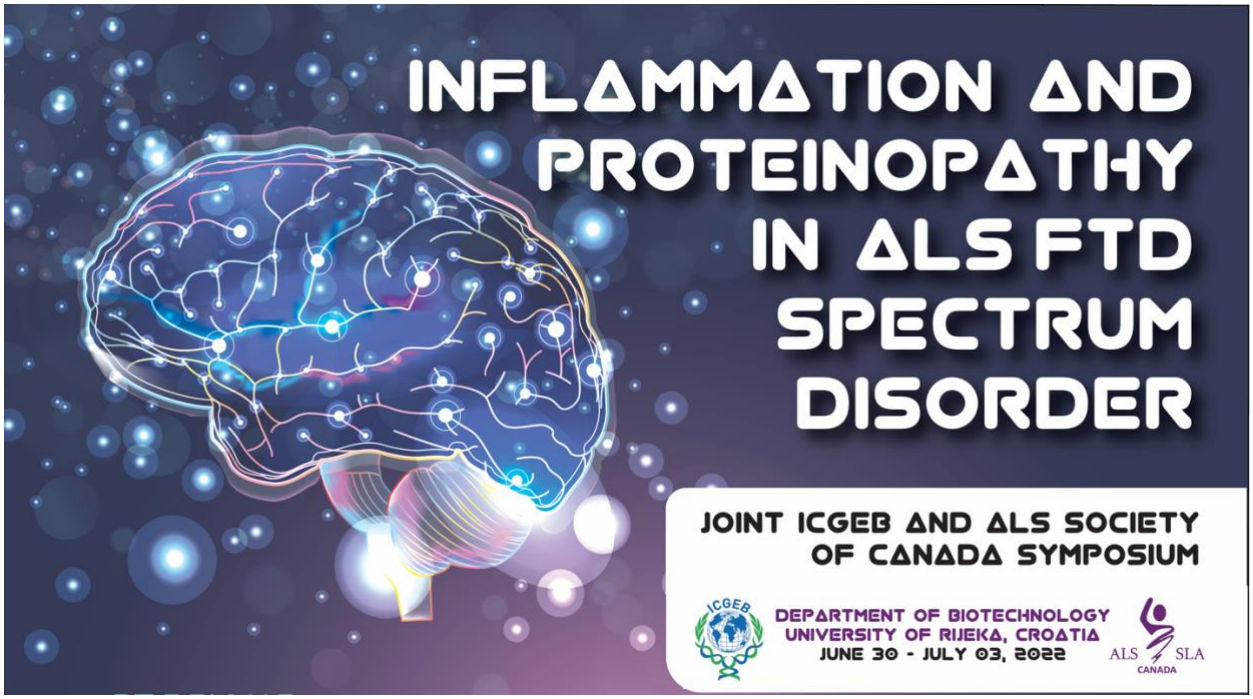

ABSTRACT BOOK

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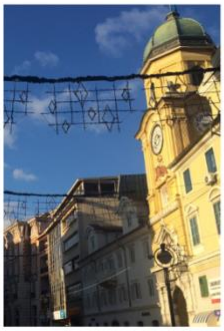
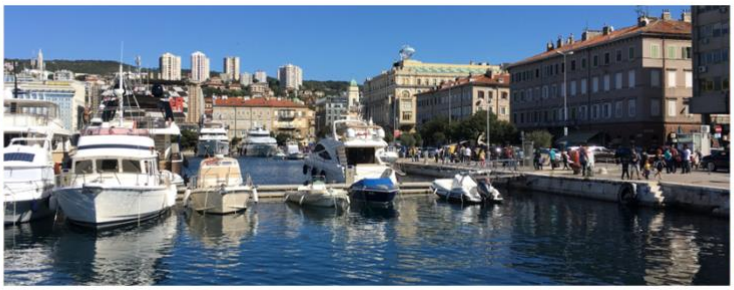


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RIJEKA, 2022

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N-Acetyl Cysteine ameliorates mitochondrial dysfunction in ischemic injury via attenuating Drp-1 mediated mitochondrial autophagy

Background and Purpose: Ischemic reperfusion (I/R) injury causes a wide array of functional and structure alternations of mitochondria, associated with oxidative stress and increased the severity of injury. Despite the previous evidence for N-acetyl L-cysteine (NAC) provide neuroprotection after I/R injury, it is unknown to evaluate the effect of NAC on altered mitochondrial autophagy forms an essential axis to impaired mitochondrial quality control in cerebral I/R injury. Methods: Male wistar rats subjected to I/R injury were used as transient Middle Cerebral Artery Occlusion (tMCAO)model. After I/R injury, the degree of cerebral tissue injury was detected by infarct volume, H&E staining and behavioral assessment. We also performed mitochondrial reactive oxygen species and mitochondrial membrane potential by flow cytometry and mitochondrial respiratory complexes to evaluate the mitochondrial dysfunction. Finally, we performed the western blotting analysis to measure the apoptotic and autophagic marker. Results: We found that NAC administration significantly ameliorates brain injury, improves neurobehavioral outcome, decreases neuroinflammation and mitochondrial mediated oxidative stress. We evaluated the neuroprotective effect of NAC against neuronal apoptosis by assessing its ability to sustained mitochondrial integrity and function. Further studies revealed that beneficial effects of NAC is through targeting the mitochondrial autophagy via regulating the GSK-3β/Drp1mediated mitochondrial fission and inhibiting the expression of beclin-1 and conversion of LC3, as well as activating the p-Akt pro-survival pathway. Conclusion: Our results suggest that NAC exerts neuroprotective effects to inhibit the altered mitochondrial changes and cell death in I/R injury via regulation of p-GSK-3β mediated Drp-1 translocation to the mitochondria. Keywords: Ischemic-Stroke, Mitochondria, Autophagy, Drp-1, Apoptosis

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Co-authors: AUTOIGG consortium

Immunoglobulins G - from humoral factors to biomarkers in Amyotrophic Lateral Sclerosis

We will present the scientific background, rationale and state of the art of the EC H2020 project AUTOIGG. The project consortium (comprised of three Academic institutions from Serbia – as coordinator, Turkey and Finland, two SMEs from France and Turkey, two research institution from USA and one from Costa Rica), strives towards the production of an innovative automated multifunctional device for diagnostics of neurodegenerative diseases, primarily amyotrophic lateral sclerosis (ALS). The background for the current project technology is inspired by the early experiments with ALS IgGs of Stanley Appel and Jozsef Engelhardt (e.g. PNAS 1991 88:647). Our studies with ALS IgGs comprised of diverse physiological phenomena in vitro: a) rise in frequency of postsynaptic currents (Andjus et al., 1996,1997); b) intracellular calcium mobilization in response to ALS IgGs on neurons and glia (Milošević et al.,2013); c) acute free radical release in a microglial cell line (Milošević et al., 2017); and d) increase in the mobility of acidic vesicles (mostly endosomes and lysosomes) in primary cortical astrocytes (Stenovec et al., 2011). Novel and preliminary results with ALS IgGs will be presented demonstrating in vitro: a) Fc-fragment dependence of the Ca2+ response in astrocytes, b) the physicochemical/metabolic alterations in astrocytes vs microglia upon ALS IgG – treatment (FTIR synchrotron light source), c) ultrastructural changes in astrocytes (SEM and AFM studies), and d)

desynchronization of neuronal network activity. Finally, we will present the rationale based on the above results of the project AUTOIGG and the state of the art of the microfluidic lab on a chip that offers better diagnostics and personalized patient stratification. Supported by the EC H2020 grant #778405 and the Innovation Fund Republic of Serbia grant #5360.

APPEL, STANLEY H.
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Regulatory T Lymphocytes Attenuate Peripheral Oxidative Stress and Acute Phase Proteins in ALS

Amyotrophic lateral sclerosis (ALS) is a multifactorial disease in which the central nervous and peripheral immune systems contribute to disease progression and burden. Increased calcium entry at axon terminals induces mitochondrial dysfunction, which in turn induces oxidative stress (OS), inflammation, and further cell damage; OS induces pro-inflammatory responses which provoke the expression of acute phase proteins (APPs). Regulatory T lymphocyte (Treg)/IL-2 therapy was assessed for suppression of OS and APP responses in longitudinal serum samples from a Phase I ALS clinical trial. The first round of Treg therapy slowed disease progression and suppressed ox-LDL levels, but during a 6-month washout period disease progression accelerated and ox-LDL increased. A second round of therapy again slowed disease progression and suppressed ox-LDL levels which rose following treatment cessation. Serum levels of APPs, soluble CD14, lipopolysaccharide binding protein, and C-reactive protein, were stabilized during Treg/IL-2 administrations, but rose when Tregs were discontinued. Thus, Treg therapy suppresses peripheral OS and the accompanying circulating pro-inflammatory induced APPs, both of which may serve as peripheral candidates for monitoring efficacies of immunomodulating therapies.

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Beneficial and Sexually Dimorphic Response to Combined HDAC Inhibitor Valproate and AMPK/SIRT1 Pathway Activator Resveratrol in the Treatment of ALS Mice

Amyotrophic lateral sclerosis (ALS) is a fatal adult-onset neurodegenerative disorder. There is no cure and current treatments fail to slow the progression of the disease. Epigenetic modulation in the acetylation state of NF-kB RelA and the histone 3 (H3) protein, involved in the development of neurodegeneration, is a drugable target for the class-I histone deacetylases (HDAC) inhibitors, entinostat or valproate, and the AMP-activated kinase (AMPK)-sirtuin 1 pathway activator, resveratrol. In this study, we demonstrated that the combination of valproate and resveratrol can restore the normal acetylation state of RelA in the SOD1(G93A) murine model of ALS, in order to obtain the neuroprotective form of NF- kB. We also investigated the sexually dimorphic development of the disease, as well as the sex-sensibility to the treatment administered. We showed that the combined drugs, which rescued AMPK activation, RelA and the histone 3 acetylation state, reduced the motor deficit and the disease pathology associated with motor neuron loss and microglial reactivity, Brain-Derived Neurotrophic Factor (BDNF) and B-cell lymphoma-extra large (Bcl-xL) level decline. Specifically, vehicle-administered males showed earlier onset and slower progression of the disease when compared to females. The treatment, administered at 50 days of life, postponed the time of onset in the male by 22 days, but not in a significant way in females. Nevertheless, in females, the drugs significantly reduced symptom severity of the later phase of the disease and prolonged the mice's

survival. Only minor beneficial effects were produced in the latter stage in males. Overall, this study shows a beneficial and sexually dimorphic response to valproate and resveratrol treatment in ALS mice.

BARALLE, MARCO
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Physiological tissue-specific and age-related reduction of mouse TDP-43 levels is regulated by epigenetic modifications

The cellular level of TDP-43 is tightly regulated; significant level variations have deleterious effects in cell viability. The predominant mechanism responsible for the regulation of TDP-43 levels is an autoregulatory negative feedback loop involving TDP 43 binding to a region of its pre mRNA 3'UTR. We have recently observed an additional regulatory level. In fact, we report here a cause-effect relationship between Tardbp gene promoter methylation, specific histone modification and the TDP-43 level in different mice tissues. Even more important there is in tissues like brain an age-related reduction of TDP 43 levels concomitant with increase promoter methylation and histone modification. Epigenetic control of TDP 43 expression in vivo was observed in mouse and also in human and mouse cultured cell lines. This may be highly relevant in the pathogenesis of neurodegenerative diseases like Amyotrophic lateral sclerosis. In this pathology the formation of TDP-43-containing brain inclusions removes functional protein from the system. This phenomenon is continuous but compensated by newly synthesized protein. The balance between sequestration and new synthesis might become critical with ageing, if accompanied by an epigenetic modification-regulated decrease in newly synthesized TDP-43. Sequestration by aggregates would then decrease the amount of functional TDP-43 to a level lower than those needed by the cell and thereby trigger the onset of symptoms.

BENDOTTI, CATERINA
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Loss of C9orf72 impairs the peripheral nervous system and anticipates symptoms in ALS mice

Repeated hexanucleotide (HRE) expansion in the intronic region of the C9orf72 gene is the most common genetic cause of familial and sporadic amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) or both, however, the mechanisms by which the mutation induces pathology in the frontal cortex or neuromuscular system are still unknown. Both gain-of-function (GOF) and loss-of-function (LOF) mechanisms have been proposed. Although several evidence in mice suggests that the loss of function of C9orf72, by itself, is not sufficient to cause the ALS phenotype, it may contribute to the pathogenesis of ALS in synergy with additional disease genetic modifiers or risk factors. Since peripheral nervous system (PNS) damage is an important determinant of disability in patients with ALS

and is involved at an early stage in the pathogenetic cascade of the disease, we aimed to study the distribution of C9orf72 in this compartment and the effect of its ablation, in control and ALS mice. We found a selective expression of C9orf72 in sciatic nerve Schwann (SC) cells that was markedly increased in SOD1G93A mice. This effect was consistent with the increase in glial fibrillar acid protein (GFAP), a marker of proliferating SC, and the reduction in myelin basic protein suggesting a role of C9orf72 in the autophagic response of SC prone to promoting regeneration of the damaged nerve in ALS mice. In keeping with this, C9orf72 ablation anticipated disease onset in SOD1G93A mice causing an aberrant accumulation of neurofilaments, a reduction of p62 and an increase in β-importin, a marker of axonal stress, in their sciatic nerve which led to the exacerbation of the neuromuscular junction denervation in ALS mice. Our findings suggest a role of C9orf72 loss of function in SC to modulate the pathogenesis of ALS. The project was co-financed using the POR FESR 2014-2020 resources of Regione Lombardia.

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Lentivirus-mediated gene modifications in mouse brain neurons and astrocytes

Introduction and aim: The introduction of genetic modifications mediated by viral vectors is a way of transferring genetic material or molecular systems into cells of interest. Sprouty (Spry) proteins are inhibitors of neural and glial growth factor dependent signaling pathways, which affect various processes in the CNS. To analyze Spry2 in the mouse brain lentiviral vectors (LVs) that encode Spry2-specific shRNA with limited tropism for astrocytes or neurons were constructed. The main aim of this study was to confirm that designed LVs can specifically target neurons or astrocytes. M&M: Male albino mice aged 12–14 weeks received one of the LVs by stereotaxic injection (STX) at predetermined coordinates in the cortex and striatum. After 7 days the mice were sacrificed by whole-body fixation. Brain tissue was isolated and prepared for immunohistochemistry. Identification of LV-transduced cells was performed by detecting the colocalization of neuronal (NeuN) or astrocyte (GFAP) markers with the eGFP transgene introduced by LV construct. Results: Confocal microscopy showed strong expression of the eGFP marker and colocalization with markers of neurons or astrocytes 7 days after STX. Astrocyte tropism was confirmed to be specific, while neural tropism LVs transduced various cell types. Conclusion: Confirmation of colocalization of eGFP with NeuN or GFAP indicated successful targeted transduction of these cells. In the future it will be necessary to determine levels of the Spry2 gene by quantifying the gene product (Western blot). The study was supported by the EU through the ERDF, under grant agreement No.KK.01.1.1.04.0085,project “Genomic engineering and gene regulation in cell lines and model organisms by CRISPR/Cas9 technology–CasMouse”;as the Scientific Centre of Excellence for Basic, Clinical and Translational Neuroscience under Grant Agreement No.KK.01.1.1.01.0007, project “Experimental and clinical research of hypoxic-ischemic damage in perinatal and adult brain".

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Strategy and activities of ALS Centre Zagreb

Aim: Referral Centre for Neuromuscular diseases and Clinical Electromyoneurography of Ministry of Health, Republic of Croatia (Centre) joined ENCALS network in 2017. Our aim was to examine if this event affected Centre's strategy and activities. Methods: interviews with team members. Results: In 2017 Zagreb ALS centre was officially formed and joined ENCALS. This turned out to be a very inspiring event. In short time Centre has started to think not just about continuing to provide up-to-date medical care, treatment, and support for patients with ALS but about actively seeking ways to contribute to the pool of knowledge about motor neuron disease. This is reflected in activities undertaken over the last five years – clinic based registry of ALS patients was created as well as blood and CSF bank. Registry provided up to date epidemiological data about Centre's patient population. Centre's team has been multidisciplinary since the beginning with recent inclusion of several new members with specialist interest in genomics and sleep disorders. Following this team expansion, genetic testing is now available for all ALS patients (whole genome sequencing). Centre has started validation and standardization of the Croatian ECAS (Edinburgh Cognitive and Behavioural ALS screen) in collaboration with Prof. Abrahams' team from University of Edinburgh. Following this same is intended with DAS (Dimensional Apathy Scale). Another aspect Centre is exploring is quality of life of patients with ALS (first results pending) as well as pregnancy and hormone influences on ALS. Centre's activities were presented at several international meetings along with invitation for further regional cooperation. Conclusion: it appears there has been a change in Centre's activity with increased productivity and improved structure since we joined ENCALS network. Although not easily quantified – change in team's attitude from mechanistic to optimistic which is sometimes even more difficult to achieve.

BILIĆ, HRVOJE
Department of Neurology Clinical Hospital Center Zagreb, University of Zagreb School of Medicine

Are we becoming familiar with familial and young onset ALS?

ALS is a progressive and fatal neurodegenerative disease predominantly affecting patient's motor functions. Mean age of disease onset is in the sixth decade, around 65 years. The majority of patients are considered as sporadic cases, while around 5-10 percent with a positive family history (having family members or close relatives diagnosed or died of ALS) are considered as cases of familial ALS (FALS). Young onset ALS cases are patients with symptom onset before an arbitrary cut-off age of 45 years, and they are considered mostly sporadic. Recent studies have reported the incidence of young onset ALS being around 5-10 percent of total ALS cases. In apparently sporadic cases of ALS, considering a polygenic disease model and higher incidence in older age groups, those affected at a younger age are considered to carry a heavier genetic risk burden. In the light of that findings specialised ALS clinics/centres around the world are offering genetic testing and counselling for young onset and familial ALS cases. In our Centre in Zagreb (University Hospital Centre Zagreb) we use NGS consisting of WGS (whole genome sequencing) for selected patients. As of today, we know that over 50 genes have been implicated in the familial form of ALS, but also those variants are being identified in apparently sporadic cases. The identified genes account for about 50-60 percent of FALS cases. In this presentation we describe a case of a young onset ALS patient from our centre with all the specifics surrounding it. Also, we touch on the specifics of patients from our centre being diagnosed with FALS (definite, probable, or possible). In the end we want to point out that the discovery of new genes implicated in ALS, alongside the broader availability and lower costs of genetic testing will increase the number of patients with definite genetic etiology and open new possibilities for targeted and personalised drugs for ALS patients in the future.

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Implication of peripheral macrophages in ALS

Microglia and peripheral macrophages, combined, have been implicated in Amyotrophic Lateral Sclerosis (ALS), but without discriminating their respective roles. Microglial cells and peripheral macrophages have distinct developmental origins and are in specific environments, therefore we hypothesized that their reaction to the disease could be different. Indeed, motor neurons have their cell bodies in the CNS surrounded by microglial cells while their axons extend at the periphery and are in contact with peripheral macrophages. We showed that macrophages along peripheral motor neuron axons of ALS mice and patients reacted to neurodegeneration. In ALS mice, peripheral myeloid cell infiltration into the spinal cord was limited and disease duration dependent. Replacing peripheral macrophages of ALS mice by macrophages more neurotrophic/ less neurotoxic reduced peripheral macrophage activation but impacted also microglial cell reactivity and led to delayed symptoms and increased ALS mouse survival. Transcriptomics analyses revealed that sciatic nerve macrophages and microglia reacted very different to neurodegeneration, with abrupt temporal changes in macrophages and progressive, unidirectional activation in microglia. Modifying peripheral macrophages suppressed proinflammatory microglial responses, with a shift towards neuronal support. Thus, modifying macrophages at the periphery has the capacity to influence disease progression and could be of therapeutic value for ALS by acting directly at the periphery.

BONETTO, VALENTINA & PASSETTO, LAURA
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Defective cyclophilin A induces TDP-43 proteinopathy: implications for ALS and FTD

Aggregation and cytoplasmic mislocalization of TDP-43 are pathological hallmarks of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) spectrum. However, the molecular mechanism by which TDP-43 aggregates form and cause neurodegeneration remains poorly understood. Cyclophilin A, also known as peptidyl-prolyl cis-trans isomerase A (PPIA), is a foldase and molecular chaperone. We previously found that PPIA interacts with TDP-43 and governs some of its functions, and its deficiency accelerates disease in a mouse model of ALS. Here we characterized

PPIA knock-out mice throughout their lifespan and found that they develop a neurodegenerative disease with key behavioural features of frontotemporal dementia, marked TDP-43 pathology and late-onset motor dysfunction. In the mouse brain, deficient PPIA induces mislocalization and aggregation of the GTP-binding nuclear protein Ran, a PPIA interactor and a master regulator of nucleocytoplasmic transport, also for TDP-43. Moreover, in absence of PPIA, TDP-43 autoregulation is perturbed and TDP-43 and proteins involved in synaptic function are downregulated, leading to impairment of synaptic plasticity. Finally, we found that PPIA was downregulated in several patients with ALS and ALS-FTD, and identified a PPIA loss-of-function mutation in a patient with sporadic ALS. The mutant PPIA has low stability, altered structure and impaired interaction with TDP-43. These findings strongly implicate that defective PPIA function causes TDP-43 mislocalization and dysfunction and should be considered in future therapeutic approaches.

BOWSER, ROBERT
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Challenges of measuring TDP-43 in human biofluids

There have been a number of publications over the past decade exploring the levels of TDP-43 protein in biofluids from ALS/FTD patients and control groups. A growing number of commercial immunoassays for TDP-43 are available, leading to variable concentrations of TDP-43 detected in either the cerebrospinal fluid (CSF) or blood of study participants. There have been wide ranges of TDP-43 protein reported in human biofluids, likely due to the lack of reproducibility and reliability of the commercial immunoassays. Efforts to identify TDP-43 species using mass spectrometry have identified a number of peptides from human brain and spinal cord tissue, yet these same TDP-43 derived peptides have been difficult to detect in human biofluids by mass spectrometry. Recent studies have used a quaking induced conversion reaction to demonstrate a TDP-43 aggregation seeding activity of CSF from ALS and FTD patients. While this method is not quantitative, it demonstrates the presence of TDP-43 within the CSF that has the capability to induce aggregates of exogenously added TDP-43. We sought to develop a sensitive and reproducible immunoassay for TDP-43 in human biofluids and make the assay methodology and reagents available to the research community as a resource from the Target ALS Foundation. We have generated and validated an immunoassay for measuring full-length TDP-43 in human CSF and blood. We are currently generating additional immunoassays for pathologic species of TDP-43. Finally, our mass spectrometry results have detected TDP-43 peptides in blood but have failed to identify these peptides in the CSF. Potential limitations of the mass spectrometry methodology will be discussed as well as future directions to create TDP-43 immunoassays for other neurodegenerative diseases.

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Glial cell adaptation to latent cytomegalovirus infection in the CNS

Congenital cytomegalovirus (cCMV) infection is a leading infectious cause of neurodevelopmental defects and hearing loss. Using a murine model of cCMV infection, it was previously shown that infection with mouse cytomegalovirus (MCMV) is associated with a strong host inflammatory response in the brain, which leads to pathological damage. Following the resolution of productive infection, the

virus establishes latency. Virus-specific T cells are retained in the brain and control reactivating virus. Whether these permanent changes in brain homeostasis affect resident glial cells is not known. To answer this question, we have performed single-cell transcriptomic analysis of microglia and astrocytes from latently infected mice. Our analysis revealed that latent MCMV infection drastically changes the composition of microglia at the single-cell level, while astrocyte homeostasis is minimally affected, indicating differential homeostatic features of these glial cells following infection. Infection induced novel subpopulations of microglia, characterized by the expression of different pro-inflammatory gene sets. Microglial subpopulations associated with MCMV latency have highly expressed genes encoding for MHC I and II molecules, and genes involved in response to interferon type I and II (Cxcl9, Cxcl10). These changes were not due to virus latency in microglia, since we did not detect viral genomes in these cells. Antiviral treatment administered early during acute infection can reduce the impact of infection on microglia, however, such treatment during latency is not effective. Altogether, our results show that latent CMV infection in the brain leads to permanent perturbation of microglial homeostasis and drives persistent neuroinflammation.

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Amyotrophic lateral sclerosis: how to deal with it?

Amyotrophic lateral sclerosis (ALS) is a progressive disabling neurodegenerative disease with a poor prognosis. Patients and their families face a number of problems and find it difficult to cope with the disease. A multidisciplinary team approach is necessary today to provide the integrated medical and mental treatment that ALS patients and their caregivers need. An important part of the team are caregivers who stay with the patient, day and night. When the first symptoms of ALS appear, the patient notices them and seeks for medical help. The first symptoms are often nonspecific and the patient wanders from one doctor to another for help. This makes the patient worried and upset. When the diagnosis is finally established, it is necessary to inform the patient.The truth needs to be told and give the patient the opportunity to come to terms with mortality and make important decisions. He needs to be supported so that he does not feel lonely. He must know that there is a way to solve the problems he will go through over the next months and years. That is stressful for the patient and his relatives. The diagnosis should be discussed with a patient in a quiet place and the healthcare professional must have enough time expecting a number of questions. Often the patient cannot remember or cannot understand everything he has been told due to stress, and the presence of a family member or a caregiver is desirable as a support. With the disease progression, the fear of immobility, sadness and depression grows. Caregivers have an important role in ALS patients. In assisting the patient, they take care of the loved person as much as possible, have a desire to do the best, but there are times when they feel helpless. Taking care of a diseased person is an unselfish act, but it can also be a great burden. Therefore, caregivers must find a way to occasionally move away from stressful situations, while maintaining their mental and physical health.

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Importance of hnRNP proteins as ALS/TDP-43 disease modifiers

In recent years, we have performed transcriptome analysis of SH-SY5Y cells silenced for DAZAP1, hnRNP Q, hnRNP D, hnRNP K and hnRNP U that were known to affect TDP-43 pathology. After cross-comparing transcriptomic profiles of cells depleted by each of these factors, we identified seven

commonly regulated transcripts: CHPF2, IGF2, IRAK2, RNF112, UBE2E3, C1orf226 and NOS1AP. Out of this list, NOS1AP (also known as CAPON) has recently emerged as an important player in brain physiology and pathophysiology. Several studies suggest that its interaction with nNOS contributes to NOS1AP-mediated excitotoxicity, the formation of neuronal processes and probably schizophrenia. Most importantly, we observed a clear correlation between the reduction of NOS1AP and the inclusion of two previously characterized cryptic exons in different brain regions of patients with TDP-43 pathology. At the functional level, using primary mouse neuronal cultures we demonstrated that decrease of TDP-43 induces a drop in NOS1AP expression and protein levels and elicited a significant down-regulation at the mRNA level of several factors predicted to interact with NOS1AP. Specifically, we found a significant decrease in several essential component of the synaptic network: PSD93, PSD95, SynGAP, and Synapsin-3. Most importantly, we observed that upregulation of NOS1AP in TDP-43 depleted SH-SY5Y cells can successfully rescue of many of these genes in the absence of TDP-43. In addition, downregulation of NOS1AP is also capable to rescue on its own the degenerative phenotype induced by TDP-43 overexpression in fly eyes. Taken together, our identification of NOS1AP as a co-regulated target by several hnRNP proteins, including TDP-43, and the role of NOS1AP in the synaptic signaling can link this gene to neurological dysfunctions associated with ALS, making it a suitable candidate for the development of novel therapeutic strategies in the context of this pathology.

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CircRNAs in Amyotrophic Lateral Sclerosis and metabolic stress

Alterations in RNA metabolism and links to cellular stress have been both described in Amyotrophic Lateral Sclerosis (ALS). In this study we investigated changes in the expression and localization of circular RNAs (circRNAs), covalently closed natural RNA molecules, in human spinal cord in ALS and in a neuronal cell line under metabolic stress. We performed RNA-sequencing (Illumina) of lumbar spinal cord samples of ALS and control individuals and in silico analysis using Partek Flow. We observed dysregulation in the expression of ~25% of circRNAs detected in spinal cord in ALS compared to controls, most of which are up-regulated and encoded within the protein coding sequence of genes. Within the group of top altered circRNAs, we confirmed the dysregulation of 5 circRNAs (circANXA1, circNEFL, circMALAT1, circSOD2_62 and circSOD2_72) using real-time PCR and compared these alterations with those observed in SH-SY5Y cells after metabolic stress. Next, we used FISH/IF to study the subcellular localization of circRNAs in SH-SY5Y cells differentiated with retinoic acid. We observed that circRNAs distribute in granular structures in the nucleus and the cytoplasm including neurites, but they barely colocalize with markers of RNA granules. We also observed colocalization of certain circRNAs with fibrillarin, a marker of nucleolus and with RSP3, a ribosomal protein, suggesting circRNAs coding properties. These findings and future experiments will help to uncover novel functions of circRNAs in neuronal death and survival, and trace RNA networks defects associated to ALS.

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Role of environmentally induced mitophagy alterations in neurodegeneration

Major neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), and Parkinson's disease (PD) have both distinct and shared features. For example, the specific nuclei affected and proteins that aggregate may be somewhat specific to individual diseases (overlapping pathology is often present). In contrast, many pathogenic pathways overlap, including oxidative stress/damage, mitochondrial dysfunction, autophagic dysfunction, and protein aggregation. Autophagy is an important cellular process that removes misfolded/aggregated proteins and clears damaged organelles. Mitophagy is an organelle specific process that is disrupted in several neurodegenerative diseases. The inability to clear toxic mitochondria is likely a key primary mechanism of neurodegeneration. This is evidenced by findings that mutations in several critical mitophagy genes (i.e., TBK1, optineurin, Parkin, PINK1) produce neurodegenerative disease, including ALS and PD. Environmental exposures have been linked to neurodegenerative disease through epidemiology and laboratory toxicology exposures (most cases are "sporadic" and likely result from gene-environment interactions). In cellular and animal model systems, we found that exposure to multiple neurotoxicants linked to neurodegeneration produces mitophagy impairments that occur prior to neuronal cell death, evidenced by biochemical, histochemical, and Western blot assays. Moreover, a newly created nematode model expressing an ALS causing mutation in optineurin preliminarily exhibits a shortened lifespan and alterations in the thrashing assay, indicating phenotypic relevance to ALS. This finding also suggests that genetic disruption of mitophagy is a primary pathology in some ALS cases. Individually, all these findings suggest mitophagy disruption as a primary pathological event. Together, these model systems will be useful in studying how gene-environment interactions modulate etiopathogenesis.

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How PQC inhibition modulates miRNA loading in large and small extracellular vesicles

Extracellular vesicles (EVs) are membrane-enclosed particles released from all eukaryotic cells that carry proteins, lipids, RNA and DNA. They are classified in two main types of EVs: large vesicles (LVs) and small vesicles (SVs). In our previous studies we observed that both LVs and SVs play a role in the disposal of neurotoxic aggregates of the TAR DNA-binding protein 43 (TDP-43) and its C-terminal fragments of 35 (TDP-35) and 25 KDa (TDP-25), associated with Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD).This release increased when the protein quality control (PQC) system [i.e. chaperone proteins, the ubiquitin proteasome system (UPS) and the autophagic pathway] was impaired, a common condition observed both in ALS and FTD. Since TDP-43 is an RNA-binding protein and it is involved in miRNA biogenesis, we wondered whether PQC impairment could also affect miRNA content in EVs. LVs and SVs were isolated from medium of NSC-34 cells, treated or not with the UPS inhibitor MG132 or with the autophagy inhibitor NH4Cl, by differential centrifugation and characterized by Nanosight. MicroRNA libraries were generated using Small RNA-Seq Library Prep Kit (Lexogen) and sequenced on a NextSeq 500/550 (Illumina). Interaction prediction was carried out on TarBase v.8 database. We found a total of 91 Differentially Expressed (DE) (log Fold Change (FC) >1 and <-1) microRNAs in treated-EVs compared to untreated

EVs. No DE miRNA were found in NH4Cl-LVs, only 7 miRNAs were DE in MG132-LVs and of the 82 miRNAs in MG132-SVs and 66 in NH4Cl-SVs, 43 were in common. Interestingly, the most enriched pathway targeted by commonly DE SVs-miRNAs is the prion disease. In conclusion our observation suggests that, in disease condition, EVs are enriched in both toxic TDP-43 species and potentially harmful miRNA, and thus they may contribute to the propagation of the disease from affected to healthy cells.

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Gene Therapies for Sporadic ALS: An Emerging Concept

Gene therapies for sporadic ALS have included ATXN2 knockdown ASOs. We have studied knockdown of TDP-43 via a misfolding-specific intrabody and RACK1 ASO. Immunization with an N-terminal domain epitope of misfolded TDP-43 gives rise to mAbs selective for pathogenic vs physiologically important forms of TDP-43. Recombinant intrabodies for the TDP-43 NTD are able to alleviate intracellular pathogenic TDP-43 when expressed through viral transduction of CNS cells. RACK1, a stress granule ribosomal protein, appears non-essential in mature cells, and RACK1 knockdown alleviates the detrimental effects of RACK1 co-aggregation with pathogenic TDP-43 and FUS. The significant amelioration of neurodegeneration by TDP-43 intrabodies and RACK1 knockdown in vitro and in vivo systems supports knockdown of these two pathogenic proteins for treating TDP-43 proteinopathy.

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Intravenous injection of AAV encoding scFv antibody to target TDP-43 proteinopathy

Abnormal cytoplasmic aggregates of TDP-43 are a pathological hallmark of degenerating neurons in many neurodegenerative disorders, including Amyotrophic Lateral Sclerosis (ALS), Frontotemporal Dementia (FTD), Alzheimer's disease, Parkinson's disease. In order to target TDP-43 and reduce its pathology, we developed an AAV vector encoding a single chain antibody (scFv) against the RNA Recognition Motif 1 (RRM1) of TDP-43. Previously, our lab (Pozzi et al.,2019) reported that this AAV-mediated delivery intrathecal of the scFv can be used to mitigate TDP-43 pathology in transgenic mice expressing mutant TDP-43. Here, we propose to further validate the antibody approach in mitigating pathology and cognitive defects in new mouse models with robust TDP-43 pathology and the use of scFv-encoding AAV vector bearing a recombinant capsid designed to achieve efficient neuronal transduction after injection directly into blood circulation. With this improved AAV vector and an injection directly into blood circulation, it is possible to reach and transduced neurons over a large portion of the nervous system. It is our hope that such AAV-delivery of scFv antibodies will succeed in TDP-43 proteinopathy. A single intravenous administration of AAV vector to achieve sustained production in neurons of therapeutic antibody mitigating TDP-43 proteinopathy is an appealing strategy for treatment of neurodegenerative diseases. Abnormal cytoplasmic aggregates of TDP-43 are a pathological hallmark of degenerating neurons in many neurodegenerative disorders, including Amyotrophic Lateral Sclerosis (ALS), Frontotemporal Dementia (FTD), Alzheimer's disease, Parkinson's disease. In order to target TDP-43 and reduce its pathology, we developed an AAV vector encoding a single chain antibody (scFv) against the RNA Recognition Motif 1 (RRM1) of TDP-43. Previously, our lab (Pozzi et al.,2019) reported that this AAV-mediated delivery intrathecal of the scFv can be used to mitigate TDP-43 pathology in transgenic mice expressing mutant TDP-43. Here, we

propose to further validate the antibody approach in mitigating pathology and cognitive defects in new mouse models with robust TDP-43 pathology and the use of scFv-encoding AAV vector bearing a recombinant capsid designed to achieve efficient neuronal transduction after injection directly into blood circulation. With this improved AAV vector and an injection directly into blood circulation, it is possible to reach and transduced neurons over a large portion of the nervous system. It is our hope that such AAV-delivery of scFv antibodies will succeed in TDP-43 proteinopathy. A single intravenous administration of AAV vector to achieve sustained production in neurons of therapeutic antibody mitigating TDP-43 proteinopathy is an appealing strategy for treatment of neurodegenerative diseases.

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Identification of new variants in patients with ALS or other Neurodegenerative Disorders by whole genome sequencing data.

To explore the missing heritability in a cohort of patients affected by Neurodegenerative disorders (NDDs), we performed WGS of 140 NDDs patients including Amyotrophic Lateral Sclerosis(ALS), Frontotemporal Dementia(FTD), Parkinson disease (PD) and Spinocerebellar Ataxia(SCA). This approach allowed us to investigate the following sequence variants: a) Single Nucleotide Variants (SNV) in coding and non-coding sequences in a panel of 855 genes implicated in NDDs. We found 16 pathogenic/likely pathogenic variants (ACMG) in genes causative of NDDs rare forms (10 in ALS) and 8 in genes causing a phenotype different from patient clinical presentation (4 in ALS). We applied SpliceAI to predict a possible effect of SNV on splicing mechanism and 102 rare variants (delta score >0.6) were identified. Among those we performed in vitro studies for 9 variants and for 6 of them, we confirmed a role in splicing alteration; b) Tandem Repeat (TR) analysis. Using Expansion Hunter, GangSTR and novel tools, 5 novel loci with a possible TR expansion were identified in ALS cohort. The results were replicated in larger independent Italian (763 ALS 1018 controls) and Dutch cohorts (3121 ALS and 1217 controls). Preliminary data confirmed one of these loci, showing that TR was present in patients and not in controls (HC) (; ITFG2: 1/352 ALS vs. 0/249 HC). Results were not replicated for the remaining 4 loci (*FRA10AC1, RFC1, HK1, INPP5B*); c) Structural variants analysis. Using Delly and CNVkit, we identified a 15q25 deletion (about 1.2Mb), previously associated with mild intellectual disability and dysmorphisms, in a PD patient. In conclusion, the prioritization pipeline identified for the first-time non-coding SNV with a possible pathogenic role in ALS. In addition, we proposed novel TR loci involved in ALS whose replication is ongoing. Moreover, we found patients with pathogenetic variants in genes involved in different NDDs, reinforcing the idea of a shared genetic cause among different NDDs.

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Investigating the molecular mechanisms involved in KIF5A-related neurodegeneration

Mutations targeting the neuron-specific kinesin KIF5A lead to neurodegenerative diseases (NDs), including amyotrophic lateral sclerosis (ALS). Distinct phenotypes develop according to which of the three KIF5A domains is affected by mutations, but the reasons behind such heterogeneity are not known yet. Our aim is to gain insight into the molecular mechanisms underlying KIF5A-related NDs by functionally characterizing four domain-specific KIF5A mutants (R17Q, R280C, R864*, N999Vfs*39). Overexpression in SH-SY5Y cells evidenced altered protein turnover for R17Q KIF5A and the ALS-related N999Vfs*39 mutant with respect to wild-type (WT) KIF5A, with the two mutants displaying shorter half-life upon cycloheximide chase. Higher accumulation was observed for R17Q and N999Vfs*39 KIF5A compared to WT KIF5A and the other KIF5A variants following proteasomal blockage, indicating that the ubiquitin-proteasome system might represent the main degradation route for the two mutants. R17Q and N999Vfs*39 KIF5A also displayed preferential partitioning in the detergent-insoluble protein fraction upon proteasome inhibition, which suggests they may form harmful inclusions when proteostasis is impaired. Altered intracellular distribution was evidenced for R864* and N999Vfs*39 KIF5A overexpressed in NSC-34 cells, with the two mutants mainly localizing at cell periphery instead of being diffused within the whole motoneuron like WT KIF5A. In particular, the ALS-related N999Vfs*39 mutant formed puncta inside cell processes, which hints at reduced protein solubility even in basal conditions, and partially sequestered WT KIF5A within them. The abnormal distribution displayed by R864* and N999Vfs*39 KIF5A was paralleled by limited colocalization between the two mutants and mitochondria, whose axonal transport is largely reliant on WT KIF5A in motoneurons. Taken together, our preliminary observations indicate that both unique and shared mechanisms might underlie the pathogenesis of KIF5A-related NDs.

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ALS/FTD-associated C9orf72 C4G2 repeat RNA disrupts phenylalanine tRNA aminoacylation

Background: Mutation in the C9orf72 gene is the most common genetic cause of ALS and FTD. Mutation is expansion of hexanuleotide repeat – GGGGCC that can reach hundreds to thousands repeats in disease, whereas up to 23 repeats are present in healthy individuals. Transcription of repeats in sense and antisense direction leads to repeat(G4C2)n and (C4G2)n RNA, which can sequester RNA binding proteins and form RNA foci pathognomonic of C9orf72 associated ALS and FTD, predicted to cause

RNA toxicity by. The aim of this work was to identify proteins that bind less studied antisense C4G2 RNA transcripts. The study focuses on cytoplasmic interaction with Phe-tRNA synthetase (FARS) and its effect on protein synthesis, as disruptions in aminoacyl-tRNA synthetases are increasingly observed in neurodegenerative disorders and can lead to protein misacylation, misfolding and aggregation. Objectives: Identification of proteins binding to antisense $(C4G2)_{32}$ RNA transcripts from C9orf72 mutation and determination of how antisense RNA-FARS interaction impact FARS aminoacylation function. Methods: We have used RNA-pull down assay from mice and human brain lysates and mass spectrometry to determine proteins that bind to $(C4G2)_{32}$. Interactions were confirmed using WB, FISH/ICC and RNA- protein PLA. Impact of antisense RNA-FARS interaction was determined using aminoacylation assay and western blots. Results: We have shown that FARSA and FARSB, interact with $(C4G2)_{32}$ in RNA-pull down assay from human and mice brain lysates. The interaction was confirmed using three different assays and interaction between FARSA and $(C4G2)_{32}$ results in significant decrease of charged $tRNA^{phe}$ in patient lymphoblasts compared to control. Additionally, expression of three phenylalanine-rich proteins was observed to be lower in C9-ALS patient lymphoblasts in comparison to control. The same was not seen for proteins low in phenylalanine. Conclusion: We found impairment of FARS catalytic function in C9 lymphoblasts, as $tRNA^{phe}$ charging is reduced compared to control. This discovery is important in highlighting the role of aminoacyl-tRNA synthetases in C9orf72 ALS/FTD, as they have been so far implicated in various neurodegenerative diseases. ARSs have multiple functions in addition to tRNA aminoacylation, and these functions should also be researched further in aspect of neurodegeneration, as they could contribute to the disease mechanisms.

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Contingent intramuscular boosting of P2XR7 axis improves motor function in transgenic ALS mice

Muscle weakness plays an important role in neuromuscular disorders comprising Amyotrophic Lateral Sclerosis (ALS), a fatal neurodegenerative disorder that leads to progressive degeneration of motor neurons and severe muscle atrophy without effective treatment. Most research on the disease has been focused on studying motor neurons and supporting cells of the central nervous system. Strikingly, recent observations have shown that the expression of the SOD1G93A mutation in skeletal muscles causes denervation of the neuromuscular junctions, inability to regenerate and consequent atrophy, all clear symptoms of ALS, suggesting that these morpho-functional alterations in skeletal muscle precede motor neuron degeneration, bolstering the interest in studying muscle tissue as a potential target for the delivery of therapies. We previously showed that the systemic administration of the P2XR7 agonist, 2′ (3′) - O - (4 - benzoylbenzoyl) adenosine 5 - triphosphate (BzATP), enhanced the metabolism, improved the innervation and promoted the myogenesis of new fibres in the skeletal muscles of SOD1G93A mice. Here we further corroborated this evidence showing that intramuscular administration of BzATP improved the motor performance of ALS mice by enhancing satellite cells and the muscle pro-regenerative activity of infiltrating macrophages. The preservation of the skeletal muscle retrogradely propagated along with the motor unit, suggesting that backward signalling from the muscle could

impinge on motor neuron death. In addition to providing the basis for a suitable adjunct multisystem therapeutic approach in ALS, these data point out that the muscle should be at the centre of ALS research as a target tissue to address novel therapies in combination with those oriented to the CNS. The project was supported by Italina Ministry of Health (project SG-2018-1236622 and the POR FESR 2014-2020 resources of Regione Lombardia.

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Long term survival of participants in the mesenchymal stromal stem cells transplantation in amyotrophic lateral sclerosis.

Background aim: Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder with very limited available treatments. The Mesenchymal Stem Cells (MSCs) can represent a promising tool for stem cell-based clinical trials in ALS patients. We conducted two phase I/II clinical trials to evaluate this treatments' safety and feasibility in the past years. Herein we report the results of the long-term survival analysis of participants in these trials. Methods: The trials aimed to evaluate safety and feasibility of autologous MSC isolated from bone marrow and implanted into the dorsal spinal cord with a surgical procedure (19 ALS patients). All the included patients were regularly monitored after transplantation with clinical, psychological, and neuroradiologic assessments at the tertiary ALS center in Novara (Italy) until death. For this analysis, we considered patients with a disease history > of 10 years from transplantation as long-survivors. Results: we enrolled 11 males and 8 females. No patients manifested severe adverse effects or increased disease progression because of the treatment also in the long term. There were no relevant radiological lesions over the disease course. Of 19 patients, 9 (47%) were considered long-survivors. The mean age of this group at transplantation was 35 years, and all patients had spinal onset. Five patients (26%) are alive, two without tracheotomy (20 years after diagnosis and 17 after transplantation) and without any bulbar sign, and three with tracheotomy but without any cranial involvement. Other four patients died after a long disease course, and they had a mean survival time of 14 years from transplantation (three of these four without tracheotomy). Discussion: No immediate clear clinical benefits were detected in these patients after the stem cells transplant, but the high percentage of patients with extended survival and no bulbar involvement can suggest that MSC can be administered safely and that a targeted patient's recruitment could be translated in a higher probability of clinical outcome achievement.

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A fragment of the RNA-binding protein RGNEF suppresses TDP-43 toxic phenotype in an animal model of ALS

Amyotrophic Lateral Sclerosis (ALS) is an adult-onset progressive disorder characterized by the selective degeneration of motor neurons. Alterations of RNA metabolism and the development of pathological neuronal cytoplasmic inclusions (NCIs) in motor neurons are hallmarks of the disease. The aggregation of the RNA binding protein (RBP) TDP-43 is a critical pathological marker of ALS and its dysregulation is causative of motor neuron death. Because of this, TDP-43 overexpression can be used to generate ALS-like phenotypes in diverse animal models, including fruit flies (Drosophila melanogaster). Previously, we described Rho guanine nucleotide exchange factor (RGNEF) as a 190 kDa RBP that forms NCIs in motor neurons of ALS patients and co-aggregate with others RBPs involved in ALS, including TDP-43. Recently, we described that an amino terminal fragment of RGNEF, containing a leucine-rich domain (LeuR), interacts in a complex and also co-aggregates with TDP-43 under metabolic stress conditions. In this study, we analyzed the effect of the co-expression in vivo of full length RGNEF or LeuR with TDP-43 in fruit flies. We observed that RGNEF overexpression increases the lifespan of flies, and notably that the expression of RGNEF or LeuR suppresses the toxic phenotype generated by TDP-43 overexpression observed through an increased lifespan, abolition of motor defects and eye degeneration. At pathological level we observed co-aggregation between LeuR and TDP-43 in the brain and optical lobe of the flies. Also, we observed that RGNEF and LeuR can directly interact with TDP-43 in vitro. This suggests that the protective mechanism of LeuR could involve the blocking of the toxic gain of function of TDP-43 aggregates. By observing that RGNEF, and particularly a critical fragment of the protein (LeuR), can modify the phenotype induced by TDP-43 in vivo, suggests a potential therapeutic pathway for this fatal disorder.

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Challenges with approaches to restoring proteostasis in ALS/FTD

Protein misfolding and aggregation are associated with multiple forms of ALS/FTD. A logical therapeutic strategy is to boost the chaperoning capacity of neural cells by inducing heat shock proteins (HSPs) to manage the load of aberrant proteins; however, motor neurons have a high threshold for inducing expression of HSPs in response to stress and poorly respond to HSP-coinducers such as arimoclomol. Histone acetylation and chromatin remodeling complexes influence expression of stress-response pathways including heat shock genes. To make matters worse, these mechanisms of nucleosome remodeling are abrogated in ALS. We hypothesized that treatment with histone deacetylase (HDAC) inhibitors might not only relieve epigenetic abnormalities, but enable HSP-inducers. Indeed, treatment with Class I HDAC inhibitors lowered the threshold for expression of stress-inducible HSPA1A (Hsp70) in cultured motor neurons and co-treatment improved efficacy of multiple types of HSP-inducing drugs. However, this effect was stress-dependent and occurred in a subset of neurons expressing $SOD1^{G93A}$ or $TDP43^{G348C}$, but did not occur in neurons expressing $FUS^{R521G/H}$. Yet the treatments were neuroprotective through other, still to be defined, mechanisms. Neither drug increased HSP expression in cortex, spinal cord or muscle of $SOD1^{G93A}$ or FUS^{R521G} transgenic mice, but as in culture, both drugs showed efficacy, most remarkably, reversing cognitive impairment in FUS^{R521G} mice. Conclusions: Therapeutic upregulation of HSPs remains elusive because of the biology of their regulation in nervous tissue. Either drugs don't induce the HSPs being assessed, their use is limited by toxicity, or disease mechanisms are suppressive. ALS is not a homogeneous disease and subtypes respond differently to treatments. The therapies we are testing have other targets and might be particularly useful in ALS associated with RNA metabolism and FTD.

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Assessing pathogenicity of OPTN variants in ALS and glaucoma by bioinformatic analysis

Amyotrophic lateral sclerosis (ALS) is a severe, rapidly progressive neurodegenerative disease which affects upper and lower motor neurons. ALS is characterized by high genetic heterogeneity – so far more than 40 genes, including OPTN, have been linked to this disease. The OPTN gene (located on chromosome 10p13) encodes for optineurin, a multifunctional, ubiquitin binding adaptor protein proposed to act in various biological processes. The OPTN mutations have also been found in another neurodegenerative disease -glaucoma – comprising a group of eye conditions in which optic nerve becomes damaged. The aim of this work was to compare OPTN variants present in ALS and glaucoma with naturally occurring variants through different in silico algorithms. We also examined if the mutations were more frequent in the evolutionary conserved regions of optineurin. The OPTN variants and subsequent protein changes were listed by expert literature curation and taken from UniProt, OMIM, ClinVar and gnomAD databases. In silico species alignment analysis of optineurin domain conservation between species was analyzed using ClustalOmega. Our results show that OPTN variants in both ALS and glaucoma predominantly affect coiled-coil regions of optineurin, but this is comparable to their length. However, ALS variants were enriched in the ZF/zinc finger. They were not enriched in ubiquitin-binding region of ABIN and NEMO (UBAN), but clustered around it. The pathogenicity of OPTN mutations was predicted using Polyphen-2, SIFT and PROVEAN algorithms. Albeit prediction of pathogenicity differed between algorithms for variants present in both ALS and glaucoma, they were consistent in assigning K59N, R83C, A93P, Q314L, M447R, E478G, K557T, D564H, L568S, H571Q as damaging protein changes present in ALS and in assigning E50K as damaging in glaucoma. Eight different species representing major vertebrate genera were taken for in silico species alignment analysis of the OPTN conservation and its impact on the mutation occurrence. Species alignment analysis showed that 26% of the OPTN variants present in ALS patients mapped to the conserved regions (G23X, Q165X, K440Nfs*8, K59N, M447R, E478G, K557T, D564H, L568S and H571Q), suggesting that they are more likely to be pathogenic. The highest degree of the conservation (total and partial) was observed in the UBAN and ZF regions of optineurin. In contrast, only 12% of the OPTN variants reported in glaucoma patients mapped to conserved regions (E50K, L54V, H486R). Although variant E322K is located on the conserved position of the optineurin protein, it is predicted to be benign according to Polyphen-2, SIFT and PROVEAN analysis. To conclude, in silico research of OPTN variants represent the basis for the further in vivo and in vitro investigations and could possibly help conceive future experimental directions relevant for resolving optineurin function in neurodegenerative diseases.

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TDP-43 depletion and cryptic exons: a therapeutic opportunity for ALS

The mislocalisation of the RNA binding protein TDP-43 from the nucleus to the cytoplasm is the pathological hallmark of sporadic ALS. As a consequence, widespread splicing changes occur in ALS, including the appearance of cryptic exons (CE) - novel toxic intronic sequences incorrectly incorporated into mature RNA. Many CEs have been described and their role in pathology, if any, is largely obscure. However, we have identified a novel CE in the synaptic gene UNC13A that is incorporated due to TDP-43 depletion in neurons. Crucially, a genetic risk variant for ALS lies within this CE. We have shown

this variant enhances the CE inclusion leading to a reduction of UNC13A RNA and protein; this event is specific for brain regions most affected in ALS. Thus, we have shown this abnormal splicing event to be associated with worse disease progression, and are working on therapeutic strategies to rescue this in order to ameliorate disease, potentially in ~97% of ALS with TDP-43 mislocalisation.

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In vivo imaging of mice after ischemic brain lesion allows insight in time dependent processes of damage and repair

The two faces of inflammation after ischemic stroke, detrimental and beneficial, are reflected differently on the brain damage and repair. The aim of this study was to follow in time the post-ischemic changes in relation to inflammation. As a model of modified neuroinflammation we used the mice with loss-of-function of Toll-like receptor 2 (Tlr2). TLRs are predominantly expressed in microglia and activated by Death-associated molecular patterns (DAMPs) after ischemic lesion. To longitudinally monitor the molecular events in the mouse brain the multimodal in vivo imaging was applied. This includes magnetic resonance imaging (MRI) with 7T preclinical scanner (Bruker) to visualize the morphology of the ischemic lesion, and bioluminescence imaging (BLI) by optical imager (IVIS Spectrum, Perkin Elmer) to get insight in gene activity in the living mouse brain using luciferase reporter. The ischemic brain lesion was achieved by middle cerebral artery occlusion (MCAO) for 60 minutes, followed by filament removal and reperfusion. The affected animals were monitored during 28 days by multiple imaging sessions, functional evaluation by neurological scoring and subsequent brain analysis at the end of the experiment. The molecular activity monitored by BLI included neuroinflammaton by Tlr2 gene, neurorepair by Gap43 gene and apoptosis by innovative approach developed in our laboratory using caged luciferin, DEVD-luciferin (VivoGlo, Promega). The analysis confirmed the dynamic differences between Tlr2-deficient and WT mice. The survival of Tlr2-deficient animals was better than of WT mice. Contrary to this, Tlr2-deficient animals performed functionally worse having higher neurological score in the acute phase. Gap43 as a marker of brain repair was higher in Tlr2-deficient animals. In conclusion, the evaluation of modified inflammation after ischemic lesion demonstrates a time-dependent combination of beneficial and detrimental effects on post-stroke damage and repair.

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Role of Metabolism in TDP-43 Pathology and its Down-Stream Toxicity

Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar dementia (FTLD) are two fatal neurodegenerative disorders with considerable molecular overlap. Cytoplasmic aggregation of transactive response DNA binding protein of 43 kDa (TDP-43) in neurons is a consistent feature of a majority of ALS and FTLD cases. Conditions associated with a conventionally risky metabolic profile, such as type 2 diabetes mellitus, high body mass index, and dyslipidaemia have been associated with delayed onset, slower disease progression, and longer survival in both ALS and FTLD. This study aimed

to investigate how TDP-43 nuclear loss of functions, which is considered an early event in the pathological aggregation of TDP-43, dysregulates cellular metabolic cascades.
TDP43 nuclear loss of function in NSC-34 mouse motor neuron-like cells revealed an alteration in neuronal energy metabolism, particularly the activity of key enzymes involved in lipid and glucose metabolism. Functional validation of these findings showed that TDP43 loss-of-function enhances energy substrate uptake but perturbs intracellular glucose metabolism by altering ratios of metabolic nucleotide factors, culminating in aberrant energy production and reduced survival of neurons. characterization of mitochondrial function also revealed an increased energetic demand of NSC-34 mouse motor neuron-like cells following TDP-43 loss of function. Furthermore, glucose manipulation of our TDP-43 pathology model exacerbates impaired neuronal health at low doses but improves neuronal viability at moderately high doses. Cumulatively, our study has underscored the putative interplay between TDP-43 loss-of-function and neuronal metabolism. Our ongoing investigations involve elucidating the subcellular mechanisms driving the purported interplay and modelling of TDP-43 pathology in brain organoids reprogrammed from induced pluripotent stem cells (iPSCs) derived from patients' fibroblasts. Keywords: TDP-43, energy metabolism, ALS, FTLD.

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Update on Clinical Trials

In this presentation I will review the current clinical trial programs in ALS and those recently completed. This will focus on current outcome measures and emerging biomarkers in addition to the trial designs and the results of the clinical trials

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"We can see clearly now the brain is washed" – Visualizing neurons and blood vessels in the cleared mouse brain using fluorescence microscopy

Light sheet fluorescence microscopy (LSFM) is method for imaging large samples or whole organs of the laboratory animals. Different tissue clearing procedures, which are necessary for imaging thick samples, are used for achieving sample transparency and the visualisation is based on labelling the structures of interest by fluorescence. The main goal of this research was to image the structures in the cleared mouse brain with a special task to verify if the clearing procedure can be beneficial even if "classical" easily available fluorescent microscopes were used for sample visualisation. The inverted fluorescence microscope (EVOS and confocal) was used for this purpose. For whole brain tissue clearing, four methods were used: ECi, iDISCO, PEGASOS and FluoClearBABB. Moreover, mouse brains were isolated from two months old animals (Thy1-YFP-16), which naturally expressed yellow fluorescent protein in neurons. As second approach, for blood vessel visualization we used L. esculentum Lectin Texas Red which was injected in the left heart ventricle of living mouse. In all three cases the mice were perfused by 1× PBS and 4 % formalin and cleared. Cleared mouse brain samples were cut on approximately 1 mm thick slices using mold, mounted on the glass slides in the drop of the final clearing solution, covered by coverslips, and imaged using inverted fluorescence microscope. The ECi method was preferred as the protocol for clearing lasted only one day and used chemicals were nontoxic. PEGASOS proved to be the most efficient method for preserving fluorescence. Even without using LSFM, it was possible to visualize fluorescently labelled structures in thick samples. In conclusion, the clearing of mouse brain produces thick slices suitable as well for imaging and analysis by fluorescence microscopy. Acknowledgement: Supported by EU project "Sinergy of molecular

markers and multimodal in vivo imaging during preclinical assessment of the consequences of the ischemic stroke."

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Deciphering molecular links between Alzheimer's disease and Niemann-Pick type C disease

Niemann-Pick type C disease (NPC) is a rare inherited lysosomal storage disorder characterized by lysosomal cholesterol accumulation leading to progressive neurodegeneration and neuroinflammation. It is intriguing that this rare monogenic disease (caused by mutations in NPC1 or NPC2 genes) shows several key features of a complex Alzheimer's disease (AD). Using NPC disease cellular and animal models our goal is to elucidate both common and specific pathways involved in neurodegeneration and/or neuroinflammation in NPC and AD. We showed that proteolysis by the key AD protease BACE1 is enhanced in NPC1-null cells as well as in NPC1-null primary mouse neurons and NPC1-null mouse brains. Increased BACE1-mediated cleavage in NPC is mostly likely due to a defect within the endolysosomal transport resulting in accumulation of BACE1 and its substrates in endocytic compartments. Moreover, BACE1-inhibition and/or genetic depletion altered neurodegeneration of Purkinje neurons, lysosomal impairment and/or neuroinflammation (activation of astrocytes and microglia), suggesting that BACE1 and/or its substrates may play a role in the pathogenesis of NPC disease as well as in AD. Furthermore, we detected impaired retromer function in NPC. Changes in retromer distribution in NPC1 mouse brains were observed already at presymptomatic stage (at 4-weeks of age), indicating that retromer defect occurs early in the course of NPC disease and may contribute to downstream pathological processes. Cholesterol depletion in NPC1-null cells and in NPC1 mouse brains reverted retromer dysfunction, suggesting that retromer impairment in NPC is mechanistically dependent on cholesterol accumulation. Defects of the endolysosomal pathway are shared by other lysosomal storage disorders as well as the more common Alzheimer's and Parkinson's disease. Hence, the knowledge gained through our work could be used to better understand rare monogenic as well as more common and complex neurodegenerative disorders.

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Single moderate traumatic brain injury in mice induces transient acute changes in the cortical iNOS expression and its cytoplasmic co-localization with TDP-43

Introduction: Traumatic brain injury (TBI) occurs as a consequence of mechanical injury to the brain and is considered to be the leading cause of death and disability in young individuals. Inflammation, one of the secondary injury pathophysiological processes, contributes to posttraumatic brain damage and lifelong cognition and memory impairments. Inducible nitric oxide synthase (iNOS) is a cytoplasmic enzyme that forms the signaling molecule nitric oxide involved in neuroinflammation. In TBI, it has been also shown that mislocalization of TAR DNA-binding protein 43 (TDP-43) contributes to post-injury pathology. We investigated acute changes in the iNOS expression following single moderate TBI in wild-type mice in different brain regions and its colocalization with TDP-43. Materials/Methods: Single moderate lateral fluid percussion injury was induced over the left parietal cortex of the male adult C57BL/J mice. Animals were sacrificed one or three days after TBI and their

brains were prepared for Western blot or immunohistological analyses. Animals of the control group were sacrificed 1 day after the sham injury procedure. Results: An increase in the iNOS expression was observed in the ipsilateral cortex one day after the brain trauma. In the tested time points, changes in the iNOS expression were not detected in the ipsilateral hippocampus and contralateral cortex, and hippocampus of traumatized animals. Also, a colocalization of iNOS and TDP-43 in the cytoplasm was observed in the most damaged part of the ipsilateral cortex one day after TBI. Conclusions: Transient acute changes in the proinflammatory marker iNOS in the ipsilateral cortex are induced by single moderate brain trauma. Cytoplasmic colocalization of iNOS and TDP-43 in the most damaged part of the ipsilateral cortex suggests the interconnection of these proteins. This work was supported by the University of Rijeka, Croatia, project number uniri-biomed-18-199 to K.P.

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Putting Brains on a Diet: Targeting Pathological Aggregation in Neurodegenerative Disorders via Metabolism

Pathological protein aggregation is a critical feature of neurodegenerative disorders (NDDs). Pathological intracellular aggregation of TAR DNA binding protein 43 kDa (TDP-43) in neurons and glia is a hallmark of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD), two NDDs with considerable clinical and molecular overlap. Intriguingly, several clinical studies, including our own work, indicate that certain metabolic conditions, such as dyslipidemia, high body mass index, and type 2 diabetes mellitus are associated with better clinical outcomes in ALS and FTLD. This disease-modifying effect of metabolic factors in neurodegenerative disease seems to be an over-arching phenomenon also implicated in other neurodegenerative conditions, such as Alzheimer disease (AD). Jawaid lab is currently focussed on investigating the effects of glucose and lipid metabolic cascades on aggregation and clearance of pathological protein aggregation in neurodegenerative disorders with a key focus on TDP-43 aggregation and beta-amyloid clearance. Preliminary results indicate that TDP-43 depletion in mouse motor-neuron like cells (NSC-37 cells) leads to broad defects in glucose metabolism suggestive of dysregulation in cellular energy sensing. Similarly, high-throughput metabolic manipulations of human microglia-like (HMC-3) cells indicate a role for lipoproteins in regulation of beta-amyloid uptake and degradation by microglia. Identification of relevant molecular cascades and their in vitro and in vivo manipulation to alter the neurodegeneration course are subjects of our ongoing investigations.

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Characterization of FUSdelta14-ALS mouse model

Mutations in FUS are causative of ALS and we have previously generated and characterised a knockin mouse model which in heterozygosity develops slowly progressive late onset neurodegeneration. We have now been able to generate viable homozygous mice, with the goal of inducing more rapid phenotypes, therefore allowing more effective experiments. We have performed histology and physiology analysis showing loss of motor neurons and denervation at one- and three-month timepoints. We have also performed in depth molecular analysis, including RNA-sequencing and RIboTag sequencing experiments, which document widespread transcription and translation changes. Lastly, we show that FMRP is altered also in adult mice, supporting a role for this important protein in FUS-ALS.

This work provides a novel tool to investigate the pathogenic mechanism of FUS mutations, and perform future translational studies.

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Antibody approaches to target TDP-43 proteinopathy

Cytoplasmic aggregates of TDP-43 are a pathological hallmark of degenerating neurons in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). With the objective to mitigate TDP-43 aggregation, we have generated antibodies (Abs), full length Abs and single chain scFv Abs against the RRM1 domain of TDP-43. One of our full-length Ab, called E6 Ab, was found to detect specifically cytoplasmic TDP-43 species, but not nuclear TDP-43. Our data demonstrated that full length Ab E6 and scFv Ab E6 can attenuate cytoplasmic TDP-43 accumulations in part by inducing degradation of TDP-43 via autophagic and proteasomal pathways. We showed that intrathecal AAV viral-mediated delivery of a scFv Ab E6 against TDP-43 RRM1 region mitigated TDP-43 pathology, and it ameliorated cognitive and motor performance of transgenic mice expressing ALS-linked TDP-43 mutants. To achieve pan-neuronal expression in the CNS of E6 scFv, we recently generated an AAV vector bearing a recombinant capsid designed to achieve efficient transduction in large neuronal populations after intravenous injection. Few weeks following single intravenous injection of AAV vector encoding scFv E6 into transgenic mice expressing TDP-43G348C, microscopy results confirmed the wide spread detection of scFv E6 antibodies in CNS neurons and preliminary results suggest an attenuation of TDP-43 mislocalization. We are also exploring a treatment based on administration of full-length Abs against TDP-43 into the cerebrospinal fluid (CSF). We are testing this approach on a new mouse model of sporadic ALS based on intracerebroventricular (i.c.v.) infusion of CSF from ALS patients. Intrathecal injection of E6 Abs in such mice infused with ALS-CSF alleviated gait deficits and increased the nuclear to cytoplasmic ratio of TDP-43 in spinal neurons. Moreover, the addition of E6 Abs to ALS-CSF before infusion into hTDP-43WT mice led to amelioration of cognitive deficits raising up the possibility that E6 Abs might neutralize the toxicity of ALS-CSF.

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Translational control of innate immune response in ALS

Microglia are the principal immune cells of the brain. The consensus today is that once activated microglia/macrophages can acquire a wide repertoire of profiles ranging from the classical pro-inflammatory to alternative and more protective phenotypes. Growing evidence suggests that optimal and timely activation of microglial cells and innate immunity is instrumental in the control of the inflammation-induced damage to CNS. We recently described a novel ribosome-based regulatory mechanism/checkpoint that controls innate immune gene translation and microglial activation involving RNA binding protein SRSF3. New evidence suggests that changes in SRSF3 expression and activation patterns could be implicated in the pathogenic transformation of microglia in amyotrophic lateral sclerosis (ALS). Using a model-system for analysis of the dynamic translational state of microglial ribosomes we show that SRSF3 binds to 3'UTR of highly regulated immune mRNAs and acts as a suppressor of translation, resulting in a chronic dissociation of mRNA and protein networks in ALS microglia. Targeted knockdown of SRSF3 using anti-sense morpholino approach, initiated at late symptomatic disease, in SOD1G93A mouse model alleviated translational arrest of selected genes,

slowed down disease progression and exerted significant disease modifying effects. Together, our findings suggest that targeting SRSF3 and mRNA translation in chronically activated microglia may open new avenues for therapeutic reprogramming of immune response in ALS.

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The impact of multidisciplinary care team on survival of Slovenian patients with amyotrophic lateral sclerosis

The Ljubljana ALS Centre, established in 2002, is the only tertiary center for amyotrophic lateral sclerosis (ALS) in Slovenia. The aim of our study was to evaluate the impact of therapeutic interventions and improvements in the multidisciplinary care on the survival of our patients. All patients diagnosed with ALS at our center during years 2003–2005 (early group) and 2011–2012 (late group) were included in this retrospective cohort study (n = 124). Kaplan-Meier survival analysis and multiple regression analysis with Cox proportional hazards model were performed to compare survival and to evaluate the differences between the two cohorts. Median survival from the time of diagnosis was 13.0 (95% CI = 10.2–15.8) months in the early group and 21.8 (95% CI = 17.2–26.4) months in the late group (p = 0.005). In the Cox proportional hazards analysis, the late group of patients was associated with better survival independently of all other prognostic factors (hazard ratio (HR) = 0.51, 95% CI = 0.32–0.81, p = 0.004). Survival was also associated with patients' age, use of noninvasive ventilation (NIV) and gastrostomy. The model fit significantly improved when the interaction between the NIV use and the observed time period was added to the model (HR = 0.34, 95% CI = 0.12–0.96, p = 0.041). Our findings suggest that improvements in the multidisciplinary care were beneficial for survival of our patients with ALS. The survival benefit in the late group of our patients could be partially explained by the improvements in the NIV use at our center. The results of the study were published in Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, 2020; 21: 203–208.

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Differential role of neurons and glial cells in cytomegalovirus infection and immune control

Human cytomegalovirus (HCMV) infection is the leading cause of congenital viral infections, which can cause a wide range of neurological sequalae. After the acute infection has been resolved, the virus remains in the central nervous system (CNS) in a state of latency. The pathogenesis of congenital CMV infection (cCMV) remains insufficiently understood. To elucidate the mechanisms of brain infection and pathogenesis during cCMV infection, we are using a murine model. As reported for HCMV, murine cytomegalovirus (MCMV) efficiently infects neurons, astrocytes and microglia. To study viral entry and dissemination in the developing murine brain, we have utilized a cell-type-specific virus labeling system. Here we show that MCMV can infect the brain independently of infection of endothelial cells, the main component of the blood-brain barrier (BBB). Following CMV entry into the CNS, infectious virus was produced by astrocytes, microglia and neurons. Even though astrocytes are initial cellular source of infectious virus in the brain, a significant proportion of the virus bypasses astrocytes and

replicate in other cells in the brain. Microglia is strongly infected during the peak of MCMV infection in brain, however, the microglia-derived virus does not spread to other cells, indicating efficient microglial mechanism of virus containment. At later time points of acute infection, when immune control is established, neurons are the main source of infectious virus, suggesting impaired immune control of virus in neurons. Furthermore, we provide evidence that neurons are potential site of MCMV latency and reactivation.

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Flail arm syndrome, a form of ALS with slow progression

ALS is a fatal neurodegenerative motor neuron disease (MND). Most frequently, ALS begins with weakness and atrophy of the upper limb muscles (classic ALS), less frequently with bulbar muscle weakness. Flail arm syndrome (FAS) is thought to be a different form of MND or ALS with slow progression. Primary muscle atrophy (PMA) is the form of MND which clinically resembles FAS. In the last 10 years, we have had three male patients with symptoms suggestive of FAS. All of them presented with progressive proximal weakness of the upper limbs. In the further course of the disease, the first patient (62 years) after 28 months had no signs of progression of muscle weakness, and soon after he died of intracerebral hemorrhage; in the second patient (69 years) we saw a progression of arm weakness and the development of leg weakness after 19 months, after a further 14 months bulbar symptoms appeared and he died 6 years after the onset of the disease. In the third patient (57 years), after 25 months, the weakness spread to the forearm and hand, but did not affect other parts of the body. He can still walk, has no bulbar symptoms. In the patients with symptoms restricted to the upper limbs weakness, the possible diagnoses include classic ALS and FAS. ALS mostly involves distal upper limb muscles, while FAS mostly involves proximal upper limb muscles. To the best of our knowledge, FAS has a better natural history than classic ALS, due to slower spread to other parts of the body. Due to the onset and the progression of muscle weakness, we believe that our patients met the criteria for FAS.

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Emerging therapeutic targets in TDP-43 proteinopathies

Alteration of RNA metabolism has emerged as a central theme in neurodegenerative diseases with mutations and/or mislocalization of RNA binding proteins, including TDP-43 and FUS, in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). TDP-43 and FUS are involved in fundamental RNA processing activities including RNA transcription, splicing, and transport. Following the recognition of their crucial role in neurodegeneration, we have used genome wide approaches to define their role in regulating expression and splicing of their RNA targets. We recently demonstrated that the human RNA most affected by reduction in TDP-43 is encoding the neuronal growth-associated factor called stathmin-2 (also known as SCG10), an essential component for neuronal regeneration and axonal maintenance. Reduced nuclear TDP-43 results in abnormal usage of cryptic splice and polyadenylation sites in pre-mRNAs from the STMN2 gene, leading to loss of stathmin-2 protein. Remarkably, although TDP-43 affects the levels or splicing of many RNAs, restoration of stathmin-2 alone was sufficient to rescue regeneration after axotomy of iPSC-derived TDP-43 depleted motor neurons. Reduced levels in stathmin-2 is a hallmark in sporadic and familial ALS/FTD, and restoration of stathmin-2 expression emerges as an attractive therapeutic strategy in the vast majority of patients with ALS/FTD. We developed approaches to block cryptic splicing of stathmin-2 by targeting dCaRx or antisense oligonucleotides (ASOs) to stathmin-2 pre-mRNA leading to rescue of axonal regeneration capacity of human motor neurons with TDP-43 deficiency. Finally, using “humanized” stathmin-2 mice

with constitutive misplicing, we established that ASO injection into cerebral spinal fluid is a therapeutically viable approach to rescue stathmin-2 mRNA levels in TDP-43 proteinopathies.

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Molecular background of amyotrophic lateral sclerosis and frontotemporal dementia: Towards therapeutic targets

Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are two devastating neurodegenerative diseases, which due to overlaps in clinical presentations, pathological features, and genetic causes, are considered two manifestations of a continuous disease spectrum. A hallmark pathological feature of both diseases is the depletion of the RNA-binding protein TDP-43 from the nucleus in the brain and spinal cord of patients. A major function of TDP-43 is to repress the inclusion of cryptic exons during RNA splicing. When it becomes depleted from the nucleus in disease, this function is lost. Recently, several key cryptic splicing targets of TDP-43 have emerged, including STMN2, UNC13A, and others. UNC13A is a major ALS/FTD risk gene. The genetic variations that increase the risk for disease seem to do so by making the gene more susceptible to cryptic exon inclusion when TDP-43 function is impaired. Here, we discuss the prospects and challenges of novel therapeutic targets and biomarkers by harnessing cryptic splicing events as novel therapeutic targets and biomarkers. These novel therapeutic strategies may be tailoring personalized medicine approaches to specific ALS and FTD patients.

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Compensatory myogenesis and acetylcholine receptor clustering delay symptoms onset and progression in SOD1 mutant mice

Amyotrophic lateral sclerosis (ALS) is a heterogeneous disease with high variability in the speed of progression even in cases with a defined genetic cause such as superoxide dismutase 1 (SOD1) mutations. $SOD1^{G93A}$ mutation on mice with distinct genetic backgrounds (C57 and 129Sv) show consistent differences in speed of disease onset and progression resembling what is observed in ALS patients. We recently hypothesized that the difference in the peripheral neuromuscular system rather than the extent of spinal motor neuron loss reflects the phenotypic difference between these two mouse models. Therefore, we redirect our attention to the skeletal muscle as an early component of ALS pathogenesis aiming to discover the molecular mechanisms contributing to the distinct phenotypes and to identify factors underlying fast and slow disease progression. In this work, we compare the functional, morphological and molecular profiles of the gastrocnemius muscle (GCM) from these two $SOD1^{G93A}$ mouse strains at the pre-symptomatic and onset stage of the disease. Data collected clearly defined the extent of NMJ stability and muscle regeneration as a discriminator between rapidly and slowing progressing ALS mice. Notably, the slow-progressing mice, despite the premature denervation and muscle atrophy, activate different compensatory mechanisms including the expression and clustering of the AChR, myogenesis and inflammatory response, that are able to delay the onset and progression of their symptoms. On the contrary, the fast progressing mice that are unable to activate

these responses in muscle exhibit a rapid deterioration of muscle force. This study highlights a set of key gene and molecular pathway indices of fast or slow disease progression, which may prove useful in identifying potential disease modifiers responsible for the heterogeneity of human amyotrophic lateral sclerosis and which may represent valid therapeutic targets for ameliorating the disease course in humans. This study is supported by AriSLA (project MUSALS-AChR).

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There's something wrong with my MAM; ER-mitochondria signaling and ALS/FTD

Signaling between the endoplasmic reticulum (ER) and mitochondria regulates a number of fundamental neuronal functions, many of which are damaged in ALS/FTD. This signaling involves close physical contacts between the two organelles that are mediated by scaffolding proteins which act to recruit and "tether" regions of ER with mitochondria. These ER regions are termed mitochondria-associated ER membranes (MAM). We have identified an interaction between the integral ER protein VAPB and the outer mitochondrial membrane protein PTPIP51 as one such tether. We and others have shown that ALS/FTD genetic insults disrupt ER-mitochondria contacts and signaling and for several of these, this is now known to involve breaking of the VAPB-PTPIP51 tethers. Since ER-mitochondria signaling regulates many of the functions that are perturbed in ALS/FTD, correcting damage to this signaling may be broadly therapeutic and our identification of the VAPB-PTPIP51 tethers reveals a molecular target for such therapy. In this seminar, the functions of ER-mitochondria signaling and the VAPB-PTPIP51 tethers will be described along with the mechanisms that regulate binding of VAPB to PTPIP51. Finally, the therapeutic potential of targeting the VAPB-PTPIP51 tethers will be discussed.
Supported by grants from the UK Medical Research Council, Alzheimer's Research UK, Alzheimer's Society and Motor Neurone Disease Association.

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Essential role of the PTPIP51 coiled-coil domain in VAPB binding and ER-mitochondria signaling

Multiple cellular and physiological processes are damaged in FTD/ALS. These include damage to mitochondria, the endoplasmic reticulum (ER), Ca2+ signaling, lipid metabolism, autophagy, axonal transport and both diseases present inflammatory responses. The biological conundrum is how so many disparate physiological processes are collectively damaged; the therapeutic challenge is determining which functions should be prioritised in drug discovery programmes. Recently, signaling between the ER and mitochondria has become of interest since this regulates all processes listed above. Moreover, numerous FTD/ALS genetic insults have now been shown to damage ER-mitochondria signaling; these include mutant TDP43, FUS, C9orf72, Sigma1 receptor and Cu/ZnSOD1. ER-mitochondria signaling requires close physical contacts between the two organelles that are mediated by the VAPB-PTPIP51 "tethering proteins". VAPB is an ER protein that binds to the outer mitochondrial membrane protein PTPIP51 to tether the organelles to permit signaling. The VAPB-PTPIP51 "tethers" regulate inositol 1,4,5-trisphosphate (IP3) receptor delivery of Ca2+ from ER stores to mitochondria, bioenergetics, lipid synthesis, autophagy and in neurons synaptic activity. Mutant TDP43, FUS and C9orf72 have been shown to disrupt ER-mitochondria signaling via breaking of the VAPB-PTPIP51 tethers. Understanding the mechanisms that regulate VAPB-PTPIP51 binding is an important area of research for FTD/ALS, and especially if correcting damaged ER-mitochondria signaling is to become a therapeutic target for these diseases. Using immunoprecipitation assays with mutant proteins, we show

here that a coiled-coil domain in PTPIP51 is essential for VAPB binding. Electron microscopy shows that the coiled-coil domain mediates ER-mitochondria tethering. Finally, we show that the coiled-coil domain is essential for IP3 receptor mediated delivery of Ca2+ to mitochondria. Supported by grants from the MRC, ARUK and Alzheimer's Society.

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Prospects for the Future of Stem Cells Therapy in Amyotrophic Lateral Sclerosis

Despite recent progress and increase interest for stem cell clinical trials in Amyotrophic Lateral Sclerosis (ALS), there is still a lack of consistent and meaningful results. Stem cells used in cellular assays and animal models have achieved certain results, but there are still many problems to be solved before they can be extended to clinical applications. Most past and ongoing stem cell clinical trials are single center phase I and II trials aimed to assess the safety and the results are still inconclusive because the majority lacked long-term follow-up and well-defined outcomes. Very few trials are controlled, as inclusion of adequate sham controls for invasive procedures is problematic and often unethical. We can conclude that some types of adult SCs are sufficiently safe and may be proposed for large clinical trials. However, it cannot yet be established which treatments provide significant benefit, and whether positive effects on the disease are sufficiently lengthy to justify the risks and costs of cell therapy. We review the current state of stem cell clinical trials in ALS and also assess future scientific, technical, ethical, regulatory and logistic challenges to be resolved in translating effective laboratory cell-based protocols to patients and the potential chances for success in developing new cell therapies to clinical application in this disease.

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The effect of quercetin on the developmental consequences of methamphetamine in fruit fly

Methamphetamine (METH) is a psychostimulant that leads to the development of addiction. METH acts on the central nervous system through various physiological pathways. Our preliminary results show that single short volatilized METH (vMETH) exposure of males leads to changes in the developmental cycle of their progeny. The development cycle is related to metabolism and the amount of reactive oxidative species. Exposure to METH induces an imbalance in the oxidative status of the cell by increasing the levels of oxidants. Quercetin (QUE) is a molecule with known antioxidant properties that could potentially act on changes in the developmental cycle after administration of METH. Our hypothesis was to test if change in oxidative status will affect the change in developmental cycle induce by METH exposure. Our approach was to supplement the food with QUE and to quantify the number of laid eggs and number of enclosed flies. We used FlyBong to administer 1 dose of vMETH or hot air as control for 1 min. After administration of vMETH we selected male flies with the highest locomotor response and mated them with virgin females. During 7 days of mating files were kept on QUE supplemented food (0.6 mM and 3.2 mM). Our results show that METH leads to a significant increase in the number of embryos in flies treated with vMETH and to moderate increase in flies treated with hot air compared to the untreated control. 3.2 mM QUE concentration reduced the number of embryos in the group of files that were exposed to vMETH and hot air, but not in the untreated control

group. These results can be explained by the effects that QUE has on the redox balance and subsequently on chromatin modification.

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Evaluation of plasmatic and cellular redox state parameters in patients affected by Amyotrophic Lateral Sclerosis

Background: Oxidative stress, the alteration of mitochondrial function, and changes in the neurovascular unit (NVU) could play a role in Amyotrophic Lateral Sclerosis (ALS) pathogenesis. Nowadays, no therapy can change the course of ALS, which suggests the need to test new approaches to slow down disease progression. Acetyl-L-carnitine (ALCAR) is a substance with a neurotrophic and protective effect, which could be considered for its possible positive role. Aim: This study aimed to analyze: 1) the plasma redox system and nitric oxide (NO) in ALS; 2) the plasma effects on peroxidation/mitochondrial function in human umbilical cord- derived endothelial vascular cells (HUVEC) and astrocytes. Method: We included 20 ALS new-diagnosed patients and five healthy controls. Each patient underwent a clinical evaluation combined with a blood sample, which was used to analyze the aforementioned parameters at diagnosis (T0). After starting ALCAR (T0), the same investigations were repeated at three months (T1) and six months (T2) from the start of administration. Results: In the plasma of ALS patients at T0, an increase in TBARS and a reduction in GSH and NO were found. In HUVEC/astrocytes, treated with a plasma of ALS patients, mitoROS increased, whereas cell viability and mitochondrial membrane potential decreased. Plasma analysis were repeated at T1 and showed an improvement in the redox state in all patients involved. In a patients' subgroup, these results were confirmed by plasma measurement at T2. Discussion: Our results show that oxidative stress and NVU play a central role in ALS and suggest that unknown plasma factors could be involved in the disease pathogenesis. Quantifiable changes in ALS plasma related to redox state alterations can be used for early diagnosis.

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Inflammatory exosomes transfer danger signal and induce glial dysfunctional calcium dynamics in naïve spinal cultured explants

Neuroinflammation is a shared hallmark of almost every pathology in the central nervous system (CNS), from ischemic or traumatic injury to neurodegenerative diseases. Despite the increasing knowledge of the molecular and cellular mechanisms contributing to neuroinflammatory pathways, the understanding of shared mediators underlying neuroinflammation for the development of novel therapeutic strategies is still lacking. Extracellular vesicles released by astrocytes, the CNS prevalent cells, are key vectorized systems able to spread and actively transfer signalling molecules, modulating target cell functions. We isolated exosomes from LPS-treated (inflamed) organotypic spinal cord slices to investigate vesicle ability to induce glial cell reactivity and inflammation in naïve organotypic spinal slices. The slices were obtained from E13 mouse embryos, cultured in vitro for two weeks and treated for 24 h with lipopolysaccharide (LPS 1 μg/ml), to induce spinal neuroinflammation. Upon medium collection and centrifugation we isolated for the first time exosomes from cultured slices which were

used to treat naive slices. LPS-induced neuroinflammation was documented by immunofluorescence and confocal microscopy quantifying glial reactivity by GFAP and Iba1 staining. Exosomes were characterised by nano-particle tracking (NTA), atomic force microscopy and western-blot analysis and their impact on healthy spinal tissue was assessed with similar approaches while dysfunctional Ca2+ signalling in reactive astrocytes was monitored by AAV5. gfaABC1D-cyto-GCaMP6f, a genetically encoded calcium indicator. Confocal microscopy and live imaging documented the ability of exosomes to activate reactive responses in healthy astrocytes. The results obtained strongly indicate that exosomes enable inflammatory danger signalling transfer from treated to naïve tissue.

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Targeting TDP-43 Autoregulation for Therapeutic Benefit In ALS-FTD

Introduction: Pathological aggregation of the DNA/RNA-binding protein TDP-43 is a characteristic of ~98% of amyotrophic lateral sclerosis (ALS) and several types of dementia including Alzheimer's disease and most notably, frontotemporal dementia (FTD). Mutations in TARDBP are seen in both FTD and ALS cases, confirming a mechanistic role for TDP-43 in dementia. We previously characterised the first TDP-43 knock-in mouse (TDP43Q331K), which harbours a human- equivalent mutation in murine Tardbp and discovered that this mutation critically disrupts TDP-43 autoregulation resulting in increased TDP-43 expression and changes in splicing, consistent with a gain of TDP-43 function. This leads to FTD-like behavioural phenotypes, frontal atrophy and cortical interneuronal loss (1). Understanding the mechanism of autoregulation offers a novel target for modulation of TDP-43 in the treatment of dementias. Objectives: 1. Study the link between TDP-43 misregulation and dementia in human cortical neurons; 2. Develop a screen to identify modifiers of TDP-43 autoregulation with translational potential. Materials and Methods: CRISPR/Cas9 mutagenesis was used to introduce both the TDP-43Q331K and M337V disease-associated mutations into individual KOLF2-C1 human iPSC lines, in addition to an inducible Neurogenin-2 transgene to rapidly differentiate cortical neurons. To measure TDP-43 autoregulation, qPCR from neuronal RNA extracts was utilised to assess the level of intron 7 splicing and pA1/pA2 polyadenylation site usage in the 3'UTR of the TARDBP transcript. To develop a stem cell line suitable to screen for modifiers of TDP-43 autoregulation, CRISPR/Cas9 was used to integrate an exogenous TDP-43 autoregulation reporter, an mScarlet fluorescent tag of endogenous TDP-43, and an inducible Neurogenin-2 transgene into the KOLF2-C1 line. Results: TDP-43Q331K and M337V cortical neurons demonstrated reduced intron 7 splicing, reduced pA2 selection and elevated TARDBP expression, confirming that these disease-associated mutations perturb autoregulation in human neurons. Integration of an exogenous TDP-43 autoregulation reporter, mScarlet fluorescent TDP-43 tag and an inducible Neurogenin-2 transgene has been successfully completed in a single iPSC line. In response to TDP-43 overexpression, splicing of the TDP-43 reporter was enhanced, indicating that it undergoes autoregulation like the endogenous TARDBP transcript. Conclusions: A variety of TDP-43 low complexity domain mutations directly perturb autoregulation and result in elevated TARDBP expression. The mechanism by which these mutations disrupt TDP-43 homeostasis in murine brain is conserved in human neurons, highlighting the relevance of these findings to human disease. We now have a stem cell line capable of rapid cortical neuronal differentiation that includes a functional TDP-43 autoregulation reporter and an endogenous TDP-43 fluorescent tag. This will be used to develop a high content imaging assay capable of screening for modifiers of TDP-43 autoregulation.

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Brain-derived neurotrophic factor, amyloid β1–42 and Apolipoprotein E genotype: Potential biomarkers for the differential diagnosis between frontotemporal dementia and Alzheimer's disease

Dementia is a chronic brain disorder that impacts cognition and mental degeneration. With dementia affecting more than 55 million people worldwide, the need for an accurate diagnosis has become an imperative. The most common forms of dementia include Alzheimer's disease (AD), frontotemporal dementia (FTD), vascular dementia (VAD), and dementia with Lewy bodies (LBD), with great similarities in symptoms among disorders. Mild cognitive impairment (MCI) is described as a transitional state between normal aging and very early dementia. It has been shown that people with MCI are at higher risk of developing dementia compared to those without MCI. Researchers are studying different biomarkers for possible use in diagnosing and tracking of different types of dementia. Accumulation and aggregation of amyloid β1–42 (Aβ1–42) is considered as one of the most potential biomarkers for AD. One of potential neuroprotective agents, interesting due to its relationship with cognitive decline, is the brain-derived neurotrophic factor (BDNF). Previous studies have reported reduced blood and brain levels of BDNF in AD and MCI. Important genetic risk factor for sporadic AD is apolipoprotein E (APOE). APOE is the regulator of lipid metabolism, and exists in three major isoforms (E2, E3, and E4) where APOE4 is associated with enhanced risk for developing AD. The aim of the study was to determine potential differences in BDNF plasma concentration and Aβ1–42 levels between subjects diagnosed with different types of dementia. Also, we aimed to investigate the difference in APOE genotype distribution between subjects diagnosed with AD, FTD, or MCI. Study enrolled 104 patients with AD, 14 with VAD, 23 with FTD, 9 with LBD, and 50 subjects with MCI. Genomic DNA was extracted using the salting-out method from blood samples. Enzyme-linked immunosorbent assay (ELISA) was used to determine the levels of Aβ1–42 in CSF and BDNF concentration in plasma. Polymorphisms in APOE gene (rs429358 and rs7412) were determined using Real Time PCR. Data was evaluated using GraphPad Prism version 4.00. Results suggest significantly lower levels of Aβ1–42 in CSF samples from AD and FTD patients, compared to individuals with MCI. Subjects with FTD were also identified as having lower levels of Aβ1–42 in CSF then patients with AD. BDNF plasma concentration were significantly lower in FTD group, compared with AD group. Analyzing APOE genotype there is a significant difference in the frequency of APOE4 allele carriers between group of patients with AD, FTD and MCI subjects. Our results confirmed that determination of BDNF and Aβ1–42 levels, and identification of the APOE ε4 allele could be used as potential biomarkers of dementia, however further research is required.

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The emerging roles of phase separation in the organization and dynamics of RNA-containing granules in ALS

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease leading to the death of upper and lower motor neurons. Mutations in RNA-binding proteins such as TDP-43, FUS and hnRNPA1that form ribonucleoproteins (RNP) granules are frequently associated with both familial and sporadic forms of ALS. While physiologically these proteins undergo liquid-liquid phase separation, ALS-associated mutations disrupt this process resulting in the formation of insoluble condensates. Yet, how cells distinguish and triage aberrant condensates from fluid-like assemblies remains poorly

understood. Using a combination of ALS-associated FUS mutations, optogenetic approach to manipulate FUS condensation and pH-sensitive reporters of organelle acidity, we systematically characterized the cause-effect relationship between the material state of FUS condensates and the sequestering of lysosomes. From our data, we have derived three main conclusions. First, regardless of whether we use wild-type or mutant FUS, protein levels in cells play a dominant role in promoting the aggregation of FUS. Second, chemically-induced FUS aggregates recruit LAMP1-positive structures. Third, mature, acidic lysosomes accumulate preferentially at FUS aggregates than at liquid-condensates both in ectopically expressed cells and in primary hippocampal neurons. Together, our data suggest that lysosome-degradation machinery actively distinguishes between fluid and solid condensates. Understanding the mechanisms underlying the clearance of aberrant RNP aggregates by the autophagy-lysosome axis provides the new therapeutic potential for treating ALS and related neurodegenerative diseases. Molecular mechanisms coupling phase separation of RNP granules and autophagy-lysosome dynamics in ALS pathology.

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Transplantation of stem cells in animal model of stroke reduces cell death

Although many preclinical and clinical trials have reported beneficial effects of stem cells on nervous tissue affected by stroke, molecular mechanisms in the background of documented improvements remain largely elusive. In this work we transplanted 1 million of neural stem cells into mice affected by stroke and compared obtained effects to animals which were placebo-treated by cell medium or were not treated. Apart from measuring volumes of stroke by magnet resonance imaging and quantifying changes in health condition, we focused on extent of pyroptosis, measured by Gsdmd and necroptosis, measured by Mlkl. While one day after cell transplantation, RNA-detected level of expression of both genes was slightly reduced, four days after transplantation downregulation of both markers in the cell-treated group was significant. Moreover, immunohistological analyses revealed that Gsdmd was primarily present in astocytes and microglia, while Mlkl was dominantly found in neurons. Their reduction correlated to significantly improved condition in only cell-treated mice, while not being accompanied with significant reduction in stroke volume. This work has demonstrated that reduction of pyroptosis and necroptosis represents one of the important molecular mechanisms by which neural stem cells improve recovery of animals affected by stroke. Interestingly, documented improvement of the general health condition after stem cell transplantation better correlates to levels of pyroptosis and necroptosis than to volume of stroke.

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FUS interactome

Neuronal degeneration has been recognized as a predominant driver of disability and disease progression in central nervous system diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Successful treatments for these disorders have yet to be developed. The aggregation of RNA binding proteins (RBPs) has been recognized as a hallmark pathological feature in these disorders, defining them as proteinopathies. Fused in sarcoma (FUS), normally a

nucleus residing RBP, is known to aggregate into physiological granules and pathological inclusions, which can impair cell homeostasis leading to neuronal cell death. Mutations in FUS that alter its C-terminal nuclear localization signal (NLS) are autosomal dominant in ALS and disrupt its nucleo-cytoplasmic shuttling leading to its cytoplasmic mislocalization. Since protein interactors of FUS and the exact signaling pathways involved in cytoplasmic toxicity of FUS remain unknown, using BioID2 proximity labeling, we aimed to identify the interactomes of FUS and FUSdNLS (lacking NLS) proteins overexpressed in a model cell line. The BioID2 technique harnesses the ability of the enzyme biotin ligase (birA) to biotinylate proximal endogenous proteins, which can thus be isolated and subjected to MS identification. Our bioinformatic analyses of proteomic data identified interaction candidates involved in RNA processing and degradation, protein translation and various signal transduction pathways, pointing to differential involvement of wt and truncated FUS in cellular processes. We validated selected interactions by pull-down assay and performed cell co-localization analyses in vitro. The interactome differences between FUS and FUSdNLS, provide detailed insight into FUS function most likely relevant to neurodegenerative diseases, that could be targeted in therapeutic interventions.

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Disruption of ER-mitochondria tethering and signalling in C9orf72-associated fronto-temporal dementia and amyotrophic lateral sclerosis

Hexanucleotide repeat expansions in C9orf72 are the most common cause frontotemporal dementia (FTD) and associated amyotrophic lateral sclerosis (ALS). The mechanisms by which the expansions cause disease are not properly understood but a favoured route involves its translation into dipeptide repeat (DPR) polypeptides, some of which are neurotoxic. However, the precise targets for mutant C9orf72 and DPR toxicity are not fully clear and damage to several neuronal functions has been described. Many of these functions are regulated by signalling between the endoplasmic reticulum (ER) and mitochondria. ER-mitochondria signalling requires close physical contacts between the two organelles that are mediated by the VAPB-PTPIP51 "tethering" proteins. Here, we show that ER-mitochondria signalling and the VAPB-PTPIP51 tethers are disrupted in neurons derived from induced pluripotent stem (iPS) cells from patients carrying FTD/ALS pathogenic C9orf72 expansions and in affected neurons in mutant C9orf72 transgenic mice. In these mice, disruption of the VAPB-PTPIP51 tethers occurs prior to disease onset suggesting that it contributes to the pathogenic process. We also show that neurotoxic DPRs disrupt the VAPB-PTPIP51 interaction and ER-mitochondria contacts and that this involves activation of glycogen synthase kinases-3b (GSK3b) a known negative regulator of VAPB-PTPIP51 binding. Finally, we show that these DPRs disrupt delivery of Ca2+ from ER stores to mitochondria which is a primary function of the VAPB-PTPIP51 tethers. This delivery regulates a number of key neuronal functions that are damaged in FTD/ALS including bioenergetics, autophagy and synaptic function. Our findings reveal a new molecular target for mutant C9orf72-mediated toxicity. This work was supported by a Fellowship to Patricia Gomez-Suaga from the Motor Neurone Disease Association and grants from the UK Medical Research Council, Alzheimer's Research UK and the Alzheimer's Society.

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Immune response in the peripheral nervous system and skeletal muscles is pivotal to counteract ALS

Several data showed that the motor neuron (MN) survival is per se not sufficient to ameliorate ALS disease progression due to the lack of preservation of the loss of connectivity between the nerve terminals and the muscles. We previously reported that MCP1 is strongly upregulated in the nervous system of slow-progressing than fast-progressing SOD1G93A mice, the latter showing a poor immune response and eventual massive nerve and muscle degeneration. To assess the MCP1-mediated therapeutic role, we boosted the chemokine along the motor unit of C57 SOD1G93A mice through a single intramuscular injection of a scAAV9 vector engineered with the Mcp1 gene. In the periphery, we provided direct evidence underlying the pivotal role of the immune response in driving skeletal muscle regeneration and thus the speed of ALS progression. Mainly, the early macrophage recruitment and phenotypic switch towards an M2 pro-regenerative phenotype sustained the activation of the myogenic program with an increased rate of muscle fiber differentiation. In the nervous system, we recorded a novel pleiotropic role of MCP1 in promoting peripheral axon regeneration and modulating neuroinflammation, ultimately preventing neurodegeneration. Our findings suggest that the peripheral immune response is delayed in ALS mice and ineffective at sustaining a substantial recovery of the compartment. Notably, the effect recorded in SOD1G93A mice pointed out the nature and temporal activation of the immune response as discriminating factors to foster skeletal muscle regeneration, slackening the dying-back degeneration and slowing down ALS course. Altogether, our observations highlight the immune response as a key determinant for disease variability and proffer a reasonable explanation for the failure of systemic immunomodulatory treatments suggesting new potential strategies to hamper ALS progression.

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TDP-43 interactome changes induced by mislocalization

TDP-43 is a DNA- and RNA-binding protein that is mainly located in the nucleus. In amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) it mislocalizes and is the predominant component of pathological cytoplasmic protein aggregates. In the present study, we investigated the interactome of wild-type TDP-43 and TDP-43 lacking nuclear localization signal (dNLS) mimicking a pathological condition in ALS. We generated inducible mammalian cell lines stably expressing the recombinant fusion protein TDP-43wt or dNLS-TDP-43 with the biotin ligase BioID2. After induction of BioID2 activity, the biotinylated proteins were isolated from the cell lysates by pull-down assay. The isolated proteins were then detected by Western blotting, silver staining, and mass spectrometry (MS). The MS analysis yielded a list of unique wtTDP-43 and dNLS-TDP-43 interactors that were further validated. Proximity-dependent biotin identification (BioID) method followed by MS revealed that wild-type TDP-43 interacts mainly with proteins of the ribonucleoprotein and spliceosome complexes, and with paraspeckles, whereas the interactors of mutant TDP-43 (dNLS-TDP-43) are components of cytoplasmic stress granules (SG) and processing bodies (P-bodies). Validation of selected interacting proteins (NONO, SFPQ, FUS, MAML1, PUM1, and ATXN2L) showed that MAML1 is unique TDP-43wt interactor, whereas NONO, SFPQ, and FUS are joint interactors of TDP-43wt and dNLS-TDP-43 and are more abundant in the TDP-43wt fraction. The interaction proteins ATXN2L and PUM1 are unique interactors of mutant TDP-43. Protein interactions in the presence and absence of NLS resulted in a list of common and unique TDP-43 interacting proteins. Our results suggest that the pathological mechanisms leading to ALS may involve loss of regulatory functions related to transcription and/or

paraspeckle function. On the other hand, the increased association of mutant dNLS-TDP-43 with proteins of P-bodies and SGs indicates that they may also have an important role in the mechanism of neurodegeneration in ALS.

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The RRM2 domain of TDP-43 is essential for its interaction with the Leucine-rich domain of Rho Guanine Nucleotide Exchange Factor (RGNEF)

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by degeneration of motor neurons. Although the cause of the disease remains elusive, a common neuropathological hallmark is the formation of neuronal cytoplasmic inclusions (NCIs) in motor neurons. Rho Guanine Nucleotide Exchange Factor (RGNEF) is an RNA-binding protein that has been observed to form NCIs in motor neurons of ALS patients and also to co-aggregate with Tar DNA-binding protein 43 (TDP-43). We previously observed that the amino terminal region of RGNEF, which contains a Leucine-rich domain (LeuR), co-localizes with TDP-43 under metabolic stress, and they are part of a high molecular weight protein complex. We also observe that LeuR and TDP-43 co-aggregate in optical lobe and brain of a Drosophila model of ALS. Since studying the mechanisms and details that underlies the formation of RNA-binding protein aggregates are critical for understanding the ALS pathology, we wanted to elucidate whether RGNEF and LeuR were able to interact directly with TDP-43. To accomplish this, we used the Nanobit protein:protein interaction system in HEK293T cells and surface plasmon resonance spectroscopy (SPR) technique. In HEK293T we observed that TDP-43 interacts with full length RGNEF and LeuR. This interaction was observed using both wtTDP-43 and TDP43-ΔNLS. We also used different segments of TDP-43 to elucidate which region of this protein interact with LeuR. Interestingly, we observed that the RRM2 domain (RNA recognition motifs 2) of TDP-43 is necessary for its interaction with LeuR. We confirmed the direct interaction between RGNEF and TDP-43, specifically with its RRM domains, using SPR. Our results provide evidence that RGNEF and its Leucine-rich domain interact directly with TDP-43 suggesting a critical role for this domain in the co-aggregation of RGNEF with TDP-43 under pathological conditions.

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Prion-like characteristics of Amyloid-β deriving clinical variants of Alzheimer's disease.

The molecular determinants of atypical clinical variants of Alzheimer’s disease, including the recently discovered rapidly progressive Alzheimer’s disease (rpAD), are unknown to date. Fibrilization of the amyloid-β (Aβ) peptide is the most frequently studied candidate in this context. The Aβ peptide can exist as multiple proteoforms that vary in their post-translational processing, amyloidogenesis, and toxicity. The current study was designed to identify these variations in Alzheimer’s disease patients exhibiting classical (sAD) and rapid progression, with the primary aim of establishing if these variants may constitute strains that underlie the phenotypic variability of Alzheimer’s disease. We employed two-dimensional polyacrylamide gel electrophoresis and MALDI-ToF mass spectrometry to validate and identify the Aβ proteoforms extracted from targeted brain tissues. The biophysical analysis was

conducted using RT-QuIC assay, confocal microscopy, and atomic force microscopy. Interactome analysis was performed by co-immunoprecipitation. We present a signature of 33 distinct pathophysiological proteoforms, including the commonly targeted Aβ40, Aβ42, Aβ4-42, Aβ11-42, and provide insight into their synthesis and quantities. Furthermore, we have validated the presence of highly hydrophobic Aβ seeds in rpAD brains that seeded reactions at a slower pace in comparison to typical Alzheimer's disease. In vitro and in vivo analyses also verified variations in the molecular pathways modulated by brain-derived Aβ. These variations in the presence, synthesis, folding, and interactions of Aβ among sAD and rpAD brains constitute important points of intervention. Further validation of reported targets and mechanisms will aid in the diagnosis of and therapy for Alzheimer's disease.

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Comparison of the Different Isoforms of Vitamin E Against Amyloid Beta-induced Neurodegeneration

Neurodegeneration is the progressive loss of structure or function of neurons. Amyloid beta could cause neurodegeneration by accumulating in the extracellular matrix. Previous studies have suggested that the dietary intake of α-tocopherol could prevent the amyloid beta aggregation and protect the brain against amyloid beta-induced neurotoxicity. However, other studies suggested tocotrienol forms of vitamin E can be used alternatively. In the presented research, from many perspectives, we compared and examined the in vitro protective effects of α-tocopherol and α-tocotrienol, which are essential isoforms of vitamin E. In this context, cell viability, cytotoxicity, amyloid beta accumulation, apoptosis, necrosis, and expressions of Calcium Channel - Alpha 1C Subunit (CACNA1C) and Beta-secretase 1 (BACE1) genes were measured on a 2D in vitro neurodegeneration model, which is formed with primary isolated neurons. Cell viability and cytotoxicity assays showed that α-tocopherol and α-tocotrienol prevent degeneration of neurons. Moreover, α-tocopherol and α-tocotrienol regulated the intracellular mechanisms that regulate calcium channel influx by decreasing the expression of CACNA1C. We also observed that the amount of amyloid beta accumulation in the extracellular matrix decreased with the application of these isoforms. Moreover, at specific time points, α-tocopherol and α-tocotrienol differ from each other in the means of protective effects. Even though this difference was not statistically significant, it was observed consistently at every measurement. In conclusion, it could be interpreted that, in more extended periods, α-tocotrienol could be a significant protective agent against Aβ-induced neurodegeneration, and it can be used as an alternative.

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Characterization of optineurin insufficiency mice during ageing

Amyotrophic lateral sclerosis (ALS) is genetically and clinically heterogenous neurodegenerative disease targeting motor neurons. Common hallmarks of ALS include chronic neuroinflammation and proteinopathy, both of which are exacerbated during ageing. A small subset of ALS patients harbours mutations in the optineurin (OPTN) gene. Optineurin is an adaptor protein in many processes, including inflammatory signalling and autophagy. To understand the role of optineurin in neurodegeneration, we

analysed a mouse model carrying OPTN^{470T} truncation, which lacks ubiquitin-binding domain, thus mimicking some ALS patient mutations. Since ALS is predominantly an adult-onset neurodegenerative disease, we analysed tissues obtained from two-year-old mice. We found no signs of neurodegeneration, microgliosis or astrocytosis the motor cortex of the brain and in the spinal cord of OPTN^{470T} mice. We have also analysed the presence of regulatory T cells (Tregs), which were shown to negatively correlate to disease progression. Interestingly, in the spleens and lymph nodes from 2-year-old OPTN^{470T} mice, we found a lower percentage of Tregs, which also had a lower level of their master transcription factor FoxP3. Protein array analysis did not show a difference in cytokine and chemokine profiles of OPTN^{470T} brains and spinal cords. OPTN^{470T} female mice did not show overt motor or memory impairments during ageing, which corresponds to the absence of signs of neurodegeneration. However, 2-year-old OPTN^{470T} male mice showed a significant drop in the motor coordination compared to 1-year-old mice, which was not observed in WT mice. Overall, OPTN insufficiency did not lead to classical ALS symptoms or neuroinflammation in aged mice but was coupled with decreased peripheral Treg numbers. Given that unmanipulated OPTN^{470T} aged mice did not show any major phenotype, we are currently developing two-hit ALS models to further elucidate the effect of optineurin insufficiency.

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Analysis of putative regulatory variants from whole genome sequencing data of 140 patients affected by neurodegenerative disorders by massively parallel reporter assay

Background/Objectives: Neurodegenerative diseases are characterized by a progressive neurological impairment. A small number of patients showed a disease family history indicating that genetic factors play a crucialrole in disease etiology. NGS has increased the rate of genetic detection, however, a missing heritability was reported which could be explained by variants in non-coding regions. Besides affecting splicing mechanism, disease-causing non-coding variants could operate deregulating gene expression. Massively Parallel Reporter Assay (MPRA) allow to analyze hundreds of thousands of regulatory variants and predict their pathogenic impact. Methods: We selected rare non-coding variants from WGS data of 140 patients affected by neurodegenerative diseases, obtaining 41 variants annotated as possible regulatory regions, using UCSC-GRCh38/hg38 ENCODE-regulation tracks. A MPRA library array was designed including a total of 60 probes for each variant in both forward and reverse strands, further divided equally into reference, alternative and scrambles and identified by a barcode. Results: A pilot study was previously conduced with a small oligonucleotide library of few variants, which demonstrated the feasibility of MPRA assay in this context. A total of 2460 probes were cloned inpMPRA vectors upstream of an ORF sequence and transfected into SHSY5Y cells. After RNA isolation and sequencing, mRNA counts and plasmid DNA ratio was performed and the bioinformatics analysis is ongoing. Conclusion: This technique can be used to analyze the pathogenic role of gene expression regulation variants in neurodegenerative diseases.

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Anti-TDP-43 antibody effect in models of sporadic ALS induced by ALS-patients CSF infusion

The majority of ALS cases (~90%) are considered sporadic (sALS) and their etiology still remains unknown. However, many studies suggest that TDP-43 could play a role in neurodegeneration. Indeed, TDP-43 proteinopathy is observed in more than 95% of ALS cases. In ALS, motor neuron degeneration is spreading, and considerable evidence suggest a prion-like disease, in which misfolded and aggregated proteins are propagating. Recently, our group obtained evidence that the cerebrospinal fluid (CSF) from sALS patients contains factors that can transmit motor dysfunction and other pathological changes. Furthermore, it is documented that removal of misfolded SOD1 by C4F6 antibody using immunoprecipitation reduces the toxicity of ALS-CSF samples toward motor neuron-like cultured cells. These findings support that TDP-43 and SOD1 proteins in the CSF of ALS patients could constitute promising targets. Finally, our team recently developed mouse monoclonal antibodies and single-chain antibodies, targeting specifically the RRM1 domain of TDP-43, that showed their therapeutic potential in ALS mice models. Our hypothesis is that the pathogenic effects of CSF from sALS in part is due to the presence of misfolded TDP-43 and SOD1, which can spread prion-like proteinopathy to healthy cells. In a first aim, we propose to test an immunotherapy based on full-length antibodies directed against the RRM1 domain of TDP-43 administered by intracerebroventricular infusion (ICV) in human TDP-43 WT transgenic mice (hTDP-43^{WT}). In a second aim, we propose to prepare, via immunocapture, ALS-CSF samples depleted of TDP-43 or misfolded SOD1. The intact and modified ALS-CSF samples will be infused ICV in mice co-expressing hTDP-43^{WT} and hSOD1^{WT} to investigate if CSF-induced pathology is alleviated. We expect that ALS-CSF samples depleted in TDP-43 or SOD1 will have reduced toxicity compared to intact ALS-CSF samples. Finally, we propose to study the long-term effect of CSF infusion on disease phenotype at different time points (1, 2, 4 and 6 months) after its cessation. If prion-like mechanisms are involved, the disease should continue to spread after stopping the infusion. These studies will provide new insights on disease mechanisms for sALS and will serve to advance the development of new treatments to halt disease propagation.

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Crosstalk of TDP-43 and Optineurin in myeloid cells

Mutations in optineurin (Optn), an ubiquitin-binding adaptor protein linked to inflammatory signaling, protein trafficking, and autophagy, have been found in a subset of amyotrophic lateral sclerosis (ALS) patients. ALS is a neurodegenerative disease marked with chronic inflammation and protein aggregation. More than 95% of ALS patients have aggregated TAR DNA binding protein 43 kDa (TDP-43) in neurons and glia, whereby TDP-43 gets ubiquitinated, hyperphosphorylated, and mislocalized to the cytoplasm. Mutations in optineurin also cause aggregation and mislocalization of TDP-43, but the putative mechanistic link between optineurin and TDP-43 pathology is still elusive. To address this, we are using: 1) optineurin knockout (KO) neuronal and microglial cell lines and 2) primary microglia from optineurin insufficiency mouse model (Optn470T) mimicking mutations found in ALS patients. We found elevated basal TDP-43 protein levels in Optn KO microglial cell line and the primary Optn470T bone marrow-derived macrophages (BMDM) and microglia. Subsequently, we analyzed TDP-43 levels upon blockage of two main protein degradation pathways: ubiquitin-proteasomal system and autophagy. TDP-43 was degraded by both pathways at a slow rate in WT, but not in Optn KO BV2 and Optn470T microglia. To test the role of inflammation on TDP-43 levels, we stimulated BV2, BMDMs, and microglia with lipopolysaccharide (LPS) to mimic bacterial infection. LPS did not affect TDP-43 transcription in neither WT nor Optn470Tcells. Notably, on the protein level, we observed a significant increase in TDP-43 in WT cells upon LPS stimulation, which was absent in both Optn KO and Optn470T cells. In the latter, TDP-43 remained at the same elevated state as in the basal conditions. We hypothesize that lack of functional optineurin leads to a state of chronic activation in the myeloid

cells but further experiments are necessary to elucidate the mechanism of crosstalk of these proteins in ALS pathogenesis.

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Endosomal fractionation of NPC1 mouse brain regions reveals sequestration of substrates of the Alzheimer's protease BACE1 within early endosomes - a mechanistic explanation for the amyloid-beta accumulation in NPC disease brains

Niemann-Pick type C (NPC) is a rare inherited lysosomal storage disorder that shares several pathological features with Alzheimer's disease (AD), including dysfunction of the endolysosomal pathway and increased levels of amyloid-beta peptides (Aß). The Aß peptides are generated by the proteolytic cleavage of the β-amyloid precursor protein (APP) initiated by the β-site amyloid precursor protein cleavage enzyme 1 (BACE1). In contrast to AD, where neurodegeneration primarily occurs in hippocampus and cortex, in NPC disease cerebellum is initially affected. We have previously shown in NPC1-null cells and in NPC1-mouse brains an enhanced cleavage by BACE1 of APP, and also exclusive BACE1 substrates, seizure protein 6 (Sez6), and seizure 6-like protein (Sez6L). We hypothesized that sequestration of BACE1 substrates within early endosomes may likely cause their increased BACE1-mediated proteolysis in NPC disease brains. To test this, we performed endosome fractionation of 10-weeks old cerebella and hippocampi from wild type (wt) and NPC1 mice using ultracentrifugation in discontinuous sucrose gradient. Fractions were collected and analysed by western blotting. We observed increased levels and different distribution of late and early endocytic markers in NPC1 vs. wt fractions from cerebellum and hippocampus. Also, Sez6 and especially Sez6L showed different distribution in NPC1 vs. wt mouse cerebella but not in hippocampus. These findings support that altered endocytic trafficking of BACE1 and its substrates, and their accumulation within early endosomes in cerebellum may likely cause their increased proteolysis by BACE1 in NPC disease brains. Further studies are needed to elucidate the potential role of BACE1 and its substrates in the pathogenesis of NPC disease.

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Genetic variability of inflammation and oxidative stress genes affects onset, progression of the disease and survival of patients with amyotrophic lateral sclerosis

Background: Inflammation and oxidative stress are recognized as important contributors to amyotrophic lateral sclerosis (ALS) disease pathogenesis. Our aim was to evaluate the impact of selected single-nucleotide polymorphisms in genes involved in inflammation and oxidative stress on ALS susceptibility and modification. Methods: 185 ALS patients and 324 healthy controls were genotyped for nine polymorphisms in seven antioxidant and inflammatory genes using competitive allele-specific PCR. Logistic regression, nonparametric tests and survival analysis were used in statistical analysis. Results: Investigated polymorphisms were not associated with ALS susceptibility.

Carriers of at least one polymorphic SOD2 rs4880 T or IL1B rs1071676 C allele more often had bulbar ALS onset (P=0.036 and P=0.039, respectively). IL1B rs1071676 was also associated with higher rate of disease progression (P=0.015). After adjustment for clinical parameters, carriers of two polymorphic IL1B rs1071676 C alleles had shorter survival (HR=5.02, 95% CI=1.92-13.16, P=0.001), while carriers of at least one polymorphic CAT rs1001179 T allele had longer survival (HR=0.68, 95% CI=0.47-0.99, P=0.046). Conclusion: Our data suggests that common genetic variants in the antioxidant and inflammatory pathways may modify ALS disease. Such genetic information could support identification of patients that may be responsive to immune or antioxidant system - based therapies.

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Neuromuscular junctions as therapeutic target in ALS.

Amyotrophic lateral sclerosis (ALS) is an adult-onset non-cell autonomous neurodegenerative disorder which causes the progressive loss of upper and lower motor neurons, leading to gradual paralysis and death in 2 to 5 years. Neuromuscular junction (NMJ) denervation is a hallmark of ALS, even preceding the death of motor neurons. Moreover, cycles of denervation and reinnervation of NMJs by motor units occurs in ALS before the final loss of motor neurons, demonstrating an active effort of remodeling of the NMJ during the symptomatic stage of the disease. Perisynaptic Schwann cells (PSCs), glial cells at the NMJ, are essential for its repair and maintenance. PSCs regulation of NMJ maintenance and repair is governed by muscarinic receptors activated by synaptic activity. While this muscarinic activation of PSCs is reduced following NMJ injury in healthy individuals, leading to PSCs changes of phenotype to support the process of NMJ repair, we observed that it is hyperactivated in mouse models of ALS. This hyper-muscarinic excitability could prevent PSCs to perform their normal functions and reduce NMJ repair, contributing to NMJ deficits and disease pathophysiology in ALS. We posit that dampening the muscarinic activity of PSCs will help maintain NMJ innervation in ALS to ultimately improve motor function. To this end, we treated SOD1G37R mice with a selective M3 mAChRs antagonist. We observed that dampening PSCs muscarinic activity in vivo improved NMJ innervation, motor neuron survival, muscle functional properties, locomotion and survival. Hence, targeting NMJs, and glial cells in particular, may be a promising therapeutic avenue in ALS.

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Antisense C4G2 repeat reduces Phe-tRNA charging and expression of Phe-rich proteins

The expanded hexanucleotide GGGGCC repeat mutation in the C9orf72 gene is the main genetic cause of amyotrophic lateral sclerosis and frontotemporal dementia. Under one disease mechanism, sense and antisense transcripts of the repeat are predicted to bind various RNA-binding proteins, compromise their function and cause cytotoxicity. Using RNA pull-down assay with disease-relevant length antisense repeat RNA we identified cytoplasmic interactions with phenylalanine-tRNA synthetase (FARS). In addition to interacting with subunit alpha (FARSA), expanded C4G2 RNA repeats are also shown to disrupt tRNA aminoacylating function of FARS in vitro and lead to decreased levels of charged tRNAPhe in patient-derived cells. Furthermore, decreased expression of Phenylalanine-rich proteins is also observed in C9orf72 patient-derived cells compared to controls. In conclusion, this research shows functional inhibition of FARSA in the presence of C4G2 RNA repeats including in C9orf72 patient-derived cells. Compromised function of tRNA aminoacylation could lead to impairments in protein synthesis and further contribute to C9orf72 mutation-associated pathology.

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NEK-1 variants in a cohort of Italian ALS patients

Introduction: Recently, large-scale whole-exome sequencing studies highlighted a significant enrichment of NEK1 loss of function (LoF) variants in amyotrophic lateral sclerosis (ALS), as well as an additional role for the p.Arg261His missense variant in the disease susceptibility. Several other missense variants have been described so far; however, their pathogenic relevance remains to be established, since many of them have been reported in both ALS patients and control cases. Objectives: The aim of our work was to further investigate the presence and impact of NEK1 variants and to explore potential genotype-phenotype correlations in a cohort of Italian ALS patients. Methods: We sequenced a cohort of 356 unrelated Italian ALS patients by Next Generation Sequencing (NGS) using using TruSeq Neurodegeneration Panel by Illumina (San Diego, CA, USA). A cohort of 380 non-neurological unrelated Italian patients, for whom NGS exome sequencing data were available from our in-house database, was selected as the control group. We considered only NEK1 variants with MAF<0.01; that were all confirmed by Sanger sequencing. Clinical significance was assessed based on the ACMG guidelines. Kaplan–Meier univariate analysis was carried out to determine the effect of NEK1 variants on survival (defined as time from symptoms onset to death/tracheostomy). Fisher's exact test and a binary logistic regression analysis, adjusted for sex and age at onset, were used to explore differences in gene variant frequencies, as well as the association of NEK1 variants with phenotype (p-value<0.05 for statistical relevance). All statistical analyses were performed using the SPSS 22.0 software (Technologies, Inc., Chicago, IL, USA). Results: Overall, we detected and confirmed, with the Sanger technique 20 different NEK1 rare variants (4 LoF and 16 missense) in 33 unrelated patients with sporadic ALS. The four LoF variants (two frameshift and two splice-site variants) were absent from all public genomic databases and from our in-house controls. The p.Arg261His missense variant, previously reported as a risk factor for ALS, was found in 13 patients and one control (p<0.001). Excluding this variant from counting, the difference in the frequency of NEK1 missense variants between patients and control was not statistically significant, in line with previous studies. Among the sixteen missense variants we found, nine were classified as variants of uncertain significance; of these, four were novel. Fifteen NEK1 variant carriers (45.4%) also harbored variants in other ALS-related genes. ALS patients carrying NEK1 variants did not differ for sex distribution, age at onset or survival from the other patients of the cohort. However, a binary logistic regression analysis, adjusted for sex and age at onset, revealed a significant higher risk for NEK1 carriers to present with upper limb involvement. Conclusions: NEK1 variants are not rare in the Italian population. The fact that we found NEK1 variants only in sporadic patients, together with the high frequency of oligogenic carriers, supports the hypothesis that some NEK1 variants confer a significant susceptibility to ALS, even though they might not be sufficient per se for disease development. These variants may however act as a phenotypical modifier.

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Diagnostic and prognostic performance of serum biomarker in upper motor neuron syndromes.

Motor neuron diseases (MND) comprise a heterogeneous group of disorders that affect the upper motor neuron (UMN) and/or the lower motor neuron, encompassing different disease phenotypes each carrying distinctive clinical and prognostic characteristics. Since primary lateral sclerosis (PLS) significantly differs from amyotrophic lateral sclerosis (ALS) in disease course, prognosis and genetic factors, several lines of evidence pointed out this phenotype may be a separate disorder, implying different pathomechanisms and potentially specific therapeutic targets. However, differentiation of PLS from ALS may represent a significant challenge for the neurologist, especially at early stages and for patients presenting with an UMN syndrome, because the initial clinical manifestation might be similar, and the differential diagnosis still relies mostly on clinical and neurophysiological follow/up. The objectives of the present study are: i) to determine the clinical features at onset that could help to differentiate between PLS and ALS; ii) to evaluate the diagnostic performance to early differentiate PLS from ALS patients presenting with UMN clinical signs of an integrated serum biomarker panel; iii) to identify the prognostic factors for patients presenting with an UMN syndrome. We selected and retrospectively analyzed the clinical data at the time of the first evaluation at our center of 128 patients presenting with selective UMN clinical involvement. These patients received a final diagnosis at follow-up of either PLS (n=74) or ALS (n=54). At the first evaluation, when available, serum neurofilament light chain (NfL), ubiquitin carboxyl-terminal hydrolase isozyme L1 (UCHL1), glial fibrillary acidic protein (GFAP), and total tau protein levels were measured using ultrasensitive single molecule array (SIMOA). PLS patients presented a longer time from symptom onset to first neurological evaluation, a slower disease progression rate and lower levels of NfL in serum compared with ALS patients ($p<0.001$). Conversely, serum levels of GFAP, total tau or UCHL1 did not differ in the two groups. Moreover, serum NfL displayed a good diagnostic performance to discriminate PLS from ALS. Binary logistic regression analysis confirmed a significant role of NfL serum levels to predict the MND phenotype. Univariate and multivariate analysis, confirmed the independent prognostic role for NfL Levels in our cohort of patients presenting with an UMN phenotype. Our study supports the role of serum NfL as a potential biomarker to discriminate between PLS and ALS at presentation and to predict prognosis in patients presenting with UMN clinical signs. These findings might help in reducing diagnostic delay, optimizing therapeutic trial design and to correctly address patients to potential tailored therapies.

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A transformed view of brain-immune system relationships: Immunotherapy to harness the immune system to help defeat neurodegenerative disease

Since the 1960s, the brain was viewed as an immune-privileged organ, unable to tolerate any immune activity. With the emerging understanding of the life-long dialogue between the brain and the immune system came an awareness of the function of systemic adaptive immunity in containing destructive factors within the central nervous system (CNS). However, as damage accumulates within the CNS, the systemic immune system loses its protective capacity and becomes an escalating factor itself, driving a vicious cycle that must be arrested. In animal models of amyloidosis and tauopathy, modestly blocking the inhibitory immune checkpoint PD-1/PD-L1 pathway was found to transiently activate the immune system and thereby drive a cascade of events that facilitates mobilization of bone-marrow-derived macrophages and FoxP3 regulatory T cells to the diseased brain. Systemic inhibition of CCR2, the chemokine receptor facilitating monocyte migration, abrogated the beneficial effect. The mobilized bone-marrow-derived macrophages express molecules associated with anti-inflammatory activity, and scavenger receptors that can remove toxic forms of misfolded proteins, dead cells, and cell debris, thereby rescuing synapses, neurons, and function. Overall, our results indicate that targeting systemic rather than CNS-specific disease-escalating factors provides a potential multi-dimensional disease-modifying therapy for neurodegenerative diseases, regardless of the primary disease etiology.

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Structural aspects and functional effects of the mutations associated to Type B Kufs disease (CLN13)

Lysosomal cysteine cathepsins belong to the C1 cysteine peptidase family (papain subfamily). They play an important role under physiological conditions, where they are tightly regulated by their endogenous inhibitors. However, if deregulated, they are involved in several pathological processes, including neurodegeneration. Among eleven human cysteine cathepsins, cathepsin F has unique biochemical characteristics and structural properties. However, even two decades after its discovery, many questions remain still unsolved, due to the challenges faced in order to get pure protein in sufficient quantities for its structural and functional characterization. On the other side, a sequence-based bioinformatics approach was crucial to evaluate the suitability of the wild-type protein from cloning until 3D structure determination by X-ray crystallography. Interestingly, our systematic approach, shows for the first time the bottlenecks that prevented earlier attempts to get this protein using different strategies and/or expression systems. Moreover, on the available 3D structure of the mature form of human cathepsin F, we evaluated the effect of the mutations found in patients, thus associated with an adult-onset neuronal ceroid lipofuscinosis, namely Type B Kufs disease (CLN13). These results, clearly showed a destabilizing effect of all evaluated mutants, thus providing the structural basis for the detrimental effect observed in functional studies.

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Chitotriosidase in CSF as biomarker for ALS

Background: Inflammation is a key pathological hallmark in amyotrophic lateral sclerosis (ALS), which seems to be linked to the disease progression. We aimed at determining prognostic potential of the putative marker of microglial activation chitotriosidase (CHIT1) in serum and CSF in newly diagnosed ALS patients. Methods Chitotriosidase-1 (CHIT1), were measured in CSF and serum of newly

diagnosed patients with ALS (n=62), disease controls (n=34). Only patients who fulfilled the diagnostic criteria for probable or definite ALS according to the El Escorial revised criteria were included in the study CSF and serum samples from age- and gender-matched patients undergoing orthopedic surgery without any neurological involvement were used as normal CSF and serum levels were correlated with several clinical parameters (age of the onset, the age of onset, sex, diagnostic delay, ALSFRS-r score, ALSFRS-r slope, number of regions displaying motor neuron degeneration, forced vital capacity. Results: In ALS, CHIT1 CSF levels were higher in patients with spinal compared with bulbar onset of the disease (p=0.017). Serum CHIT1 levels were not different in ALS compared to controls. Positive correlation was observed between CHIT-1 CSF levels and ALS-FRS slope (r = +0.3; p=0,035), indicating that patients with high disease progression have high level of CHIT-1. We did not register any significant correlation when we compared CHIT1 CSF and serum levels with the age of onset, sex, diagnostic delay, ALSFRS-r score, number of regions displaying motor neuron degeneration, forced vital capacity. Conclusion: CHIT1 concentrations in the CSF of patients with ALS may reflect the extent of could be a potentially useful marker for prediction of disease progression in ALS and, therefore, seems suitable as a supplemental marker for patient stratification in therapeutic trials.

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Human cathepsin F: an unusual cysteine protease involved in Type B Kufs disease (CLN13)

Human cathepsin F is a lysosomal cysteine protease containing an unusually long propeptide region (251 amino acids) with an unknown function. We predicted that human cathepsin F contains three natively disordered regions within the enzyme's propeptide and various aggregation-prone amino acid stretches. The recent discovery of novel pathogenic variants within the human cathepsin F gene further confirms the link of this enzyme with the adult-onset neuronal ceroid lipofuscinosis namely, Type B Kufs disease (CLN13). Noteworthy, these novel pathogenic variants exhibit impairments as seen in patients clinically diagnosed with early-onset Alzheimer's disease and/or mimicking frontotemporal dementia-parkinsonism. Using a neuronal cellular model, we were able to recapitulate many of the features reported in the cathepsin F-deficient mouse and a mechanism of action was proposed.

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SARS-COV-2, global aging and neurodegeneration: is this the perfect storm?

Over 159M people world-wide have been reported to have had COVID-19, resulting in over 6.3M deaths (WHO data, 2020-21). While significant gains have been made in understanding the SARS CoV-2 virus that underlies this, the long-term consequences of SARS-CoV-2 human exposure (known as the post-COVID-19 syndrome or long-COVID) remain to be fully understood. Despite this, there is emerging consensus that some combination of the residual of direct viral damage (neurotropism based on ACE2 receptor-dependent and independent mechanisms), aberrant immune response (e.g., immune dysfunction, cytokine storm consequences, nonspecific inflammation, antineuronal autoimmune dysfunction) and microvascular endothelial damage contribute to both persistent and/or progressive dysfunction. Nowhere is this more evident than in the post-COVID-19 syndromes of neurological dysfunction. Complicating this has been the occurrence of the pandemic on a background of a rapidly aging population, with many countries now having more individuals over the age 65 than those under the age of 65. It is predicted that this trend will continue with the proportion of individuals in the healthy aged group having the greatest gains. This represents a significant challenge for a segment of the population centered about the 5th through 7th decades of life in which death rates were significantly less than those above the 8th decades of life. In essence, defining an ‘at-risk’ group who, having a greater likelihood of longevity and then having had SARS-CoV-2 exposure, are at a potentially greater

likelihood of either accelerated neurodegeneration or the de novo development of a neurodegenerative process. Early indications that this may indeed be the case can be found in recent neuroimaging studies showing focal areas of brain atrophy and reduced metabolic activity in patients with post-COVID-19 syndrome, the evidence of acceleration of Parkinson's disease and the development of persistent cognitive impairment and/or dementia. There is recent evidence to suggest that the SARS-CoV-2 nucleocapsid protein can seed the formation of amyloid fibrils, either with α-synuclein or stress granules. Such observations should prompt longitudinal, age-stratified, international studies in which COVID-19 affected patients undergo deep endophenotyping across a range of clinically and biologically relevant markers to fully understand the pathophysiology and thus potential mitigating strategies of the post-COVID-19 syndrome.

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Trappc9 deficiency in mice impairs learning and memory by causing imbalance of dopamine D1 and D2 neurons

Genetic mutations in the gene encoding transport protein particle complex 9 (trappc9), a subunit of TRAPP that acts as a guanine nucleotide exchange factor for Rab proteins, cause intellectual disability with brain structural malformations by elusive mechanisms. Here, we report that trappc9-deficient mice exhibit a broad range of behavioral deficits and postnatal delay in growth of the brain. Contrary to volume decline of various brain structures, the striatum of trappc9 null mice was enlarged. An imbalance existed between dopamine D1 and D2 receptor containing neurons in the brain of trappc9-deficient mice; pharmacological manipulation of dopamine receptors improved performances of trappc9 null mice to levels of wild-type mice on cognitive tasks. Loss of trappc9 compromised the activation of rab11 in the brain and resulted in retardation of endocytic receptor recycling in neurons. Our study elicits a pathogenic mechanism and a potential treatment for trappc9-linked disorders including intellectual disability. Genetic mutations in the gene encoding transport protein particle complex 9 (trappc9), a subunit of TRAPP that acts as a guanine nucleotide exchange factor for Rab proteins, cause intellectual disability with brain structural malformations by elusive mechanisms. Here, we report that trappc9-deficient mice exhibit a broad range of behavioral deficits and postnatal delay in growth of the brain. Contrary to volume decline of various brain structures, the striatum of trappc9 null mice was enlarged. An imbalance existed between dopamine D1 and D2 receptor containing neurons in the brain of trappc9-deficient mice; pharmacological manipulation of dopamine receptors improved performances of trappc9 null mice to levels of wild-type mice on cognitive tasks. Loss of trappc9 compromised the activation of rab11 in the brain and resulted in retardation of endocytic receptor recycling in neurons. Our study elicits a pathogenic mechanism and a potential treatment for trappc9-linked disorders including intellectual disability.

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Structural characterization of the TDP43 / RGNEF hetreooligomer in neuron inclusion bodies

The formation of TDP-43 protein aggregates in motor neurons is a well-characterized phenotype of ALS. Another RNA-binding protein, RGNEF, has also been found to co-aggregate with TDP-43 within inclusions, in vivo, suggesting a potential pathophysiological role alongside TDP-43. Co-localized aggregation of TDP-43 and RGNEF has been shown to be dependent on the presence of specific structural domains in both proteins, suggesting they may directly interact. Such interactions within protein inclusions, that drive aggregation, may serve as potential targets for early diagnosis and clinical intervention of ALS. Here we investigate the possibility that co-aggregation is mediated by a direct interaction between RGNEF and TDP-43. Potential interacting residues were identified by evolutionary co-variation and used to construct a model of an RGNEF-TDP-43 complex. In this model, the RRM1 and RRM2 domains of TDP-43 form significant contacts with the LeuR (residues 1-222) domain of RGNEF. This interaction was observed using 3 independent computational docking algorithms, suggesting that the interaction may represent an association that occurrs in vivo. In order to characterize the interaction, we initially expressed and purified recombinant RGNEF from E.coli. Unfortunately, all attempts at obtaining recombinant LeuR yielded insoluble protein, suggesting the domain is inherently instable on its own. Given the predicted interaction interface between RGNEF and TDP-43, we hypothesize that co-expression of TDP-43 may stabilize the structure of RGNEF. This approach is being pursued with the aim of generating sufficient RGNEF-TDP-43 complex to permit further biophysical analysis including structure determination via X-ray crystallography.

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Mechanism of paroxetine-mediated autophagic induction in cell models of ALS/FTD

Protein aggregation is a hallmark of neurodegenerative diseases (NDs), including Amyotrophic Lateral Sclerosis/Frontotemporal dementia (ALS/FTD). The C9ORF72-hexanucleotide expansion G4C2 represents one of the most frequent genetic causes of ALS/FTD diseases. One molecular mechanism underlying C9ORF72-pathology is the accumulation of dipeptide repeats (DPRs) encoded by G4C2 expansion. Also, TDP-43 inclusions are present in the 98% of ALS and 50% of FTD cases. It has been postulated that aggregates represent a protective response of the cells, aimed to compartmentalize harmful substrates for subsequent removal. However, persistent aggregates cause physical damage on intracellular components, and sequester key factors of the protein quality control (PQC) system. PQC enhancement for aggregates clearance represents a therapeutic approach under investigation in ALS/FTD. Here, we aimed to dissect the mechanism of autophagic induction of the antidepressant paroxetine in NeuroblastomaXSpinal Cord 34 (NSC-34) motoneuron-like cells. We show that paroxetine induced the expression of autophagic markers LC3, SQSTM1/p62 and LAMP1, by RT-qPCR. Also, an enhancement of the autophagic flux was observed, with appearance of LC3 and SQSTM1/p62 puncta in immunofluorescence, increased protein levels and LC3-I/LC3-II conversion in western blot. Paroxetine is a cationic amphiphilic drug, known to induce lysosomal membrane permeabilization (LMP). Indeed, we observed an induction of lysosomal damage upon paroxetine treatment using galectin-3 puncta assay. We found that LMP triggered the activation of Transcription Factor EB (TFEB), a master regulator of autophagy, suggesting lysophagy induction for damaged lysosomes turnover. Therefore, we tested if paroxetine-mediated autophagic induction favoured the clearance of protein aggregates. Indeed, we observed a decrease in high molecular weight insoluble species and aggregates of ALS/FTD substrates, such as the toxic fragment of TDP-43 (TDP-25) and

the DPR polyGA. In conclusion, these results suggest that paroxetine, by inducing a cell protective response, may be beneficial in the removal of harmful aggregates in ALS/FTD.

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A multi-omics approach to study monozygotic twins discordant for amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, characterised by progressive death of upper and lower motor neurons, whose aetiology is still partially understood. The majority of ALS cases are sporadic, while 10% are familial. To investigate genetic and epigenetic factors underlying ALS, we studied a monozygotic twin pair discordant for ALS with a multi-omics approach, combining whole exome sequencing with genome-wide methylome- and transcriptome data from whole blood and PBMCs. For methylation, we used the Illumina EPICArray and ChaAMP software for the analyses, while for gene expression study Illumina TruSeq Stranded mRNA sequencing was performed. Results of the three omics were considered independently and in combination. We identified 59 differentially expressed genes (p.adj < 0.1; |log2FC| >1) and confirmed the up or downregulation for 6 of them by ddPCR. Functional analyses on DEGs performed by GSEA, IPA and G-Profiler revealed the involvement of adaptive and innate immune system pathways. After QC, we found 2 differentially methylated probes (p.adj ≤ 0.1) in CACNA1G and VAX1 genes; while filtering by delta beta (Δβ) values, we identified 2 probes with Δβ ≤ - 0.25 (in an intergenic region and RUSC1-AS1) and 2 probes with Δβ ≥ 0.25 (in AARS and KPNA4). None of them fell into the 59 DEGs. For exome analyses, ExomeDepth and ClassifyCNV identified 3 deletions and 1 duplication of uncertain significance in the ALS twin. Analyses of SNV, after filtering for frequency (≤ 0,00005) and QC (PASS), identified 25 variants classified as VUS (n=18) or likely benign (n=7). In conclusion, we integrated different omics performing functional analyses with several bioinformatic tools that underlined a possible role of the immune system in the disease. Further understanding of these immunological results and the validation of methylation results are ongoing to elucidate possible somatic genetic factors that could underlie susceptibility to sporadic ALS.

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TDP-43 mediated regulation of RNA binding proteins in ALS

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the selective loss of motor neurons. Efforts to design effective therapies are crippled by our lack of understanding the molecular lesions and aberrant processes that lead to disease pathogenesis. While several new players in ALS pathogenesis have recently emerged, RNA binding proteins, such as TDP-43 and hnRNP A1, have become a primary focus. However, little is understood about how these proteins interact and/or coordinate RNA processing and metabolism. RBPs such as TDP-43 and hnRNP A1 are both mutated in familial ALS cases and mislocalized into cytoplasmic aggregates in the motor neurons of affected patients. In the case of TDP-43, cytoplasmic inclusions are accompanied by a depletion of nuclear TDP-43. It is our hypothesis that mislocalisation of TDP-43 disrupts physiological functions within the nucleus. We have examined the downstream consequences of nuclear TDP-43 depletion on various aspects of RNA metabolism. One particular focus is on the stress granule mechanism as it serves as an interface between genetics and potential environmental contributions to disease. Our data support

that a compromised stress granule response, due to TDP-43 nuclear depletion, may contribute to neuronal vulnerability in ALS.

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Genetic depletion of BACE1 alters neuroinflammation in a knock-in mouse model of Niemann-Pick type C disease

Niemann-Pick type C disease (NPC) is a rare neurodegenerative disorder affecting mainly children. Mutations in genes coding for cholesterol transport proteins NPC1 or NPC2 result in accumulation of cholesterol and other lipids in late endosomes and lysosomes, causing dysfunction of endolysosomal pathway, neurodegeneration (primarily of Purkinje neurons), neuroinflammation and tau hyperphosphorylation. Interestingly, NPC shares several features with Alzheimer's disease (AD). Previously, we identified enhanced proteolysis by BACE1, a key AD enzyme, as additional common feature. Since BACE1 is considered a potential therapeutic target against AD, we hypothesized that enhanced BACE1-mediated proteolysis in NPC may play a role in NPC pathogenesis and that BACE1-directed therapeutic strategies may ameliorate and/or decrease progression of NPC. We crossed NPC1-I1061T knock-in mice (NPC1-KI, B6.129-Npc1tm1Dso/J) with BACE1-knock out (BACE1-KO, B6.129-Bace1tm1Pcw/J) to generate a new NPC1 mouse model with one or both BACE1 alleles deleted. Generation of NPC1-KI mice with both BACE1 alleles deleted was not successful, with only few mice surviving, indicating that NPC1 dysfunction and BACE1 loss of function are interconnected, resulting in poor survival. Analyses of 9 weeks old NPC1-KI mice with only one BACE1 allele deleted (NPC1-KI/BACE1-HET) showed decreased neurodegeneration of Purkinje neurons, decreased activation of astrocytes and lower accumulation of lysosomes in the brains of NPC1-KI/BACE1-HET vs. NPC1-KI mice. Interestingly, partial deletion of BACE1 in NPC1-KI mice seemed to further activate microglia but did not affect tau hyperphosphorylation. Our findings indicate that BACE1 and its substrates could be implicated in neuroinflammation in NPC. Further analyses are underway to determine if these changes occur in pre-symptomatic stage of the disease, as well as how the long term BACE1 depletion affects survival and other pathological features of NPC.

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Shaky hands are a part of motor neuron disease phenotype: clinical and electrophysiological study of 77 patients

Background: In the sharp contrast with the existing literature, we frequently observe minipolymyoclonus, tremor and pseudodystonic thumb posturing in patients with motor neuron disease. We conducted a clinical and electrophysiological study to describe phenomenology, prevalence and pathophysiology of involuntary movements in motor neuron disease. Methods: We included 77 consecutive patients. Involuntary movements were assessed at rest and on action. Patients were videotaped. Arm muscle tone, power and deep tendon reflexes were evaluated. Accelerometry with electromyography was recorded in a subset of patients. Results: Involuntary movements were observed in 68.9 % of patients and could be separated into rest minipolymyoclonus, thumb tremor,

pseudodystonic thumb posture, action minipolymyoclonus, and action tremor. One-third of patients reported negative impact of involuntary movements on hand use. Logistic regression showed that rest minipolymyoclonus and thumb tremor were more likely to occur in patients with more prominent distal muscle weakness and less spasticity. Similarly, action involuntary movements were more likely to appear in weaker patients. Patients with brisk tendon reflexes were more likely to display action tremor than action minipolymyoclonus. Action tremor was characterized by accelerometer and corresponding electromyography peak frequency, which decreased with mass loading, suggesting a mechanical-reflex tremor. Conclusions: Involuntary movements are common, but poorly recognized feature of motor neuron disease that may add to functional impairment. Results of our study suggest that involuntary movements are likely of peripheral origin, with a non-fused contraction of enlarged motor units being a common driving mechanism. Minipolymyoclonus appears if no synchronization of motor units occurs. When synchronization occurs via stretch reflex, mechanical-reflex tremor is generated.

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Computational Study of the Monoamine Oxidase B Mechanism-Based Irreversible Inhibitors

Monoamine oxidase B (MAO B) is a flavoenzyme responsible for the metabolism of endogenic and exogenic amines such as monoamine neurotransmitters whose disturbed homeostasis is implicated in the wide range of neurodegenerative pathogenesis. MAO B represents a primary pharmacological target for the treatment of Alzheimer's and Parkinson's disease. Commercial drugs, selegiline, and rasagiline, are administrated with dietary restrictions and in high doses are associated with more frequent and greater intensity side effects. There is constant market pressure for the development of new, mechanism-based MAO B inhibitors with more favorable pharmacokinetic profiles. An innovative approach was developed for the drug design which involves binding of the scaffolds with propargylamine core which is present in commercial drugs which target MAO enzymes. More favorable thermodynamic profiles are obtained using methods of script based molecular docking and molecular dynamics simulations. A more favorable kinetic profile of the inhibitory activity was obtained and characterized via the quantum chemical cluster approach.

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TNF-α, IL-1α and IL-10 - potential inflammatory biomarkers in Alzheimer´s disease

Mild cognitive impairment (MCI) is defined by memory deficits that do not significantly impact daily functioning. However, around 10-15% of subjects with MCI develop Alzheimer´s disease (AD) each year and 80% of MCI subjects will be subsequently diagnosed with AD. AD is the most common cause of dementia characterized by the accumulation of senile plaques and neurofibrillary tangles in the brain, which result in neurodegeneration and progressive deterioration of cognitive functions. At present, clinical diagnosis of (probable) AD is established through a combination of clinical symptoms, cognitive screening tests, detailed neuropsychological testing and imaging techniques. Moreover, so far, there are only symptomatic treatments that are trying to counterbalance the neurotransmitter

disturbance in AD. Therefore, a wide range of multidisciplinary research has focused on the AD pathophysiology, specific biomarkers, and new therapeutic options for its prevention and treatment. More recently, the presence of a sustained immune response in the brain has emerged as a significant factor underlying the AD pathology. The complex interaction between pro-inflammatory (TNF-α, IL-1α) and anti-inflammatory (IL-10) cytokines, and their imbalance could be the critical risk factor for AD development. The aim of this study was to investigate possible associations of TNF-α (rs1800629), IL-1-α (rs1800587) and IL-10 (rs1800896) gene polymorphisms with AD, as well as to determine serum TNF-α, IL-1α and IL-10 levels in AD patients and subjects with MCI. Study enrolled 74 patients with AD and 96 subjects with MCI. Genomic DNA was extracted from peripheral blood by a salting out method. Genotyping was performed using Real-time PCR. The concentrations of TNF-α, IL-1α and IL-10 cytokines were determined by ELISA. Data was evaluated using GraphPad Prism version 4.00. No significant associations of TNF-α, IL-1α and IL-10 polymorphisms with AD were observed. However, patients with AD had significantly lower IL-1α and IL-10, as well as significantly higher TNF-α serum levels compared to subjects with MCI. Dementia severity (MMSE scores) was positively (IL-1α and IL-10) or negatively (TNF-α) correlated with the serum levels of investigated cytokines. The study confirmed the important role of the immune system in AD, suggesting dysregulation in the pro- and anti-inflammatory response. Further research is needed to clarify the potential of IL-1α, TNF-α and IL-10 as peripheral biomarkers in AD.

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The role of microbiota in Parkinson's disease

Parkinson's disease (PD) is the second most common chronic age-related, progressive neurodegenerative disorders. In PD, despite remarkable advances in our insight into the responsible mechanisms, the etiology remains unknown. The key neuropathology in PD is Lewy body (LB) deposition (abnormal aggregates of a misfolded protein called α-synuclein) and consequently neuronal dysfunction, involving many other brain areas and neurotransmitter systems. It is proposed a staging scheme based on rostro-caudal pathological progression and it was suggested that in the earliest stages, PD damage is confined to non-dopaminergic structures in the lower brainstem, the olfactory bulb or perhaps the peripheral autonomic nervous system, accounting for the early appearance of non-motor symptoms. Neuropathology of PD has also been found in the enteric nervous system (ENS). Many studies have reported significant PD-related alterations of gut microbiota. There is a new suggestion that PD started when a pathogen enters the body via the nose or the GI system, leading to the formation of LBs and spreading from the enteric nervous system (ENS) to the central nervous system (CNS) through the vagus nerve. Growing evidence has indicated that the abnormality of gut microbiota and its metabolic products may be triggers for the formation of LBs in the ENS. With all those new insights and discoveries, a lot of possible therapeutic possibilities are coming in focus. In conclusion, current evidence suggests that abnormalities in gut microbiota may contribute to neuro-inflammation and motor progression of PD. Further studies are needed and in this lecture an overview of studies considering microbiota and PD will be presented.

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hnRNPH in nuclear G4C2 foci and cytoplasmic stress granules of C9ORF72 ALS and FTD

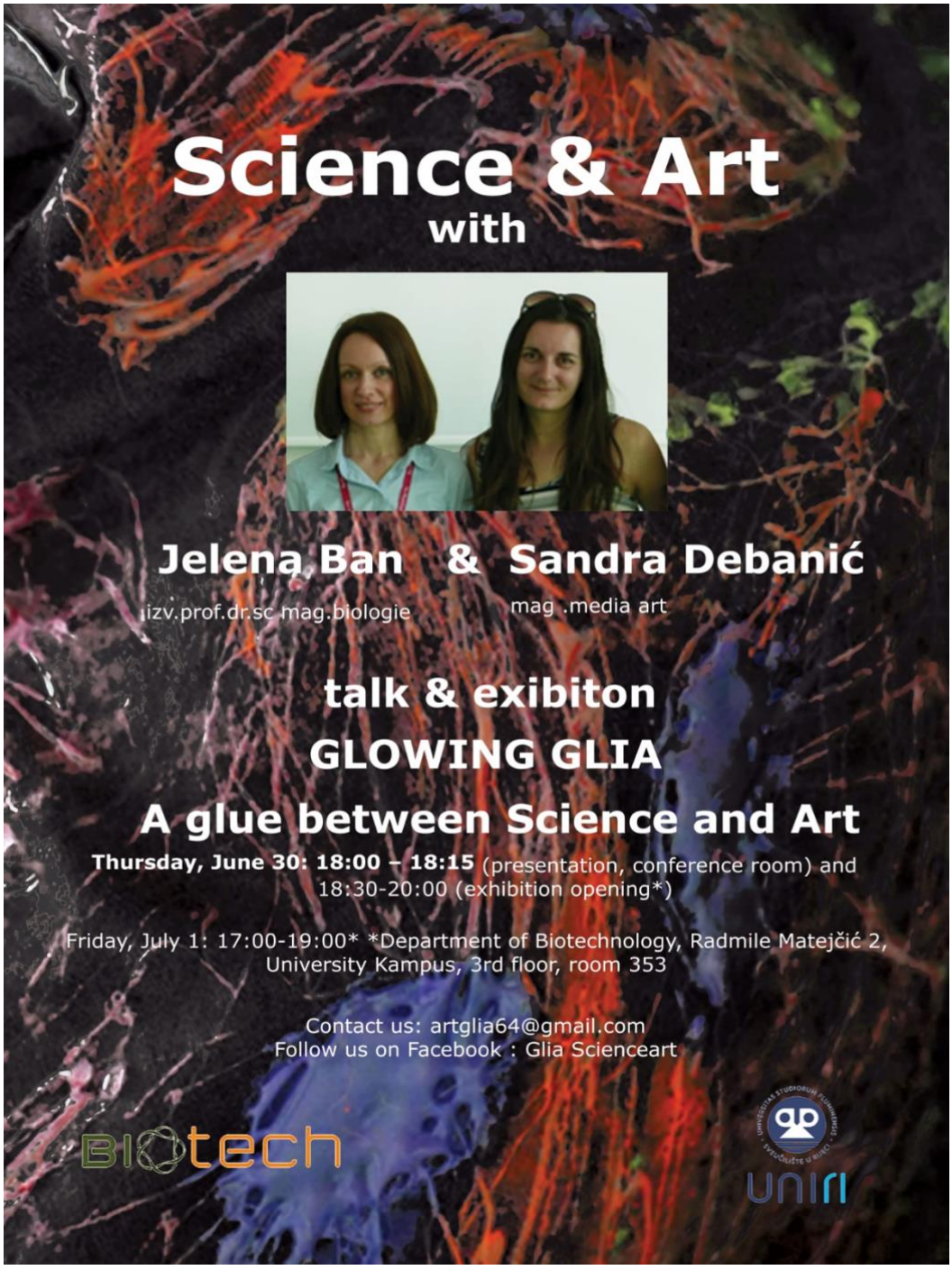
Hexanucleotide G4C2 repeat expansion in the first intron of C9orf72 is the most common known cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). One of the hallmarks is the formation of RNA foci in the nucleus, G4C2 foci, which contain aberrant repeat transcripts and sequester a variety of RNA-binding proteins (RBP). In many neurodegenerative diseases, including ALS, increased oxidative stress characterised by the formation of stress granules (SG) is a concomitant pathological factor. hnRNPH is a member of a large protein family of RBPs involved in the regulation of alternative splicing, mRNA stabilization, transcription, and translation. In ALS and FTD brain tissues, hnRNPH colocalizes with nuclear RNA G4C2 foci, whereas under cellular stress conditions, it is localized in cytoplasmic stress granules. Sequestration of hnRNPH in insoluble RNA aggregates correlates with dysregulation of splicing and may contribute to neurodegeneration. Our goal was to decipher which domains of hnRNPH determine its localization in G4C2 foci and stress granules. Nuclear foci share a group of interacting proteins with stress granules and their simultaneous presence in ALS neurons could have further pathological implications. We designed a series of hnRNPH1 protein constructs based on its domain structure and introduced mutations into individual qRRM domains to disable their RNA-binding activity. Quasi (q)RRM2 and qRRM3, but not qRRM1, are sufficient for localization of hnRNPH in stress granules. Localization of hnRNPH in G4C2 foci is independent of the RNA-binding activity of any individual qRRM domain. Using RBDmap, we show that the putative ZnF domain of hnRNPH may have RNA-binding activity. RNA binding may not be the only driving force for sequstration of hnRNPH into the G4C2 foci associated with C9orf72 ALS, as hnRNPH protein is still localized in G4C2 foci even when the RNA binding activity of the qRRM and ZnF domains have been genetically removed.

NeuroArt Exhibition

Jelena Ban & Sandra Debanić:

GLOWING GLIA: The Glue Between Science & Art, presentation and exhibition of a new project

We will talk about how the art installation "Glia - Forgotten Nerve Cells on Canvas" was created within the Micro-Macro Art project, in collaboration with Prof. Ingeborg Fülepp, Center for Innovative Media, Academy of Applied Arts Rijeka and we will present our new project „GLOWING GLIA: the glue between Science and Art“. After the short introductory lecture, we will open the exhibition in an attractive exhibition space of the laboratory at the Department of Biotechnology.



Public Awarness Campaign: Swim & Walk for Brain Health

Main organizers:
Andrea Markovinović (Department of Basic and Clinical Neuroscience, King's College London)
Nikolina Prtenjača and Ivana Munitić (Department of Biotechnology, University of Rijeka)
Vladimira Vuletić (Clinical Hospital Centre, Rijeka)

NeuroArt Contest

Main organizers:
Marta Kolarić & Ivana Munitić (Department of Biotechnology, University of Rijeka)

Open call for microscopic photographs, comics, drawings, or videos about the brain.

For those researchers with an artistic zeal and/or original ideas on **popularizing neuroscience**, clarifying how the brain functions, and raising awareness of the importance of maintaining brain health.

Deadline: June 15, 2022

Prizes:

- 1st place 200 €; 2nd place 150 €; 3rd place 100 € (or a corresponding voucher from a travel agency, as preferred)
- 10-15 best works will be exhibited at the international Symposium in Rijeka: Joint ICGEB – ALS Society of Canada Symposium on Inflammation and Proteinopathy in ALS/FTD Spectrum Disorder (June 30 - July 3)

Technical specifications:

- Microscopic photographs, comics, drawings, or videos are accepted.
- Up to 3 works can be submitted. The organizer reserves the right to conduct a selection of the works. The works could have been created at any time and could have been exhibited before. They can originally be in any digital or analogue technique.

Application instructions:

Works are sent in a digital form to: alsrijeka2020@gmail.com and cc: ivana.munitic@biotech.uniri.hr (please indicate your **name, surname, year of birth, occupation and title(s) of the submitted artwork** in the email. Also, it is necessary to enclose **consent for the publication** of this information, e.g. *"I consent to the publication of my name, surname, and occupation."*

Works that are not originally digital need to be scanned to be of sufficient print quality, at least 200dpi. Large files (>10 MB) can be sent via Wetransfer (https://wetransfer.com/) or other services.

Competition and artwork exhibition is organized by the Department of Biotechnology. By applying, the author allows the publication of artwork at the premises and website of the Department of Biotechnology, University of Rijeka related to the symposium: http://www.optineurin-neuroimmunology.uniri.hr/als-rijeka-2022 - symposium.html