: 0.69 (95%CI: 0.51-0.86), C-terminus: 0.81 (95%CI: 0.67-0.95).

Conclusion: The novel β-synuclein-specific ELISAs have robust analytical performance and cover almost the entire β-synuclein molecule, suggesting that truncations or post-translational modifications do not affect clinical performance. These assays can thus be used to reliably distinguish AD from control CSF samples. Further validation of these ELISAs across various clinical AD stages is underway to assess their full diagnostic and prognostic utility.

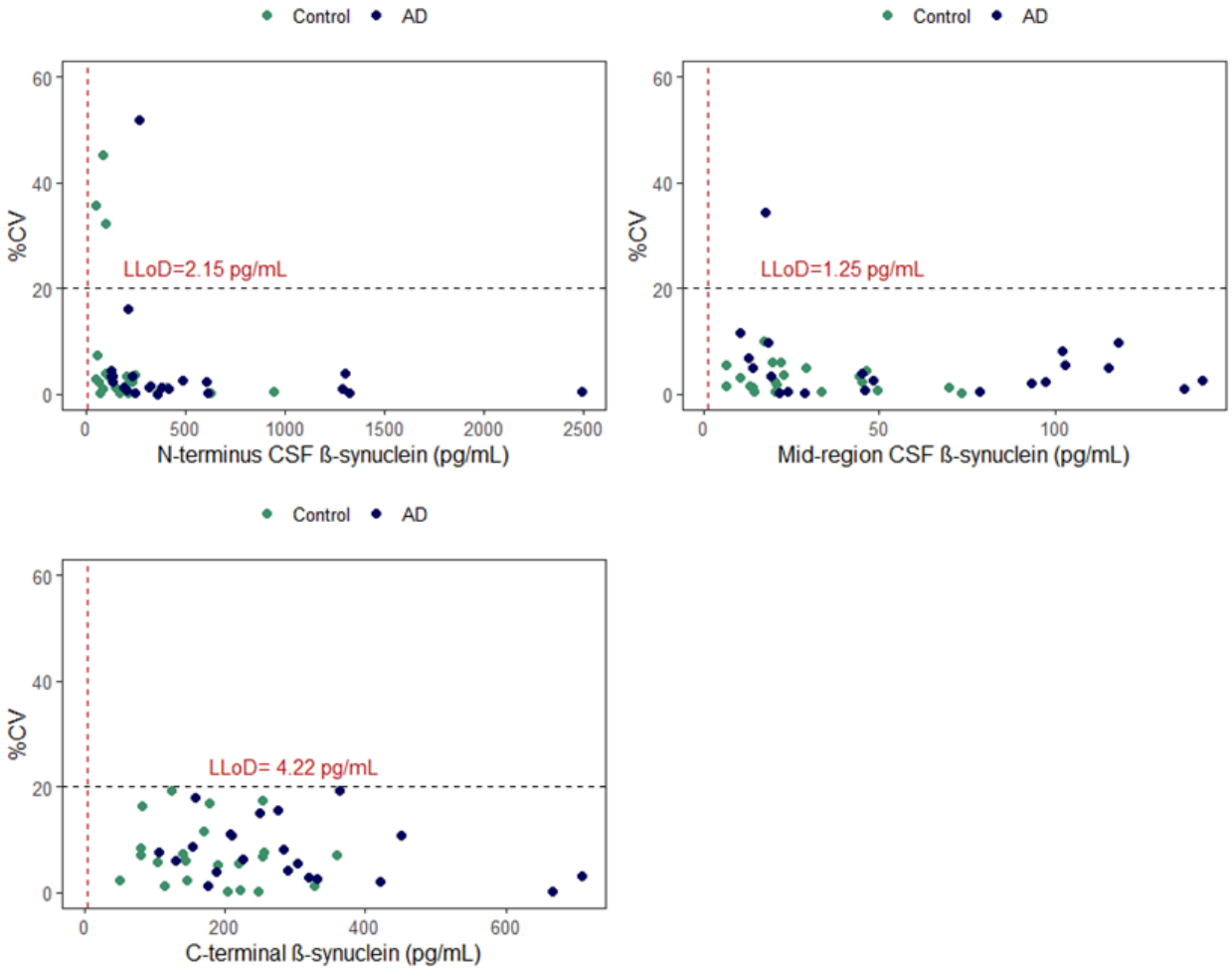

Figure 1. Precision plots of the novel CSF β-synuclein ELISAs. The plots demonstrate β-synuclein concentrations for each assay on the x-axis against the variation of duplicate measurements as percentage coefficient of variation (%CV). The clinical samples were color-coded per group as AD (mean age 69.8±7.1 years: 55% F) and control (68.8 ±7.4 years: 20%F). The vertical dashed lines represent the lowest limit of detection (LLoD) (i.e., mean of 16 blanks*10x standard deviation of the blanks), and the horizontal dashed lines represent a CV of 20% for each assay.

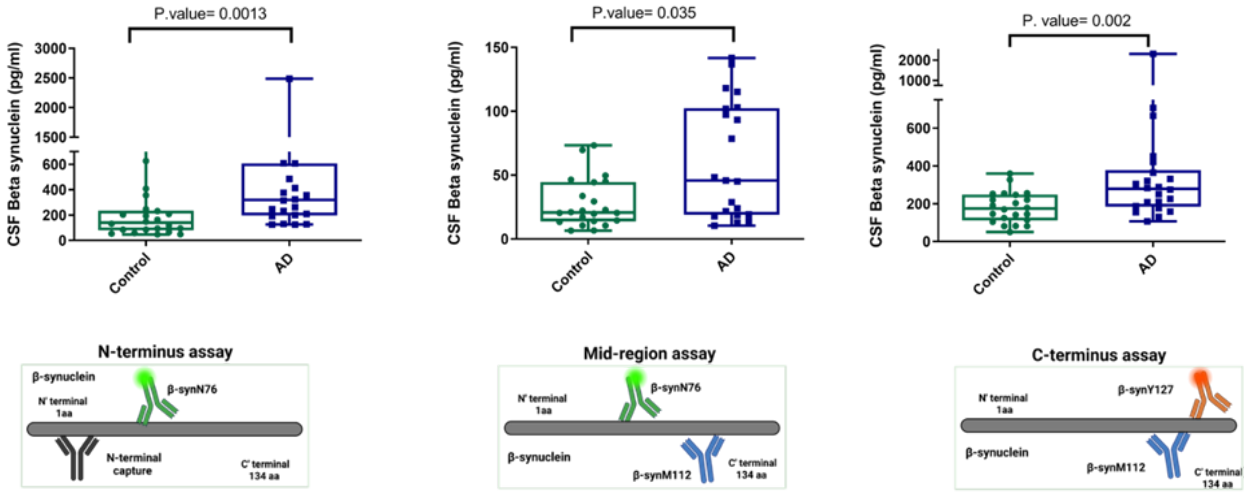

Figure 2. A) Box plots of the novel CSF β-synuclein ELISAs. A) N-terminal-β-synuclein ELISA, B) mid-β-specific-synuclein ELISA, C) C-terminal-β-specific-synuclein ELISA. Group differences were calculated using non-parametric Mann-Whitney U Tests, and results are shown with p-values within the boxplots. All assays showed significant differences between the groups with p-values of <0.05.

Reference

1. Barba, L., et al., Cerebrospinal fluid β-synuclein as a synaptic biomarker for preclinical Alzheimer's disease. J Neurol Neurosurg Psychiatry, 2023. 94(1): p. 83-86.
2. Halbgebauer, S., et al., Beta-synuclein in cerebrospinal fluid as an early diagnostic marker of Alzheimer's disease. J Neurol Neurosurg Psychiatry, 2021. 92(4): p. 349-356.
3. Oeckl, P., et al., Alpha-, Beta-, and Gamma-synuclein Quantification in Cerebrospinal Fluid by Multiple Reaction Monitoring Reveals Increased Concentrations in Alzheimer's and Creutzfeldt-Jakob Disease but No Alteration in Synucleinopathies. Mol Cell Proteomics, 2016. 15(10): p. 3126-3138.

Word Lists for Speech Audiometry: A Comparison Between Human and Synthetic Speech

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Objectives: The objectives of this study were (1) to determine whether the standard Dutch word lists for speech audiometry are equally intelligible in normal hearing listeners, (2) to compare the intelligibility of synthetic and human speech.

Design: Participants performed speech recognition tests in quiet with the original (human) word lists and synthetic word lists. The latter were created using the Google Cloud text-to-speech (TTS) system. Speech recognition functions were estimated for all human and synthetic lists.

Study sample: Twenty-four young adults with normal hearing.

Results: The variability in intelligibility among word lists was significantly higher in human speech material than in synthetic speech material, with list differences up to approximately 20% at fixed presentation levels in the former. The average speech recognition threshold (SRT) of the human speech material was 1.6 dB lower (better) than the SRT of the synthetic speech material.

Conclusions: The original Dutch word lists show large variations in intelligibility. These list effects can be greatly reduced by combining two lists per condition. Synthetic speech is a promising alternative to human speech in speech audiometry in quiet.

Maternal factors during pregnancy and pubertal timing in offspring: A systematic review of the literature

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Introduction: Although maternal exposure to endocrine-disrupting chemicals (EDCs) has been well documented, evidence on other non-genetic maternal factors during pregnancy that may contribute to pubertal timing in their offspring is fragmentarily and inconclusively addressed. Therefore, the aim of this systematic review is to gather and critically appraise all relevant literature on this topic.

Methods: The search was conducted in Medline, Embase, PsycInfo and Web of Science. Observational studies investigating maternal factors influencing puberty in offspring were included. The quality of the studies was assessed using the Newcastle-Ottawa Scale (NOS). Subsequently, a best-evidence approach was applied to summarize the findings and draw conclusions on the level of evidence. This systematic review has been registered on PROSPERO (CRD42023394102).

Results: The literature search identified 4199 articles, of which seventy-three were included in the review. The data were predominantly from high-income countries, comprising forty-three studies focusing on girls, twenty-five on boys and five on both sexes. The methodological quality of most included studies ranged from moderate to high. In both boys and girls, there is strong evidence of a positive association between maternal gestational weight gain (GWG) and an earlier pubertal timing, while no association was found with maternal substance use, thyroid dysfunction or gestational hypertension. In addition, there is insufficient evidence of an association with maternal psychological factors, smoking, diet, physical activity, pre-pregnancy weight/BMI, diabetes, menstruation related disorders and steroid medication use.

Conclusion: Our findings provide a comprehensive overview of the quality and consistency of existing evidence regarding maternal factors during pregnancy that may be associated with the pubertal timing in their offspring. This review may serve as an orientation for future research initiatives, with a particular focus on exploring these associations among male offspring and in low- and middle-income countries.

Low-dose Naltrexone (LDN) extends healthspan and lifespan through activation of the transcription factor SKN-1/NRF2 in C. elegans

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Introduction: Aging is a topic of urgency and importance, particularly as the world's aging population continues to grow. Numerous studies have been conducted to identify potential interventions that can improve health and promote longevity, however few are close to implementation. One promising approach to accelerate the implementation is drug repurposing, or using existing drugs for new indications.

Methods: Here, we selected naltrexone by repurposing existing drugs from the Library of Integrated Network-based Cellular Signatures (LINCS) with several selection criteria. In recent decades, there has been increasing attention and use of low-dose naltrexone (LDN) as an adjunct treatment modality for cancers, autoimmune diseases, chronic pain and mental health issues. Then we studied the potential benefits of low-dose naltrexone (LDN) in promoting healthy aging using Caenorhabditis elegans as a model organism.

Results: We found that a low, but not high dose of naltrexone extended both healthspan and lifespan in C. elegans worms. Further analysis revealed that LDN treatment-induced longevity was dependent on SKN-1 (NRF2 in mammals) signaling. Moreover, LDN treatment not only increased the expression of innate immune genes but also activated the oxidative stress response in worms, which could be abolished by inhibition of SKN-1/NRF2.

Conclusion: Overall, paired with LDN's low side effects profile, our study highlights the great potential of LDN to be repurposed as a geroprotector for promoting healthy aging and suggests further research in humans is warranted.

Comprehensive analysis of tumor associated macrophages in glioblastoma (GBM)

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Introduction: Glioblastoma (GBM) is the most common and extremely aggressive primary tumor of the central nervous system. Macrophages can take up around 50% of cells in the tumor microenvironment (TME) of GBM and are thought to play a role in therapy resistance by creating an immunosuppressive TME. Researchers have used the M1/M2 dichotomy to describe the pro- and anti-inflammatory states of macrophages, respectively. However, an expanding number of studies have found that this classification strategy oversimplifies the nature of macrophages as seen in patients. Therefore, a more comprehensive method to represent the continuous spectrum of cellular states of macrophages is essential. In this study, we aim to develop a bioinformatic pipeline to distinguish the different cellular states of macrophages, and explore their impact on clinical outcomes.

Methods: (max 125 words) A bioinformatic pipeline based on principal component analysis (PCA) and macrophages single-cell RNA sequencing (scRNA-seq) datasets was developed to identify the cellular states of macrophages, which addressed the high inter-tumoral heterogeneity of GBM. Image mass cytometry (IMC) and multiplex immunohistochemistry (mIHC) are used to verify the existence of the macrophage states. The ssGSEA algorithm was applied to calculate state scores for macrophages, which were further classified into “positive” and “negative” cells by fitting into a mixture model with two peaks (“mixtools” R package). To identify the clinical relevance of the macrophage states, the correlation analysis and survival analysis were conducted at the patient level. In our own GBM cohort, the mIHC will be applied to measure the macrophage states and verify our findings.

Results: (max 125 words) Four public GBM scRNA-seq datasets with 94 samples were input into our pipeline (Figure A), and four macrophage states together with a proliferating status were identified. Reanalysis of the public IMC dataset confirmed the existence of these first 3 states (no marker for PC_HSP in the dataset; Figure B). By mapping macrophages into a butterfly plot, we revealed the continuous spectrum of macrophages(Figure C). Also, it’s easy to find macrophages with more than one states, which reveals the complexity of macrophages. Prognosis analysis found that respondersto neoadjuvant anti-PD-1 therapy had a higher mean PC_ExtracellularMatrix score ratio, compared with that in non-responders (Figure D). Also, the percentage of PC_Classic positive macrophages is a protective factor for progression-free survival (P = 0.011; Figure E).

Conclusion: (max 125 words) The continuous spectrum of macrophages in GBM could be represented by these 4 macrophage states identified in the study. PC_ExtracellularMatrix is a

predictor for neoadjuvant anti-PD-1 therapy response. And PC_Classic is a protective factor for progressionfree survival. Further analysis will measure these macrophage states in our cohort and address their role in TME.

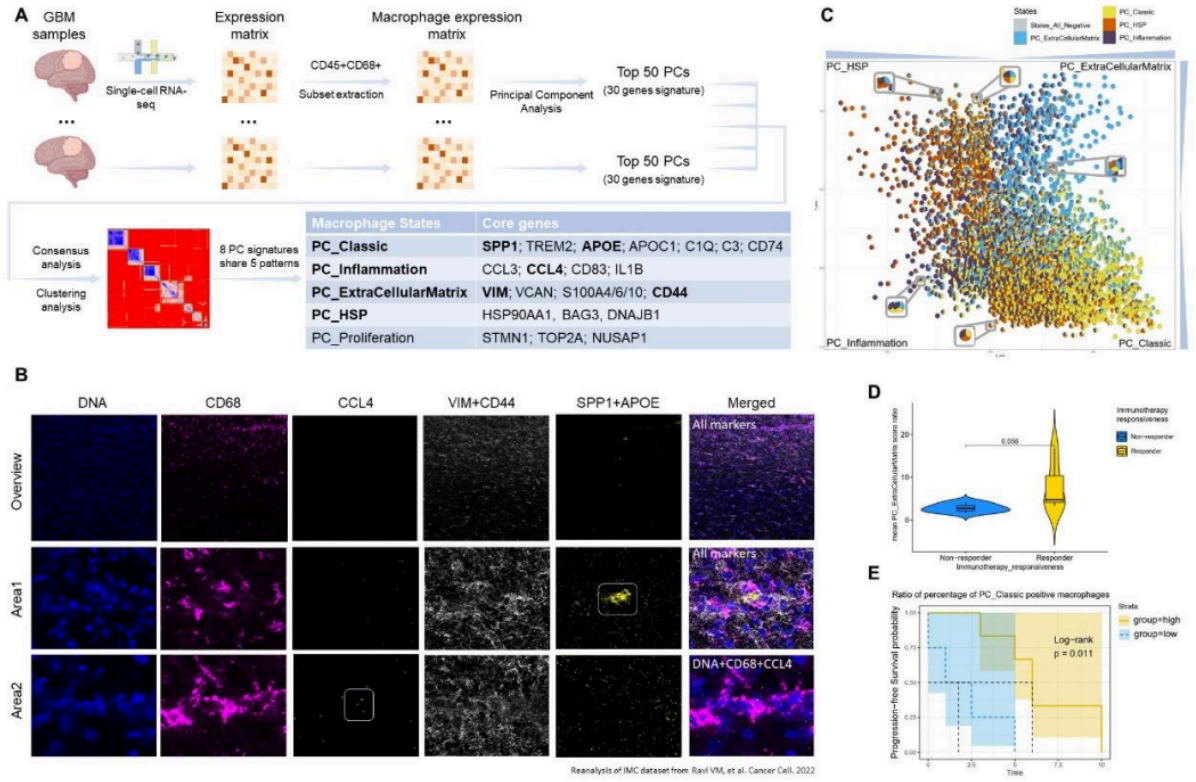

Figure A The developed pipeline, which was used to identify macrophage states. Four macrophage states, together with a proliferating status, were identified. Four public scRNA-seq datasets were used (GSE154795, GSE163120, GSE182109, and OMIX003593). **Figure B** Public GBM IMC dataset (Ravi VM, et al. Cancer Cell. 2022) was reanalyzed to validate the existence of three identified macrophage states. Macrophage with PC_Classic could be found in Area 1 (white rectangle). Macrophage with PC_Inflammation could be found in Area 2 (white rectangle). Macrophage with PC_ExtraCellularMatrix could be found in both areas. **Figure C** Butterfly plot of macrophages. The closer the location of cells to the corner, the larger the states score the cell has. **Figure D** Violin plot of the mean PC_ExtracellularMatrix score ratio, grouped by responsiveness to neoadjuvant anti-PD-1 therapy. The mean status score ratio was calculated as (mean(status scores of macrophages with positive status))/(mean(status scores of macrophages with negative status)). **Figure E** Survival curve of GBM patients treated with neoadjuvant anti-PD-1 therapy, grouped by the percentage of PC_Classic positive macrophages.