Poster abstracts - Stop Alzheimer Young Researcher Congress 2026 (SAYRS) 2nd edition

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FAMILIARITY-RELATED PROCESSES AND THE TRANSENTORHINAL CORTEX VOLUME: INSIGHTS INTO THE COGNITIVE FUNCTIONS OF AN EARLY SITE OF ALZHEIMER'S PATHOLOGY

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Abstract

Objectives: The transentorhinal cortex (tErC), which includes the medial portion of the perirhinal and the anterolateral portion of the entorhinal cortices, is among the first brain regions affected by Alzheimer's Disease (AD). Yet, its precise cognitive role remains controversial. Recent models propose that this region supports *episodic familiarity* (i.e., the sense of recognizing something based on a specific prior encounter) and *lifetime familiarity* (i.e., the gradual accumulation of familiarity with object concepts over the course of one's life), and that it is particularly involved in tasks requiring fine conceptual discrimination between similar objects. The present study examined episodic and lifetime familiarity across three tasks to understand how the tErC supports familiarity, using materials with varying conceptual overlap and lifetime familiarity levels in both normal and pathological aging, encompassing different levels of tErC integrity.
Methods: Fifty-eight older adults (aged 55+), including healthy individuals and patients with Mild Cognitive Impairment or subjective cognitive decline, completed familiarity tasks. tErC volume was measured via high-resolution MRI and was associated with tasks performance using repeated measures ANCOVAs or stepwise linear regressions.
Results: The tErC volume was associated with familiarity performance selectively in task conditions involving high conceptual overlap between targets and lures. Exploratory cluster analysis identified three subgroups: one mostly composed of healthy individuals, another including mainly patients with memory impairment, and a third heterogeneous group. The latter group relied on familiarity to compensate for impaired recollection but committed more false alarms and showed reduced accuracy as conceptual overlap increased.
Conclusions: Results indicate that tErC integrity is selectively related to familiarity-based performance when recognition requires resolving interference between conceptually similar items. We discuss how combining tasks assessing different aspects of familiarity could help identify early cognitive decline. We suggest this approach should betested in patients with AD biomarkers.

SAYRC-002

DISCRIMINATION TASKS INCREASE THE VALUE OF PLASMA P-TAU217 TO PREDICT PRECLINICAL ALZHEIMER'S DISEASE

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Abstract

Objectives: Alzheimer's disease (AD) can be diagnosed using amyloid (Aβ) and tau-PET imaging. These techniques are expensive and invasive, making them hardly practical for screening in clinically unimpaired populations. Research is therefore focused on developing more affordable methods to detect pre-symptomatic AD pathology. Cognitive tests evaluating discriminative abilities and plasma biomarkers appear to be a promising method for overcoming these limitations.
Methods: Eighty-one older adults underwent a blood test enabling the measurement of plasma biomarkers (pTau217), tau-PET, Aβ-PET, 3D T1-weighted brain MRI, standard neuropsychological assessment, the Conceptual Matching Task (CMT), and the Visual-Short Term Memory Binding Test (VSTMBT). The two latter tasks evaluate conceptual and perceptual discrimination abilities, respectively. Participants were classified based on their Aβ status: 56 Aβ-negative (Aβ-; Centiloid<20) cognitively unimpaired (CU) and 25 Aβ-positive (Aβ+; Centiloid>20) CU.
Results: CMT and VSTMBT performance was lower in Aβ+ CU than in Aβ- CU individuals and correlated with Aβ and tau-PET imaging. These tasks were more sensitive than standard neuropsychological assessment for detecting Aβ or taupathology in CU individuals. Moreover, combining discrimination tasks and plasma pTau217 slightlyimproved the prediction of the amyloid and tau-PET status in CU individuals, compared to using pTau217 or cognition alone. Overall combining pTau217, standard cognitive assessment and VSTMBTperformance achieved perfect sensitivity for predicting tau-PET status (AUC=0.98, Sn= 1, Sp=0.89).
Conclusions: Cognitive tests assessing discrimination abilities appear promising in the diagnosis of preclinical AD and are even morepredictive when combined with plasma measurements.

SAYRC-003

SIX-YEAR LONGITUDINAL EVALUATION OF SYNAPTIC DENSITY PET IN PATIENTS WITH AMNESTIC MILD COGNITIVE IMPAIRMENT

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Abstract

Objectives: This study aimed to investigate long-term longitudinal changes in synaptic density (SV2A) PET and their correlation with cognitive decline in patients with a baseline diagnosis of amnestic mild cognitive impairment (aMCI).

Methods: Thirty aMCI patients (73 8yrs, 10M/20F, MMSE 21-30, 28 A +/1A -/1unknown) underwent baseline [^{11}C]UCB-J PET, of which 21 aMCI (8M/13F, MMSE 18-29, 20 A +) underwent follow-up after 2.1 0.1 years and 6 aMCI (2M/4F, MMSE 2-19, all A +) underwent another follow-up session at 5.7 1.1 years from baseline. [^{11}C]UCB-J images were acquired from 60-90 minutes post injection and standardized uptake value ratios (SUVR) were calculated for 6 Braak regions using the whole cerebellum as a reference region. Linear mixed effect models were used to investigate the longitudinal change in [11C]UCB-J SUVR as well as the correlation with MMSE in the 6 aMCI patients who underwent 3 assessments.

Results: We found a significant effect of time on [^{11}C]UCB-J binding (p=0.003), indicating a longitudinal decrease across all Braak regions (Figure 1). When investigating the different regions, we found that the effect was most pronounced for Braak regions I-IV, while being non-significant for Braak V and VI. The effect remained significant after partial volume correction (p=0.01) in Braak regions I-III. MMSE scores decreased significantly over time (p=0.01). We found a significant correlation between decrease in MMSE and partial volume corrected [^{11}C]UCB-J binding in Braak region IV (p=0.01) but not in the other Braak regions.

Conclusions: We show a long-term longitudinal decrease in [^{11}C]UCB-J binding over the progression from aMCI to advanced AD which was correlated to the decrease in MMSE in Braak region IV. Further patient and healthy control follow-up data is being collected to increase the sample size in the next months to enable the use of a joint propagation model to test the correlation between tracer binding and cognitive decline.

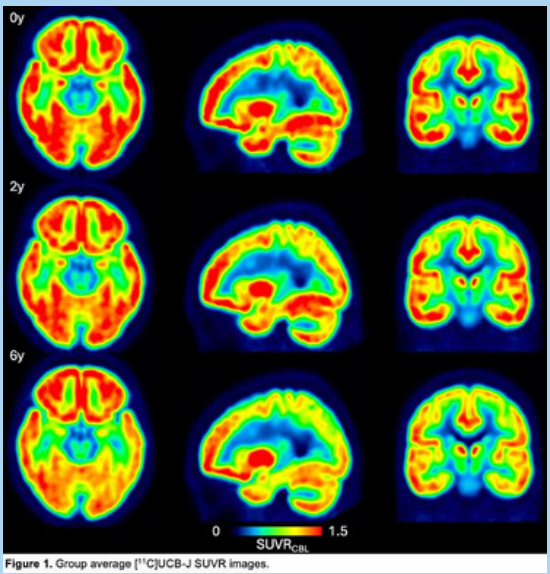

Figure 1. Group average [^{11}C]UCB-J SUVR images.

A NEUROPSYCHOLOGICAL TASK TO ASSESS GIST AND DETAIL MEMORY IN HEALTHY VERSUS PATHOLOGICAL AGING: PRELIMINARY RESULTS

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Abstract

Objectives: This project aims to develop a neuropsychological test to screen for early cognitive markers of Alzheimer'sDisease(AD).Considering that the transentorhinal cortex (tERC) is among the first brain areas affected by AD neuropathology,we aim to target the functions of this region. We based our neuropsychological approach on a recent model that identified two parallel networks within the medial temporal lobe for the processing of detailed and global (gist) information in memory. In this view, the tERC would belong to the gist network,leading to the hypothesis that extracting gist representations from memory would be affected in very early AD.We thus built a neuropsychological test measuring gist and detail memory and tested its reliability in healthy young and older adults.
Methods: First,we conducted several methodological validation steps in 120 participants. Second, we administered the test to 50 young and 50 older adults in a behavioral study.
Results: Older adults were impaired in the detail memory task but had preserved memory for gist information. These results were not explained by education level, task difficulty, or image type.
Conclusions: The test developed here reliably evidenced th eage-related decline in detail memory typically reported in the literature. Furthermore,our results support the assumption that preserved memory for gists is a marker of healthy aging, which makes it a suitable candidate for differentiating typical from pathological aging. The next step of this work is to administer the memory test to aging participants (55 and older), combined with plasma AD biomarkers measurements and high-resolution neuroimaging to assess its sensitivity to early AD changes. This second study is ongoing.

NEURAL NETWORKS AND EEG TO TRACK COGNITIVE DECLINE : A COMPARATIVE STUDY

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Abstract

Objectives: Electroencephalography (EEG) provides a low-cost, non-invasive window into brain activity and has shown promise in the study of neurodegenerative conditions such as Alzheimer's disease, particularly for the detection of early stages. Building on this potential, recent advances in deep learning have enabled the extraction of subtle biomarkers from EEG signals, further enhancing their utility for early detection. However, EEG signals are inherently noisy, and their analysis depends strongly on methodological choices, which can substantially influence the extracted representations and resulting performance. We therefore conduct a systematic evaluation of EEG-based cognitive impairment classification by systematically varying task formulation, preprocessing pipelines, and model architectures to assess their impact on distinguishing healthy controls (HC), patients with subjective cognitive decline (SCD), mild cognitive impairment (MCI), and dementia due to Alzheimer's disease (AD).
Methods: We designed a factorial experiment on a public EEG dataset. Four deep learning architectures were evaluated across five preprocessing pipelines and three clinical task formulations: one 4-class task (HC-SCD-MCI-AD), one binary task(AD-HC), and one 3-class task (HC-SCD-MCI). Hyperparameters were optimized using Optuna and evaluations were performed at the subject level using cross-validation with strict subject independent splits.
Results: Performance dropped sharply as tasks became more clinically realistic, with mean accuracy decreasing substantially from binary AD–HC classification to 4-class staging. Our findings also indicate that both the choice of neural network architecture and the preprocessing strategy influence classification outcomes.
Conclusions: Our results highlight the promise of EEG data in identifying early indicators of Alzheimer's disease and emphasize the importance of systematically evaluating both preprocessing strategies and task formulations to ensure clinically meaningful results.

SAYRC-006

HIGH-RESOLUTION EX-VIVO MRI REVEALS CA1 PYRAMIDAL LAYER VULNERABILITY TO EARLY TAU AND TDP-43 PATHOLOGY

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Abstract

Objectives: Tau pathology affects the medial temporal lobe (MTL) early in Alzheimer's disease (AD), making it critical for understanding this pathology. The MTL is also the initial site of TDP-43 pathology in limbic-predominant age-related TDP-43 encephalopathy (LATE) and frontotemporal lobar degeneration (FTLD). Although in-vivo MRI detects MTL atrophy, its sensitivity to subfield-specific changes and the contributions of tau and TDP-43 at early stages remain unclear. We aimed to identify MTL subregions affected by early tau pathology using high-resolution ex-vivo MRI and histopathology to assess the impact of tau and TDP-43 on MTL atrophy.
Methods: High-resolution ex-vivo 11.7T MRI scans of human MTLs were acquired from 16 donors with early tau pathology (Braak NFT stage ≤ II), including three cases with LATE neuropathologic changes (LATE-NC) and two FTLD-TDP cases. The anterior MTL was manually segmented into 12 subfields, including subiculum (SUB), CA1, CA2/3, dentate gyrus (DG), entorhinal cortex (ERC), and transentorhinal cortex (TEC). Cortical thickness was estimated using a semi-automated approach. pTau/pTDP-43-positive neurons and tau pathological burden were quantified in ERC/TEC, CA1/SUB, and CA3/DG.
Results: Comparisons between Braak 0/I (N=8) and Braak II (N=7), adjusted for age, sex, and co-pathologies (α-synuclein/TDP-43), showed reduced CA1 pyramidal layer thickness in Braak II cases ($p<0.01$). TDP-43-positive individuals (N=5) showed reduced thickness of the CA1 pyramidal layer and the CA2/3 stratum radiatum-lacunosum-moleculare (SRLM). Partial Spearman correlations showed associations between CA1 pyramidal layer thickness and tau and TDP-43 measures. When controlling for tau/TDP-43 burden, only TDP-43 remained significant ($R\leq-0.61;p\leq0.05$), while tau showed a non-significant trend ($R\leq-0.47;p\leq0.24$).
Conclusions: Combining high-field ex-vivo MRI with histopathology shows that early tau pathology is associated with CA1 pyramidal atrophy, identifying CA1 as a key target for in-vivo detection of preclinical AD. Importantly, TDP-43 contributes to CA1 atrophy, highlighting the need to account for TDP-43 when interpreting early MTL structural changes.

SAYRC-007

MOLECULAR INSIGHTS INTO HYPERSPECTRAL RETINAL IMAGING AS A NOVEL BIOMARKER FOR ALZHEIMER'S DISEASE

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Abstract

Objectives: Alzheimer's disease (AD) is the leading cause of dementia and affects over 50 million people worldwide. Increasing evidence highlights retinal imaging as a potential biomarker for AD, as the retina is a part of the centra lnervous system and shares pathological hallmarks with the brain, including amyloid-β (Aβ) deposition. Hyperspectral retinal imaging (HSRI) offers non-invasive, high-resolution, and cost-effective detection of retinal changes associated with AD. Although HSRI has demonstrated potential in distinguishing PET-confirmed early AD patients from controls, the molecular basis of the HSRI signals still remains unclear.Therefore, this study aims at investigating the molecular and pathological correlates of HSRI signals to establish HSRI as a biomarker for AD.
Methods: *In vitro* and *in vivo* models were used to characterize hyperspectral signatures of distinct Aβ conformations, combined with machine learning to classify spectral features .Synthetic aggregation assays were combined with HSRI to assess conformational-state-dependent spectral signatures. *Ex vivo* HSRI was performed on retinal tissue from App^{NL-G-F} (amyloid, AD) and (Thy1)-h[A30P]αSYN (α-synuclein, Parkinson's disease) mice and compared with wild-type controls at 4 and 18 months of age. HSRI signatures were correlated with soluble and insoluble Aβ levels.
Results: Pilot data of the synthetic aggregation assays showed distinct hyperspectral signatures for each Aβ42 conformational state. The signatures return to baseline upon aggregation, supporting the role of soluble Aβ oligomers in generating AD-specific HSRI signals. *Ex vivo* HSRI, revealed genotype- and age-dependent spectral differences, with HSRI signals correlating with soluble and insoluble Aβ42 levels.
Conclusions: These findings provide a first molecular and pathological insight into the origins of AD-associated HSRI signals and support the development of HSRI as a non-invasive imaging biomarker for AD. Further insights from these studies will be essential for the rational implementation of HSRI as an imaging biomarker for AD in the clinic.

SAYRC-008

DETERMINANTS OF FRACTAL MOTOR ACTIVITY REGULATION: INSIGHTS FROM >9000 DAYS OF ACTIGRAPHY IN A COMMUNITY-DWELLING MULTI-ETHNIC POPULATION

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Abstract

Objectives: In humans, motor activity exhibit a complex regulation, yielding fractal patterns that reflect the system's adaptability. Alterations of fractal motor activity regulation (FMAR) patterns have been shown to be a predictor for Alzheimer' s disease and their reduction follows the progression of the disease. Nevertheless, to improve the clinical relevance of such information, researchers need to further understand the factors that might affect FMAR in community-dwelling older individuals, including but also beyond age and sex.
Methods: We analysed actigraphy recordings, covering > 9000 days, from >1500 individuals from the U.S (MESA dataset) to quantify FMAR alterations. Using Bayesian linear mixed statistical modelling, we searched for associations between these alterations and demographic factors (age, sex, body mass index), as well as physical activity levels and, for the first time, ethnic origins.
Results: First, we confirmed that more altered FMAR is observed with increasing age, as well as in women compared to men. In agreement with results obtained in rodents, we observed that higher activity levels were linked to less degraded FMAR, opening up possibilities for interventional studies targeting physical activity. Finally, we showed, for the first time, that ethnic minorities displayed more altered FMAR than white participants.
Conclusions: Our study, the largest cross-sectional study on FMAR in a multi-ethnic population sample, provided evidence for effects of age and sex on FMAR, compatible with the expected age- and sex-dependent neuronal integrity loss of the underlying brain network responsible for FMAR. Besides, we reported for the first time in humans evidence for an effect of activity levels on FMAR, confirming results obtained in rodents. Finally, our results highlighted differences in FMAR alterations between ethnic minorities and white participants. Overall, by clarifying the determinants of FMAR alterations, our study will help in establishing fractal regulation as an additional marker for health monitoring.

SAYRC-009

EXPLORATORY EVALUATION OF PLASMA TDP-43 IN LATE AND OTHER TDP-43 PROTEINOPATHIES USING A SIMOA ASSAY

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Abstract

Objectives: Limbic-predominant age-relatedTDP-43 encephalopathy (LATE) is characterized by TDP-43 pathology primarily affecting medial temporal structures and episodic memory impairment in adults over 75 years. Currently, no validated in vivo biomarker exists for LATE. Plasma TDP-43 is a candidate biomarker, however, its behavior in LATE and TDP-43–related disorders remains unclear. We evaluated plasma TDP-43 concentrations in probable and possible LATE, amyotrophic lateral sclerosis (ALS),and frontotemporal lobar dementia (FTLD) using the Simoa™TDP-43assay.

Methods: Forty-two participants were included: 5 probable LATE (78.7±9.0years), 16 possible LATE (73.4 ±9.0), 6 ALS/FTLD (62.3±4.4), and 15 young controls (25.8±3.9). Suspected LATE or ALS/FTLD participants underwent 3DT1-weighted MRI, [18F]-MK-6240 tau PET, amyloid PET, and neuropsychological assessment. Probable LATE was defined by A−/T−status, hippocampal atrophy ≤ 5th percentile, memory impairment (<−1.5SD), and negative plasma pTau217 (<0.193pg/mL). Possible LATE met similar criteria but showed Alzheimer's biomarker positivity. ALS and FTLD diagnoses were established clinically. Controls underwent MMSE screening and blood sampling only. PlasmaTDP-43 was measured in duplicates using the Simoa™ kit. Group differences were assessed using the Kruskal–Wallis test followed by pairwise Wilcoxon rank-sumtests.

Results: Plasma TDP-43 differed across groups. ALS/F TLD showed the highest levels(5096.9±3143.7 pg/mL), followed by possible LATE (3675.2±2774.7pg/mz). Young controls exhibited variability (4687.7 ±3797.4 pg/mL). Unexpectedly, probable LATE showed the lowest concentrations (1039.9±1024.7 pg/mL), significantly lower than all other groups ($p<0.05$). Assay precision was acceptable (coefficient of variation 5.81%).

Conclusions: Plasma TDP-43 levels differ significantly between diagnostic groups.Reduced concentrations in probable LATE may reflect decreased physiological TDP-43, however, divergence from ALS/FTLD remains unexplained. Although limited by the small probable LATE sample, these findings support investigation of plasma TDP-43 as a potential LATE biomarker. Ongoing quantification of TDP-43 in post-mortem brain-derived insoluble fractions aims to clarify these findings.

SAYRC-010

CROSS-LINGUISTIC DETECTION OF FRONTOTEMPORAL DEMENTIA USING QUANTITATIVE FEATURES OF SPEECH AND LANGUAGE FOR ENGLISH AND DUTCH PATIENTS

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Abstract

Objectives: Speech and language deficits present as some of the first symptoms of Frontotemporal Degeneration (FTD). Our recent systematic review suggests that some changes in speech and language are similar across different languages (Coppietersetal.,2024). The objective of this study was to use quantitative features of speech and language to differentiate Dutch-andEnglish-speaking patients with FTD from healthy controls in a non-invasive and widely accessible way.
Methods: The participants are all recruited as part of the Genetic Frontotemporal Dementia Initiative (GENFI) Consortium (Rohreret.al.,2015). Asymptomatic carriers and patients in this study have a familial form of FTD and have a known FTD-causing gene.Their first-degree relatives are included as controls. This study includes 395 English-speaking participants, and 192 Dutch-speaking participants. Participants performed the Cookie Theft Picture Description task for 1 minute. Speech and language features were extracted using the Winterlight protocol (Robinet.al.,2021). A subset of features shown to be the most altered longitudinally in patients with FTD in previous research were investigated (Robin et. al., 2021). This included total words, mean unfilled pauses, information units, graph diameter, and graph density. To create a composite feature per participant combining these metrics, a principal component analysis was performed on the selected features within the separate languages, with the first component being considered the“composite feature”.
Results: The Receiver Operating Characteristic (ROC) curve shows a AUC of 0.756 when comparing patients and controls in English .Comparison of carriers and patients showed a high AUC of 0.73. Interestingly the AUC for comparing patients and controls in the Dutch dataset the AUC remained high at 0.798, and 0.726 when comparing carriers and patients.
Conclusions: A limited number of quantitative features derived from a short speech sample can successfully differentiate patients with FTD from controls across languages.

SAYRC-011

THE Γ-SECRETASE PARADOX: WHEN ENZYME INHIBITION INCREASES TOXIC AB PRODUCTION

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Abstract

Objectives: Γ-secretase-mediatedcleavage of APP generates amyloid-β (Aβ) peptides of different length and impairmentsinthisprocess that lead to generation of longer Aβs play a central role in Alzheimer's disease (AD)pathogenesis. Pharmacological inhibition of γ-secretase was therefore extensively pursued inADtherapy.However, the phase 3 clinical trial testing the γ-secretase inhibitor (GSI) Semagacestat failedtodemonstrate cognitive benefit and was prematurely stopped due to adverse effects. Giventhekeyrole of γ-secretase in NOTCH signaling, GSIs were subsequently repurposed for oncology.TheGSINirogacestat has received FDA and EMA approvals for the treatment of desmoid tumors, arareandbenign yet locally aggressive fibromatosis. Evidence suggests that certain GSIs (including Semagacestat)reduce secretion of Aβ while promoting the accumulation of longer Aβ species intracellularly.Inlightofour recent finding implicating shifts in Aβ production towards longer forms as pathogenic,weinvestigated whether clinical GSIs share similar properties to semagacestat.
Methods: We performedcell-freeassays using purified γ-secretase and APP-C99 to assess inhibitory capacity. Nirogacestatwasevaluated alongside Crenigacestat (phase I), AL-102 (phase II/III), and Semagacestat. The active-sitedirected GSI L685,458 was used as reference.
Results: Cell-free assaysdemonstrated incomplete inhibition for all GSIs, except L685,485, and in most this was accompaniedbyproduction of longer Aβs. In parallel, HEK293, SH-SY5Y and iPSC-derived neurons overexpressing$APP_{Swedish}$ were treated with 1 µM inhibitors. Secreted Aβ species were quantified by ELISA, whereasintracellular Aβ was analyzed by immunoprecipitation followed by western-blotting analysis.Cell-based assays showed that while secreted Aβ levels decreased upon treatment, as expected,Nirogacestat and AL-102 induced intracellular accumulation of long Aβ peptides, detectable evenatlownanomolar concentrations, whereas Crenigacestat did not. These findings indicate that Nirogacestat exhibits pseudo-inhibitory behavior: partially inhibiting Aβ production, while enhancinggeneration of longer Aβ peptides.
Conclusions: These resultsdonotestablish clinical risk, and no conclusions regarding patient safety can be drawn from these dataalone.Nonetheless, these findings warrant awareness among clinicians managing patients on long-termnirogacestat therapy, although their physiological relevance remains to be established.

SAYRC-012

LEXICAL-SEMANTIC RETRIEVAL IN THE EARLY STAGE OF ALZHEIMER'S DISEASE: A COMPARISON OF THREE INTERVENTIONS BASED ON DIFFERENT THEORETICAL APPROACHES

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Abstract

Objectives: Patients with Alzheimer's disease (AD) experience early lexical-semantic difficulties, such as anomia, which impact their ability to interact socially and their quality of life. This research aims to investigate the benefits of three treatment methods based on different theoretical approaches: the Elaborated Semantic Feature Analysis (ESFA) method, derived from semantic network models; the Treatment by Embodied Reactivation of Memory (TERM) ,based on the theory of embodied cognition; and the Holistic Intervention of Semantic Memory (HOLISM), built on the hub-and-spoke model.
Methods: Twenty-nine patients in the early stage of AD were recruited (MMSE ≥20) and divided into three groups: ESFA (N=10), TERM (N=9), and HOLISM (N=10). A four-phase experimental design was followed: pretest (A;3weeks), intervention (B;8weeks) ,posttest (A; 1 week), follow-up (A; 1 week). During phases A, patients attended two sessions per week for cognitive assessment based on episodic memory task, lexical-semantic tasks including an experimental naming task, executive function task, and speech task. During the intervention phase ,patients attended two individual sessions per week. Some of the concepts that they failed to name in the pretest experimental naming task were then trained using the respective methods.
Results: Initial results from the comparison of the three assessment phases (A) indicate improvements in naming in all groups (ESFA: $\chi^2(2) = 19.538$, $p<.001$; TERM: $\chi^2(2) = 7.588$, $p = .023$; HOLISM: $\chi^2(2) = 13.282$,$p=.001$). At this stage, the generalization of effects to other untrained concepts seems to occur only with the ESFA method ($\chi^2(2)=6.222$;$p=.045$) (data collection in progress for HOLISM).
Conclusions: The three methods appear to have different therapeutic effects. The results will be interpreted and discussed in the context of theoretical approaches.

SAYRC-013

ENGINEERING CAR-MICROGLIA TO TARGET AB PATHOLOGY *IN VIVO*

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Abstract

Objectives: Microglial dysfunction in amyloid-β (Aβ) recognition and clearance is a central event in Alzheimer's disease (AD) pathogenesis. Although anti-Aβ antibodies slow cognitive decline by indirectly favouring Fc-mediated clearing of Aβ plaques by microglia, they are limited by inefficient brain delivery and adverse events such as ARIA. We aimed to engineer induced pluripotent stem cell (iPSC)-derived microglia expressing a chimeric antigen receptor (CAR) that directly links Aβ binding to protective intracellular signaling, thereby enhancing plaque clearance while avoiding non-specific and deleterious inflammatory activation.
Methods: We generated iPSC-derived CAR-microglia expressing an extracellular Aducanumab single-chain variable fragment fused to the intracellular signaling domain of DAP12, modeling TREM2-associated protective pathways. CAR-microglia were evaluated for Aβ uptake and degradation *in vitro*, on *ex vivo* amyloid mouse brain slices, in human 3D brain assembloids, and *in vivo* following xenotransplantation into amyloid mouse models using our established microglial engraftment platform. Microglia phagocytosis and activation states were assessed by single-cell RNA sequencing, flow cytometry and immunohistochemistry.
Results: CAR-microglia efficiently and specifically internalized and degraded Aβ *in vitro* and *ex vivo*. CAR-microglia robustly engrafted human assembloids and the mouse brain after xenotransplantation *in vivo*, achieving up to 80% plaque clearance within colonized areas. Transcriptomic and histological analyses revealed selective activation of phagocytic and antigen-presenting (HLA) programs without induction of interferon or pro-inflammatory pathways previously observed in microglia following peripheral antibody treatment.
Conclusions: Directly coupling Aβ recognition to DAP12-dependent microglial signaling promotes efficient, localized plaque clearance while avoiding inflammatory signatures. Unlike systemic antibody therapies, transplanted microglia can be delivered directly into the brain parenchyma, bypass the blood–brain barrier, self-renew, and achieve widespread colonization after a single administration. CAR-microglia represent therefore a programmable, long-lived cellular therapeutic platform with the potential to provide sustained and spatially controlled amyloid removal in AD.

SAYRC-014

REDUCTION OF TAU PATHOLOGY PROPAGATION BY TAU SILENCING IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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Abstract

Objectives: In this study we aimedto assess the effects of siRNA targeting tau in a mouse model of tau pathology propagation when thereduction of tau level started immediately at the induction of tau pathology, or when tau reductionstarted after some development of tau pathology.
Methods: We used a mouse modelexpressing the 6 non-mutated human tau isoforms in the absence of endogenous murine tau (htau).We induced the propagation of tau aggregates in htau mice by the intracerebral injection of pathological tau from human Alzheimer's cases (AD tau) which will recruit and seed endogenous humantau and will further propagate in a prion-like manner via neuroanatomical pathways. hTaumice were treated with siRNA tau or siRNA non-targeting concomitantly with or aftertheinjection of AD tau. After 3 months, the spatial learning of mice was assessed by Barnes maze andtaupathology was analyzed by immunolabelling.
Results: When starting the reduction of tau level simultaneously with the induction of tau pathology we have observed a better performance in solving the Barnes maze and a decrease in tau pathology formation. However, treatment with siRNA in mice already developing tau pathology performed at similar levels as mice treatedwithsiRNA non-targeting in solving the Barnes maze.
Conclusions: Taken together,our data suggest that siRNA targeting tau administered concomitantly with the induction of tau pathology rescues spatial learning and reduces tau pathology, but if administered when tau lesions are already present in the brain it has no effect on spatial learning.

SAYRC-015

DECIPHERING ALLOSTERIC MODULATION OF γ-SECRETASE ACTIVITY BY SMALL MOLECULES

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Abstract

Objectives: Alzheimer's disease (AD) is associated with the accumulation of longer amyloid-β (Aβ) peptides generated by γ-secretase (GSEC) cleavage of the amyloid precursor protein (APP). Pathogenic mutations in PSEN1/2, which encode the catalytic core of GSEC, or in APP itself increase the production of longer Aβ species by destabilizing GSEC–APP interactions. γ-Secretase modulators (GSMs) promote the generation of shorter, non-toxic Aβ peptides and represent a genetically supported therapeutic strategy. However, previous cryo-EM studies identified binding modes of imidazole-based GSMs only in the presence of active-site inhibitors, complicating the interpretation of GSM-specific conformational effects on GSEC. Here, we aim to establish a comprehensive structural and mechanistic understanding of the mode(s) of action of preclinically tested GSMs using cell-based and cell-free assays combined with cryo-electron microscopy (cryo-EM) structure determination in a native membrane-like environment.
Methods: We investigated the mechanisms of action of chemically distinct imidazole-based GSMs using a combination of cell-based and cell-free assays, complemented by cryo-EM structure determination.
Results: Our data showed that structurally diverse GSMs differentially modulate GSEC activity at nanomolar concentrations. Cryo-EM structures revealed that, in the absence of active-site inhibitors, GSMs alone induce significant conformational changes in GSEC. Furthermore, functional analyses showed that GSMs rescue mutation-driven impairments in both overall catalytic activity and processivity.
Conclusions: These findings provide mechanistic insight into the mode of action of GSMs and offer direct structural evidence for allosteric modulation of GSEC, supporting their further development as therapeutic strategies for AD.

SAYRC-016

INFLUENCE OF EMOTIONAL CONTEXT ON EXECUTIVE FUNCTIONS IN SPONTANEOUS SPEECH IN HEALTHY AGING AND ALZHEIMER'S DISEASE

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Abstract

Objectives: This study examines the impact of emotiona lcontexts on three executive functions (EFs), inhibition, cognitive flexibility and planning, in healthy aging and in moderate to severe Alzheimer's disease (AD), using an ecological spontaneous speech task.
Methods: Two phases will be conducted: (1) development of ExEcoLang Scan, an automated Python-based tool to analyze executive language components in spontaneous speech under different emotional contexts; (2) application of this tool in AD patients. In the first part, 60 cognitively healthy older adults (MMSE ≥ 27 ; 79.81 ± 5.50 years) completed standardized executive tasks and discussed topics with positive, negative, and neutral valence. Half of the speech were used to train the software to (1) automatically transcribe the recordings, (2) complete a executive components of language grid, and (3) determine a performance level for each EF base on standardized test scores. The remaining data were analyzed blindly by the model to assess its ability to classify executive performance solely from linguistic indicators. In the second part, 108 patients with moderate (MMSE: 10–20) or severe AD (MMSE < 10), will participate in a semi-structured interview covering the three valences conditions. EF indicators identified in these productions using the ExEcoLang Scan will be compared with those of healthy participants.
Results: Findings from the first phase will be presented at the congress. EF indicators extracted from speech are expected to correlate with standardized scores. Moderate AD patients should show greater EF mobilization than severe patients,with stronger engagement in positive and negative contexts.
Conclusions: If the hypotheses are confirmed, the findings would support the clinical relevance of EFs preserved within spontaneous speech as a foundation for developing psychoeducational strategies aimed at reducing behavioral disturbances in patients with AD. Such approaches could ultimately contribute to improving quality of life for patients, caregivers, and relatives.

SAYRC-017

CYTOSOLIC PHOSPHOLIPASE A2 AS A PROMISING TARGET FOR INNOVATIVE THERAPEUTIC APPROACH IN ALZHEIMER'S DISEASE

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Abstract

Objectives: Alzheimer's disease (AD) remains the most common cause of dementia worldwide and still represents a major public health challenge.Despite decades of research, no curative treatment is currently available, underscoring the need for innovative therapeutic strategies. In this context, we investigated a newly developed peptide complex (PC) designed to inhibit cytosolic phospholipase A2 (cPLA2), a key enzyme potentially implicated in neurodegeneration, and engineered to cross the blood-brain barrier.
Methods: The inhibitory potential of this PC was assessed in vitro by quantifying arachidonic acid release as an indicator of cPLA2 function. Its cellular distribution and keyAD-related biomarkers (APP, Aβ and GSK3β-pY216) were examined by immunofluorescence in neuronal and glial cell models. In vivo, we examined the effects of PC in APP/PS1 mice on amyloid plaque burden and Tau phosphorylation patterns.
Results: Our PC significantly reduced arachidonic acid release and modulated cPLA2 activity in both astrocytes and neurons. Our observations also indicate that glutamate excitotoxicity promotes cPLA2 translocation to membrane processes and is associated with increased APP expression, enhanced Aβ production and elevated GSK3β-pY216 activation, which contributes to both Aβ generation and Tau phosphorylation. In APP/PS 1mice, treatment with PC led to a marked decrease in amyloid plaque burden. It also restored asomatic, physiological-like distribution of PTau (Ser199), preventing the axonal filamentous pattern observed in untreated APP/PS1 mice.
Conclusions: Together, these findings support the therapeutic potential of this peptide complex and its relevance as a candidate disease-modifying strategy for AD.
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References: André S, Verteneuil S, Ris L, Kahvecioglu ZC, Nonclercq D, De Winter J, Vander Elst L, Laurent S, Muller RN, Burtea C, J. Alzheimers Dis. Rep. 2023, 7(1):1395-1426. doi: 10.3233/ADR-230075.

SAYRC-018

INVESTIGATING AMYLOID PLAQUE HETEROGENEITY IN COGNITIVE RESILIENT VERSUS ALZHEIMER'S DISEASE BRAINS

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Abstract

Objectives: Alzheimer's disease (AD) is neuropathologically characterised by extracellular amyloid-β (Aβ) plaques, intracellular tau tangles and glial reactivity. Genetic and clinical evidence establishes amyloid aggregation as a key upstream driver of disease. However, amyloid pathology is frequently observed in cognitively unimpaired older individuals, indicating that plaque presence is necessary but not sufficient for clinical manifestation. In genetic, early-onset AD shifts in the ratio of longer Aβ species, relative to shorter less aggregation-prone peptides, are sufficient to cause disease. We hypothesize that plaque molecular composition determines their neurotoxic potential by eliciting differential glial reactivity, which in turn remodels amyloid plaque composition. Our recent ex vivo analyses support the existence of distinct amyloid plaques that differentially populate the (postmortem) brains of AD versus amyloid-positive cognitively unimpaired (AP-CU) individuals. Our ongoing studies aim to comprehensively define plaque subtype composition and determine how their molecular features shape interaction with the surrounding cellular environment.
Methods: Human post-mortem cortical tissue from AD and AP-CU donors was analysed by single-plaque mass spectrometry imaging (MSI) to profile individual amyloid plaques and define plaque subtypes. These findings were validated by immunohistochemistry (IHC).
Results: Single-plaque analysis identified two distinct plaque populations across AD and AP-CU brains. One subtype displayed low ganglioside content and reduced Aβ38; the second was enriched in both gangliosides and Aβ38. The first plaque subtype was more prevalent in AP-CU cases, while the second was more abundant in AD cases. Recent IHC analysis identified Aβ38+ and Aβ38- plaque subtypes and confirmed their differential prevalence in AP-CU versus AD cases.
Conclusions: Our data support the concept that amyloid plaque composition, rather than plaque load, is a key determinant of disease outcome. The identification of molecularly distinct plaque subtypes provides a framework to understand why some individuals remain cognitively resilient despite amyloid pathology.

SAYRC-019

UNVEILING TAU POST-TRANSLATIONAL MODIFICATIONS IN ALZHEIMER'S DISEASE PROGRESSION

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Abstract

Objectives: Alzheimer's Disease (AD) is a neurodegenerative disorder responsible for 60% of dementia cases. AD is considered as a secondary tauopathy, marked by pathological Tau aggregation driven by an additional factor: the amyloid pathology. Their development is a multistep process, probably driven by alterations in post-translational modifications (PTMs). Beyond improving our understanding of tauopathy pathophysiology, PTMs also represent valuable biomarkers. Tau biochemistry in advanced stage of AD has already been explored by our group. However, the precise roles of Tau PTMs remain incompletely understood. We sought to extend these investigations across the AD spectrum and multiple brain regions. Our project aims to characterize the temporal sequence of Tau PTM changes, both as diagnostic tools and as mechanisms underlying Tau accumulation.
Methods: We studied both soluble and insoluble protein fractions from human brains spanning different "ABC score" throughout AD progression, corresponding to Aβ plaque score (A), neurofibrillary tangles (NFTs) score (B) and neuritic plaque score (C). Several brain regions (hippocampus, inferior frontal gyrus and inferior temporal gyrus) affected at distinct Braak stage, were examined. Tau PTMs (phosphorylation, ubiquitination, methylation, acetylation) were assessed using Immunoprecipitation followed by mass spectrometry. An absolute quantification of Tau isoforms was performed using SureQuant method. Post-analysis was conducted using Proteome Discoverer Software and R.
Results: This approach enabled mapping Tau PTMs dynamics during AD progression across different brain regions. Certain PTMs appeared as promising early biomarkers, including pTau217 and pTau262, whereas others, such as UbiTau311 and UbiTau317, were associated with more advanced disease stage. We also identified PTMs specifically linked to insoluble Tau accumulation. Finally, combinations of PTMs within the protein fractions were examined to further elucidate the functional role of well-characterized modifications.
Conclusions: These findings provide new insights into the evolution of Tau biochemistry across AD progression and contributed toadeeper understanding of tauopathy pathophysiology.

SAYRC-020

APOE DEFICIENCY RESTORES LIPID HOMEOSTASIS AND MITIGATES TAU PATHOLOGY

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Abstract

Objectives: The most important riskfactor for the development of late onset AD is ApoE, the major lipid transporter in the CNS. Recen treports of putative protective mutations within the ApoE gene indicate that delay of symptom onset by modulatingApoE is possible. Furthermore, lipid changes are associated with these mutated forms. Finally, lipids have important (intra) cellular functions, constituting all cellular membranes and being involved in signalling pathways crucial for dealing with pathological insult. Here, we investigated lipid metabolism within tauopathy models and how ApoE deficiency influences these lipid profiles.
Methods: 11 month old TauP301S mice (T+) were used, crossed with ApoE KO mice. Immunochemical stainings and biochemical analyses were used to investigate tau pathology and. Moreover, lipidomic analysis was performed. A complementary SH-SY5Y tauP301L cellular model was used for *in vitro* experiments.
Results: T+ ApoE deficient mice showed decreased tau pathology compared to ApoE expressing mice. Moreover, downstream neurodegeneration was also ameliorated by ApoE deficiency, highlighting the therapeutic potential of ApoE based therapies.Furthermore, lipidomics analysis showed vastly altered lipid profiles in T+ mice compared to healthy, age-matched WT mice, including decreased phospholipids. Interestingly, T+ ApoE KO mice showed a (partial) restoration towards WT, which correlated with decreased tau pathology and neurodegeneration. Increase in lysosomal signal was observed in the T+mice, correlated with neurodegeneration, and was mitigated by ApoE deficiency. Finally, *in vitro* experiments showed that when signalling phospholipids where altered, similarly to the T+ lipidome, lysosomal remodelling occurred.
Conclusions: These findings show that ApoE deficiency ameliorates disease progression and prevents shifts in lipid composition. And that this shift in lipid composition alters lysosomal functioning. Further research will focus on characterizing the exact link between Tau, lipids how ApoE affects these factors. These insights will open new avenues for therapeutic targets for tauopathies and AD in particular.

MITOCHONDRIAL LOCALIZATION OF AMYLOID BETA DURING AGING AND DEVELOPMENT OF AN INDUCED ALZHEIMER-LIKE MODEL IN KILLIFISH

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Abstract

Objectives: Alzheimer's disease (AD) is characterized by amyloid beta (Aβ) accumulation and progressive neurodegeneration. Although mitochondrial dysfunction is a key feature of AD pathology, the intracellular localization of Aβ and its impact on mitochondrial processes remain unclear. In addition, there is a need for experimental models enabling controlled investigation of Aβ-driven pathology and therapeutic interventions. This study aimed to investigate the localization of spontaneous Aβ and its association with mitochondrial dysfunction during aging in killifish (*Nothobranchius furzeri)*, and to develop an AD-like model through Aβ42 injection.
Methods: Telencephalon samples were collected from young (8 weeks post-hatching, wph), middle-aged (20 wph), and aged (32 wph) fish. Aβ localization was assessed by immunofluorescence. Gene expression related to amyloid processing and microglial activation was analyzed by qPCR. Microglial abundance and synaptic integrity markers were evaluated. Cognitive performance was assessed using an active avoidance assay. Aβ accumulation was experimentally induced by cerebroventricular microinjection of Aβ42.
Results: Aged fish exhibited increased Aβ accumulation, elevated microglial levels, synaptic alterations, and cognitive decline compared to young fish. Aβ signal increased with age and was predominantly detected in proximity to mitochondria. Amyloidogenic gene expression remained unchanged, whereas microglia-associated genes were significantly upregulated. Phosphorylated tau levels did not show significant changes across age groups. In the induced AD-like model, Aβ42 administration increased microglial activation and synaptic alterations, without inducing detectable plaque-like deposition. Genes involved in amyloid processing were downregulated.
Conclusions: Aβ accumulates in association with mitochondrial compartments during brain aging in killifish. This model supports the role of mitochondrial dysfunction in AD and provides a framework for studying early pathological mechanisms. The absence of plaque formation after Aβ42 injection may reflect the non-aggregated state of the peptide.

SAYRC-022

CAN SPECIFIC DIMERIC ORIENTATIONS SHAPE THE DEVELOPMENT OF THE AMYLOID PATHOLOGY IN ALZHEIMER'S DISEASE?

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Abstract

Objectives: Abnormal deposition of β-amyloid peptides (Aβ) is an early hallmark of Alzheimer's disease (AD), yet FDA-approved Aβ-targeting therapies reduce plaque burden without improving cognition. Moreover, amyloid plaque load poorly correlates with disease severity, suggesting that alternative Aβ species drive AD ppathology. Aβ is produced from the C99 fragment by γ-secretase, The exact cleavage outcome is dictated by its presenilin (PS) subunit: PS1 mainly produces extracellular Aβ40, whereas PS2 favors intracellular production of the more aggregation-prone Aβ42, which forms toxic oligomers prior to plaque deposition. Increasing evidence suggest that **soluble Aβ oligomers**, rather than amyloid plaques, are the primary drivers of cellular toxicity in AD. However, the molecular structure of these toxic species and their contribution to disease progression remain poorly understood. We previously demonstrated that C99 can form dimers whose conformations influence downstream processing. This study investigates whether specific C99 dimeric conformations selectively promote the formation of amyloid assemblies that seed toxic Aβ oligomers.
Methods: To generate defined dimeric conformations, **asparagine (N) mutations** were introduced at selected positions within the transmembrane domains of C99. These **HA-tagged** constructs were expressed in SH-SY5Y cells to assess how dimeric conformations shape the development of the amyloid pathology
Results: Two specific conformations harboring the **A30N** and **V36N** mutations, significantly influenced the production of amyloidogenic assemblies. The A30N mutation promoted selective interaction with PS2-dependent γ-secretase, increased the Aβ42/Aβ40 ratio, and enhanced intracellular Aβ42 production, leading to elevated formation of pathological Aβ oligomers and higher-order assemblies. In contrast, the V36N mutation favored a less amyloidogenic profile, maintaining Aβ42 primarily in a non-aggregating monomeric state.
Conclusions: Together, these findings identify specific APP dimerization interfaces as molecular switches regulating presenilin-dependent cleavage, Aβ aggregation propensity, and toxic oligomer generation. Targeting APP dimerization emerges as a promising therapeutic strategy by modulating early, potentially reversible stages of AD pathology.

SAYRC-023

Aβ SEEDING INDUCED PYROPTOSIS ACTIVATION IN APP23 MICE

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Abstract

Objectives: Alzheimer's disease (AD) is characterized by the accumulation of amyloid-β peptide (Aβ) and abnormal phosphorylated tau proteins in the brain, leading to neurodegeneration. The prion-like seeding potential of Aβ aggregates has been well-documented in the brain. Several researchers confirmed that Aβ induces microglia activation, mediates inflammatory cytokines release and inflammasome activation, leading to caspase-1-dependent pyroptosis. In this study we aimed to investigate whether Aβ seeding in the brain contributes to the pyroptosis activation.
Methods: Human-derived brain lysates (n = 4 AD and n= 5 non-AD) or a PBS vehicle (n = 6) were stereotactically injected into the hippocampus of two-month old female APP23 mice. Pyroptotic activity was assessed by identifying Aβ plaques surrounded by triple-positive microglia (IBA1, GSDMD, and Caspase-1), indicating Aβ-associated pyroptotic microglia. For each mouse, scanned images of the hippocampal region were taken from both the injected and the non-injected (contralateral) hemisphere, which lacked Aβ plaques. The quantification included the number of Aβ plaques per hippocampus and the number of pyroptotic microglia associated with Aβ plaques.
Results: Only the group injected with AD brain lysates showed seedings with Aβ plaque deposition in the hippocampal region (one-way ANOVA, Dunnett's test: p=0,0405). Quantification revealed elevated pyroptotic microglial counts in AD-injected mice compared to PBS and non-AD controls (one-way ANOVA, Dunnett's test: p=0,0324).
Conclusions: In the brain, pyroptotic cell death is increasingly recognized as a driver of neuroinflammation and neurodegeneration in AD, and our findings further support its relevance in plaque-associated microglial dysfunction.

SAYRC-024

UNRAVELLING AMYLOID-INDUCED TAU PATHOLOGY IN ALZHEIMER'S DISEASE VIA TRANSCRIPTOMIC PROFILING OF MOUSE HIPPOCAMPAL BRAIN TISSUE

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Abstract

Objectives: Alzheimer's Disease (AD) is characterized by amyloid beta (Aβ) peptide accumulation and hyperphosphorylated tau aggregation, respectively, as Aβ plaques (A) and neurofibrillary tangles (T), and is associated with neurodegeneration (N), framed within an ATN axis. Evidence suggests a causal link between amyloid and tau pathology, initiating AD symptoms and neurodegeneration. However, the mechanisms by which Aβ drives tau pathology remain unclear. This study aimed to identify gene expression pathways and molecular targets involved in amyloid-induced tau pathology, using mouse models that recapitulate different aspects of AD pathology.

Methods: Bulk RNA sequencing was performed on hippocampal tissue from wild-type (F-T-), amyloid-only (5xFAD; F+), tau-only (TauP301S; T+), and amyloid-tau (5xFAD x TauP301S; F+T+) mice. Differential gene expression and pathway enrichment analyses were conducted to identify transcriptional changes associated with amyloid, tau, and their interaction.

Results: Bulk RNA sequencing revealed transcriptomic profiling differences among the various mouse models, recapitulating distinct aspects of AD pathology. F+T+ mice demonstrated gene expression changes not observed in F+ or T+ mice alone, suggesting a specific transcriptional response to the co-occurrence of amyloid and tau pathology. Several pathways were uniquely enriched in the amyloid and tau pathology combined mouse model. Notably, a subset of genes dysregulated in F+T+ mice may act as molecular mediators of amyloid-induced tau pathology. These findings highlight potential targets for disrupting the pathological cascade from amyloid to tau and tau-associated neurodegeneration.

Conclusions: These results yield detailed insights into the intricate interplay between amyloid and tau pathology in AD. The identification of specific genes and pathways involved in this process provides new insights into the molecular mechanisms of AD progression and contributes significantly to the development of innovative therapeutic interventions for halting tau-driven neurodegeneration.

SAYRC-025

THE INTERACTOME OF SOLUBLE AMYLOID-β IN HUMAN ALZHEIMER'S DISEASE BRAINS

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Abstract

Objectives: In Alzheimer's disease (AD), amyloid-β (Aβ) forms aggregates, ranging from soluble oligomers to insoluble plaques. There is currently limited knowledge about proteins interacting with soluble Aβ in human brain tissue, which is hypothesized to be relevant for initiation of Aβ deposition and/or Aβ toxicity. This study aimed to systematically characterize proteins interacting with soluble Aβ in human AD brain tissues.

Methods: Soluble Aβ interactors were assessed in human occipital cortex from symptomatic AD (n=3) and asymptomatic AD (n=6) cases using affinity purification mass spectrometry under an Aβ-specific binding condition (4G8 Aβ17–24 antibody) and a non-specific bead control. Proteins were classified as differentially enriched (DE; present in both conditions but enriched in antibody samples) or Aβ-exclusive (absent from bead controls). Candidate DE interactors were prioritized through differential analysis, whereas candidate Aβ-exclusive proteins were prioritized using unsupervised clustering, principal component analysis (PCA), ROC curve analysis, and a composite score integrating detection frequency, enrichment (median log2 intensity), and stability (MAD/median). In addition, Significance Analysis of INTeractome (SAINT) was applied to the full dataset to identify proteins preferentially

bound to Aβ. Functional enrichment was performed using the Database for Annotation, Visualization, and Integrated Discovery (DAVID). **Results:** The combined Aβ17-24 interactome consisted of 59 proteins, which were associated with mitochondrial and metabolic processes, CCT/TRiC chaperone complex, cytoskeletal remodeling, and synaptic plasticity. **Conclusions:** The soluble Aβ interactome comprises proteins associated with biological pathways previously implicated in AD, as well as non-amyloidogenic Aβ interactors spanning key neuronal systems. These findings indicate that soluble Aβ directly engages and perturbs multiple cellular networks central to neuronal homeostasis, supporting its contribution to AD pathophysiology.

SAYRC-026

EFFECT OF APOE AND MURINE APOE-CHRISTCHURCH MUTATION ON AMYLOID AND TAU PATHOLOGY IN ALZHEIMER'S DISEASE MICE

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Abstract

Objectives: To evaluate the impact of APOE and its variants on Alzheimer's disease (AD) pathology and the associated immune response. We systematically investigated how APOE and APOE variants influence amyloid and tau pathology, neurodegeneration, and microglial activation in preclinical models.
Methods: We generated *Apoe* knockout (KO) mice, *ApoeCh* (R146S), and mimic *Apoe3Ch*(R146S, R122C) mice, and crossed them with combined 5xFAD amyloidosis and PS19 tauopathy models (ATN). We used stereotaxic brain injections, immunohistochemistry, biochemical assays, and single-cell RNA sequencing to examine the APOE, APOECh, and APOE3Ch on pathological effects of AD.
Results: APOE deficiency led to diffuse plaques, reduced amyloid-induced tau pathology, and decreased disease-associated microglial (DAM) phenotypes in the ATN model. Ongoing studies are assessing the impact of APOEch on tau pathology and neurodegeneration in ATN mice, as well as the effects of APOE3Ch on amyloid burden, tau pathology, neurodegeneration, lipid/cholesterol metabolism, and microglial activation.
Conclusions: These findings demonstrate that APOE deficiency alters both amyloid and tau pathology while reducing DAM activation inATN models. Using our different models we aim to disentangle APOE-dependent functions and their contributions to AD pathology and progression in the ATN framework.

SAYRC-027

MOLECULAR BASIS OF IFITM3-DRIVEN AMYLOID-B PRODUCTION: A NOVEL INTERACTION WITH APP

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Abstract

Objectives: Dysregulation of amyloid β (Aβ) production and/or clearance initiates pathogenic cascades leading to Alzheimer's disease (AD). Interferon-induced transmembrane protein 3 (IFITM3), an innate immune protein upregulated during viral infections, has been identified as an endogenous interactor of γ-secretase (GSEC), and its overexpression increases Aβ production. The molecular basis of this modulation remains unclear. We aimed to elucidate how IFITM3 regulates Aβ generation and to characterize its interactions with key components of the amyloidogenic pathway.
Methods: IFITM3 overexpression and knockdown were performed in vitro to assess effects on Aβ production and γ-secretase processivity. Protein–protein interactions were analyzed by co-immunoprecipitation. Structure–function studies examined the role of the N-terminal GXXG motif implicated in IFITM3 dimerization. IFITM3 steady-state levels were evaluated in cells with varying amyloid precursor protein (APP) expression and following APP knockdown. IFITM3 expression was further analyzed in cortex samples from Down syndrome (DS) and DS-associated AD (DS-AD) patients and in Dp16 mice under conditions of APP gene dose reduction.
Results: IFITM3 overexpression increased Aβ levels without altering γ-secretase processivity nor product line preference, whereas IFITM3 knockdown did not reduce Aβ production. IFITM3 interacted with γ-secretase via presenilin and additionally with the mature, glycosylated form of APP. APP expression regulated IFITM3 steady-state levels in vitro. Elevated IFITM3 levels were observed in DS and DS-AD cortex and in Dp16 mice, and were normalized upon reduction of APP gene dosage. The GXXG motif was not required for the amyloid-promoting effect, supporting a role for monomeric IFITM3.
Conclusions: Our findings identify a previously uncharacterized interaction between IFITM3 and mature APP and suggest reciprocal regulation between these proteins. We propose that monomeric and dimeric IFITM3 coexist, andtheir equilibrium regulates Aβ production by modulating GSEC-APP enzyme-substrate interactions,linking innate immunity to amyloid pathology and altered APP gene dosage.

SAYRC-028

ROLE OF CONFORMATIONAL DYNAMICS OF PRESENILIN IN Γ-SECRETASE SUBSTRATE PROCESSING

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Abstract

Objectives: The γ-secretase (GSEC) complex, with presenilin (PSEN) as an active subunit, generates amyloid-β (Aβ) peptides through sequential cleavage of the amyloid precursor protein (APP). Mutation-driven impairments in this sequential proteolysis, arising from the destabilization of GSEC-Aβ complexes, cause Alzheimer's disease. The structural mechanisms controlling this stability and substrate processing remain unclear. Here, we combine computational and experimental approaches to define mechanistic principles of APP/Aβ processing by GSEC and to dissect potential differences between PSEN isoforms.
Methods: Molecular dynamics simulations were performed on PSEN1- and PSEN2-containing GSEC complexes. Key residues and regions were tested experimentally using site-directed mutagenesis and cell-based assays, followed by quantitative assessment of processivity by ELISA-based Aβ profiling or mass spectrometry. Structures of GSEC complexes were determined by cryo-EM.
Results: Molecular dynamics simulations reveal that TM6a of PSEN undergoes a conformational change in the absence of substrate near the catalytic site. This transition occurs more frequently in PSEN1- than in PSEN2-containing complexes. A similar TM6a conformation is observed in cryo-EM structures of PSEN1-GSEC bound to a small molecule in the absence of substrate, supporting the relevance of this state.
Conclusions: Our data supports a "tug-of-war" model for APP/Aβ processing. The polar ectodomain exerts an outward pulling force that destabilizes the GSEC–Aβ complex and promotes product dissociation, whereas inward pulling associated with substrate threading facilitates repositioning for subsequent cleavage. We propose that TM6a dynamics contribute to this inward movement and promote sequential substrate processing. The higher frequency of this transition in PSEN1 compared to PSEN2 suggests reduced efficiency in PSEN2, potentially contributing to isoform-specific differences in processivity. Together, these findings identify TM6a dynamics as a structural element linking PSEN conformational change to GSEC processivity.

SAYRC-029

ALZHEIMER'S DISEASE-ASSOCIATED PRESENILIN 2 INFLUENCES THE LIPIDIC PROFILE

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Abstract

Objectives: Alzheimer's disease (AD) is the most prevalent neurodegenerative dementia. Presenilins (PSs) 1 and 2, the catalytic subunits of γ-secretase, are key regulators of amyloid-β (Aβ) production and amyloid pathology. Mutations in PSs found in familial forms of AD (FAD) are thought to induce a partial loss-of-function effect. Pathogenic effects of PSs extend beyond Aβ production. PS2 in particular plays a critical role in mitochondrial function and cellular bioenergetics. Here, we investigate the role of PSs in cellular lipid metabolism.
Methods: The role of PS2 was studied using PS2 homozygous knock-out (-/-) mice. The effect of an FAD mutation was studied using a transgenic mouse model expressing the human PSEN2 gene carrying the N141I mutation on a murine PS2 heterozygous knock-out (+/-) background (PS2N141I). WT and PS2 -/- primary neurons, as well as PS2 +/- and PS2N141I primary neurons, were cultured in a lipid-deficient medium, with or without supplementation with a lipid concentrate. Cellular lipidic profile was assessed using the PhenoVue Nile Red lipid stain.
Results: PS2 deletion was associated with a decreased lipidic content, which could not be rescued by exogenous lipid supplementation. Interestingly, the N141I mutation showed the same deficit. However, in this case, lipid levels were restored upon lipid concentrate addition.In parallel, RNA-Sequencing results identified Gbf1, a gene involved in vesicular trafficking, as a downstream target of PS2, with consistent downregulation at the mRNA and protein levels in PS2 -/- and PS2N141I models.
Conclusions: These findings support a key role for PS2 in lipid homeostasis, with a partial loss-of-function effect of the N141I mutation. The identification of Gbf1 as a downstream target of PS2 is particularly compelling, given its emerging role in recruiting enzymes involved in lipid metabolism. Ongoing studies using siRNA approaches aim to directly link Gbf1 downregulation to the altered lipid profile observed in these models.

SAYRC-030

INTEGRATING AGING INTO MODELS OF ALTERED PLD3 FUNTION TO ELUCIDATE BRAIN CIRCUIT AND ALZHEIMER'S DISEASE VULNERABILITIES

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Abstract

Objectives: Phospholipase D3 (PLD3) is a lysosomal exonuclease that mainly degrades mitochondrial DNA. Loss-of-function triggered by late-onset Alzheimer's disease (LOAD)-linked variants causes an autophagic/lysosomal catabolic bottleneck[1]. Spatial transcriptomic data now reveals a higher PLD3 expression in specific brain areas including the hypothalamus and hippocampus. How altered PLD3 expression herein affects these brain circuits is currently unknown. We first examined PLD3 loss-of-function in neuronal models lacking aging signatures. Building on this, we now aim to assess its impact in age-relevant neurons derived from directly converted fibroblasts.
Methods: We generated a PLD3 knockout mouse and crossed it with $APP^{N\text{-}L\text{-}GF}$ knock-in mice. Histological analyses were performed at 3, 6, and 9 months, with 6 months used for electrophysiology and ultrastructural electron microscopy (EM). *In vivo* data were complemented by studies on primary hippocampal cultures and iPSC-derived neurons from AD patients. We examined molecular pathways including STING signaling, lysosomal homeostasis, and transcriptomics to uncover mechanisms driving synaptic alterations.
Results: $APP^{N\text{-}L\text{-}GF}$xPLD3KO mice showed an altered amyloid plaque pathology from 6 months onwards. We replicated the observed lysosomal and autophagic pathology[1] in primary mouse neurons and in human-derived neurons. MEA-recordings of CA3 long-term potentiation showed that PLD3 deficiency significantly lowered the amplitude, corresponding to the observed synaptic alterations on EM.
Conclusions: We link lysosomaldysfunction caused by altered PLD3 activity to disrupted hippocampal circuit function, a region relevant to clinical AD features. Ongoing work aims to integrate aging phenotypes into these modelstobetter capture the complexity of LOAD.
Ref. 1 Van Acker, Nat Commun.(2023) doi:10.1038/s41467-023-38501-w

SAYRC-031

MIRNAS IN ALZHEIMER'S DISEASE: EXTENDING PROFILING DATA TO PROBE THEIR PATHOLOGY ASSOCIATION AND REGULATORY FUNCTIONS

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Abstract

Objectives: Alzheimer'sdisease(AD) leads to memory loss and cognitive decline in the elderly, with no cure currentlyavailable.Uncovering new treatments is crucial, and microRNAs (miRNAs), which are dysregulated in AD and have multifaceted targeting, represent one of the promising candidates for therapeutic intervention.
Methods: We analyzed miRNA expression in post-mortem brain tissue from the superior temporal T1 isocortex of AD patients (n=19; Braak stages V–VI) and controls (n = 11), as well as the hippocampus of AD patients (n=14;Braakstages IV–VI) and controls (n = 5; Braak stages I–II), using next-generation sequencing (NGS). Differentially expressed miRNAs were validated by RT-qPCR. We further examined associations between miRNA expression and tau pathology, assessed the modulatory effects of specific miRNAs on tau seeding in vitro, and investigated their functional roles in cellular models.
Results: We profiled differentially expressed miRNAs in the temporal cortex and hippocampus from post-mortem brains (ADvs.controls). In the temporal cortex, we identified six significantly downregulated miRNAs(miR-132-3p, miR-132-5p, miR-212-3p, miR-129-5p, miR-129-1-3p, and miR-542-3p), where as in the hippocampus, an early affected region, we did not find any statistically significant miRNAs. However, these six miRNAs identified in the cortex showed similar downregulated expression trends in the hippocampus. Notably, members of the miR-132 and miR-129 families were associated with taupathology (with neuropathological Braak stages and with biochemically quantified tau phosphorylationlevels) and were consistently altered in both regions. In tau seeding assays in vitro, miR-146b-5p and miR-151a-5p, another two differentially expressed miRNAs in the temporal cortex, significantly modulated tau seeding in a tau biosensor HEK-FRET cell model; however, miR-132 and miR-129 had no effect. In cellular models (HEK-FRET, HT22, and N2a), biogenesis and regulation of miR-132 and miR-129 were independent of each other. In N2a cells, miR-129-5p increased tau expression levels and showed neuronal differentiation properties, in contrast to miR-132-3p.
Conclusions: Our study provides insights into miRNA dysregulation in AD, highlighting the involvement of specific miRNAs in the progression of tau pathology and their regulatory effects on tau-related mechanisms.

SAYRC-032

INVESTIGATING COMPARTMENT-SPECIFIC RNA MISLOCALIZATION DRIVEN BY ALTERNATIVE POLYADENYLATION IN TDP-43 PROTEINOPATHIES

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Abstract

Objectives: Cytoplasmic mislocalization and aggregation of nuclear RNA-binding protein TDP-43 is a major pathological hallmark of Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). TDP-43 regulates different steps of RNA metabolism, including alternative polyadenylation (APA) at the 3'UTR of mRNAs, a key process that modulates transcript stability, localization and translation. Disease-related 3'UTR lengthening or shortening of TDP-43-bound transcripts have been reported in postmortem ALS/FTD brains with TDP-43 proteinopathy and in TDP-43-depleted neuronal cultures[3-5]. Beyond its nuclear role, TDP-43 is also present in axons, where its loss impairs mRNA transport and local translation. Whether TDP-43-dependent APA changes in neurons impact mRNA subcellular localization, and thus axonal transcriptome, remains unknown. We aim to characterize compartment-specific APA events in iPSC-derived cortical neurons and determine whether TDP-43-dependent APA dysregulation could lead to alterations in mRNA distribution in axons.
Methods: iPSC-derived cortical neurons were cultured in microfluidic devices, to isolate pure axons, and transduced with TDP-43 shRNA-expressing lentivirus to model TDP-43 loss-of-function. Neuronal and axonal transcriptomes were profiled using SMART-Seq2, and data analysis is ongoing to validate previously reported APA events linked to TDP-43 silencing, and identify novel axon-specific events. These APA events will be validated using real-time quantitative PCR (RT-qPCR) with primers targeting distinct 3'UTR lengths.
Results: We validated the purity of extracted human axonal transcriptomes with RT-qPCR by the absence of somatic markers. Lentiviral-mediated TDP-43 silencing was optimized, achieving robust downregulation of TDP-43 at transcript and protein level. RNA-seq analysis of compartment-specific transcriptomes revealed the presence of previously reported APA events, which were further validated with RT-qPCR, as well as *de novo* APA events.
Conclusions: Our findings provide a platform to study compartment-specific APA events upon TDP-43 pathology including nuclear loss-of-function. Future work will determine whether TDP-43-dependent APA dysregulation could underlie axon demise and neuronal vulnerability in TDP-43 proteinopathies.

SAYRC-033

A NOVEL ROLE FOR PRESENILIN-2/ɣ-SECRETASE IN NEURONAL ORGANELLE HOMEOSTASIS: LINK TO ALZHEIMER'S PATHOGENESIS

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Abstract

Objectives: Mutations in presenilin-2(PSEN2) cause familial Alzheimer's disease (FAD), yet the molecular mechanisms remain poorly understood. Endolysosomal and mitochondrial dysfunctions are early features of Alzheimer's disease (AD),preceding β-amyloid deposition and neuronal loss. However, these processes have largely been studied inisolation. Here, we uncover a role for PSEN2 in regulating neuronal organelle homeostasis through its restricted localization to late endosomes/lysosomes (LE/Lys). Beyond their degradative function,LE/Lys actas hitchhiking platforms for RNA granules and are emerging regulators of localized protein synthesis in axons.
Methods: We analyzed axonal compartments in iPSC-derived human neurons (iNeurons) with PSEN2 knockout (PSEN2KO) or an FAD-linked mutation (FADPSEN2). We performed super-resolution and electron microscopyon axons to study endolysosomal and mitochondrial homeostasis in both live and fixed cells (e.g.organellar dynamics or morphology) and analyzed the axonal transcriptomes.
Results: PSEN2 disruption impairs LE/Lys trafficking, resulting in significantly fewer lysosome-mitochondria contacts. Potentially, triggering downstream mitochondrial dysfunction and morphologic alalterations, as observed by electronic microscopy. Transcriptomic analysis of isolated axons revealed over 500 upregulated transcripts in PSEN2KO and FADPSEN2axons compared to wild-type, with mitochondrial-related processes among the top enriched gene ontology terms. This was accompanied by elevated local protein synthesis, consistent with the transcriptomic profile. Interestingly, some of these translation events colocalized with LE/Lys–mitochondria contact sites
Conclusions: We hypothesize that reduced lysosome–mitochondria contacts, may impair the transfer of newly synthesized proteins into mitochondria. The increase in translation may represent a compensatory response to sustain mitochondrial protein supply at axons, since efficient delivery is hindered by the reduced number of contacts. To conclude, we propose that endolysosomal dysfunction, a nearly hallmark of AD, may additionally drive downstream translation and mitochondrial defects, offering new insights into AD pathogenesis.

SAYRC-034

DESIGNED AND PATHOLOGICAL TDP-43 ALS AND MYOPATHY VARIANTS: AGGREGATION, NEURO- AND MYOTOXICITY SCREENING IN DROSOPHILA MELANOGASTER AND CELLULAR MODELS

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Abstract

Objectives: A hallmark in neurodegenerative diseases is the accumulation of misfolded proteins, leading to cellular toxicity and eventual neurodegeneration. In ALS, TDP-43 mislocalizes to the cytoplasm resulting in neuronal TDP-43 postive inclusions. Pathological mutations in the TARDBP gene are predominantly clustered in the C-terminal prion-like domain, a region crucial for aggregation propensity and liquid–liquid phase separation. Most mutations in TARDBP-gene are neuropathological. However, recently, some mutations have been linked to rimmed vacuole myopathies, without neuropathy. These TDP-43 variants differ in prion-like amino acid composition (PLAAC) scores, with myopathy-linked variants predicted to have the strongest aggregation potential. Therefore, we hypothesise that in neurons, highly aggregating variants remain cytoplasmic, allowing WT TDP-43 to function normally in the nucleus. In muscle cells, cytoplasmic accumulation of strongly aggregating variants is predicted to be highly toxic, explaining myopathic phenotypes. By contrast, many ALS-associated variants, with lower PLAAC scores, enter the nucleus and interact with WT TDP-43 exerting a dominant-negative effect and introducing neurotoxicity.
Methods: *Drosophila* expressing different TDP-43 variants will be phenotypically assessed. In parallel, cell models co-transfected with WT and mutant TDP-43 will be used to examine co-localization, subcellular distribution, and aggregation dynamics with confocal microscopy.
Results: In *Drosophila*, ALS variants exhibit more severe neurotoxic phenotypes than myopathy variants, such as developmental defects. The co-localization experiments show that the myopathy-linked variants have impaired nuclear translocation, with increased nuclear WT localization. ALS-associated variants co-localize to both the cytoplasm and nucleus, together with WT TDP-43.
Conclusions: Our data suggest that variant-specific aggregation and localization of TDP-43 determine whether pathology manifests in muscle or neurons. ALS-linked variants disrupt nuclear TDP-43 function, leading to neurotoxic outcomes, whereas myopathy-linked variants predominantly remain in the cytoplasm, allowing WT TDP-43 to function normally in the nucleus.

SAYRC-035

A KETOGENIC APPROACH IN THE NEW APPNL-G-F/MAPT MOUSE MODEL OF ALZHEIMER'S DISEASE: CHARACTERISATION OF DIETS IN MALES AND FEMALES

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Abstract

Objectives: Glucose brain metabolism impairment is one of the earliest hallmarks of Alzheimer's disease (AD). It starts to decrease several years before the first clinical symptoms. A new hypothesis states that high consumption of fructose, could contribute to glucose hypometabolism. On the contrary, ketone body metabolism in the brain remains intact in early AD. A ketogenic diet (KD) could then be useful to counteract glucose hypometabolism by enhancing brain energetic metabolism through the production of ketone bodies. This study aims to investigate the effect of both an early KD and a high fructose diet on the development of AD in male and female APPNL-G-F/MAPT mice.
Methods: A KD and a high fructose diet were introduced at 3 months of age for 3-4 months against a control chow. Brains were harvested to test synaptic activity and plasticity in the hippocampus by slice electrophysiology, metabolomics in cortex by 1H-NMR and neuroinflammation by cytokine quantification in the hippocampus and anterior cortex. Both males and females were used in this study.
Results: At 6 months of age, there was clear neuroinflammation in the APPNL-G-F/MAPT model with an increase in IL-1β, IL-6 and TNF-α among others. There were no differences in long-term potentiation but a slight deficit in short-term plasticity was observed in the AD model. Males also showed a higher excitability than females, independently of the strain. 1H-NMR revealed an increase in unsaturated fatty acids, lactate, succinate and glutamine in AD mice compared to wild-type mice but in a sex-specific manner. Importantly, the KD reduced the amount of TNF-α and KC/GRO in the hippocampus and the cortex respectively.
Conclusions: There were not many deficits in the APPNL-G-F/MAPT model at 6 months of age but there are indeed important sex differences to consider. The KD showed promising results by managing to reduce neuroinflammation.

SAYRC-036

PRESENILIN-2 INTERACTS WITH LAMTOR1 AT LATE ENDOSOME/LYSOSOME-ENDOPLASMIC RETICULUM CONTACT SITES TO GUARD ENDOLYSOSOMAL HOMEOSTASIS

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Abstract

Objectives: Mutations in Presenilin-2 (PSEN2), a catalytic subunit of γ-secretase, cause familial Alzheimer's disease (FAD). Beyond its role in β-amyloid generation, we hypothesized that PSEN2 sustains essential endolysosomal functions whose disruption contributes to early neuronal vulnerability. Given the preferential localization of PSEN2/γ-secretase to late endosomes and lysosomes (LE/Lys), we investigated its role in endolysosomal homeostasis and inter-organelle communication, processes increasingly implicated in neurodegenerative disorders including Alzheimer's disease (AD).
Methods: We performed proximity-dependent biotinylation using APEX2-tagged PSEN2 in PSEN-deficient cells to map its spatial interactome. Organelle-associated candidates were validated through biochemical, imaging, and functional analyses in PSEN2-deficient and FAD-linked mutant models.
Results: Lamtor1, a dual regulator of lysosomal positioning and mTORC1 signaling, emerged as a specific PSEN2 interactor. Super-resolution microscopy localized PSEN2-Lamtor1 complexes to LE/Lys-ER membrane contact sites (MCSs), positioning PSEN2 at hubs coordinating organelle dynamics and nutrient sensing. Altered PSEN2 expression disrupted Lamtor1-dependent signaling, promoting aberrant BORC recruitment to LE/Lys and driving their peripheral repositioning. This redistribution was accompanied by increased mTORC1 recruitment at LE/Lys, elevated basal mTORC1 activity, and loss of nutrient responsiveness. These phenotypes were recapitulated in primary hippocampal neurons, where PSEN2 deficiency or FAD mutation caused enlarged LE/Lys, altered axonal transport, and abnormal accumulation of mTORC1-positive LE/Lys at growth cones.
Conclusions: These findings identify PSEN2 as a regulator of endolysosomal dynamics and inter-organelle communication. Disruption of LE/Lys-ER contact site integrity may represent an early vulnerability pathway in AD, linking PSEN2 dysfunction to endolysosomal collapse. Given that endolysosomal and mTORC1 alterations are common features of sporadic AD, this mechanism may extend beyond genetically-defined FAD and represent a broader pathogenic axis.

SAYRC-037

AMYLOID-B ENGAGES PERK-ATF4 SIGNALLING DURING PATHOLOGICAL TAU PHOSPHORYLATION IN HUMAN NEURONS

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Abstract

Objectives: Alzheimer's disease is a progressive neurodegenerative disease characterized by amyloid beta aggregation, Tau hyperphosphorylation and neuronal loss. Among these pathological features, the misfolding and propagation of abnormal Tau species show the strongest correlation with cognitive decline. However, the mechanism by which Tau pathology is initiated and subsequently amplified in the human brain remain poorly understood, limiting the development of effective disease modifying therapies.
Methods: Here, we establish an in vivo human model of amyloid driven Tau pathology using xenografted human cortical organoids without additional genetic manipulation.
Results: Spatial transcriptomics and single cell RNA sequencing demonstrate the formation of canonical layer-like cortical architecture and good neuronal maturation within the grafts. Upon exposure to pathological Aβ, transplanted organoids develop extensive extracellular amyloid accumulation at the graft border regions. This is accompanied by a pronounced loss of human neurons and the emergence of multiple hyperphosphorylated Tau epitopes following implantation. Transcriptomic profiling reveals a prominent induction of oxidative and proteostatic stress responses, with activation of the PERK ATF4 signalling pathway. Pharmacological interference with PERK dimerization using GSK2606414 is associated with a partial reduction of Tau hyperphosphorylation across multiple pTau epitopes.
Conclusions: Together, these findings demonstrate that sustained amyloid exposure is sufficient to induce core Alzheimer's disease neuropathological features in human neurons in vivo and implicate PERK ATF4 signalling as a stress responsive pathway that contributes to Tau hyperphosphorylation during disease progression.

SAYRC-038

MULTI-OMICS PROFILING REVEALS MOLECULAR PATHWAYS INVOLVED IN RNF216-MEDIATED NEURODEGENERATION

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Abstract

Objectives: Bi-allelic loss-of-function mutations in RNF216, encoding an E3 ubiquitin ligase, cause a rare recessive neurodegenerative disorder (ND) characterized by early-onset dementia, white matter lesions, movement abnormalities, and hypogonadotropic hypogonadism. NDs often share clinical, genetic, and neuropathological features, suggesting common mechanisms. Beyond this rare disorder, RNF216 is implicated in other NDs: rare damaging variants are enriched in early-onset Parkinson's disease, decreased RNF216 expression is observed in Alzheimer's disease, and it may mediate tau aggregation[1,2]. Patients with RNF216-mediated neurodegeneration also show ubiquitin- and p62-positive intranuclear inclusions, further linking RNF216 dysfunction to broader neurodegenerative processes. Our objective is to elucidate the molecular pathways regulated by RNF216 to uncover mechanisms relevant to both rare and common NDs.
Methods: The main objective of this project is to unravel the molecular pathways involving RNF216-mediated neurodegeneration. To achieve this, we employed a proximity-dependent biotinylation assay to identify RNF216 interaction partners. In parallel, we applied a multi-omics approach: antisense oligonucleotides (ASOs) were used to generate RNF216 knockdown (KD) cells for both transcriptomic and proteomic profiling. The transcriptomic analysis was further extended to include splicing analysis.
Results: RNF216 forms nuclear speckles and interacts with 159 partners, suggesting nuclear hubs involved in splicing, transcription, and chromatin organization. Loss of RNF216 disrupts gene and protein expression, downregulating cytoskeletal organization, cell adhesion, intracellular trafficking, vesicle transport, and autophagy, while upregulating transcription, splicing, chromatin processes, and cell cycle, likely reflecting compensatory responses. RNF216 KD also alters splicing, particularly skipped exons and retained introns. Notably, 35 proteins in our downregulated list after RNF216 KD are known to be down in AD.

Conclusions: RNF216 acts as a multifunctional hub coordinating transcription, nuclear RNA processing, chromatin dynamics, ubiquitin signaling, and cytoplasmic organization. Its loss disrupts cellular homeostasis and connects rare and common neurodegenerative processes.

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SAYRC-039

RESTORING THE NEURON-OLIGODENDROCYTE UNIT IN ALZHEIMER'S DISEASE: A CENTRAL ROLE FOR PDE4D SIGNALLING

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disorder classically defined by the accumulation of amyloid-β (Aβ) plaques and tau tangles. Recent findings, however, suggest that early white matter alterations, impaired adaptive myelination, and oligodendrocyte dysfunction play a key role in AD pathogenesis. The dynamic interaction between oligodendrocytes and neurons, essential for signal conduction, metabolic support, and plasticity, is increasingly recognized as a critical component of memory function and is profoundly disrupted in AD. Strategies aimed at restoring this oligo-neuron unit by promoting both neuro-regeneration and remyelination could therefore provide a novel therapeutic avenue.

The second messenger cAMP regulates essential regenerative processes such as neurite outgrowth, synaptic plasticity, and oligodendrocyte maturation. Intracellular cAMP levels are tightly controlled by phosphodiesterases (PDEs), of which the PDE4D family plays a prominent role in the central nervous system. Our previous work demonstrates that specific PDE4D subtype inhibition enhances oligodendrocyte differentiation and neurite growth. Distinct PDE4D isoforms mediate the observed cellular effects: long PDE4D isoforms (e.g. PDE4D3/5/7/9) drive neuronal plasticity, while short and super-short isoforms (e.g. PDE4D1/2/6) promote OPC differentiation and myelination.

Consistent with a central role of cAMP-driven trophic support, PDE4D inhibition in iPSC-derived OPCs upregulates neurotrophic and pro-regenerative gene programs, supporting its remyelinating and neuro-restorative potential. Transcriptomic profiling under Aβ stress further highlights molecular vulnerabilities and repair entry points: in human iPSC-OPCs, Aβ exposure enriches molecular functions related to (sphingo)lipid transfer, calcium binding, ion-channel modulation, and extracellular matrix regulation, while Aβ-treated mature iPSC-neurons show enrichment in cytoskeletal binding, phosphatase activity, and calcium-associated interactions. Importantly, iPSC-derived OPCs and neurons display PDE4D isoform expression patterns closely mirroring those of primary human brain cells, reinforcing their validity as translational disease models. By further dissecting the molecular mechanisms underlying PDE4D-mediated repair, this work aims to uncover more downstream targets that can strengthen the neuron–oligodendrocyte unit in AD.

SAYRC-040

TMEM106B HAS A ROLE IN THE LYSOSOMAL–ER CROSSTALK AND DENDRITIC SPINE DEVELOPMENT

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Abstract

Objectives: *TMEM106B* was initially identified as a major genetic modifier for FTLD-TDP in *GRN* mutation carriers and has since been implicated in multi pleneurodegenerative diseases (AD, LATE, PD) and aging. The risk haplotype correlates with elevated TMEM106B protein levels, which has gained extreme relevance with the latest findings showing that the C terminal domain of TMEM106B can be shed and form amyloid-like fibrils. This highlights the need to understand its function and cleavage mechanisms.

Methods: We generated *TMEM106B* knockout (*TMEM106B*$^{-/-}$) iPSC-derived neurons and studied them *in vitro* and via NPC xenografts in *Rag2*$^{-/-}$mice. Lysosomal health was assessed by imaging, ICC, and enzymatic assays; neuronal health by proteomics, interactomics, neurite outgrowth analysis and multielectrode array (MEA) recordings. Morphology and lysosomal content of the transplanted neurons was examined by IHC at 9 months post transplantation.

Results: *TMEM106B*$^{-/-}$neurons showed normal lysosomal trafficking but increased Cathepsin D activity and soma-localized lysosomes, confirmed in xenografts. Interestingly, proteomics revealed reduced synaptic proteins and increased extracellular contact proteins, which was functionally confirmed by increased neurite outgrowth but defective network bursting upon*TMEM106B* loss-of-function. Most strikingly, transplanted*TMEM106B*$^{-/-}$ neurons exhibited longer, less complex neurites and lacked mature dendritic spines. To understand the link between the lysosomal phenotypes and the impact in synapses, we analyzed the TMEM106B interactome, which suggested roles in lysosome–ER contacts and/or local translation (i.e. ribosomal subunits, ER proteins and RNA binding proteins).

Conclusion: We identify TMEM106B as a regulator of dendritic spine development and maintenance, linking it directly to synaptic health. Further studies will include confirming the implication of TMEM106B in lysosome-ER contacts or with local translation.

SAYRC-041

THE PLD3-STING-STIM1 AXIS LINKS LYSOSOMAL DYSFUNCTION TO SYNAPTIC FAILURE AND MEMORY IMPAIRMENT IN ALZHEIMER'S DISEASE

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Abstract

Objectives: Phospholipase D3 (PLD3) is a neuronal lysosomal exonuclease linked to late-onset Alzheimer's disease (AD). By degrading mitochondrial DNA, PLD3 maintains lysosomal integrity and prevents activation of the cGAS-STING pathway (Van Acker et al., Nat.Comm. 2023), yet its mechanistic contribution to neuronal dysfunction and AD etiopathogenesis remained unclear.
Methods: A knockout mouse model was generated to investigate PLD3 function *in vivo*. Neuronal lysosomal integrity, calcium signalling, and gene expression changes were assessed, alongside analyses of synaptic structure and function in hippocampal circuits. Behavioural testing evaluated memory performance. Pharmacological inhibition of STING was used to examine the potential for rescuing cellular, synaptic, and cognitive deficits.
Results: PLD3 deficiency impaired mitochondrial DNA clearance, causing lysosomal leakage, cholesterol and APP-CTF accumulation, and cGAS-STING activation. RNA-seq revealed downregulation of synaptic plasticity and glutamatergic signalling genes, involving altered calcium regulators such as STIM1; linking lysosomal stress to disrupted calcium homeostasis. Co-immunoprecipitation revealed that STING binds STIM1 and that this interaction is reduced in PLD3 KO neurons, leading to enhanced store-operated calcium entry and impaired axonal lysosomal transport. These molecular changes converged at hippocampal mossy fiber-CA3 synapses, producing smaller synapses, shorter postsynaptic densities, increased vesicle heterogeneity, impaired long-term potentiation, as well as memory deficits (6 months old gender-balanced cohort). Pharmacological STING inhibition restored STING-STIM1 dynamics, rescued lysosomal motility and synaptic transmission, and improved spatial memory.
Conclusions: By uncovering the crosstalk between PLD3 and STING-STIM1 signalling, we provide mechanistic insight into how lysosomal dysfunction can impact Ca^{2+}regulation that may drive synaptic and cognitive impairments in the absence of overt extracellular amyloid pathology. Future studies should address how PLD3 interacts with other lysosomal AD risk genes and whether its modulation could offer synergistic benefits in combination therapies.

SAYRC-042

NEURON-SPECIFIC NANOPORE METHYLOME PROFILING REVEALS NOVEL TARGETS IN ALZHEIMER'S DISEASE

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Abstract

Objectives: Alzheimer's disease (AD) is the leading cause of dementia, yet cell type-specific epigenetic signatures in AD and its prodromal stage, Mild Cognitive Impairment (MCI), remain largely unexplored. This study aimed to identify neuron-specific DNA methylation and hydroxymethylation changes, integrating these epigenetic signatures with transcriptional data from the same neuronal population to identify novel disease-related gene targets.
Methods: We performed neuron-specific methylation and hydroxymethylation analysis using post-mortem middle frontal gyrus tissue from a large cohort (CTL n=66, MCI n=43, AD n=59). Neuronal nuclei were isolated via NeuN-based fluorescence-activated nuclei sorting. Oxford Nanopore reduced representation methylation sequencing allowed for the assessment of over 7 million CpG sites.
Results: Using the Limma weighted least squares analytical pipeline, we identified differentially methylated regions (DMRs; CTL-AD: 15, CTL-MCI: 8, MCI-AD: 19) and differentially hydroxymethylated regions (DhMRs; CTL-AD: 9, CTL-MCI: 3, MCI-AD: 13). Further target selection was based on DMR significance, gene set enrichment results, overlap between AD and MCI vs CTL, neuronal expression levels, and regulatory functions of the DMR. Gene expression of 15 target genes was quantified using qPCR on RNA derived from the same neuronal nuclei. Three target genes, i.e. *SYNJ2*, *SATB2,* and *TRIM16*, showed reduced expression in AD neurons.
Conclusions: This is the first epigenetic implication of *SYNJ2*, *SATB2,* and *TRIM16* in AD. Our findings demonstrate the importance of cell type-specific epigenomic analysis for identifying disease-related methylation patterns that are masked in bulk tissue analysis. Future work includes CRISPR-dCas9-mediated epigenetic editing in neuronal cultures to assess the causal contribution of these methylation changes.

SAYRC-043

ELECTROPHYSIOLOGICAL MAPPING OF HUMAN STRIATAL MICROCIRCUITS ENABLES PARKINSON'S DISEASE STRATIFICATION

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Abstract

Objectives: Different molecular alterations lead to neuronal network defects converging into the motor symptoms of Parkinson's disease. While symptoms alone do not reveal the underlying molecular etiology, the affected circuits may encode characteristic functional signatures that enable mechanistic stratification of the disease.
Methods: We built human striatal microcircuits on high-density multielectrode arrays comprising human induced pluripotent stem cell-derived cortical, medium spiny and nigral dopamine neurons.
Results: We demonstrate that neurons establish appropriate connectivity, with glutamate driving network activity and dopamine modulating neuronal excitability. We then generated two series of isogenic circuits carrying *LRRK2* and *GBA* mutations associated with disease. Predictive modeling revealed mutation-specific electrophysiological signatures, enabling accurate (>85%) genotype-specific classification.
Conclusions: These findings show that *LRRK2* and *GBA* mutant circuits display early and distinct defects, and combining stem cell-based circuit models with AI-powered computational approaches enable to connect molecular etiologies of disease with motor circuit dysfunction. Our work thus enables to stratify disease beyond clinical symptomatology.

SAYRC-044

MODELING HUMAN BRAIN MATURATION USING 3D CORTICAL ORGANOIDS

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Abstract

Objectives: Human brain maturation extends from late embryonic development until early adolescence[1]. Previous mouse models have contributed to the body of knowledge on brain maturation, however, there are species-specific differences between human and mouse brain development[2,3].
Methods: Here, we aim to study human brain maturation from embryonic to early postnatal life through a long-term human pluripotent stem cell-derived human brain organoid model (hBOs) up to 6-9 months *in vitro*.
Results: Our immunostainings and transcriptomic data show a time-dependent appearance of neuronal and glial subtypes, similar to the *in vivo* human brain. Comparative analysis between mid-stage (3.5 months) and early stage (1 month) hBOs showed transcriptional shifts consistent with early neuronal maturation, including upregulation of synaptic and cytoskeletal pathways and downregulation of cell cycle-related genes associated with progenitor populations. Moreover, our immunostainings late stage hBOs show the presence of mature neuronal features such as expression of synaptic proteins and presence of synapses, complex mature neuronal dendritic trees, and functional changes in axonal transport and activity patterns in the late stage. We also show the involvement of alternative splicing as an instrumental step in human brain maturation. Among these transcriptomic changes, long-term hBOs exhibit differences in TAU isoform expression, which mimic what is observed in the in vivo human newborn brain.
Conclusions: Thus, our long-term hBO model mimics brain maturation features present in the late stages of human brain development and as such it may be a useful model to study human brain maturation in a dish.

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SAYRC-045

WHEN MYELIN FAILS: GREY MATTER VULNERABILITY IN ALZHEIMER'S DISEASE

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Abstract

Objectives: Alzheimer's disease (AD) is characterized not only by amyloid-β plaques and tau neurofibrillary tangles, but also by alterations in non-neuronal cell types essential for neuronal support. Oligodendrocytes and their myelin sheaths play a central role in maintaining axonal function, yet detailed profiling of myelin dynamics in the human AD brain remains limited. Although neuroimaging studies increasingly highlight white matter degeneration as an important contributor to AD pathophysiology, the status of myelin within cortical grey matter is less well understood.
Methods: In this study,we examined myelin integrity and oligodendrocyte dynamics in the middle temporal gyrus (MTG) of post-mortem tissue from control and AD cases (n = 15) to track myelin alterations and relate them to classical neuropathological markers.
Results: Our results reveal a compartment-specific vulnerability of cortical grey matter myelin in AD, accompanied by an active yet likely insufficient regenerative response. The grey-matter-restricted changes and their strong associations with tau pathology suggest that myelin disruption in AD is closely tied to local neuronal and synaptic dysfunction.
Conclusions: These findings position oligodendrocyte dynamics within cortical grey matter as a critical, previously underrecognized component of AD progression.

SAYRC-046

EFFECTS OF HEARING LOSS ON THE HIPPOCAMPUS, HIPPOCAMPAL NEUROGENESIS, AND COGNITIVE DECLINE: A SCOPING REVIEW OF PRECLINICAL ANIMAL STUDIES

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Abstract

Objectives: A literature review was carried out to systematically evaluate the effects of hearing loss on hippocampal neurogenesis and cognitive decline.
Methods: Following PRISMA methodology, an initial PubMed and Web of Science search was performed on October 28, 2025, to review preclinical animal studies examining the effects of hearing loss on the hippocampus and cognition. Data extraction was carried out in accordance with the Animal Research Reporting of In Vivo Experiments (ARRIVE) guidelines, and a risk of bias assessment was performed using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) criteria.
Results: Of the 2,818 records screened, 40 studies met the inclusion criteria. All included studies employed experimental animal models. Preliminary results suggest a link between hearing loss, hippocampal neurobiological alterations, and cognitive deficits.
Conclusions: Outcomes of this systematic review give more insight into the link between auditory dysfunction, altered hippocampal neurogenesis and cognitive decline, offering new perspectives on shared mechanisms underlying hearing loss and cognitive decline.

SAYRC-047

UNRAVELING THE LINK BETWEEN CIRCADIAN RHYTHM-NEURAL ACTIVITY AND ALZHEIMER'S DISEASE WITH HUMAN CORTICAL ORGANOID MODELS

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Abstract

Objectives: Alzheimer's disease (AD) is the most common form of dementia among the elderly. Disruption of the circadian rhythm (CR) can occur early during AD progression and further, may control neural activity patterns, amyloid beta (Aβ) and Tau-phosphorylation levels in the brain. However, the mechanisms underlying the link between CR, neural activity patterns and AD in specific brain cell types are still unknown.

Methods: This project investigates how cellular CR contributes to AD pathogenesis using human cortical organoids (hCOs) derived from induced pluripotent stem cells (hiPSCs).

Results: We demonstrate the presence of CR in in vitro hCOs, attested by the oscillatory expression of CR modulators. Further, pharmacological activation of cholinergic and noradrenergic receptors led to increased c-Fos expression and calcium dynamics in hCOs following 6 hours agonist exposure, with basal levels recovered 5-day post-exposure, suggesting an increase in neural activity in hCOs. Interestingly, this treatment increased the clearance rate of soluble Aβ from hCOs medium, suggesting that neural activity may promote Aβ degrading mechanisms in the cell. Next, to assess alterations in neural rhythms and molecular pathways in AD brain cells, we generated hCOs from familial AD (FAD) patient cells. FAD hCOs showed increased c-Fos+ cells located around Aβ aggregates, suggesting local hyperactivity. Interestingly, most c-Fos+ cells had an astrocyte identity, highlighting a potential early role for astrocytes in AD.

Conclusions: These observations suggest that CR-neural activity alterations may be an early mechanism in AD pathogenesis, highlighting that modulation of CR-neural activity patterns may be a promising target for preventive and therapeutic AD strategies. Further, hCO models could be a useful tool to study the link between CR-neural activity and AD.

SAYRC-048

THE EFFECT OF NEURODEGENERATION ON ULTRASONIC VOCALISATIONS (USV) AND THEIR NEURONAL SUBSTRATESIN MICE AND RATS: A SYSTEMATIC REVIEW

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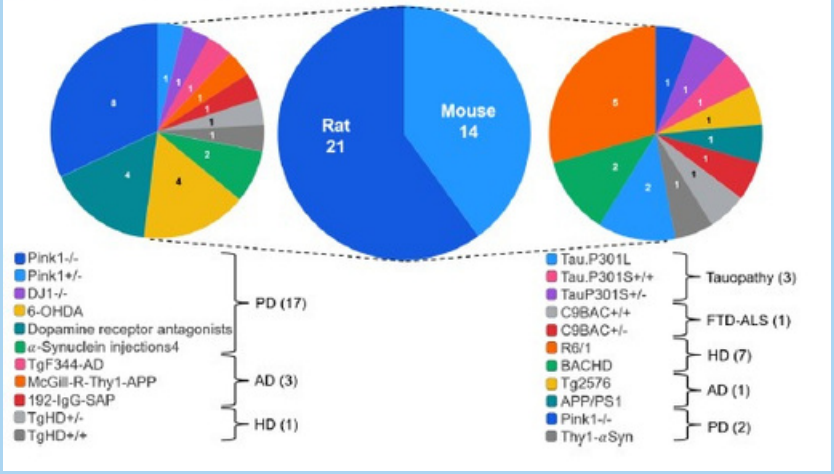
Abstract

Objectives: Neurodegenerative diseases such as Alzheimer's disease (AD), frontotemporal degeneration (FTD), Huntington's disease (HD), and Parkinson's disease (PD) frequently affect speech production, including increased pausing, dysarthria and/or apraxia of speech. With emerging therapies, early non-invasive markers are urgently needed for timely diagnosis and monitoring. Rodent vocalisations offer a promising translational model, yet the neural circuits underlying early speech changes remain poorly characterised, and a comprehensive overview of these rodent models and their outcomes is lacking. We therefore conducted a systematic review to identify affected ultrasonic vocalisation (USV) features and implicated neural circuits in well-characterised rat and mouse models.
Methods: This review was prospectively registered in PROSPERO (ID: CRD420250615249). We systematically searched PubMed, Embase, and Web of Science on January 16, 2025, yielding 3,649 records after duplicate removal. Following abstract and full-text screening, 34 reports were included. Extracted data includes first author, year, species and strain, USV paradigms, molecular characterisation of brain tissue, and corresponding outcomes.
Results: The review included 14 mouse and 21 rat studies, examining AD, PD, HD, FTD-ALS, and tauopathy models (Figure 1). The most commonly used paradigm to elicit USVs was courtship, in which male vocalisations were recorded following exposure to a female in oestrus. Across studies, consistent alterations were observed in USV features. In AD models, the number of calls was consistently reduced across species and was detectable as early as 3 months of age in transgenic mouse models; reduced call duration was also reported in rat models. In tauopathy models, call number, frequency, duration, and complexity of USVs were altered in an age- and model-dependent manner, with early increases in vocalisations in Tau.P301S mice pups and later reductions across multiple USV parameters in adult Tau.P301L mice. These changes were associated with tau pathology in brainstem and midbrain regions involved in vocal motor control.
Conclusions: Across rat and mouse models of AD, PD, HD, and tauopathies, alterations in call number and duration show consistent results. Although fine-grained cross-species comparison is currently lacking, these findings facilitate comparison between early vocal changes in rodents and speech alterations in patients and provide insight into affected neural circuits.

Figure 1: Number of papers



SAYRC-049

CHARACTERIZING NEURONAL SUBPOPULATIONS ACROSS FTLD-TDP SUBTYPES USING SNRNASEQ

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Abstract Objectives: Frontotemporal lobar degeneration with TDP-43 aggregates (FTLD-TDP) comprises three
major subtypes (A–C), defined by the type and distribution of pathology. Bulk RNA-seq studies have suggested subtype-specific differences in gene expression and splicing; however, whether these patterns are conserved within individual brain cell types remains unclear. Given that neurons are the most vulnerable cell type in FTLD-TDP, we aimed to characterize their transcriptional profiles across subtypes.
Methods: We generated short-read single-nucleus RNA-seq (10x Genomics) data from 107 frontal cortex samples spanning FTLD-TDP subtypes A, B, and C, GRN mutation carriers, FTLD-FET, ALS-FUS, and controls. Following demultiplexing, quality control, and doublet filtering, we performed broad cell-type annotation and detailed subannotation of neuronal populations.
Results: A total of 1M nuclei were sequenced, of which ~870k passed QC. Glutamatergic neurons were classified into 12 subclasses (~212k cells), and GABAergic neurons into 19 subclasses (~85k cells). No significant differences in cell-type abundance were observed between FTLD-TDP subtypes and controls. Among GABAergic neurons, FTLD-TDP GRN cases exhibited the highest number of differentially expressed genes, followed by FTLD-TDP type A, with PVALB+ subclasses showing the strongest transcriptional alterations in all FTLD-TDP subtypes. Pathway analyses in Inh PVALB HTR4 neurons revealed downregulation of metabolic and oxidative phosphorylation pathways, processes essential for these fast-spiking, high-energy-demand neurons.
Conclusions: Ongoing analyses aim to identify the most vulnerable neuronal subtypes and uncover subtype-specific molecular pathways, providing insights into selective vulnerability and potential therapeutic targets in FTLD-TDP subtypes.

SAYRC-050

POLYGENIC RISK FOR ALZHEIMER'S DISEASE SHAPES MICROGLIAL INFLAMMATORY AND ANTIGEN-PRESENTATION PROGRAMS IN VIVO

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Abstract

Objectives: The risk of developing Alzheimer's disease (AD) is largely dictated by our genetic profile, with a large portion of genetic variants affecting the expression of microglial genes. The same genes also change expression when microglia face amyloid-beta (Aβ) pathology, making it likely that our genetic background largely determines how well microglia can cope with amyloid stress and subsequently influence the surrounding brain milieu. Here we aimed to unravel how genetic variation can impact the microglial transcriptome and function.

Methods: We developed a 'human microglia village' approach, where we simultaneously xenotransplant human iPSC-derived microglia from multiple donors in the same mouse (*App*^{NLGF} or *App*^{HuHu}). By assessing the donor-specific microglial transcriptomes with and without exposure to amyloid, this platform allows to disentangle the intrinsic genetic effects from those induced by the brain environment.

Results: Marked inter-donor transcriptomic differences were observed in hMG derived from homeostatic, non-amyloid brain environments, demonstrating divergent baseline states across individuals. Amyloid exposure induced highly varied expression of MHC class II genes across donors, up to 5-fold differences, which correlated with individual's AD polygenic risk scores.
Conclusions: These findings demonstrate that polygenic risk can be decoded into functional immune phenotypes in human microgliaand establish a scalable *in vivo* platform to dissect the genetic regulation of cellular responses in complex brain disorders.

SAYRC-051

PARTNERS IN CRIME: THE LANDSCAPE OF COMPLEX CELLULAR INTERACTIONS OF MICROGLIA IN ALZHEIMER'S DISEASE

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Abstract

Objectives: Alzheimer's disease (AD) is a complex neurodegenerative disease, presenting pathological hallmarks of amyloid-beta (Aβ) plaques and tau tangles, combined with neuronal loss, gliosis and inflammation. Most genetic risk factors for AD reside in microglia, the immune cells of the brain. These cells are likely to be the hub of the neuroinflammatory events occurring in the AD brain, although they presumably do not act alone. Here we plan to dissect the array of complex cellular interactions between both mouse and human microglia with brain cell states *in vivo* and how these interactions can change in the context of AD.
Methods: To this end we sampled brain cells in the AD mouse model ($App^{NL\text{-}G\text{-}F}$) and its control ($App^{hu/hu}$), alongside mice depleted of microglia between 3-6 months of age, and of mice in which iPSC derived human microglia were xenotransplanted into the brain.
Results: We found increased GFAP immunoreactivity in brains composed of human microglia, with the AD model displaying the highest levels. To determine transcriptomic shifts as well as detect interaction networks, cells were extracted from the cortex, hippocampus and choroid plexus. We obtained a transcriptomics atlas of 150K cells across both mouse and human microglia, astrocytes, pericytes, endothelial cells, choroid plexus epithelial cells, oligodendrocytes and its precursor cells, monocytes, neutrophiles and neurons. We will subcluster an annotate substates in each individual cell type and determine the shifts in cell states based as well as specific pathways of communication from mouse or human microglia. Cellular interactions will be inferred based on transcriptomic profiles and validated *in vitro*.
Conclusions: We believe that understanding the extent of cellular interactions from microglia will fundamental to grasp the complex landscape of alterations in AD.

SAYRC-052

UNRAVELLING THE ROLE OF LYSOSOMAL EXONUCLEASE PHOSPHOLIPASE D3 IN THE DISEASE-ASSOCIATED MICROGLIA PHENOTYPE IN ALZHEIMER'S DISEASE

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Abstract

Objectives: The high regional specialization and complexity of the brain provides microglia with a range of diverse signals, requiring different responses. One of these includes the disease-associated microglia (DAM) phenotype which is acquired by microglia surrounding amyloid plaques in Alzheimer disease (AD) brains. The late-onset AD risk factor Phospholipase D3 (PLD3) is systematically upregulated in DAMs. PLD3 is a lysosomal exonuclease that regulates inflammatory responses by degrading single-stranded DNA; i.e., the substrate of toll-like receptor 9. In neurons, PLD3 loss-of-function majorly impacts lysosomal proteostasis, suggesting PLD3 could play a role at the crossroad between inflammation and the microglial degradative capacity.
Methods: Using complementary models, we studied the effect of PLD3 *in vitro* using iPSC-derived patient cell lines carrying a PLD3 AD variant and differentiated to human microglia, as well as *in vivo* models of PLD3KO mice in an APPNLGF background. Single cell omics and histological assessments were performed at various timepoints, as well as functional assays *in vitro*.
Results: In an initial characterization of the PLD3KOxAPPNLGF model, we observed that PLD3 deficiency results in an altered amyloid plaque organisation towards fewer plaques of larger size, and a redistribution of microglia which cluster less efficiently around plaques. Moreover, microglial activation was reduced with PLD3 deficiency and led to alterations in degradative compartments within these microglia.
Conclusions: Further investigation will determine the extent of the phenotypic change in both mouse and human microglia and to which degree this affects microglial functions. The acquired knowledge will unveil a functional link between a dysfunctional exonuclease/PLD3 activity and microglial activities, and how this impacts the AD pathology.

SAYRC-053

ENGINEERING A MULTICELLULAR NEUROIMMUNE COMPETENT HUMAN BRAIN ORGANOID MODEL FOR ALZHEIMER'S DISEASE

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Abstract

Objectives: Alzheimer'sdisease (AD) is neuropathologically characterized by amyloid-beta (Aβ) plaques and neurofibrillary tangles. AD occurs as familial or sporadic (genetic and environmental factors). Increasing evidence highlights microglia as key mediators in AD pathogenesis, with human-specific factors crucial for modeling the disease spectrum, emphasizing the need for improved human models.

Methods: Here,we optimized a protocol to derive human microglia from pluripotent stem cells (hPSC) through exposure of primitive macrophage precursors (PMPs) to an IGMT cytokine cocktail (IL34, GM-CSF, M-CSF,andTGFβ1) that results in a population with enriched microglial-specific gene expression and typical microglia ramified morphology.

Results: classical cultures using FBS showed endothelial-like contamination. Transcriptomic analysis revealed upregulated microglial gene signatures, enhanced cytokine response, cytoskeletal reorganization, and extracellular matrix remodeling. Compared to PMPs and H9 hPSCs, IGMT microglia displayed a canonical microglial transcriptome. Upon exposure to Aβ fibrils, microglia showed higher motility, rapid recruitment, aggregation, and efficient phagocytosis. Transcriptomic profile of Aβ-treated microglia revealed enriched pathways associated with AD, as well as Interferon response, TNF-alfa signaling and mitochondrial-associated pathways. However, phagocytosis capacity, cytoskeleton and extracellular matrix-associated pathways were downregulated, suggesting a state of progressive microglial dysfunction. Finally, we established a human multicellular brain organoid (hmBOs) model that incorporates microglia.Optimal integration and survival was achieved by a combination of PMPs and IGMT cytokines,which led to high neuronal viability and microglial densities comparable to the *in vivo* cortex.

Conclusions: We developed neuroimmune-competent hmBOs that could serve to study neurodegenerative disorders characterized by an important immune component, such as AD.

SAYRC-054

SECRETED SIGNALS: TRACING HUMAN MICROGLIAL COMMUNICATION IN ALZHEIMER'S DISEASE IN VIVO

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Abstract

Objectives: The discovery of numerous microglial genetic risk factors in Alzheimer's disease (AD) shifted the field's focus towards understanding the change in functional roles of these cells. Single-cell transcriptomic analyses revealed prominent heterogeneity among human microglia, raising critical questions about their contributions to pathology. However, a major challenge remains: translating transcriptomic profiles into functional insights. Microglia influence their environment and intercellular communication via secretion of soluble factors, some of which (i.e., C1q, IL-1β, and TNFα) implicated in neuronal dysfunction and degeneration. Yet, the full spectrum of secreted proteins remains poorly defined, limiting our understanding of their roles in AD.

Methods: By tracing different subcellular organelle compartments inside microglia, we target a multitude of cellular processes that change in AD. For example, targeting the classical secretory pathway via ER-localised proximity-labelling (TurboID), we capture protein synthesis and the proteins getting shuttled out of the cell. This allowed us to identify endogenously secreted proteins in an unbiased manner using affinity-purification mass spectrometry both *in vitro* and *in vivo*. We combined TurboID labelling with xenotransplantation of human iPSC-derived microglia into the mouse brain, enabling profiling of the microglial secretome in the brain, CSF and plasma.

Results: We are investigating microglial responses to AD's key pathological features, amyloid-β and tau aggregates (App^{NL-G-F} andTHY-Tau22 mice). This form of protein-tracing allows us to isolate cell-type specific molecules from CSF and brain parenchyma to map secreted mediators relevant to AD.

Conclusions: By capturing the human microglial language within the living brain, this tool has the potential to redefine how we study intercellular communication in health and disease. We are now able to deconvolute complex environments and the cellular origin of their components, unlocking new frontiers in neurodegeneration research and therapeutic discovery.

SAYRC-055

UNRAVELING A ROLE FOR PSEN2/Γ-SECRETASE IN MICROGLIA DURING ALZHEIMER'S DISEASE PROGRESSION

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Abstract

Objectives: For decades, γ-secretases have been central to Alzheimer's disease (AD) research both genetically and functionally, due to their intricate involvement in the production of Aβ and autosomal dominant mutations irrevocably lead to AD. These large intramembrane proteases consist of four subunits of which Presenilin (PSEN) provides the catalytic activity. Two homologues of PSEN exist, PSEN1 and PSEN2 and the one present in the complex dictates its subcellular localization. PSEN1/γ-secretase complexes are more broadly distributed including the plasma membrane and recycling endosomes whereas PSEN2/γ-secretase is more restricted in its localization (1)**.** This, inevitably, leads to complex specific substrate pools underscoring potential different functional impacts of the individual complexes. In neurons, the absence or mutation of PSEN2 leads to endolysosomal defects that impact synaptic homeostasis ultimately impairing cognitive function (2). Recently, γ-secretase was shown to play an integral role in microglia, where 85 microglia proteins were identified as bona fide γ-secretase substrates (3).
Methods: This drove us to investigate the role of PSEN2/γ-secretase complexes in microglia during AD pathogenesis
Results: In mouse microglia, PSEN2 deficiency leads to earlier plaque recruitment, increased expression of disease associated microglia (DAM) markers and higher proliferation. Curiously, microglia carrying a familial AD (FAD) mutation showed the opposite. However, mouse microglia do not fully recapitulate the human genetic aspects of AD nor do they mirror human microglia physiology. Therefore, we opted to transplant PSEN2KO and FADPSEN2 human microglia progenitors into immunodeficient APPNLGF mice(4). Examining, simultaneously, the surface proteome and single-cell transcriptome showed a shift of FADPSEN2 microglia towards the highly activated HLA cluster whereas PSEN2KO microglia shift more towards the secretory cytokine response microglia (CRM).
Conclusions: We highlight the functional differences between PSEN2 deficiency and mutation that we aim to further unravel.
References: 1. Sannerud R, et al. Restricted Location of PSEN2/γ-Secretase Determines Substrate Specificity and Generates an Intracellular Aβ Pool. Cell. 2016 Jun 30;166(1):193–208. **2.** Perdok A, et al. Altered expression of Presenilin2 impacts endolysosomal homeostasis and synapse function in Alzheimer's disease-relevant brain circuits. Nat Commun. 2024 Nov 29;15(1):10412. **3.** Hou P, et al. The γ-secretase substrate proteome and its role in cell signaling regulation. Mol Cell. 2023;83(22):4106–22. **4.** Mancuso R, et al. Xenografted human microglia display diverse transcriptomic states in response to Alzheimer's disease-related amyloid-β pathology. Nat Neurosci. 2024 May 1;27(5):886–900.

SAYRC-056

A SYSTEMS IMMUNOLOGY ANALYSIS OF ALZHEIMER'S DISEASE REVEALS AN AGE- AND ENVIRONMENTAL EXPOSURE-INDEPENDENT DISTURBANCE IN B CELL MATURATION

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Abstract

Objectives: Alzheimer's disease is a severe neurodegenerative disorder, with multifactorial mechanisms of disease development and progression. Evidence from genetic association studies, animal models, and clinical investigation suggests a neuroimmunological component to disease, with links to the peripheral immune system. To comprehensively characterize peripheral immune system alterations in individuals withAlzheimer's disease.
Methods: Here we applied a systems immunology approach to determine the immunological correlates of Alzheimer's disease. Using high-dimensional flow cytometry and machine learning, we comprehensively assessed the cellular component of the peripheral immune system in a well-characterized cohort of 184 Alzheimer's patients and 105 healthy spouses.
Results: Using this approach, Alzheimer's patients demonstrated a disturbance in B cell maturation, a feature that was associated not only with disease status but also with cognitive decline. This effect was age and environmental exposure independent, suggesting a disease-intrinsic relationship between Alzheimer's disease and B cell maturation.
Conclusions: These results provide an underexplored avenue for improving both mechanistic understanding and therapeutic design in Alzheimer's disease

SAYRC-057

UNRAVELLING THE ROLE OF MICROGLIA T-CELLS INTERACTION IN ALZHEIMER'S DISEASE PATHOLOGY

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Abstract

Objectives: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by amyloid-beta (Aβ) plaque accumulation, tau tangles, neuroinflammation, and brain atrophy. While traditionally linked to neuronal dysfunction, increasing evidence implicates the immune system in disease progression. Genome-wide association studies (GWAS) have identified AD risk variants enriched in immune-related genes, particularly those expressed by microglia, the brain's resident macrophages. Exposure to amyloid drives microglial activation, which is paralleled by accumulation of T cells in the brain parenchyma and in the cerebrospinal fluid.
Methods: Using spectral flow cytometry and immunohistochemistry, we profiled the adaptive and innate brain-resident immune populations in the App^{NLGF} mouse model of amyloidosis and correlated the expression of antigen-presenting machinery in microglia with the adaptive immune response. Regulatory T cells (Tregs) are a subset of T cells with immunomodulatory and anti-inflammatory functions. Expansion of Tregs has been proposed as a therapeutic approach in neuroinflammation and neurodegeneration. Treg therapy in AD has proven efficacy in ameliorating amyloid pathology and neuroinflammation in mice.
Results: To test the impact of Treg therapy on microglial activation and brain T cell recruitment, we induced brain-specific Treg expansion using the PHP.BaCamKII-IL2 vector. In our model, IL2-mediated Treg expansion modified the microglial phenotype by inducing MHC-II expression and promoting phagocytic activity.
Conclusions: These preliminary findings suggest that Tregs modulate microglial responses and support targeting microglia-T cell interactions as a potential AD therapy.

SAYRC-058

APOE GOVERNS THE MICROGLIAL TRANSITION TO A DISEASE-ASSOCIATED STATE AND MODULATES AMYLOID PLAQUE ENGAGEMENT

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Abstract

Objectives: Dementia is projected to affect over 153 million people by 2050, primarily due to Alzheimer's disease (AD). While certain *APOE* mutations are known to delay symptom onset by decades, the mechanistic basis for this protection remains poorly understood. Given that ApoE is a putative "master regulator" of microglial activation, this study aimed to determine how ApoE deficiency influences the transition of microglia from a homeostatic to a disease-associated state and how this shift impacts amyloid pathology.
Methods: We employed a multi-modal approach comparing wild-type (WT), amyloid-producing (5xFAD), and 5xFAD/ApoE-knockout (KO) mice. Single-cell RNA sequencing (scRNA-seq) was used to profile transcriptional landscapes and perform trajectory inference of microglial populations. These high-dimensional data were validated using immunofluorescence microscopy to assess microglial-plaque interactions and amyloid morphology.
Results: scRNA-seq revealed that while 5xFAD mice exhibit a robust shift toward reactive and disease-associated microglia (DAM), the absence of ApoE significantly blunts this transition. Trajectory inference identified a "maturation arrest" in ApoE-KO microglia, characterized by a significant pseudotime delay along the homeostatic-reactive-DAM lineage. This was corroborated by a failure to upregulate key genes involved in lipid metabolism, energy production, and cytoskeletal remodelling. Consequently, immunofluorescence showed that ApoE-KO microglia exhibit impaired plaque engagement and reduced phagocytic capacity, resulting in a shift from compact to diffuse amyloid plaque morphology.
Conclusions: Our findings establish ApoE as the essential molecular switch governing the microglial response to amyloid pathology. By demonstrating that the loss of ApoE prevents the full acquisition of the DAM phenotype, we identify the ApoE-DAM signaling axis as a driver of neuroinflammation in AD. These data suggest that therapeutic modulation of ApoE-dependent microglial pathways may offer a precise strategy to alter disease progression and preserve neural homeostasis.

SAYRC-059

BACTERIAL AMYLOID-INDUCED PERIPHERAL INFLAMMATION AMPLIFIES NEUROINFLAMMATION IN THE APPNL-G-F/MAPT DOUBLE KNOCK-IN MOUSE MODEL OF ALZHEIMER'S DISEASE

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Abstract

Objectives: Dysbiosis and intestinal inflammation are important comorbidities of Alzheimer's disease (AD), but the causality and exact mediators of this gut-brain interplay remain to be characterized. In our previous work, we identified the bacterial amyloid curli,produced by the gut microbiome, as a potent trigger of enteric immune responses, typified by a lasting, systemic production of serum amyloid A3 (SAA3). Since serum amyloids (SAAs) can penetrate the blood-brain barrier and are elevated in AD patients, we now asked to what extent peripheral exposure to curli would influence brain pathology in the $APP^{nl\text{-}g\text{-}f}$/MAPT knock-in mouse model of Alzheimer's disease.
Methods: Eight-month-old $APP^{nl\text{-}g\text{-}f}$/MAPT mice received an intraperitoneal injection of either curli or PBS. Circulating SAA levels and *Saa3* expression in brain were quantified. Additionally, CD45+/CD11b+ myeloid cells were isolated from the brain for bulk RNA sequencing, and key transcriptional changes were validated using RNAscope.
Results: Intraperitoneal curli injection induced both elevated SAA levels in the circulation as well as local *Saa3* expression in meninges, choroid plexus, and perivascular cells. Since SAAs promote their cognate gene expression, we hypothesize that these brain regions are directly exposed to the SAAs generated in the periphery.CD45+/CD11b+ myeloid cells,isolated from the mouse brain of curli-injected $APP^{nl\text{-}g\text{-}f}$/MAPT mice, displayed a pro-inflammatory transcriptional signature as compared to their PBS-injected counterparts. Validation with RNAscope revealed a significant influx of calprotectin-positive (*S100a8/9* expressing) cells and upregulation of microglia activation markers *Fcgr2b*, *Spp1,* and *Msr1* adjacent to amyloid deposits. Currently, we are investigating the causal contribution of SAAs to this response using genetic modulation,and evaluating the impact on AD pathology progression.
Conclusions: Thus, our findings reveal a first mechanistic connection between bacterial amyloids, peripheral immune signalling, and central inflammation.

SAYRC-060

OTULIN CONTROLS MICROGLIA ACTIVATION AND SUPPRESSES THE DEVELOPMENT OF ALZHEIMER'S-LIKE PATHOLOGY IN MICE

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Abstract

Objectives: Inflammatory signaling pathways are subjected to tight regulation to avoid chronic inflammation and the development of inflammatory pathology. One of the proteins involved in such regulation is the deubiquitinating enzyme OTU deubiquitinase with linear linkage specificity (OTULIN), a deubiquitinating enzyme that exclusively hydrolyzes linear ubiquitin chains from proteins modified by the linea rubiquitin chain assembly complex (LUBAC). Loss-of-function mutations in OTULIN underlie a severe early-onset human autoinflammatory disease and severe pathology in experimental mouse models. However, little is known about a role for OTULIN in the central nervous system (CNS) homeostasis.
Methods: Here, we have investigated the role of OTULIN in CNS homeostasis and during Alzheimer's disease (AD)pathology.
Results: Mice lacking OTULIN in CNS myeloid cells ($OTULIN^{Cx3Cr1\text{-}KO}$) show increased microglial cell numbers that acquire a disease-associated microglia (DAM) phenotype according to single cell transcriptome analysis Moreover,OTULIN deletion in CNS-resident myeloid cells exacerbates AD pathology in *APP/PS1*miceandin$APP^{NL\text{-}G\text{-}F}$ mice, as shown by a higher Aβ burden and an increased neuron loss in the hippocampus and cortex of OTULIN deficient mice upon aging.
Conclusions: Together, our data suggest that microglial OTULIN acts as an important regulator of microglial activation, protecting mice from developing AD pathology.

SAYRC-061

ASTROCYTIC CERULOPLASMIN DEFICIENCY TRIGGERS IRON TOXICITY AND NEURODEGENERATION IN A LRRK2 PARKINSON'S TRI-CULTURE MODEL

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Abstract

Objectives: Astrocytes and microglia carrying the LRRK2-G2019S mutation contribute to non-cell-autonomous dopaminergic neuron (DAn) degeneration in Parkinson's disease (PD), yet the mechanisms underlying their crosstalk remain unclear. Here, we sought to define how LRRK2-G2019S mutant astrocyte-microglia interactions drive DAn vulnerability.
Methods: We developed a novel induced pluripotent stem cell (iPSC)-derived tri-culture system comprising healthy DAn together with either LRRK2-G2019S mutant or isogenic control iPSC-derived astrocytes and microglia. We combined transcriptomic profiling with functional assays to assess DAn degeneration, glial reactivity, and pathway alterations across conditions.
Results: LRRK2-mutant astrocytes emerged as the primary drivers of the observed phenotype, adopting a hyperreactive state that promoted microglial activation and subsequent DAn degeneration. Mechanistically, we identified a selective downregulation of ceruloplasmin (CP), a copper-dependent ferroxidase, in LRRK2-G2019S astrocytes. Reduced CP expression disrupted iron homeostasis, leading to Fe^{2+} accumulation and increased reactive oxygen species (ROS), which in turn drove microglial reactivity and neurodegeneration. Pharmacological restoration of CP re-established iron and redox homeostasis, reduced microglial activation, and protected DAn from degeneration.
Conclusions: Our findings uncover a novel astrocyte-microglia-neuron axis driving PD pathogenesis and highlight iron dysregulation as a central mechanism. Our novel stem cell-derived tri-culture platform provides a powerful system for dissecting disease mechanisms and identifying therapeutic targets.

SAYRC-062

MICROGLIA INTEGRATION ACCELERATES PATHOLOGICAL PHENOTYPES IN A HUMAN MULTICELLULAR BRAIN ORGANOID MODEL OF ALZHEIMER'S DISEASE (AD HMBO)

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Abstract

Objectives: Alzheimer's disease (AD), the predominant cause of dementia in the elderly, is neuropathologically characterized by the presence of amyloid-beta (Aβ) plaques and neurofibrillary tangles in the brain. Increasing evidence supports a central role of the inflammatory component driven by microglia cells (MC) in AD pathogenesis and highlights the importance of human species-specific factors to accurately model the disease. Recent data suggests that MC could contribute to Aβ plaque formation at early stages of the pathology in mouse models (Baligács et al., 2024). However, their role in a fully human genetic background remains poorly understood.
Methods: Here, we generated human pluripotent stem cell (hPSC)-derived MC and examined their responses to AD-related pathology both in monoculture and within a human multicellular brain organoid model (hmBO) carrying the familial AD-associated London mutation within the APP gene.
Results: MC exposed to soluble Aβ peptides and to fibrillar Aβ resulted in increased MC recruitment, clustering, and phagocytic activity, demonstrating that hPSC-derived MC functionally respond to Aβ similarly to microglia in the human AD brain. hPSC-London hBOs without MC recapitulate the formation of small, non-fibrillar Aβ aggregates and Tau hyperphosphorylation without significant cell death. Notably, the introduction of hPSC-derived MC (AD hmBOs) led to their active recruitment to Aβ deposits and resulted in a significant increase in both the number and size of Aβ aggregates, alongside alterations in Tau phosphorylation levels.
Conclusions: Together, these results establish hmBOs as a valuable human platform for investigating microglia-driven mechanisms inneurodegenerative diseases, such as AD.

SAYRC-063

OLIGODENDROCYTE PRECURSOR CELLS SHOW DISTINCT DNA METHYLATION PATTERNS ACROSS DIFFERENT AGES IN APPSWE/PS1D9 MICE

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Abstract

Objectives: Accumulating evidence implicates oligodendrocyte precursor cell (OPC) dysfunction in Alzheimer's disease (AD). We hypothesize that this dysfunction is due to altered DNA methylation (DNAm), which regulates both oligodendrocyte development and myelination. The APPswe/PS1d9 mouse model is commonly used to investigate AD, with transgenic mice showing amyloid beta plaque deposition at 4-6 months and cognitive decline at 8-10 months. Here, we aimed to profile DNAm patterns of OPCs derived from APPswe/PS1d9 mice at different ages.
Methods: A2B5+OPCs were isolated from female APPswe/PS1d9 mice and age-matched wild-type littermates at the age of 2months (pre-onset; nWT = 7, nAPPswe/PS1d9 = 8), 6 months (amyloid plaque formation; nWT = 8, nAPPswe/PS1d9 = 8), and 10 months (cognitive decline; nWT = 4, nAPPswe/PS1d9 = 5). Genomic DNA was subjected to bisulfite conversion and analyzed using the Illumina Mouse Methylation Beadchip, which covers over 285,000 CpG sites across the genome. Differential methylation analysis was performed on a locus and region level, comparing wildtype to APPswe/PS1d9 within each age group.
Results: Linear modeling revealed DNAm changes in genes related to OPC differentiation and myelination (Lrp1 ,Opcml, Tyro3, Bmpr1b, and Zhx2) at 6 months and immune function (Lair1, Otud3) and DNA repair (Mlh3, Cux1) at 10 months. Differentially methylated regions were observed in genes related to the transgenicmodel (App and Prn) and mitochondria (Trak1 and Dnajc19). Only 13 genes showed altered DNAm in more than one age group, with most genes (98%) being unique to one group.
Conclusions: APPswe/PS1d9 OPCs show altered DNAm patterns compared to wildtype, and these patterns change with advancing pathology. The identified differentially methylated genes may offer new insights into the epigenetic landscape of AD and may serve as targets for future research.

SAYRC-064

THE ROLE OF MIRNAS ON TAU PATHOLOGY PROPAGATION IN ALZHEIMER'S DISEASE

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Abstract

Objectives: To identify the di[erentially expressed miRNAs in Alzheimer's disease (AD) brain and to study their association with tau pathology.
Methods: RNA next generation sequencing was performed from postmortem temporal cortex (TCX) and hippocampal tissue of AD patients and neurocognitively healthy controls. Target prediction and pathway analyses were carried with the di[erentially expressed (DE) miRNAs. DE miRNAs were studied in tau biosensor cells exposed to tau seeds to study their e[ect on tau seeding by FACS and immunoblotting was used to evaluate their regulatory e[ect. Tau seeds were obtained from AD postmortem brain tissue.
Results: miR-129-5p, miR-129-1-3p, miR-132-3p, miR-1-3p, miR-133a-3p and novel-miR-71 were downregulated and miR-151a-5p, miR-151b, miR-3065-3p, miR18a-5p, and novel-miR-122 upregulated in TCX, whereas in the hippocampus only miR-545-3p and miR-3611 were increased. We found that miR-129-5p and miR-3065-3p correlated negatively and positively with AD progression (by Braak staging), respectively, and that miR-129-5p and miR-3065-3p negatively correlated with each other in TCX and hippocampus. We also observed a nonlinear change of miR-545-3p in the hippocampus, with the highest peak at Braak 4. We did not observe any di[erence in tau seeding by the presence of miR-129-5p or miR-3065-3p mimics, but we observed a downregulation of LRP1 by miR-129-5p mimic and an upregulation of EEA1 by miR-3065-3p mimic. However, the inhibition of miR-129-5p increased tau seeding. Pathway enrichment analysis and gene ontology revealed that di[erentially expressed miRNAs are involved in several processes relevant for tau spreading.
Conclusions: The expression levels of miR-129-5p and miR-3065-3p are correlated with AD progression and with each other. Both miRNAs seem to regulate di[erent stages of the endocytic pathway, from internalization to ligand degradation, which could impact the spreading of tau and its pathologic forms. Yet, more studies are needed to fully comprehend their regulatory role.