ABSTRACT BOOK

BEB DAY 2025

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SEMINÁRIO MAIOR DE COIMBRA

FROM MICRO-SCALES TO MACRO-WORLDS

TRANSCENDING THE LIMITS OF SCIENCE

FLASH-TALKS

C4BP as a Novel Interactor of APRc: A Complement Evasion Mechanism in Rickettsia

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Rickettsia are obligate intracellular bacteria transmitted by arthropod vectors that cause mild to life-threatening diseases. The lack of vaccines and the increased geographical distribution of these pathogens are causing increased concern due to their impact on global health. C4BP is a crucial complement inhibitor that is a prime target for pathogens in immune resistance. Recently, we have shown that rickettsial APRc - a highly conserved membrane-embedded aspartic protease - binds immunoglobulins, mediates serum resistance, and targets other human serum components. Herein, we provide evidence on C4BP as an APRc interactor. C4BP interaction with rickettsiae was analysed by surface-binding assays and whole-cell ELISA using NHS and HRP-labelled C4BP. APRc interaction with C4BP was evaluated by pull-down and ELISA. APRc-C4BP interaction domains were mapped through ELISA assays using C4BP WT and CCP mutants. C4BP cofactor activity was assessed in the presence of APRc and purified C4b by Western blot. APRc impact on complement activation was assessed by measuring C5b-9 using ELISA. We confirmed C4BP-binding at rickettsial surface in the presence of NHS and purified C4BP and confirmed C4BP cofactor activity at the surface of Rickettsia. We demonstrated that APRc binds to C4BP in a concentration-dependent manner and mainly through CCP5 and CCP7 C4BP domains. Our results showed that C4BP maintains its cofactor activity in the presence of APRc by cleaving C4b into the inactive form C4d. Additionally, APRc inhibits the activation of the classical and lectin complement pathways. With this work we confirmed that APRc targets C4BP, maintaining its cofactor activity and impacting complement activation. We confirmed Rickettsia recruitment of C4BP at its surface, revealing a novel immune evasion mechanism used by Rickettsia.

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AMPK dynamics in hypothalamic astrocytes determines circadian behavior in mice

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The intricate interplay between metabolism and circadian physiology involves the molecular clock—a ubiquitous circuit in every cell that governs genes driving rhythmic processes like feeding and sleep-wake cycles. Circadian behavior is regulated by two pacemakers: the suprachiasmatic nucleus (SCN), entrained by light, and the food-entrainable oscillator (FEO), synchronized by feeding signals, though its precise location remains unknown. Astrocytes, acting as sensors of peripheral metabolic signals, function as competent oscillators essential for generating and coordinating circadian outputs. This study explores the role of astrocytic AMPK and its link to metabolic homeostasis and circadian regulation.

We found that rhythmic AMPK phosphorylation is endogenously regulated by the circadian clock in primary hypothalamic astrocytes and the mouse hypothalamus in the absence of entrainment signal (light or food). This suggests phospho-AMPK exhibits intrinsic, energy-independent rhythmicity. To assess AMPK signaling's impact in astrocytes, we created mouse models with deletion of AMPKγ2 or AMPKα1 in GLAST-positive astrocytes. Notably, AMPKγ2 deletion impairs food anticipatory activity (FAA), while AMPKα1 deletion enhances it, indicating AMPK modulation affects feeding-related entrainment. Moreover, AMPKγ2 deletion lengthens the free-running period and reduces SCN intercellular coupling while AMPKα1 deletion produces opposite effects. Interestingly, both models result in positive energy balance. Using virogenetic tools and deletion of AMPK subunits in SF1 neurons, we show that ventromedial hypothalamic astrocytes—but not neurons—are key components of the FEO.

In summary, AMPK oscillation in astrocytes is crucial for aligning circadian rhythms with light and feeding cues.

Modulation of fear extinction performance by social hierarchy

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Exaggerated fear and impaired fear extinction are key features of anxiety and fear-related disorders. Importantly, individual differences in fear extinction performance in a population may set vulnerability risk to anxiety disorders. Recently, we have developed in our lab a behavioural model in mice to study interindividual variation in fear extinction performance, based on the contextual fear conditioning and extinction paradigm. Using this C57Bl/6J strain, we observed that while a subset of animals subjected to extinction training is able to successfully extinguish fear, others fail. Because these are inbred animals, with no other genetic or pharmacological manipulation, we hypothesize that social hierarchy, an environmental phenomenon conserved across most animal species, as a factor underlying performance in fear extinction. Evidence from the literature links social hierarchy to anxiety behaviours, though results vary across studies. Moreover, the effects of social hierarchy on fear extinction have not yet been evaluated. In this study, we aim to explore the interaction between social hierarchy and fear extinction by investigating the interaction between different behavioural metrics using deep learning tools, and the underlying circuit dynamics. Using the tube test and the contextual fear conditioning and extinction paradigm, we categorized mice housed in groups of four animals according to their social rank and performance in extinction. Preliminary data suggests that dominant animals exhibit worse extinction learning performance when compared to subordinates. Additionally, using c-FOS staining, we observe a higher neuronal activation in the anterior cingulate cortex and prelimbic regions of the medial prefrontal cortex in dominant animals during extinction memory retrieval. Together, results highlight a possible mechanism at the circuit level by which social rank influences fear extinction.

Small Particles, Big Impact: Extracellular Vesicles in Sleep Apnea

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Obstructive Sleep Apnea (OSA) is a prevalent sleep disorder worldwide, especially among the elderly, and may accelerate aging. Untreated, OSA has been associated with several chronic comorbidities, as metabolic, cardiovascular disorders, and neurodegenerative disorders. Extracellular Vesicles (EVs) act as important mediators of intercellular communication, being involved in several physiological and pathological processes. The hypothesis that OSA-related EVs contribute to accelerated aging through a senescence-associated secretory phenotype warrants further exploration. This study investigates the impact of EVs from OSA patients on aging hallmarks.

Plasma-derived EVs were isolated from 12 men with severe OSA (54±10 years) at diagnosis and after 24 months of CPAP treatment, and from control groups: age-matched (n=14; 49±8 years) and young controls (n=12; 24±2 years). EVs were isolated using size-exclusion chromatography and characterized for concentration, size and cargo content – with transcriptomic miRNA analysis at 8 a.m. and 10 p.m. Functional assays were conducted by incubating human fibroblasts with EVs from OSA patients and controls, assessing aging hallmarks, including senescence markers and biological clock regulation. Ethical approval was obtained from FMUC and Coimbra Hospital committees.

Results show successfully characterized plasma-derived EVs, verifying size and protein composition. Nanoparticle Tracking Analysis showed that EV concentration was significantly elevated in OSA patients, before and after treatment, compared to controls. EV-associated miRNA profiling was analysed in the four different groups at 8 a.m. and 10 p.m.

In functional assays, EVs from OSA patients appeared to dysregulate the NRF2 pathway and disrupt biological clocks. These results suggest OSA might aggravates/promotes some hallmarks of aging, some of which are not reverted by CPAP treatment.

Pathogenic mechanisms mediated by anti-GABAAR-autoantibodies in autoimmune epilepsy

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Epilepsy is a chronic brain disorder characterized by recurrent unprovoked seizures due to abnormal hypersynchronous neuronal activity. Despite efforts, about one-third of epilepsy cases remain of unknown cause, and a substantial proportion of patients develop drug resistance to the available medication.

Interestingly, autoantibodies (Abs) targeting neuronal proteins have been identified in drug-resistant epilepsy and status epilepticus, suggesting an autoimmune basis for epileptogenesis and. Specifically, human Abs against GABAAR subunits are linked to acute refractory seizures and severe status epilepticus in autoimmune encephalitis; however, these are not routinely tested in clinical practice, limiting patient's diagnosis and the understanding of their pathogenic role.

This work combines functional and molecular analyses to examines the effects of GABAAR-aAbs on hippocampal neuronal cultures to elucidate their role in epilepsy pathogenesis. Briefly, hippocampal neurons were exposed to patient's serum containing GABAAR-aAbs and neuronal network activity analysed with microelectrode arrays (MEA), revealing an increase in the strength and rhythmicity of networks excitability and connectivity, resembling patients' clinical seizures. We also disclosed a mechanism of internalization of GABAAR-aAbs in hippocampal neurons preceding the observed functional alterations, pointing to a pathogenic effect of GABAAR-aAbs mediated by their internalization. Besides, the incubation of hippocampal neuronal cultures with GABAAR-aAbs serum significantly increased γ2-GABAAR internalization rate, as revealed by a fluorescence assay for receptor internalization.

Our findings reveal pathogenic mechanisms mediated by GABAAR-aAbs that might underlie epileptogenesis in autoimmune epilepsy. These insights enhance our understanding of disease pathophysiology and highlight potential biomarkers for diagnosis, paving the way for targeted therapeutic strategies.

Addressing cerebellum defects in a mouse model of schizophrenia harbouring a human mutation in the CACNG2 gene encoding stargazin

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Cerebellar dysfunction has been implicated in several neuropsychiatric disorders. Both connectivity impairments and structural alterations in this brain region have been found in schizophrenia patients, suggesting a potential role for the cerebellum in the expression of schizophrenia-related phenotypes. However, despite these findings, the precise cerebellar defects that result in cognitive impairment and social behaviour abnormalities remain poorly understood.

This project aimed to study cerebellar defects in a mouse model harbouring a human mutation in the CACNG2 gene, which was identified in association with schizophrenia. Stargazin, the CACNG2-encoded protein, is a synaptic protein highly expressed in the cerebellum, where it plays non-redundant functions in targeting AMPAR to the synapse. Knock-in mice harbouring a stargazin mutation associated with intellectual disability show abnormal cognitive and social behaviours, as well as impaired motor learning. Therefore, we aimed to assess social behaviour and sensorimotor gating in knock-in mice expressing a schizophrenia-associated variant of stargazin (STGSN-KI mice), as well as to evaluate cerebellar neuronal excitability.

Behavioural characterization revealed that STGSN-KI mice show depressive-like behaviour, deficits in prepulse inhibition of the acoustic startle response and in social behaviour. Overall, these results indicate that STGSN-KI mice recapitulate phenotypes observed in schizophrenia patients and other animal models of schizophrenia. Expression of mutant stargazin led to aberrant intrisic pattern of firing and higher excitability of Purkinje cells in the Crus I region of the cerebellum of STGSN-KI mice, compared to wild-type animals. Altogether, this work unveiled specific behavioural features altered in STGSN-KI mice, shedding light into the excitability deficits in Crus I Purkinje cells that might contribute to the behavioural abnormalities observed in this animal model.

POSTERS

Unravelling and treating the ageing phenotype of the blood-brain barrier

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The blood-brain barrier (BBB) is a metabolic and physical barrier made of brain endothelial cells (BECs). It is a crucial biological barrier that can become dysfunctional with aging. This dysfunction is characterized by phenotypic alterations in BECs (changes in tight-junctions and transport systems) and increased permeability, which contribute to cognitive decline. Senescence has been linked to BBB deterioration in recent years. Sadly, the impact of senescent BECs on BBB structure and function is not fully understood. Thus, this work aimed to study the BBB senescence program, establish an in vitro senescent BBB model, and evaluate the consequences of senescent BECs pharmacological treatment on BBB properties.

Recently, we have demonstrated that a senescence phenotype in BECs is a key factor in BBB dysfunction. We compared brain sections of young (3 months) and aged (24-27 months) mice. Aged mice showed an increase in senescence markers (p21+, p16+, and HMGB1-) on BECs compared to young. Concomitantly, a rise in BBB permeability and inflammation was noted in aged mice, with a drop in the tight-junction occludin. This was partly rescued by senolytic treatments.

Additionally, we developed an in vitro senescent BBB model by exposing endothelial cells to radiation. Increases in p21+ and SA-□-galactosidase, and HMGB1 extravasation were detected. Moreover, a rise in permeability with a decline in TEER and occludin levels were noticed. These results reveal BBB dysfunction in the presence of senescent BECs and mimic alterations found in aged mice BBB.

Finally, we explored pharmacological strategies to reverse the dysfunctional alterations of the in vitro senescent BBB model. We screened 14 senotherapeutics to identify compounds that specifically target senescent BECs. Further testing in our model led to the identification of two senotherapeutics that significantly improved BBB function. Overall, this study presents new avenues for understanding and addressing BBB aging.

Quercetin's Neuroprotective Potential in Cocaine Exposure: A Longitudinal easyPET.3D and Histopathological Study in Mice

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Cocaine addiction presents a significant public health challenge, evidenced by a 20% increase in global usage over the last decade, reaching an estimated 23 million users in 2022. The absence of effective treatments underscores the urgency for novel therapeutic strategies.

This study evaluated the potential of quercetin, a flavonoid, to counteract cocaine-induced neurobiological changes in two mouse strains (BALB/c (+) and C57BL/6J). Using [18F]-FDG and a microPET imaging (easyPET.3D), the metabolic activity in brain regions critical to cocaine addiction were assessed: hippocampus, amygdala, prefrontal cortex, thalamus, and striatum. Complementing this functional imaging data, detailed histopathological analyses to evaluate structural integrity and identify potential cellular damage within these same brain regions were performed.

Cocaine elicited significant metabolic and structural alterations, with C57BL/6J mice exhibiting higher sensitivity. Quercetin administration showed a significant restorative effect on baseline metabolic activity in BALB/c (+) mice, suggesting a potential neuroprotective role. However, the efficacy of quercetin was limited in C57BL/6J mice, particularly in the amygdala and prefrontal cortex, regions critical for emotional regulation and executive function, respectively. While quercetin partially mitigated the histopathological damage induced by cocaine, it did not fully reverse the structural deficits. Nonetheless, the observed ability of quercetin to normalize metabolic function suggests a potential therapeutic avenue for promoting neuronal recovery and improving clinical outcomes in the treatment of cocaine addiction. This research highlights the complex interplay between genetic factors and drug responses, and the potential for natural compounds like quercetin to contribute to novel therapeutic strategies.

Keywords: Cocaine, Flavonoids, Quercetin, easyPET.3D, [18F]-FDG

Liposome-Mediated T Cell Engineering for CAR T Cell Therapy

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Chimeric Antigen Receptor (CAR) T cell therapy has revolutionised cancer treatment by engineering T cells to recognise and eliminate tumour cells. While viral vectors remain the standard for gene delivery, their high production costs and safety concerns drive the need for alternative approaches. Liposomes have emerged as a promising alternative due to their biocompatibility and ability to protect and efficiently deliver genetic material. This study aims to develop and characterise liposome formulations to mediate gene delivery for CAR T cell production.

Five formulations (F1–F5), with different lipid compositions, were prepared using the thin-film hydration method followed by extrusion. Lipid quantification was performed using the Liebermann-Burchard (F1, F2, F5) and Fiske-Subbarow (F3, F4) assays. Lipoplexes were prepared by complexing liposomes with plasmid DNA at the charge ratios (+/-) 1/1, 2/1, 4/1, and 8/1. Dynamic light scattering was used to characterise lipoplexes in terms of size, polydispersity index, and zeta potential. Cytotoxicity was assessed using the Alamar Blue assay. Transfection efficiency and cellular uptake were evaluated by flow cytometry at 48h and 3h post-transfection, respectively. The results revealed that the prepared liposomes have a size between 98.3 and 152.7 nm and positive surface charge (34.2–47.8 mV), confirming their cationic nature, which facilitates DNA complexation. Lipoplexes prepared at lower charge ratios (1/1; 2/1) displayed larger particle sizes, suggesting less DNA complexation, while higher ratios (4/1; 8/1) resulted in smaller, more stable complexes. However, at the 8/1 ratio, lipoplexes induced higher cytotoxicity, suggesting a relationship between stability and cellular viability. Lipoplexes prepared with liposomes F4, at the charge ratio 4/1, achieved the highest transfection efficiency and high cellular internalisation, highlighting their potential as a non-viral gene delivery system for CAR T cell production.

Boosting therapeutic accumulation of senotherapeutics in the brain

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The world's population is ageing at an accelerated pace. New strategies to tackle brain ageing and improve a healthy lifespan are imperative. Senescent cells have been proven to accumulate in the brain over time, leading to cognitive and memory impairments. Senotherapeutics have shown promising results in fighting ageing deleterious effects, and their positive impacts on the aged brain are being explored. Microglial cells are crucial players in the brain ageing process, promoting the brain's susceptibility to dysfunction and cognitive impairment. The clearance of senescent brain cells by the action of senotherapeutics has shown an improved cognition in animal models and an amelioration in the context of age-related disorders. However, due to its inherent properties, the blood-brain barrier (BBB) remains a significant hurdle to brain drug delivery. There is still limited knowledge on the brain penetration of senotherapeutics and selective action on senescent brain cells.

Our goal is to boost brain accumulation of senotherapeutics by engineering a method to locally open the BBB and promote the passage and therapeutic activity of senotherapeutics into the brain.

We were able to induce and characterize the senescence-induction process in microglial cells and select a senotherapeutic with selective impact in senescent cells versus healthy cell populations. We also validate the properties of the photothermal nanoparticles to be applied as BBB-opening mechanism and characterize the impact of temperature in brain endothelial cells.

Our initial findings are an inspiration to modulate BBB permeability and refine brain delivery strategies for anti-ageing drugs.

mRNA formulation for fibroblast targeting in the context of spinal cord injury

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Spinal Cord Injury (SCI) disrupts the communication between the brain and the peripheral body leading to loss of motor, sensory and/or autonomic functions. Current therapeutic options are limited to symptomatic treatments. In the first hours after the injury, pro-fibrotic events are activated where fibroblasts form a fibrotic tissue that blocks neural regeneration, representing potential targets for innovative therapies.

Herein, we load previously identified cationic polymeric nanoparticles (PNP) with GFP mRNA (GFP-PNP) and prove their tropism towards fibroblasts in the central nervous system (CNS). Meningeal fibroblasts (MF), glia, or neural progenitors (NP) were transfected with GFP-PNP, and efficiency was measured as the percentage of GFP positive cells (GFP+). Also, we develop a spinal cord (SC) organotypic model by culturing slices of rat SC in an air-liquid interface and assessing the viability of the tissue. The PNP formulation was tested in this model similarly to that described for cell cultures. The transfected cell types were characterized by evaluating the overlap of GFP + with immunostaining of specific cell markers (PDGFRa, vimentin, aSMA, GFAP).

Our results show 50-70% of GFP+ MF in contrast to <10% glia and ~15% NP. The SC organotypic model tissue posed good viability for up to 18 days. In this model, the GFP expression pattern indicated transfection selectivity. About 40-55% of the GFP+ co-stained with typical fibroblast markers (PDGFRa, vimentin), in contrast with only ~10% expressing the astrocytic marker GFAP. Remarkably, around 50% of GFP+ expressed aSMA, a marker of pro-fibrotic fibroblasts, suggesting preference for activated fibroblasts.

With this work, we developed a promising platform for specific delivery of mRNA to fibroblasts that opens possibilities for fibrosis control upon SCI by adapting the mRNA sequence to virtually any target, and we created a powerful SC organotypic model for in vitro testing of formulations and therapeutics.

Cationic Glycopolymer Nanosystems for Non-Viral T Cell Engineering

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Gene-engineered T cell immunotherapies have shown great promise in the treatment of hematological malignancies. However, the high production costs and limited scalability associated with viral-based gene modification remain significant challenges. As an alternative, non-viral gene delivery systems, particularly those based on cationic polymers, have emerged as viable and cost-effective strategies. This work focuses on the development of novel non-viral gene delivery nanosystems based on cationic glycopolymers for T cell engineering. A library of copolymers composed of 2-aminoethyl methacrylate (AMA) and 2-lactobionamidoethyl methacrylate (LAMA) was synthesized via ARGET ATRP. These glycopolymers were complexed with plasmid DNA encoding the green fluorescent protein (GFP) at polymer/DNA N/P ratios of 8/1 and 10/1. GFP expression was evaluated after 48 h using flow cytometry, while particle size and zeta potential were assessed via dynamic light scattering (DLS) and electrophoresis, respectively. Three distinct glycopolymers with varying carbohydrate-to-cationic ratios and monomer distributions (random vs. block) were tested: PAMA90-b-PLAMA113, PAMA92-co-PLAMA95 and PAMA114-co-PLAMA20. All formulations formed stable polyplexes with physicochemical properties conducive to cellular internalization. Enhanced transgene expression was observed at the higher N/P ratio (10/1). Notably, all glycopolymer-based systems outperformed standard polyethylenimine (PEI)-based polyplexes, with PAMA114-co-PLAMA20 exhibiting the most pronounced effect: a threefold increase in GFP-positive cells, coupled with reduced cytotoxicity. This superior performance can be attributed to the increased cationic content of PAMA114-co-PLAMA20, which plays a key role in facilitating both efficient polyplex formation and endosomal escape. Overall, these findings highlight the potential of cationic glycopolymer-based nanosystems as promising platforms for non-viral gene delivery in T cell engineering.

Cytokine plasma profiling as a potential predictor of clinical stage transition in first-episode psychosis

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Psychotic disorders are among the leading causes of disability globally. Although early intervention in first-episode psychosis (FEP) may improve long-term outcomes, predicting illness trajectory remains a major clinical challenge. To address this, we conducted low-level cytokine and chemokine profiling on plasma samples from 35, stage 2 [1] FEP patients. After at least one year of follow-up, patients were classified based on clinical transition to stage 3 (relapsing episodes): transition (T, n=17) and non-transition (noT, n=18). Univariate statistical analysis identified a significant increase of IL-17A in the T group ($p < 0.05$). Multiple logistic regression using IL-17A, IFN-γ, and MIP-3α achieved strong group discrimination (AUC = 0.853). The optimal cut-off, determined via Youden's index, yielded a cross-validated sensitivity of 76.47%. These findings suggest that a specific cytokine/chemokine panel may serve as a promising predictive tool for psychosis relapse in FEP, warranting validation in larger, independent cohorts.

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MicroRNA-enriched extracellular vesicles: a strategy for heart repair

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Cardiovascular diseases, including myocardial infarction (MI), are the leading cause of death and morbidity in developed countries. Patients who survive an MI often experience a reduced quality of life due to: (i) uncontrolled and excessive cardiac fibrosis, which is a primary contributor to heart failure-related mortality, (ii) insufficient regenerative potential of the injured heart, cause by low proliferation of cardiomyocytes (CMs), and (iii) a high propensity for the development of ventricular arrhythmias, which may lead to a sudden death. Current clinical therapies for MI have significant shortcomings, such as failing to control excessive cardiac fibrosis and not promoting cardiac regeneration.

To address these issues, we propose to use pro-regenerative formulations based on small extracellular vesicles (sEVs) enriched with microRNAs to attenuate cardiac fibrosis and induce CM proliferation. For that, activated neonatal rat cardiac fibroblasts, that are mainly responsible for myocardial fibrosis and extracellular matrix (ECM) deposition, were isolated, cultured and treated with sEVs enriched with miR-29b-3p. The results show a reduction in both alpha-smooth muscle actin fibres (10 %) and gene expression of ECM proteins (e.g., type III collagen and elastin). On the other hand, by treating neonatal rat CM with sEVs enriched with miR-199a-3p, we were able to increase in 5 % the number of CMs with proliferative capacities as evaluated by EdU (5-ethynyl 2´-deoxyuridine) staining. Our results suggest that these two microRNAs can be used in combinatorial interventions to simultaneously target different pathways involved in cardiac regeneration.

Modulation of Brain Cholesterol Metabolism via CYP46A1 in a Mouse Model of Spinocerebellar Ataxia Type 3

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Machado-Joseph disease (MJD), also known as Spinocerebellar Ataxia Type 3 (SCA3), is the most common autosomal dominant ataxia worldwide leading to severe clinical manifestations and premature death. SCA3 is caused by a CAG-repeat expansion in the MJD1 gene, resulting in an expanded polyQ tract in the coding region of the ataxin-3 (atx3) protein, which gains a toxic function and leads to neuronal degeneration.

Dysregulation of brain cholesterol metabolism has been implicated in several neurodegenerative disorders, including SCA3. We have previously shown that systemic administration of AAV-PHP.eB encoding CYP46A1, a key enzyme in cholesterol turnover, was neuroprotective in post-symptomatic SCA3 Tg mice, enhancing motor function and reducing mutant atx3 accumulation, 10 weeks post-injection (in prep). Thus, CYP46A1-treated animals revealed an upregulation of CYP46A1 levels (turnover) and cholesterol synthesis markers, including HMGCR, HMGCS, SREBP-2, DHCR24 and DHCR7 , compared to non-treated SCA3 Tg mice. However, the mechanisms underlying the neuroprotective effects mediated by CYP46A1 are still unknown.

Here, we aimed to characterize brain cholesterol metabolism markers in SCA3 Tg mice, at four different disease progression stages: 2–3; 4–5; 9–10 and 17–18 weeks of age in comparison to WT animals of the same strain by RT-qPCR analysis. Results demonstrated a general reduction of cholesterol synthesis, transport (ApoE and Abca1) and turnover in SCA3 Tg mice, particularly at timepoint 4-5 weeks with a stabilization of expression at timepoint 9-10 weeks, similar to levels determined for age-matched WT animals.

In conclusion, we demonstrate for the first time that the SCA3 Q69 transgenic mouse model exhibits disrupted brain cholesterol homeostasis, which can be modulated through systemic CYP46A1 gene delivery, leading to a general improvement in disease phenotype and reinforcing the neuroprotective potential of CYP46A1 in SCA3 pathophysiology.

CRISPR activation as a new gene therapy strategy for Machado-Joseph disease

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Machado-Joseph disease, also known as Spinocerebellar ataxia type 3 (MJD/SCA3), is a polyglutamine (polyQ) neurodegenerative disorder caused by the abnormal expansion of the CAG repeats in the ATXN3/MJD1 gene, resulting in accumulation of mutant ataxin-3. Ultimately, this results in neurodegeneration in brain regions, such as cerebellum and brainstem, and progressively evolve to debilitating symptoms. Currently, there is no treatment available.

Previously, our group identified sirtuin-1 as a neuroprotective factor in MJD/SCA3-associated neuropathology. Both MJD/SCA3 mouse models and patient-derived fibroblasts showed lower sirtuin-1 levels, while its overexpression reduced mutant ataxin-3 aggregation and blocked neurodegeneration. These findings suggest Sirt1 upregulation as a potential therapy for MJD/SCA3.

In this study, we explored CRISPR activation (CRISPRa) gene therapy approach to selectively enhance endogenous sirtuin-1. We developed and validated an adeno-associated virus (AAV)-delivered CRISPRa system to upregulate Sirt1 in vitro and in vivo.

Also, we expanded the CRISPRa approach to co-activate a second known neuroprotective gene. This co-activation resulted in significant reduction of mutant ataxin-3 and preservation of neuronal function. Our findings underscore the potential of AAV-delivered CRISPRa as a gene therapy tool for polyQ disorders, such as MJD/SCA3 and highlight the potential of multigene activation in targeting neurodegeneration mechanisms.

Non-Viral Gene Delivery Using Redox-Responsive Glycopolymer Nanogels for Immunotherapy

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Cancer continues to represent a major global health burden, being characterized not only by uncontrolled cell proliferation but also by significant immune dysregulation. Current treatment modalities, including chemotherapy, radiotherapy, and surgery, frequently result in adverse side effects and therapeutic resistance. In contrast, immunotherapies, particularly Chimeric Antigen Receptor T cell (CAR T cell) therapy, represent a personalized approach to

redirect the immune system against tumors. However, the success of this technique relies on the development of efficient, safe, and non-viral gene delivery systems. This study explores the potential of redox-responsive nanogel formulations, based on a cationic glycopolymer, as non-viral platforms for gene delivery. The nanogels were prepared at RT for 18 h, combining a previously developed glycopolymer via ARGET ATRP, a plasmid DNA encoding the green fluorescent protein (GFP), and a crosslinker. Different nanogels were prepared at various polymer/DNA (N/P) ratios in PBS or purified water at pH 8.5. Following incubation with Jurkat cells, GFP expression was assessed via flow cytometry after 48 h. Additionally, we characterized the particle size and surface charge using dynamic light scattering and electrophoresis. The solvent environment significantly influenced nanogel properties, as nanogels formed in purified water at pH 8.5 exhibited smaller hydrodynamic diameters and higher surface charges than those in PBS, indicating enhanced colloidal stability due to electrostatic repulsion. These results highlight the critical influence of solvent choice on the formulations' physicochemical properties. Furthermore, transfection assays confirmed that all nanogels successfully delivered DNA into T cells with minimal cytotoxicity. Together, these findings demonstrate how adaptable our nanogel platform is, combining precise physicochemical features with effective biological performance.

Inn

Proteomic Profiling of Cortical Neurons: Insights for Modeling Ischemic Brain Injury

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Ischemic stroke and neonatal hypoxic-ischemic encephalopathy (HIE) share brain ischemic injury as a common pathological feature but differ in the developmental context. In vitro models using oxygen-glucose deprivation (OGD) in primary neuronal cultures are widely used to mimic ischemic conditions. However, the proteomic differences between immature and mature neurons—commonly modeled at different days in vitro (DIV)—remain underexplored. Understanding how the neuronal proteome evolves, for example, from DIV8 to DIV15 can improve our interpretation of ischemic models. In this work, we established and optimized a proteomic workflow to compare the proteomic profiles of cortical neuronal cultures at DIV8 and DIV15. Two approaches—short-gel C and on-beads digestion—were compared, and the most efficient was used for label-free quantitative proteomic analysis. This comparison provides insights into how neuronal maturation shapes the baseline proteome and supports the selection of the appropriate in vitro model to better reflect disease stage, improve the understanding of ischemic mechanisms, and guiding to the evaluation of therapeutic strategies.

ovative Therapies

Interplay between Store-Operated Calcium Entry (SOCE) and Mitochondria-Associated Membranes (MAMs) in neuronal-like cells

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Mitochondria-associated membranes (MAMs) modulate calcium (Ca2+) homeostasis, endoplasmic reticulum (ER) stress response and mitochondrial function, with dysregulation linked to neuropsychiatric disorders. These subcellular structures mediate ER-mitochondria Ca2+ transfer via sarcoplasmic/ER Ca2+-ATPase (SERCA), inositol 1,4,5-trisphosphate receptor (IP3R) at the ER membrane, voltage-dependent anion-selective channel (VDAC) in the outer mitochondrial membrane, and mitochondrial calcium uniporter (MCU) in the inner mitochondrial membranes. If these processes are disrupted, they impair mitochondrial function and dynamics, promoting apoptosis. Store-operated calcium entry (SOCE), activated by ER Ca2+ depletion, is involved in the response of the ER and mitochondria to stress. However, the link between SOCE and MAMs in neurons exposed to stressful stimuli remains unclear and was examined in this study using the SH-SY5Y neuronal cell line treated with the ER stress inducer thapsigargin (Tg) for 3 or 6 hours in the presence or absence of the SOCE inhibitor 2-APB (24 hours pre-incubation). Under stress conditions, cell viability was assessed via MTT assay, SOCE activity was monitored by single-cell calcium imaging (SCCI) with Fluo-4/AM, MAM structure was analysed by proximity ligation assay (PLA) and Western blot (WB), and MAM function was assessed via SCCI with Rhod-2/AM. Tg treatment induced a significant depletion of ER Ca2+ levels and activated the SOCE pathway. MAM morpho-functional alterations under Tg-induced stress conditions, namely changes in the number of ER-mitochondria contacts, reduced levels of mitofusin 2 (MFN2), a key MAM tethering protein, and increased ER-to-mitochondria Ca2+ transfer, were shown to be affected in the presence of 2-APB. These findings highlight SOCE's essential role in Ca^{2+} regulation and ER-mitochondria interactions at MAMs in neuronal cells. This study provides a basis for future exploration of SOCE´s role in brain health and disease.

Oxidative Stress-Induced Mitochondrial Dysfunction Promotes Nucleophagy and DNA-Enriched Amphisome Formation in Parkinson's Disease

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra. It has been described that several molecular mechanisms lead to dopaminergic neurodegeneration.

Mitochondrial and lysosomal dysfunction are well described alterations in PD but its association with DNA damage and micronuclei formation has not been well characterized.

In this study, we investigated the mechanism involved in the formation of DNA-enriched amphisomes, promoted by mitochondrial dysfunction and their potential role in neuroinflammation and PD progression. We used human dermal fibroblasts from control and sporadic Parkinson's Disease (sPD) to evaluate the mitochondrial reactive oxygen species (ROS) production and the expression of autophagic and endosomal-lysosomal pathways. Additionally, we analysed PD cortical brain tissue to evaluate the involvement of these pathways in disease pathology.

Our findings revealed that sPD cells exhibited increased mitochondrial ROS levels, DNA damage, altered expression of nuclear envelope proteins and increased micronuclei formation. Furthermore, we observed an impairment in the autophagosomal-lysosomal pathway leading to the fusion of autophagosomes with endosomes and an accumulation of DNA-enriched amphisomes.

In summary, our findings highlight a dysfunctional mitochondrial-lysosomal axis in PD, contributing to genomic instability and the formation of DNA-enriched amphisomes. This pathway may play a key role in neuroinflammation and disease progression, offering new insights into PD pathophysiology.

Defining the putative role of IELs in mediating protective exercise effects for intestinal immunology

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The aggravating burden of gastrointestinal diseases, metabolic syndrome and cardiovascular diseases represents a major public health concern (Motillo et al., 2020; Wang et al., 2023). Lifestyle factors, such as exercise and diet, are key modulators of these conditions, albeit through unclear underlying mechanisms. Intestinal intraepithelial lymphocytes (IELs) are crucial for the gut, rendering them as putative intermediates of exercise in disease and health.

Our previous work uncovered that maternal vitamin A levels regulate innate lymphoid cells (ILC) development and long-term immunity of the offspring (van de Pavert & Ferreira et al., 2014). Recently, we gathered solid preliminary data showing retinoid signals control IELs and their thymic precursors (Ferreira et al., unpublished). Interestingly, IELs modulate the bioavailability of the enteroendocrine hormone glucagon-like peptide-1 (GLP-1) by its receptor membrane expression, hinting their role in diabetes and cardiovascular diseases (He et al., 2019). Moreover, IELs exhibit a unique metabolic profile, enriched in intestinal absorption and lipid pathways (Brenes et al., 2021 and Ferreira et al., unpublished). Thus, IELs functions surpass local immunity and epithelial homeostasis maintenance, expectedly acting as systemic regulators. By sensing environmental cues, IELs may influence whole-body metabolism.

In our current work, we are testing whether IELs adopt a protective phenotype in response to exercise. Using a voluntary running wheel model combined with gene expression profiling, flow cytometry, and advanced immunometabolic analyses, we are determining IEL responses in normal and IEL-deficient animals. In this way we test if IELs sense and are shaped by exercise, probing the role of exercise-regulated IELs in systemic metabolic diseases. Although preliminary, our findings open new perspectives to target IELs in gastrointestinal and metabolic diseases, linking cellular metabolism to whole-body health.

CLN3 loss affects lysosome distribution and nuclear homeostasis in Batten Disease

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Neuronal Ceroid Lipofuscinoses (NCLs), known as Batten Disease, are defined by mutation in CLN proteins, including CLN3, a lysosomal membrane protein. Despite advances, CLN3-Batten disease remains fatal due to limited understanding of CLN3 function. Recently, we showed that CLN3 depletion induced a YAP1-dependent pro-apoptotic phenotype with an increase in DNA damage. Considering that vision deterioration is one of the first signals in Batten disease, we investigate the effect of CLN3 loss of function in nuclear homeostasis using a retinal pigmented cell line, ARPE-19. Here, we show that the loss of function in CLN3 knockout ARPE-19 cells (CLN3KO) upregulated the expression of pro-apoptotic genes, with increased levels of γ-H2Ax, p-ATM and p-p53, markers of DNA damage, as well as altered nuclear morphology. We investigate if CLN3 loss could affect lysosomal movement and lysosome-nucleus interactions with consequences in the delivery of molecules to the nucleus. By quantification the lysosomal cellular distribution, we found that CLN3KO cells exhibit an increased percentage of peripheral lysosomes than control cells. Next, we assess if alterations of lysosomal distribution could revert the effect of CLN3 loss of function in nuclear homeostasis. Interestingly, by promoting the peripheral or perinuclear distribution of lysosomes using kinesin-based constructs, we observed increased DNA damage in control cells, being exacerbated in condition of higher levels of peripheral lysosomal population. Of note, KIF5B overexpression, a kinesin motor protein, increased rolling movement between lysosomes and nucleus, a phenotype shared with cells overexpressing CLN3. Although any significant DNA damage increase was observed in CLN3KO cells with peripheral lysosomes, perinuclear lysosome relocation reduced DNA damage. These findings suggest that lysosomal cellular distribution impacts nuclear homeostasis and CLN3 appears to play a pivotal role in lysosome-nucleus interactions.

Impact of Polyethylene Microspheres on Inflammation and Senescence Markers in Skin Cells

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Microplastics (MPs) have been raising concerns about environmental and human health. Polyethylene (PE) is a synthetic organic polymer and one of the main constituents of plastics. PE MPs are widely used in cosmetics and personal care products due to their cost-effectiveness, versatility and durability. However, its effects on skin cells remain unclear. Dermal exposure to PE MPs may induce cellular and molecular changes associated with skin ageing and disease.

This study aims to investigate the potential cytotoxic effects of PE-MPs in human dermal fibroblasts (NHDFs) and murine macrophages (RAW 264.7), focusing on inflammatory responses and senescence.

Cultured cells were incubated with different concentrations of MPs (25-500µg/ml) for 24 and 48 hours. Cellular metabolic activity was measured using the resazurin assay in both cell lines. In macrophages, nitric oxide (NO) production was quantified using the Griess assay, interleukin-1 beta (IL-1β) secretion was measured by ELISA and the expression of pro-IL-1β, and inducible nitric oxide synthase (iNOS) was analysed by Western blot. In fibroblasts, collagen mRNA levels were measured by RT-PCR and the morphology was analysed by microscopy. The senescence markers H2Ax and Lamin B1 were monitored by immunocytochemistry and β-galactosidase activity was quantified by a cytochemical assay.

Preliminary findings indicate that exposure to PE-MPs compromises cellular metabolism in both cell types. In macrophages incubated with MPs, increased NO production and IL-1β secretion and a slight decrease in pro-IL-1β expression with no changes in iNOS content were observed. Fibroblasts also exhibited changes in morphology and in senescence markers.

In summary, our data suggests that PE MPs can trigger inflammation and morpho-functional alterations in fibroblasts from the dermis, contributing to their senescence. Further research is needed to clarify their role in promoting skin ageing.

Mitochondrial complex I deficiency dysregulates lipid metabolism leading to altered peroxissomal function

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Mitochondrial dysfunction is a hallmark of aging and age-related disorders. Despite the interdependence between mitochondria and peroxisomes, their crosstalk remains elusive, and direct evidence assessing peroxisomal function in the context of a pre-existing mitochondrial defect remains scarce. We characterized peroxisomal biogenesis in mouse embryonic fibroblasts (MEFs) lacking mitochondrial respiratory complex I-deficient (NDUFS4-KO). Transcript levels of key peroxins involved in peroxisomal biogenesis, maturation and β-oxidation pathways, were upregulated in NDUFS4-KO. Despite an increased number of peroxisomes, they exhibited reduced levels of peroxisomal matrix proteins and β-oxidation, suggesting impaired peroxisomal function. KO lipidomic profiling revealed alterations on multiple lipid classes, including triacylglycerols (TAG), accompanied by an increase in lipid droplet area. These cells also exhibited dysregulated mitochondrial β-oxidation markers, potentially due to reduced substrate availability from peroxisomes. Also, an accumulation of long-chain fatty acids was observed, indicating a dual impairment in mitochondrial-peroxisomal β-oxidation, which may contribute to the altered lipid profile. Furthermore, supplementation with very long-chain fatty acids, which rely on peroxisomal β-oxidation, impaired the cellular response to mitochondrial uncoupling and impacted mitochondrial morphology. Our findings provide novel insights into the intricate mitochondria-peroxisome interplay and highlight how mitochondrial dysfunction can perturb peroxisomal homeostasis, lipid metabolism, and β-oxidation.

Physiologic shear stress promotes endothelial nitric oxide synthase activation and reduces OXPHOS complexes' subunits expression in neonatal endothelial progenitor cells

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Introduction: Endothelial progenitor cells (EPCs) support endothelial homeostasis and release nitric oxide (•NO), which mediates, among others, mitochondrial respiration, and shear stress response. While steady laminar flow (SLF) can exert atheroprotection in mature endothelial cells, its effect remains unknown in EPCs. Mitochondria support EPCs proliferation and migration. Aim: Unravel if SLF influences endothelial nitric oxide (eNOS)-dependent signaling and mitochondrial oxidative phosphorylation system (OXPHOS) complex subunits in neonatal EPCs. Methods: EPCs were isolated from umbilical cord blood from healthy donors. Static EPCs were exposed to VEGF-165 25ng/µL for 5,15,30 and 60 min. EPCs were subjected to 48h-SLF (20 dynes/cm2) in parallel-slide flow chambers. SLF-cell alignment was quantified with OrientationJ. Protein levels were assessed by immunoblotting. Normality was assessed with Shapiro-Wilk test and data analyzed with the most suitable paired test. Results: SLF-induced cell orientation distribution revealed increased area under the curve (p=0.01) and maximum peak (p=0.002) values vs. static. ERK1/2 and eNOS activation were increased in SLF-EPC vs. static (p=0.04;0.0012) and vs. VEGF-treated static (p=0.006;0.004). Mitochondrial OXPHOS complexes subunits SHDB and COXII were decreased in SLF vs. static cultures(p=0.04). Complexes III (UQCRC2) and V (ATP5A) subunits' expression correlated negatively with TOM20 (r2=-0.9,-0.9;p=0.03,0.03). Conclusion: SLF induced both ERK1/2 and eNOS activation and decreased mitochondrial OXPHOS complexes II and IV subunits expression in EPCs, suggesting that SLF could impact EPCs' function. SLF might be more effective in improving the signaling pathway involving ERK1/2 and eNOS than VEGF. These open a new avenue for improving therapies targeting endothelial dysfunction.

SFRH/BD/11934/2022,UIDB/04539/2020,UIDP/04539/2020,LA/P/0058/2020;UIDB/00617/2020;UIDP/04378/2020,UIDB/04378/2020,LA/P/0140/2020.PASGRAS:101080329.

ENGAGEMENT OF PUPIL-LINKED AROUSAL DURING REINFORCEMENT LEARNING TASK

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Changes in pupil size under conditions of isoluminance are a sensitive marker of activity in the ascending neuromodulatory nuclei: noradrenergic, acetylcholinergic, dopaminergic and serotonergic nuclei. In the reinforcement learning (RL) tasks, where individuals adapt their behavior based on feedback from rewards and punishments, pupil responses provide informative insight into the underlying neurocognitive mechanisms. In this study, we investigated the relationship between pupil responses and reward prediction errors (RPEs), value updating, and decision uncertainty, during a probabilistic learning task with three difficulty levels (easy-medium-hard).

Participants were 73 healthy older people aged 54-77 years. The task was performed in a dark room with constant luminance. Participants completed a probabilistic reinforcement learning task while pupil size was continuously recorded using an eye-tracking system.

Behavioral analyses showed that participants were able to learn the task and that difficulty levels had a statistically significant effect on behavioral outcomes such as accuracy and reaction time (RT). Results revealed that mean accuracy decreases and RT increases when the task difficulty increases. Mean accuracy was calculated for easy, medium and hard levels as 64%, 62%, and 57%, (SD:3.07, 1.46, and 2.91) respectively. Mean RT was also calculated for easy, medium and hard levels as 1.13s, 1.17 s, and 1.23 s, (SD:0.059, 0.066, and 0.056) respectively. Analyses of pupil size fluctuations revealed two pupil dilation responses. One evoked by stimulus onset and a second response that started with the onset of the feedback. Pupil response to feedback was larger for negative than positive feedback suggesting that pupil size reflects arousal-related responses to unexpected outcomes. Future analyses will explore the relationship between the engagement of pupil-linked arousal responses and learning through reinforcement.

Early Redox Disturbances Across Aging Tissues Reveal Sex-Dependent Plasma FTIR Signatures

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The global aging population is increasing, highlighting the need to understand early age-related alterations to better identify aging determinants, risk factors, and enable early diagnosis of altered aging trajectories. This study aimed to identify early changes in metabolically active tissues in a sex-dependent manner and explore correlating plasma traits.

Visceral adipose tissue (AT), heart, kidney, liver, pancreas, and blood plasma were collected at 6, 16, and 32 weeks (w) of age (n≥4/timepoint) from male and female Sprague-Dawley rats. Oxidative damage (lipid peroxidation, protein carbonylation), total antioxidant capacity (TAC), catalase activity, and free thiols were measured by spectrophotometry, ATP levels by luminescence, and untargeted plasma screening by Fourier Transform Infrared (FTIR) spectroscopy followed by machine learning in Python. Two-way ANOVA was applied ($p<0.05$ significant).

Overall, TAC peaked at 16w in both sexes. At 16w, males showed a cumulative increase in free thiols, especially in liver and heart, while females showed a marked increase in catalase activity, more evident in AT, liver, and heart. These changes corresponded to peaking oxidative damage in males, particularly in pancreas, liver, and heart, while in females, a milder rise was observed from 6 to 16w. Redox alterations were accompanied by age-related increases in cardiac ATP levels, with modest variations in other tissues. FTIR screening revealed significant age-dependent alterations in several spectral regions, some correlating with tissue-specific redox patterns.

Aging disrupts redox homeostasis and energy metabolism in a tissue- and sex-dependent manner, with measurable signatures in plasma. Identifying early age-related alterations is key to uncovering aging determinants and potential early interventions.

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Selective Mitochondrial Redox Disruption Points to Early Oxidative Stress in Parkinson's and ALS Models

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Neurodegenerative diseases (NDs) are associated with mitochondrial dysfunction, oxidative stress, and thiol redox imbalance, contributing to neuronal death. Mitochondria possess an independent antioxidant system that can serve as the first line of cellular defense, playing a key role in redox homeostasis. This study aimed to investigate whether disruption of the mitochondrial antioxidant system could represent a common early marker of NDs.

To test this hypothesis, fibroblasts from Parkinson's disease (PD) patients and lymphoblasts from Amyotrophic Lateral Sclerosis (ALS) patients were used, along with healthy controls. These peripheral cells were selected for their accessibility compared to central nervous system neurons, offering practical models for biomarker discovery. Cells were treated with MitoCDNB, a mitochondria-targeted compound derived from CDNB (1-chloro-2,4-dinitrobenzene) that selectively disrupts mitochondrial redox balance by depleting glutathione (GSH) and inhibiting thioredoxin reductase 2 (TrxR2). Following treatment with low concentrations of MitoCDNB—previously validated in B104 and NSC-34 cell lines—cell viability, metabolic activity, and reactive oxygen species (ROS) production were assessed.

Preliminary results show that although disease-affected cells exhibited similar viability to control cells, there was a trend toward increased mitochondrial superoxide production in the disease groups. These findings suggest an early redox imbalance potentially linked to impaired mitochondrial antioxidant defenses in PD and ALS. This redox sensitivity may serve as an early biomarker of NDs, though further studies are needed to confirm these observations and elucidate the underlying mechanisms.

Novel Synaptic Bridge: The Characterization of the NMDA-NRXN2 Complex

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Schizophrenia (SCZ) is a severe mental disorder affecting approximately 1% of the global population, characterized by a complex symptom spectrum including positive symptoms, negative symptoms, and cognitive impairments including memory and learning deficits. The intricate mechanisms underlying neuronal communication depend critically on precise postsynaptic receptor localization and synaptic cell adhesion molecules, which modulate receptor function.

We are investigating the functional interaction between the GluN2B subunit of N-methyl-D-aspartate receptors (NMDARs) and the presynaptic protein Neurexin2 (NRXN2) to understand potential molecular contributors to SCZ-related cognitive deficits. Prior research in our lab revealed a significant reduction in postsynaptic density NRXN2 levels in cortical neurons lacking GluN2B (GluN2B-/-), a prominent NMDAR subunit in the hippocampus and neocortex. Notably, levels of Neuroligin, a known NRXN binding partner, remained unchanged in GluN2B-/- neurons, suggesting a potential direct interaction between NRXN2 and NMDARs.

First, to biochemically characterize this molecular complex, we performed immunoprecipitation (IP) assays which demonstrated a direct interaction between NRXN2α and NMDARs. This finding was validated in heterologous HEK293T cells and a dense co-culture system, confirming co-immunoprecipitation of endogenous GluN2A and GluN2B-containing NMDARs with NRXN2α. Further IP experiments using mouse brain lysates confirmed this interaction and highlighted the importance of NRXN post-translational modifications for NMDAR binding. Lastly, immunocytochemical analysis revealed a higher interplay between NRXN2α and GluN2B-containing NMDARs in more mature neurons, with changes in both the distribution and morphology of both NRXN2α and GluN2B clusters.

Together, these findings establish a novel molecular link between NRXN2 and NMDARs, offering potential insights into synaptic dysregulation mechanisms in schizophrenia.

Investigating Dietary Strategies in Machado-Joseph Disease Models

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Spinocerebellar ataxia type 3, also known as Machado-Joseph Disease (MJD), is an autosomal dominantly inherited ataxia caused by an abnormal CAG expansion in the ATXN3 gene. This mutation results in an expanded polyQ tract in the resultant protein, which aggregates, leading to progressive neurodegeneration and motor impairment. Despite advances in understanding disease mechanisms, no cure is available. This study aims to investigate the potential benefits of dietary interventions - Caloric restriction (CR), Mediterranean diet (MeDi), and Ketogenic Diet (KD) - using a transgenic mouse model of MJD (YAC-MJD84.2; MJD-Tg) and an in vitro MJD model.

Mice's body weight and food intake were monitored over 11 weeks. Wild-type (WT) and MJD-Tg mice steadily gained weight on the control diet. Under CR, both groups initially lost weight, with MJD-Tg mice experiencing significantly greater loss before partial recovery. Although food intake remained stable, KD-fed MJD-Tg and WT mice initially lost weight but began gaining steadily after two weeks. Mice fed with MeDi exhibited similar patterns to those on the control diet. The impact of these diets on MJD-Tg cerebellar neuropathology is currently under investigation. We used mimetic conditions of CR, MeDi, and KD in HEK-293T cells expressing mutant ATXN3 to explore the underlying molecular mechanisms. Without compromising cell viability, oleic acid (a MeDi mimetic) increased SIRT1 expression. CR slightly increased SIRT1 levels, while 3-hydroxybutyric acid (a KD mimetic) reduced mutant ATXN3 levels and increased SIRT1 expression. These findings suggest that dietary interventions may have the potential to modulate MJD pathology.

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Hippocampal Region-Specific and Age-Dependent Zinc, ROS and Autofluorescence Responses to Hypoxia

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Synaptic zinc and reactive oxygen species (ROS) regulate hippocampal function, influencing neurotransmission, plasticity, and oxidative stress responses. Maintaining their balance is essential for neuronal health, as disruptions can contribute to neurodegeneration. Hypoxia, whether physical or chemical, alters hippocampal function and may differentially affect zinc and ROS dynamics. Given the distinct functional roles of the dorsal and ventral hippocampus, it remains unclear whether they respond differently to hypoxia. To investigate this, zinc, ROS, and autofluorescence changes were recorded in dorsal and ventral hippocampal slices under both types of hypoxia. Hippocampal slices from young adult and middle-aged female Crl:WI(Han) rats were incubated with the fluorescent zinc or ROS probes NG-DCFDA and H2DCFDA, respectively. Zinc and ROS signals were obtained by subtracting FAD-linked autofluorescence (measured from indicator-free slices) from the detected fluorescence signals. These were recorded at CA3 mossy fiber synapses, chemically stimulated with 20 mM KCl, both during physical or chemical hypoxia. In middle-aged rats, FAD-linked autofluorescence decreased with increasing physical hypoxia severity, but only in the dorsal region. The decrease in zinc signals detected was higher under moderate physical hypoxia, in both regions. Furthermore, there were no significant dorsal-ventral differences in FAD-linked autofluorescence or zinc changes in each hypoxic condition. However, ROS changes rose during chemical hypoxia but not in physical hypoxia. In mixed hippocampal slices, aging was associated with increased FAD autofluorescence and decreased zinc and ROS changes under the same hypoxic conditions. These findings revealed no regional differences in hippocampal FAD autofluorescence, zinc and ROS responses to hypoxia. However, dorsal hippocampus was slightly more sensitive to changes in hypoxic conditions than ventral hippocampus.

Investigating circadian rhythm disturbances in the hypothalamus of progeria mouse model

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Hutchinson-Gilford progeria syndrome is a rare, fatal genetic condition due to a mutation in LMNA gene, leading to premature and accelerated aging, resulting in a lifespan of approximately 15 years. The deleterious impact of this genetic condition on the central nervous system is not completely understood. Given the role of the hypothalamus and circadian rhythm dysfunction of whole-body aging, we hypothesise that LMNA mutation may impact the hypothalamus, including circadian rhythm regulators, in a progeria mouse model (LmnaG609G/G609G mice). This study aims to investigate and characterise the hypothalamus and clock genes in Lmna+/+, LmnaG609G/+ and LmnaG609G/G609G mice with 3, 11 and 18 months of age.

In this study we use quantitative PCR to assess the expression levels of core circadian rhythm genes (Bmal1, Per1-3, Clock, Cry1 and 2, Rev-erb a) and and neuropetides of orexinogenic and anorexinogenic pathways in the hypothalamus. Immunohistochemical analysis is used to visualise neuropeptidergic markers (Hcrt, Avp, Vip, Pomc, Agrp, Npy) within the different nuclei of the hypothalamus of progeria, heterozygous and wild-type mice. By investigating the hypothalamus, a key regulator of aging and sleep-wake homeostasis, and the expression of clock genes, this study seeks to establish a potential link between circadian rhythm disturbances and the accelerated aging process in progeria. Understanding these mechanisms could pave the way for future therapeutic interventions to mitigate the accelerated aging phenotype in progeria. Furthermore, this research may enhance our understanding of chronobiological dysfunction, not only in the context of progeria but also in the normal aging process.

TARPs in the regulation of M-channel function and neuronal excitability

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Neuronal excitability dysfunction is a key feature of neurodevelopmental disorders like epilepsy. Understanding the underlying mechanisms associated with this dysfunction is crucial for developing therapeutic strategies. Excitability is regulated by ion channels, including M-type (Kv7) channels, which are low-threshold, slowly activating potassium channels that modulate neuronal activity and network function. Due to their role in seizure suppression, M-channels are an important drug target; however, available M-channel activators have very adverse side effects and have been withdrawn from clinical use. An alternative approach is to target channel interactors to modulate their activity indirectly and bypass side effects.

M-channels are tetrameric and assemble in the brain as Kv7.2 homomers or Kv7.2/Kv7.3 heteromers. We identified a novel Kv7.2 interactor, Stargazin, a TARP (Transmembrane AMPA receptor regulatory protein) family member essential for AMPA receptor regulation and fast excitatory neurotransmission. Our previous studies demonstrated that Stargazin affects M-currents. Here, we developed new tools to investigate this interaction further and assess whether other TARPs regulate Kv7.2.

Using bioinformatical tools, we were able to map out the interaction sites and through SplitFAST, a fluorescence complementation assay that allows the visualization of protein-protein interactions in live cells, we confirmed the Stargazin-Kv7.2 interaction and showed its regulation upon cellular depolarization and PKC activity. Our findings also revealed that additional TARP family members interact with and differentially regulate Kv7.2.

Overall, this study provides strong evidence of the Stargazin-Kv7.2 interaction and identifies TARPs as bona-fide auxiliary subunits of Kv7.2. The SplitFAST assay enabled real-time analysis, offering spatial-temporal insights. These findings lay the foundation for exploring modulators targeting newly characterized Kv7.2 regulatory mechanisms.

Lactobacillus rhamnosus extracellular vesicles for immune-mediated rescue of sporadic Parkinson's disease

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Introduction: Gut-brain axis dysfunction alongside immune impairment are key features in the development of sporadic Parkinson's disease (PD), supported by a mouse model induced by patient-derived fecal material previously established in our group, in which PD features develop in line with gut-brain progression. It is now a priority to dissect new strategies to harness PD at the early stages to prevent caudo-rostral progression. Here we assess the benefit of bacterial extracellular vesicles (BEVs) secreted by beneficial microbiota members which help regulate intestinal inflammation and, through systemic interactions, may impact brain function, therefore potentially delaying disease progression.

Methods: PD pathology was induced in C57/BL6 mice by oral administration of fecal samples from patients. BEVs were isolated from Lactobacillus rhamnosus cultures and similarly fed to mice. Intestinal and brain dysfunction were assessed by behavior tests, immunofluorescence staining and ELISA kits.

Results: Therapeutic supplementation of BEVs in PD mice resulted in a rescue of ileal immune balance, reflected by cytokine levels and abundance of specific immune populations including CD11+ cells, and CD4+ and CD8+ lymphocytes, which mitigated systemic and brain inflammation, blood-brain barrier permeability, measured by IgG extravasation, TH+ neuronal loss and motor performance deficits.

Discussion: Our results suggest that BEVs supplementation protects against intestinal dysfunction in PD mice, while limiting immune activation, thus counteracting the existing inflammatory environment. Additionally, BEVs prevented the development of brain pathology, supporting Brundin's theory that sequential exposure to triggers, facilitators and aggravators is necessary for PD development. By addressing gut inflammation, BEVs may block disease progression at this stage, preventing neurodegeneration and symptom onset.

Extracellular Vesicles in Insomnia: Optimized Workflow and Preliminary Insights

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Chronic insomnia is the most prevalent sleep disorder worldwide. One of its main characteristics is sleep deprivation, a symptom that has also been associated with the development of various comorbidities, such as cardiovascular diseases, diabetes type II, obesity, neurodegenerative disorders and anxiety.

Extracellular vesicles (EVs) play a critical role in intercellular communication and may reflect pathophysiological changes in various diseases due to their capacity to protect microRNAs from damage when circulating in biological fluids, such as plasma. Studying EVs in the context of insomnia may uncover molecular alterations associated with chronic sleep disruption and provide insights into its biological impact. Despite their potential, standardized and reproducible EV isolation protocols that are easy to implement in clinical settings are still lacking, limiting their translational application. In this study, we have optimized a size exclusion chromatography-based protocol for EV-isolation from human plasma, ensuring high purity and yield. Isolated fractions were characterized using Nanoparticle Tracking Analysis (NTA) for size distribution and concentration, microBCA for protein quantification, and Western Blotting for the detection of EV-associated markers (e.g., Flotillin-1, LAMP2). Results confirm successful isolation of EV-enriched fractions from fraction 8 to 11, with a concentration of 2,01 x 10^9 and 3,37 x 10^9 particles/mL, respectively. The isolated EV-population showed a mean size of 164,8 nm and was positive for EV-associated markers and negative for cellular markers. This optimized and reproducible method lays a robust foundation for subsequent comparative analyses between patients with chronic insomnia and healthy controls, potentially unravelling key EV-based molecular differences that could deepen our understanding of the pathophysiology of insomnia.

Exploring The Molecular Pathogenic Mechanisms of Anti-CASPR1 Autoantibodies in the Central Nervous System

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The significant increase in autoimmune diseases has prompted intensive research into their pathogenic mechanisms. In the last decade, contactin-associated protein 1 (CASPR1) has been identified as a self-antigen in painful and debilitating cases of demyelinating neuropathies and different clinical studies have been published. Recently, autoantibodies against CASPR1 (CASPR1-Abs) were found in a Chronic Demyelinating Polyneuropathy patient with central nervous system (CNS) symptoms, especially memory loss.

Despite the large expression of CASPR1 and other similar antigens in the brain, and studies detecting high protein count in the CSF (sign of brain inflammation) of patients presenting with anti-CASPR1 or other antibodies, CNS symptoms and implications are not screened by clinicians.

In this study, CNS implications of CASPR1-Abs antibodies were explored, addressing a critical gap in understanding the full spectrum of their effects. Based on previous evidence of CASPR1-mediated regulation of excitatory synapses in the hippocampus, and the large expression of CASPR1 in the cortex, cerebellum and hippocampus, it was hypothesized that CASPR1-Abs could reach the brain and cause synaptic disturbances that potentially lead to unknown CNS dysfunction.

Here, we found that CASPR1-Abs bind to and are internalized by rat hippocampal neurons. Studies using micro-electrode arrays, calcium imaging, and patch-clamp electrophysiology showed alterations in neuronal excitability and network synchrony caused by exposure to CASPR1-Abs. Additionally, changes in AMPA and GABA receptors and synaptic composition was evaluated by immunocytochemistry. This study offers the first comprehensive analysis of the molecular mechanisms underlying CASPR1-Ab effects in the brain, calling the attention of clinicians to screen for CNS involvement in cases of autoimmune neuroinflammation.

Other

Clock gene signature predicts Insomnia and links to sleep/circadian parameters

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Chronic Insomnia is a prevalent and complex sleep disorder that is challenging to diagnose due to reliance on self-reported symptoms and diverse clinical presentations. The most severe form, insomnia with short sleep duration (ISSD), is defined by a total sleep time (TST) of less than six hours on polysomnography. However, current diagnostic guidelines rarely recommend objective assessments, highlighting the need for alternative biomarkers. Emerging evidence suggests a link between chronic insomnia and disruptions in the circadian clock system, potentially altering clock gene expression, though the extent of these effects remains unclear.

Here, we investigate sleep and circadian rhythm-related alterations in chronic insomnia and its subtypes, ISSD and insomnia with normal sleep duration (INSD), by assessing plasma cortisol, wrist and axillary temperature, and clock gene expression in peripheral blood mononuclear cells (PBMCs). Additionally, we use machine learning to identify the most relevant clock genes for detecting insomnia and classifying its subtypes.

Patients with chronic insomnia exhibited reduced body temperature rhythms, higher nighttime cortisol levels, and significant alterations in clock genes expression, including in BMAL1, PER1-2, REV-ERBα, and REV-ERBβ, compared to controls. Most alterations were more significant in the ISSD. Moreover, associations between clock gene expression, sleep-related parameters and insomnia severity index (ISI) scores were identified. Using machine learning, we identified three genes as sensitive biomarkers distinguishing chronic insomnia from controls and differentiating between ISSD and INSD subtypes.

Our findings highlight the potential of integrating circadian markers and machine learning to enhance our understanding of chronic insomnia pathophysiology and identify novel biomarkers for diagnosis and treatment strategies.

Other

DLC Coatings for Orthodontic Implants: in vitro and in vivo Biocompatibility Assessment

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Pure metals and first-generation metallic biomaterial alloys, predominantly stainless steel (SS) and nickel-titanium (Ni-Ti) alloys, are vital in Orthopaedics and Orthodontics. However, their in vivo corrosion, particularly in the aggressive oral environment with fluctuating pH, leads to the release of potentially cytotoxic ions like nickel (Ni) and chromium (Cr). This raises concerns about long-term safety due to possible allergic and inflammatory responses. Diamond-like carbon (DLC) coatings are investigated as a solution to enhance corrosion resistance and improve biocompatibility.

This collaborative study between iCBR/CiBB(FMUC) and CEMMPRE (FCTUC) aimed to evaluate the efficacy of DLC coatings in functionalizing orthodontic alloys. In vitro assessments included saliva immersion tests to produce eluates. MTT assay measured fibroblast, macrophage and their co-culture viability, following incubation with the eluates, providing insights into cellular compatibility. In vivo biocompatibility was evaluated through subcutaneous implantation in Balb/c (+) mice, with inflammatory response monitored using easyPET.3D imaging and subsequent histopathological analysis.

The MTT assays revealed that DLC1 exhibited superior cell viability. All DLC coatings demonstrated improved corrosion resistance compared to uncoated SS316L. However, the Si/DLC2 coating showed higher standardized uptake values (SUV) in microPET imaging, suggesting potentially a reduced biocompatibility. Despite observing a mild acute inflammatory response, the overall results indicate that DLC coatings hold substantial promise for enhancing the longevity and reducing adverse reactions associated with orthodontic biomaterials. This research underscores the potential of DLC coatings to minimize metal ion release and improve the clinical performance of metallic implants.

Keywords: DLC, SS316L, in vitro studies, in vivo studies, easyPET.3D

Other

FISETIN AND CMS121 PROTECT AGAINST PALMITIC ACID-RELATED LIPID DYSMETABOLISM AND CELL DEATH IN SH-SY5Y NEURONAL-LIKE CELLS

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Stroke, the primary cerebrovascular disease (CVD), is a leading cause of mortality worldwide. The rising incidence of lifestyle-linked cardiovascular risk factors (e.g., unhealthy hypercaloric diets) may further worsen the stroke burden. Hypercaloric diet-derived palmitic acid (PA) may cause brain damage and enhance CVD risk, while healthier lifestyles (e.g., the neuro-/cardioprotective Mediterranean diet (MedDiet)) may mitigate CVD-related brain injury. Thus, we aimed to analyze the neuroprotective effect of the MedDiet-derived flavonol Fisetin and its more powerful synthetic derivative CMS121 against PA-induced metabolic alterations and cell death in differentiated SH-SY5Y neuronal-like cells.

First, we assessed the most efficient, non-toxic concentrations of Fisetin and CMS121 in protecting against PA-induced decline in SH-SY5Y neuronal-like cellular metabolic activity by the resazurin assay. Then, we utilized cellular lysates and colorimetric techniques to quantify the levels of cholesterol, free fatty acids (FFA), and the caspase-3-like activity.

We observed that 50 nM Fisetin or CMS121 were the most efficient in recovering the neuronal-like metabolic activity after PA exposure ($P<0.001$ and $P=0.003$). This was accompanied by a reduction in neuronal-like levels of cholesterol ($P<0.001$ and $P=0.002$) and FFA ($P<0.001$ and $P<0.001$). Moreover, our preliminary data suggest that Fisetin and CMS121 may decrease caspase-3-like activity by 52% and 48% after PA treatment.

In sum, Fisetin and CMS121 may protect against PA-induced changes in neuronal-like metabolic activity, lipid (dys)metabolism, and apoptotic death. Further studies are required to elucidate these issues.

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Other

NEUROPROTECTIVE ROLE OF FISETIN AND CMS121 AGAINST PALMITIC ACID-INDUCED OXIDATIVE STRESS IN SH-SY5Y NEURONAL-LIKE CELLS

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Stroke is a cerebrovascular disease resulting from the interplay of genetic, environmental, and lifestyle factors, including diet, physical activity, and oxidative stress, which may contribute to vascular dysfunction and neuronal injury. Among lifestyle-related risk factors for stroke, hypercaloric diets rich in saturated fatty acids, such as palmitic acid (PA), appear to play an important role, possibly involving oxidative stress, inflammation and neurodegeneration. Hence, healthier lifestyles, e.g., the Mediterranean diet (MedDiet), and particularly the flavonol Fisetin and its derivative CMS121, may be neuroprotective. However, their role against stroke remains unclear. Hence, we aimed to investigate the neuroprotective role of Fisetin and CMS121 against PA-induced oxidative stress in SH-SY5Y-derived neuronal-like cells.

We used cellular lysates from SH-SY5Y neuronal-like cells, either treated or not with PA, and with the most efficient, non-toxic concentrations of Fisetin or CMS121, to assess the levels of the oxidative stress markers thiobarbituric acid reactive substances (TBARS) and carbonyls groups (for lipid and protein oxidation, respectively), by colorimetry.

We observed that Fisetin and CMS121 significantly reversed the PA-induced neuronal lipid (by 59% and 66%, respectively) and protein oxidation (by 67% and 72%, respectively).

Our preliminary findings suggest that MedDiet-related bioactive compounds may exert strong anti-oxidative stress and neuroprotective potential. However, further studies are needed to clarify this issue.

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Anemia in Inflammatory Bowel Disease Treated with Biologics in Pediatrics

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Introduction: Anemia is among the most frequent complications of pediatric Inflammatory Bowel Disease (IBD). To assess the impact of biological therapy on anemia, we evaluated its prevalence and etiology in pediatric patients with IBD, before and after anti-Tumor Necrosis Factor-alpha treatment.

Material and Methods: In this cross-sectional, observational study, pediatric IBD patients naïve to biological therapy with analytical assessments done at diagnosis, treatment initiation, and 6 months after treatment initiation were included. We analyzed anemia prevalence, etiology, and hemoglobin trends across the three timepoints, along with demographic and clinical factors influencing these outcomes.

Results: The study included 61 patients, 75.4% of which were diagnosed with Crohn Disease. Infliximab the primary treatment (93.4%). Iron supplementation was provided to 32.8% before and 14.8% after starting biologics. Anemia was present in 77.1% at diagnosis, decreasing to 41.0% pre-treatment and further to 6.6% after 6 months. Iron deficiency anemia affected 68.1% at diagnosis, 52.0% before, and 50.0% after treatment; anemia from other causes rose from 31.9% to 50.0%. Hemoglobin increased markedly, especially post-treatment. Among the 22 patients whose anemia resolved after biological treatment, only 22.7% had received iron. No clinical or demographic factor significantly predicted anemia at any time point.

Discussion and Conclusions: Hemoglobin levels improved significantly after 6 months of biological therapy, with anemia prevalence dropping even without iron supplementation. These findings highlight the role of biologics in resolving anemia through inflammation suppression. While iron deficiency remained prevalent, other causes gained prominence over time, underlining the need for ongoing evaluation to optimize treatment strategies. Biological therapy plays a key role in anemia management in pediatric IBD, despite the persistent impact of inflammation in some patients.

Other

MLPA probe design for congenital deficiency of Factor XIII

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Congenital deficiency of Factor XIII is an autosomal recessive disease characterized by delayed bleeding after haemostatic challenges like tooth extractions, and has an incidence of 1 in 2.000.000 people, although this is higher in regions with high inbreeding. The phenotype severity is correlated with the activity level of Factor XIII. When these values are lower that 5%, the patient has a severe haemorrhagic clinical presentation, whilst when the values are higher than 20%, some individuals can be asymptomatic or have excess bleeding after challenge. Complex genetic inheritance hinders the diagnostic of these disorder, usually performed by functional testing. Despite recent advances in sequencing technologies, many cases are not detected by this method, considering the possible existence of large deletions driving pathology, which are not detected by sequencing. Here we aimed to develop and validate an MLPA assay to target copy number variations in these two genes. The study followed a standardized protocol aimed at designing two sets of probes that obeyed to key conditions including molecular size constraints, percentage of C/G nucleotides and genomic specificity. Preliminary results show that all the probes were able to recognize the target regions, which were appropriately amplified. However, one of the probe mixes still requires optimization, specifically regarding probe/DNA ratio. Overall, our results support the potential of our MLPA design to be used in clinical diagnosis, specifically in patients whose genetic diagnosis cannot be reached using the currently available methods.