



FIRST NBG-TIGEM JOINT MEETING

# IX NEAPOLITAN BRAIN GROUP MEETING

BOOK OF ABSTRACTS

12.12.19 • TIGEM, POZZUOLI (NAPLES)

## Under the patronage of:



# SINPIA

Società Italiana di Neuropsichiatria  
dell'Infanzia e dell'Adolescenza  
Sezione Campano-Molisana



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### Promoters

Ennio Del Giudice, Neapolitan Brain Group, President  
Andrea Ballabio, Telethon Institute of Genetics and Medicine, Director

### Local Organization Office

TIGEM Scientific Office: +39 08119230682

## Presentation of the Meeting

The next meeting of the Neapolitan Brain Group (NBG) will be held on 12 December 2019 at the Telethon Institute of Genetics and Medicine (Tigem) in Pozzuoli and will be the first meeting jointly organized from Tigem and NBG.

English has been chosen as the official language of the meeting to allow full participation of foreign researchers working in our region.

The program of the event is made of two lectures by Prof. Zurzolo and Prof. Gennarino, two discussion sections ("From the bedside to the bench" and "From the bench to the bedside"), 12 oral presentations selected by a committee of international reviewers and 41 posters. Overall, more than 50 research contributions in the field of neuroscience will be presented during the day.

All the contributions come from Research centers and Universities located in Campania (e.g. University of Naples Federico II, University of Campania Luigi Vanvitelli, University of Salerno, Tigem, CEINGE, IBBR, IBB, ISPAAM, ISASI, IGB), but also from Research centers outside the region. Most of the research presented is in collaborations with Italian and international research institutes.

The selected communications will be presented in most of the cases by the youngest members of the research groups. In fact, one of the goals of the NBG group is to promote the active participation of younger researchers to stimulate their scientific skills and productive confrontation with peers and more experienced and older colleagues.

*The Organizing Committee*

## Telethon Institute of Genetics and Medicine

The Telethon Institute of Genetics and Medicine (TIGEM) ([www.tigem.it](http://www.tigem.it)) is an international reference centre for research on genetic diseases. It was created in 1994 by the Telethon Foundation, one among major Italian non-profit organizations, to promote the advancement of research aimed at the diagnosis, prevention and cure of human genetic diseases. TIGEM's mission is to understand the mechanisms underlying genetic diseases and to develop therapeutic and preventive strategies.

In the first activity period, research at TIGEM was focused primarily on disease gene identification and on the characterization of the molecular defects underlying genetic diseases. The successful identification of several disease genes, each involved in a different biological pathway, created the need and the interest to perform functional studies to elucidate disease mechanisms. Several mouse models of genetic diseases have been generated and/or used at TIGEM to fulfil this purpose.

Since its foundation, the Institute has also been at the forefront of genomic research, exploiting available genomic resources by using bioinformatic and functional genomics approaches. More recently, viral gene delivery technology was introduced at TIGEM and used for in vitro and in vivo gene therapy approaches.

The Institute counts with 14 independent research groups and over 220 people from 8 different countries including graduate students, post-docs, technical and administrative staff. The scope of the science currently covered at TIGEM is focused on three strategic research programs: Cell Biology and Disease Mechanisms, Genomic Medicine, and Molecular Therapy.

TIGEM is currently located in the upper town quarters of Pozzuoli, at the "Comprensorio Olivetti", an ex-factory built in the 1954. The Institute counts with 5000 square meters converted in laboratories, office and research services: 4 "open space" laboratories, 4 meeting rooms, over 28 offices for researchers and administrative staff, dedicated spaces to microscopy, cell culture and bioinformatics. An auditorium capable of seating 150 people for business, training and scientific dissemination. TIGEM counts with strong collaborations with National Universities such as Università degli studi di Napoli Federico II, and the Università della Campania Luigi Vanvitelli.

Several research and training programmes are available at TIGEM, spanning from undergraduate training to doctoral and post-doctoral positions. Graduate students join TIGEM by way of two different programmes: Open University-OU and European School of Molecular Medicine-SEMM. The goal of the graduate programs is to prepare doctoral students for research careers in human genetics, functional genomics and molecular medicine.

TIGEM activity is supported by ten core facilities that provide cutting edge technology as well as "house-keeping" assistance. Each core is supervised by a TIGEM investigator and is composed of specialized technical staff. Seven research core facilities (Vector Core, Advanced Microscopy and Imaging Core, Bioinformatics Core, Medaka Fish Facility, Next Generation Sequencing Facility, High Content Screening Facility, Behavioural Core) offer high-quality and rapid scientific and technical services that help to improve and speed up the work of TIGEM investigators. Moreover, the Cell Culture and Cytogenetics Core provides fully-equipped cell culture facilities and technical support for cellular and cytogenetic studies to TIGEM investigators. Finally, the Informatics Core and the General Services Core provide maintenance for the Institute's informative resources and general activities. An animal facility is located in a separate building, located close to the main TIGEM building. The facility, 700sqm, is able to host 11000-13000 animals and also includes suites for surgical, procedures as well as dedicated rooms for in-vivo retina phenotyping and behavioural tests. TIGEM's Scientific Office (SO) assists and guides research scientists during grant application preparation and management. The SO assists researchers in keeping track of milestones and deliverables set in the research plan and in the coordination of the administrative assistance to researchers for the management of scientific proposals. This office offers TIGEM scientists support in transforming their research ideas into a successful grant application.

## Neapolitan Brain Group

The NBG was born in 2015 from an idea of Professor Ennio del Giudice (University of Naples Federico II) to foster the interaction between basic and clinical researchers in the Neapolitan area and in Campania, interested in the study of physiology and pathologies of the nervous system.

The group wants to be an opportunity to meet, in an informal atmosphere, all fans of clinical and basic research in the field of Neuroscience who intend to improve mutual knowledge and, as far as possible, explore moments of fruitful collaboration with the purpose to create a cultural network among the Research centers and Universities located in Campania dedicated to the study of the physiology and pathology of the nervous system.

The NBG is under the patronage of the University of Naples Federico II.

Recently the NBG has also established itself as a non-profit association with the aim of improving its cultural offer. The group is open to all those who are interested in the basic and translational themes of neuroscience and, in particular, to young people from the different Universities and Research Institutes in Campania.

Until today the NBG held the following meetings:

1st Meeting on 6/4/2015 at the Department of Biotechnology, University of Naples Federico II.

2nd Meeting on 2/4/2016 at the Department of Biotechnology, University of Naples Federico II.

3rd Meeting on 28/4/2016 at Department of Biotechnology, University of Naples Federico II.

4th Meeting on "Molecular, physiopathological and clinical mechanisms in neuroprotection of neonatal hypoxia", 9/6/2016 at the Department of Biotechnology, University of Naples Federico II.

5th Meeting on 15/12/2016 at CEINGE Naples.

6th Meeting on 12/14/2017 at Stazione Zoologica Anton Dohrn, Naples.

7th Meeting with ECM Course on "The diseases of the nervous system: pathogenetic bases and new therapeutic approaches", 31/5/2018 at CESTEV, University of Naples Federico II.

8th Meeting on 12/13/2018, at CESTEV, University of Naples Federico II.

9th Meeting on 12/12/2019 organized jointly with Tigem, at Tigem (Pozzuoli).

At those meetings, more than 200 participants, including basic researchers, doctors, doctoral students, post-docs, post-graduates, trainees and undergraduates from the universities and research institutes of Campania presented their results.

The group's mailing list currently counts about 300 members.

The group has a dedicated web page (<http://www.neapolitanbraingroup.it>), a Facebook page (<https://www.facebook.com/NBG2000/>) and has been several times cited by the F2 Magazine UNINA:

<https://www.unina.it/-/12300029-gruppo-di-confronto-nbg-neapolitan-brain-group>

<http://www.unina.it/-/13439443-5-meeting-del-neapolitan-brain-group>

<http://www.unina.it/-/18143154-viii-meeting-del-neapolitan-brain-group->

## Scientific Programme

### Morning Session

8:15	<i>Transfer from Pozzuoli Solfatara Metro Station to TIGEM</i>
8:30	Registration – presenters can hang their posters
9:00	Opening: Welcome Addresses - TIGEM and NBG Arturo De Vivo, Pro-Rettore, University of Naples Federico II Luigi Califano, President, School of Medicine of the University of Naples Federico II Gennaro Piccialli, CESTEV Director- University of Naples Federico II Antonio Simeone, IGB – CNR Director
9:30-10:15	<b>Keynote Lecture</b> <b>Chiara Zurzolo</b> introduced by <b>Lucio Nitsch</b> : <i>Understanding progression of neurodegenerative diseases: the role of lysosomes and tunneling nanotubes</i>
10.15-10.55	<b>From the bedside to the bench - Gioacchino Tedeschi, Maria Giuseppina Miano</b> Brunetti-Pierri: <i>Whole exome sequencing in undiagnosed disease patients</i> Nigro: <i>How to approach the unsolved (Telethon Undiagnosed program)</i>
10.55-11.10	Discussion
11.10-11.30	<i>Coffee break</i>
11.20-11.50	<i>TIGEM visit</i>
11.30-12.30	Poster session
12.30-14.00	<b>Selected talks - Chairpersons: Carmela Bravaccio, Luisa Cigliano</b> Esposito: <i>Burden of rare variants in 26 candidate genes predicts the risk of late onset Parkinson's disease</i> Vitiello: <i>Thick corpus callosum: neuroradiological, clinical and genetic characterization of an Italian series</i> Pontillo: <i>Resting-state functional MRI analysis of brain connectivity in Charcot-Marie-Tooth 1A</i> Russo: <i>Cortico-striatal pathway integrity in Fabry Disease: a diffusion MRI connectometry analysis</i> Indrieri: <i>miR-181a/b downregulation as new therapeutic strategy in mitochondria-associated neurodegeneration</i> Carrella: <i>MicroRNAs miR-181a and miR-181b (miR-181a/b) downregulation as potential therapeutic approach for Inherited Retinal Diseases (IRDs)</i>
14.00-15.00	<i>Lunch break</i>

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## Scientific Programme

### Afternoon Session

- 15:00-15:40     **Keynote Lecture**  
**Vincenzo Alessandro Gennarino** introduced by **Sandro Banfi**: *Protein Dosage: A New Lens on Neurological Disorders*
- 15:40-16:20     **From the bench to the bedside - Giangennaro Coppola, Brunella Franco**  
*Early-onset potentially treatable epileptic encephalopathies*  
Maurizio Taglialatela: *Personalized therapies for developmental epileptic encephalopathies*  
Pasquale Striano: *The role of the child neurologist*
- 16:20-16:30     Discussion
- 16:30-17:15     **Selected talks - Chairpersons: Goffredo Scuccimarra, Carla Perrone-Capano**  
Santonicola. *Modulation of CSPA/DNJ-14 ameliorates SMA related neurodegeneration in C. elegans*  
Verrillo. *Phytocannabinoid treatment in a mouse model of West syndrome with spontaneous seizures*  
Maglione. *Pharmacological inhibition of Sphingosine-1-phosphate degradation is therapeutically effective in a Huntington's disease animal model*
- 17:15-17:30     Coffee break
- 16:30-17:15     **Selected talks - Chairpersons: Andrea de Bartolomeis, Alessandro Fraldi**  
Damiano. *Evidence of increased oxidative stress in Pompe disease. A new therapeutic target?*  
De Risi. *Synaptic Mechanisms governing the pro-cognitive effects of Spermidine in ageing*  
Morra. *Role of the PI4,5P2 5-phosphatase OCRL at the synapse*
- 18:15-18:30     Closing remarks: A. Ballabio, E. Del Giudice
- 18:30-19:00     NBG members General Assembly
- 19:15             Transfer to Pozzuoli Solfatara Metro Station

**Oral Presentations**

(in alphabetical order of the presenting author)



Invited lecture

### **Whole exome sequencing in undiagnosed disease patients**

Cappuccio G.<sup>1,2</sup>, Pinelli M.<sup>1,2</sup>, Torella A.<sup>2,3</sup>, Fecarotta S.<sup>4</sup>, Musacchia F.<sup>2</sup>, Castello R.<sup>2</sup>, Mutarelli M.<sup>2</sup>, Parenti G.<sup>1,2</sup>, Casari G.<sup>2</sup>, Nigro V.<sup>2,3</sup>, Brunetti-Pierri N.<sup>1,2</sup>

<sup>1</sup>Department of Translational Medicine, Section of Pediatrics, Federico II University, Naples, Italy

<sup>2</sup>Telethon Institute of Genetics and Medicine, Pozzuoli, Naples, Italy

<sup>3</sup>Medical Genetics, Department of Biochemistry, Biophysics and General Pathology, University of Campania 'Luigi Vanvitelli', Naples, Italy

<sup>4</sup>DAI Materno Infantile, Federico II University, Naples, Italy

Starting from April 2016, the Pediatrics division of Federico II University Hospital is participating to the Telethon Undiagnosed Program (TUDP) aiming at achieving a molecular diagnosis in childhood-onset and unknown genetic disease by whole exome sequencing (WES). The program enrolled undiagnosed patients through a nation-wide network of pediatric centers. Patients with unknown genetic syndromes underwent clinical evaluation and selected cases were discussed with other participating TUDP clinicians in plenary meetings. Cases were prioritized according to pre-defined criteria, including severity of the disease and lack of a diagnosis after comprehensive diagnostic evaluations. Trios (parents plus patient) were analyzed by WES and in some case by whole genome sequencing. Candidate variants were filtered based on the inheritance pattern, frequency in normal controls and predictions of their damaging effect at the protein level. In selected cases candidate variants were evaluated by functional studies to elucidate the disease causality and pathogenic mechanisms. The results were shared with other international centers involved in undiagnosed diseases initiatives. Of the 153 enrolled cases in our unit of the TUDP, 125 completed the entire diagnostic WES workflow. In 56 families, causative variants were detected and in 10 cases strong candidate variants were identified. Genes affecting more families included ADNP (2 families) followed by *ASXL3*, *DDX3X*, *GRIN1* and *GRIN2B*. 16 patients showed a phenotype that was extended or more severe (*NMNAT1*, *SMARCA2*, *WDR81*, *KARS*, *DST*, *RAB3GAP1* and *ATP6V1B2*) leading to the identification of allelic disorders. Moreover, we identified several new disease genes, such as *POLR2A*, *DHX37*, *SMPD4*, *RAB10* and *B4GALT5*. Overall, the diagnostic rate of WES was 48%, that is higher compared to the rate previously reported. This higher rate of diagnosis is likely a consequence of stringent criteria for enrollment of undiagnosed disease patients. In conclusion, WES allowed expansion of the phenotypes of known disorders and led to the discovery of novel disease-causing genes.

## **MicroRNAs miR-181a and miR-181b (miR-181a/b) downregulation as potential therapeutic approach for Inherited Retinal Diseases (IRDs)**

Carrella S.<sup>1,2</sup>, Indrieri A.<sup>1,3</sup>, Piccolo D.<sup>1</sup>, Ciampi L.<sup>1</sup>, Pizzo M.<sup>1</sup>, Barbato S.<sup>1</sup>, Marrocco E.<sup>1</sup>, Ezhova Y.<sup>1</sup>, Franco B.<sup>1,3</sup>, Surace E.M.<sup>3</sup>, Banfi S.<sup>1,2</sup>

<sup>1</sup>Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy

<sup>2</sup>Medical Genetics, Department of Precision Medicine, University of Campania "L. Vanvitelli", Caserta CE, Italy

<sup>3</sup>Medical Genetics, Department of Translational Medical Science, University of Naples "Federico II", Naples, Italy

IRDs are a group of highly genetically heterogeneous disorders characterized by progressive photoreceptor (PR) cell death and blindness. The broad genetic heterogeneity of IRDs represents an important limitation to the development of gene-specific therapies. Therefore, there is a strong need for the implementation of gene-independent therapeutic strategies that can be effectively used in a large number of IRDs both to slow down disease progression and to support gene-dependent procedures. In this respect, microRNAs are promising therapeutic tools due to their capability to simultaneously modulate multiple molecular pathways involved in disease pathogenesis and progression. Over the last decades, evidence linking mitochondrial dysfunction to neurodegenerative disorders, included those affecting the outer retina, is increasing. Importantly, since mitochondrial dysfunction seems to be an early event in these conditions, therapies targeting basic mitochondrial functions hold great promise. Recently, we discovered that miR-181a/b inactivation leads to increase of mitochondrial turnover in the retina and protects neurons from cell death in several in vivo models of mitochondria-mediated neurodegeneration. We now demonstrate that reduction of miR-181a/b levels in a mouse model for an autosomal dominant form of Retinitis Pigmentosa preserves PRs from death, ameliorates PR outer segment structures and mitochondria morphology, which results in improvement of visual function. Moreover, we also found that miR-181a/b downregulation significantly decreases lipofuscin accumulation in the retinal pigment epithelium both in in vitro and in in vivo model for Stargardt disease. Overall our data indicate that the use of miR-181a/b inhibitors could be an effective and innovative gene-independent therapeutic strategy for IRDs.

### **Evidence of increased oxidative stress in Pompe disease. A new therapeutic target?**

Tarallo A.<sup>1,2</sup>, Damiano C.<sup>1,2</sup>, Minopoli N.<sup>1,2</sup>, Zappa F.<sup>1</sup>, Coletta M.<sup>2</sup>, Porto C.<sup>2</sup>, Strollo S.<sup>1</sup>, Baldi R.<sup>2</sup>, Monti D.<sup>3</sup>, De Matteis M.A.<sup>1</sup>, Parenti G.<sup>1,2</sup>

<sup>1</sup>Telethon Institute of Genetics and Medicine, Pozzuoli, Italy

<sup>2</sup>Department of Translational Medical Sciences, Federico II University, Naples, Italy

<sup>3</sup>Department of Chemical Science, Federico II University, Naples, Italy

**BACKGROUND:** Pompe disease (PD) is a metabolic myopathy caused by the deficiency of acid alpha-glucosidase. Typical pathologic features of PD are generalized intralysosomal glycogen storage and impairment of the autophagic pathway. Considering the important role of autophagy in the selective clearance and disposal of damaged mitochondria, we speculated that oxidative stress is present in PD and contributes to the disease pathophysiology.

**METHODS:** We studied oxidative stress by using biochemical assays (ROS generation, lipid peroxidation, GSH levels) and by evaluating stress markers by western blot analysis in PD patients' cells and in tissues from the PD mouse model in comparison with the respective controls. We also studied the effect of stress on recombinant human GAA (rhGAA) uptake and intracellular trafficking of the mannose-6-phosphate receptor (M6PR), and the potential of antioxidants in rescuing the deleterious effects of oxidative stress

**RESULTS:** We found high levels of ROS and lipid peroxidation and reduced GSH levels in all samples examined. We also found dysregulation of stress and autophagy markers. Immunofluorescence analysis showed abnormal localization and trafficking of M6PR in PD fibroblasts. The level of correction of GAA activity by rhGAA inversely correlated with oxidative stress. Antioxidants attenuated stress levels, improved M6PR localization/trafficking, and enhanced rhGAA internalization. Autophagy-modulating drugs also reduced oxidative stress and improved internalization of rhGAA.

**DISCUSSION:** Our results suggest that secondary abnormalities to PD pathophysiology. Increased oxidative stress appears to affect ERT efficacy. These abnormalities represent potential therapeutic targets in order to improve the ERT efficacy and the outcome of patients.

## **Synaptic Mechanisms governing the pro-cognitive effects of Spermidine in ageing**

De Risi M.<sup>1,2</sup>, Torromino G.<sup>1,2</sup>, Treves A.<sup>3</sup>, Middei S.<sup>1</sup>, Ammassari-Teule M.<sup>4</sup>, Mele A.<sup>5</sup>, Ballabio A.<sup>1</sup>, Settembre C.<sup>1</sup>, De Matteis A.<sup>1</sup>, De Leonibus E.<sup>1,2</sup>

<sup>1</sup>Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy

<sup>2</sup>Institute of Cellular Biology and Neurobiology (IBCN-CNR), Monterotondo, Italy

<sup>3</sup>Scuola Internazionale Superiore di Studi Avanzati (SISSA), Trieste, Italy

<sup>4</sup>Fondazione Santa Lucia, Rome, Italy

<sup>5</sup>Dipartimento di Biologia e Biotecnologie "C. Darwin", Università di Roma "La Sapienza", Rome, Italy

Mild cognitive impairment (MCI) is an intermediate condition occurring in some individuals before the development of dementia or Alzheimer's Disease (AD). In humans MCI pinpoints increased beta amyloid (A $\beta$ ) load, which is one of the major pathological marker of AD.

MCI is often characterized by memory capacity (MC) decline, a function which we previously demonstrated to recruit hippocampal glutamate 1 receptors (GluA1) in young mice.

A promising pharmacological strategy for prevention of age-related insults is Spermidine (SPD), a polyamine naturally present in living organisms and declining with ageing. SPD is known to stimulate autophagy, but the mechanisms through which it exerts its beneficial effects are still unknown.

We developed a model of pathological ageing in mice by identifying the inter-individual differences in MC of a middle-aged population of mice. By using the Different Object Task (DOT) we observed that a portion of mice naturally end up in impaired performance as compared to age-matched preserved subjects. Concomitantly, impaired subjects reported increased soluble and insoluble isoforms of A $\beta$ -42, associated to autophagy blockade and GluA1 synaptic trafficking impairments.

Treating impaired subjects with SPD not only rescued MC, but it also promoted A $\beta$  clearance and rescued the GluA1 trafficking impairment through autophagy re-activation.

Thus, we provided for the first time a direct link between SPD and GluA1 trafficking, through autophagy re-activation and A $\beta$  clearance.

### **Burden of rare variants in 26 candidate genes predicts the risk of late onset Parkinson's disease**

Gialluisi A.<sup>1</sup>, Reccia M.G.<sup>1</sup>, Modugno N.<sup>1</sup>, Nutile T.<sup>2</sup>, Lombardi A.<sup>1</sup>, Di Giovannantonio L.G.<sup>2</sup>, Pietracupa S.<sup>1</sup>, Ruggiero D.<sup>1,2</sup>, Scala S.<sup>1</sup>, Gambardella S.<sup>1,3</sup>, International Parkinson's Disease Genetics Consortium (IPGDC)<sup>4</sup>, Iacoviello L.<sup>1,5</sup>, Gianfrancesco F.<sup>2</sup>, Acampora D.<sup>2</sup>, D'Esposito M.<sup>2</sup>, Simeone A.<sup>2</sup>, Ciullo M.<sup>1,2</sup>, Esposito T.<sup>1,2</sup>

<sup>1</sup>IRCCS INM Neuromed, Pozzilli, IS, Italy

<sup>2</sup>Institute of Genetics and Biophysics "Adriano Buzzati-Traverso", National Research Council, Naples, Italy

<sup>3</sup>Department of Biomolecular Science, University of Urbino Carlo Bò, Urbino, Italy

<sup>4</sup>International Parkinson's Disease Genetics Consortium (IPGDC)

<sup>5</sup>Research Center in Epidemiology and Preventive Medicine (EPIMED). Department of Medicine and Surgery. University of Insubria, Varese, Italy

Keynote lecture

## **A new Lens on Neurological Disorders**

Gennarino V.A.

Department of Genetics & Development, Pediatrics and Neurology, Columbia University Medical Center, New York, USA

The family of neurodegenerative disorders known as proteinopathies, whose most famous members are Alzheimer's disease, Parkinson's disease, and Huntington's disease, involve problems with protein folding and clearance. In most cases there is a mutation that changes the conformation of the disease-causing protein in a way that alters its function and causes it to accumulate over time in neurons. Recent work, however, has shown us that even wild-type proteins can wreak havoc when they are expressed at too-high levels. For example, duplication of the amyloid precursor protein (APP) locus causes autosomal dominant early-onset Alzheimer's disease, and duplications or triplications of alpha-synuclein (SNCA) are associated with familial Parkinson's disease. In animal models, extremely high (30x-40x) levels of wild-type ataxin1 (Atxn1) cause disease reminiscent of spinocerebellar ataxia type 1 (SCA1). Could modest elevations of protein levels also cause disease? In particular, could aberrant RNA processing and post-transcriptional regulation of specific proteins alter their steady-state levels sufficiently to cause neurodegeneration or neurodevelopmental diseases? Evidence suggests the answer is "yes." The coordinated activities of microRNA (miRNA) and RNA-binding proteins (RBPs) regulate mRNA turnover, localization and translation, and orchestrate hundreds of circuits that are responsible for proper cognitive function. Yet little is known about the role of miRNA and RBPs in brain development or disease. Pursuing this line of thought, I investigate the regulation of two RNA binding proteins involved in neurological diseases: PUM1 and NUDT21.

We recently discovered that PUM1 is important for brain development leading to two different neurological diseases with different phenotypes grouped as Spinocerebellar Ataxia 47 (SCA47) with autosomal dominant inheritances with incomplete penetrance. The early-onset PADDAS Pumilio1-Associated Developmental Disability, Ataxia and Seizure causes variable degrees of developmental delay, motor coordination, and sometimes intellectual disability. The adult-onset PRCA Pumilio1-Related Cerebellar Ataxia, on the other hand, is a mild, late-onset pure cerebellar ataxia that strikes in the fourth or fifth decade of life. As of October 2019, we have identified a total of 36 PADDAS and PRCA patients, and clinicians around the world continue to send us new cases. My lab is now studying the mechanisms to understand the two different diseases caused by different mutations in PUM1.

Loss of function of MECP2 causes Rett syndrome, whereas duplications of the gene cause an equally devastating neurodevelopmental disease known as MECP2 duplication syndrome. This got us wondering about how neurons regulate MeCP2 levels and noticed that MECP2 has a long 3'UTR that contains two poly-adenylation (p(A)) sites leading to a short and long messenger mRNAs, respectively. These isoforms are bound by the CFIm protein complex, part of which (CFIm25) is encoded by a gene called NUDT21. We identified individuals who have copy-number variations spanning NUDT21—and these individuals phenocopy Rett syndrome. CFIm25 accounts for at least part of the ~5% of Rett syndrome cases that have no MECP2 mutations. My lab is now working to understand how other proteins in the CFIm complex are involved in the same disease, and our preliminary data are quite exciting.

## **miR-181a/b downregulation as new therapeutic strategy in mitochondria-associated neurodegeneration**

Indrieri A.<sup>1,2</sup>, Carrella S.<sup>1,3</sup>, Barbato S.<sup>1</sup>, Spaziano A.<sup>1</sup>, Marrocco E.<sup>1</sup>, Fernandez-Vizarra E.<sup>4</sup>, Volpe M.G.<sup>1</sup>, Pizzo M.<sup>1</sup>, Ezhova Y.<sup>1</sup>, Golia F.M.<sup>1</sup>, Ciampi L.<sup>1</sup>, Tammaro R.<sup>1</sup>, Giordano N.<sup>1</sup>, Carboncino A.<sup>1</sup>, Zeviani M.<sup>4</sup>, De Leonibus E.<sup>1,5</sup>, Surace E.M.<sup>2</sup>, Banfi S.<sup>1,3</sup>, Franco B.<sup>1,2</sup>

<sup>1</sup>Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli (NA), Italy

<sup>2</sup>Medical Genetics, Department of Translational Medical Science, University of Naples "Federico II", Naples, Italy

<sup>3</sup>Medical Genetics, Department of Precision Medicine, University of Campania "L. Vanvitelli", Italy

<sup>4</sup>MRC Mitochondrial Biology Unit, University of Cambridge, Cambridge, UK

<sup>5</sup>Institute of Cellular Biology and Neurobiology "ABT", CNR, Roma, Italy

Mitochondrial dysfunction underlies the pathogenesis of neurodegenerative diseases (NDs), either directly, as in mitochondrial diseases (MDs), or indirectly, as in more common NDs, such as Parkinson's disease (PD). Despite the efforts, effective therapies are still not available for these conditions.

We demonstrated that microRNAs miR-181a and miR-181b (miR-181a/b) control key genes involved in mitochondrial biogenesis and function, and that downregulation of these miRNAs enhances mitochondrial turnover in the central nervous system through the coordinated activation of mitochondrial biogenesis and mitophagy.

We thus tested the effect of miR-181a/b inactivation in NDs associated with mitochondrial dysfunction. We first showed that miR-181a/b downregulation effectively protects neurons from cell death ameliorating the disease phenotype in several animal models of MDs, i.e. Microphthalmia with Linear Skin Lesions, Leber Hereditary Optic Neuropathy (LHON) and Leigh Syndrome. We then tested whether miR-181a/b downregulation could also be effective in 6-OHDA-induced PD models. Remarkably inactivation of miR-181a/b reduces the extent of death in nigrostriatal dopaminergic neurons in 6-OHDA-treated medaka fish and mice, and results in improved motor performances in mice.

Finally, we tested whether the modulation of miR-181a/b could be therapeutically exploited in NDs by using Adeno Associated Viruses (AAV) vectors encoding miR-181a/b "sponges", which allow long-term downregulation of miR-181a/b in vivo. Interestingly AAV-miR-181a/b-sponges are effective in mice and their administration ameliorates the phenotype of Ndufs4KO mice, a LHON genetic model.

We propose that miR-181a/b represent novel gene-independent therapeutic targets for a wide-range of NDs caused by mitochondrial dysfunction, and that miR-181a/b inactivation could be translated for clinical application.

## **Pharmacological inhibition of Sphingosine-1-phosphate degradation is therapeutically effective in a Huntington's disease animal model**

Di Pardo A.<sup>1</sup>, Amico E.<sup>1</sup>, Pepe G.<sup>1</sup>, Capocci L.<sup>1</sup>, Martinello K.<sup>1</sup>, Marracino F.<sup>1</sup>, Madonna M.<sup>1</sup>, Fucile S.<sup>1,2</sup>, Maglione V.<sup>1</sup>

<sup>1</sup>IRCCS Neuromed, Pozzilli (IS), Italy

<sup>2</sup>Sapienza University, Rome, Italy

Huntington's disease (HD), is the most common neurodegenerative disorder with no effective cure currently available. The disease is caused by an expansion of polyglutamine tract in Huntingtin (Htt) a widely expressed protein with a plethora of functions.

Over the past few years our research has shown that alterations in sphingolipid metabolism represent a critical determinant in the pathogenesis of the disease. In particular, we have provided the first evidence of aberrant metabolism of sphingosine-1-phosphate (S1P), with a consequent reduction of its bioavailability, in multiple disease settings including human post-mortem brains from HD patients. Importantly, we have also demonstrated that pharmacological interventions, aimed at reducing S1P degradation, by inhibiting S1P-Lyase (SGPL1), resulted beneficial in a HD cell model.

Here, we investigated whether the SPGL1 inhibitor 2-Acetyl-5-tetrahydroxybutyl imidazole (THI), may result therapeutically effective in-vivo, in the R6/2 mice, one of the most useful HD animal model.

Our results demonstrate that chronic administration of 0.1 mg/kg THI preserves both motor and cognitive functions in HD mice. Biochemical, electrophysiological and histological analyses revealed that treatment ameliorates neuronal and myelin homeostasis and reduces mutant Htt toxicity in the brain of HD mice.

In this study, we consolidate the evidence that sphingolipid metabolism is a "druggable" target in HD with a great therapeutic potential. Considering that several drugs, that modulate S1P pathways, are already on the market or in advanced phases of clinical trials for the treatment of human disorders, we believe that our findings may facilitate their repositioning in HD and/or promote the development of new compounds.



## **Role of the PI4,5P2 5-phosphatase OCRL at the synapse**

Morra V.<sup>1</sup>, Staiano L.<sup>1</sup>, Di Tullio G.<sup>1</sup>, Polishchuk E.<sup>1</sup>, Nussbaum R.<sup>2</sup>, Devuyst O.<sup>3</sup>, De Matteis M.A.<sup>1,4</sup>

<sup>1</sup>Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy

<sup>2</sup>Invitae Corp, San Francisco, California, USA

<sup>3</sup>Institute of Physiology and NCCR Kidney.CH, University of Zurich, Zurich, Switzerland

<sup>4</sup>Dept. Molecular Medicine and Medical Biotechnology, Federico II Univ., Naples, Italy

Invited lecture

**How to approach the unsolved (Telethon Undiagnosed program)**

Nigro V.

Università della Campania "Luigi Vanvitelli", Naples, Italy  
Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy

The next generation sequencing (NGS) has revolutionized our approach to genetic diseases, from a single genetic test to whole genome studies. In 2016, Telethon launched in Italy the first program for undiagnosed diseases (UDP), a pilot program aimed at standardized clinical and genetic analysis of pediatric patients with serious unresolved conditions. This program made it possible to develop strategies to offer to 350 families the most accurate and effective diagnostic path.

Patients with genetic syndromes receive a detailed annotation with Phenotips and cases are usually studied with high coverage WES of the entire trio / quartet. Cases that remain negative are shared online via platforms such as Phenome Central to find phenotypic similarities. Furthermore we have developed a new approach for still negative cases with the 10x Genomics platform. This produces a partition of high molecular weight DNA fragments (HMW-gDNA) in micelles, together with an adapter molecule and a barcode sequence. With this strategy, long sequences are linked and in phase and even small structural changes can be detected.

We show some selected cases that have been solved thanks to these technologies.

## **Resting-state functional MRI analysis of brain connectivity in Charcot-Marie-Tooth 1A**

Pontillo G.<sup>1</sup>, Coccozza S.<sup>1</sup>, Russo C.<sup>1</sup>, Perillo T.<sup>1</sup>, Dubbioso R.<sup>2</sup>, Tozza S.<sup>2</sup>, Manganelli F.<sup>2</sup>, Quarantelli M.<sup>3</sup>, Brunetti A.<sup>1</sup>

<sup>1</sup>Department of Advanced Biomedical Sciences, University "Federico II", Naples, Italy

<sup>2</sup>Department of Neurosciences and Reproductive and Odontostomatological Sciences, University "Federico II", Naples, Italy

<sup>3</sup>Institute of Biostructure and Bioimaging, National Research Council, Naples, Italy

### Introduction

Evidences collected in different neuropathies demonstrated that peripheral nerve pathology can broadly influence brain connectivity, far beyond the involvement of the sensorimotor network. Aim of this study was to investigate brain functional connectivity (FC) changes in Charcot-Marie-Tooth type1A (CMT1A).

### Methods

In this cross-sectional study, we enrolled 18 right-handed patients with genetically confirmed CMT1A, and 20 comparable healthy controls (HC). Resting-State (RS)-fMRI data were analyzed with a seed-based approach sampling 32 ROIs included in the SPM-based CONN toolbox and characterizing an extended set classical RS networks. Between-group differences for distinct seeds were tested using the standard General Linear Model implemented in SPM12, covarying for age, sex and mean motion and correcting for multiple comparisons at cluster level. Results of each analysis were considered significant for  $p < 0.05$ , Bonferroni-corrected for multiple comparisons (0.05/32).

### Results

CMT1A patients presented several clusters of increased FC of seeds within the Default Mode, Dorsal Attention, Language and Salience Networks, encompassing different supra-tentorial cortical areas ( $p < 0.001$ ). Furthermore, a cluster of reduced FC relative to the occipital cortex was found in the left lentiform nucleus.

### Conclusion

CMT1A patients showed extensive rearrangements in brain connectivity. In particular, FC increase could reflect a reduction of the physiological anticorrelation between the Default Mode (DMN) and other networks, as well as possible compensatory mechanisms. On the other hand, reduced FC between occipital cortex and lentiform nucleus could represent a maladaptive disruption of the visual cortico-striatal functional loop.

**Cortico-striatal pathway integrity in Fabry Disease: a diffusion MRI connectometry analysis**Russo C.<sup>1</sup>, Pontillo G.<sup>1</sup>, Coccoza S.<sup>1</sup>, Paoletta C.<sup>1</sup>, Battocchio M.<sup>2</sup>, Schiavi S.<sup>2</sup>, Pisani A.<sup>3</sup>, Daducci A.<sup>2</sup>, Brunetti A.<sup>1</sup><sup>1</sup>Department of Advanced Biomedical Sciences, University "Federico II", Naples, Italy<sup>2</sup>Department of Computer Science, University of Verona, Italy<sup>3</sup>Department of Public Health, Nephrology Unit, University "Federico II", Naples, Italy**Introduction**

Recent evidences suggested possible presence of extrapyramidal system involvement in Fabry Disease (FD), a lysosomal storage disorders mainly characterized by cerebrovascular events. Aim of this study was to investigate microstructural integrity of cortico-striatal connections in FD.

**Methods**

Forty-seven FD patients and 49 matched healthy controls underwent a 3T MRI scan, including Diffusion Tensor Images (DTI) sequence (voxel size 2.2×2.2×2.2mm<sup>3</sup>, 64 directions with b-value=1000s/mm<sup>2</sup> and nine b=0s/mm<sup>2</sup>). Fractional Anisotropy (FA), axial (AD), radial (RD) and mean diffusivity (MD) maps were computed for each subject using MRtrix. Anatomically-Constrained Tractography (ACT) with iFOD2 algorithm was performed to obtain 1 million streamlines. Connectomes were built using the standard FreeSurfer Desikan-Killiany atlas; DTI metrics and connectomes were combined to carry on diffusion MRI connectometry. Finally, values corresponding to bundles connecting the precentral gyrus (PreCG) with the striatum were extracted.

**Results**

We found microstructural impairment of cortico-striatal tracts predominantly affecting the left side. A significant reduction in mean FA values of left cortico-striatal fibers ( $0.43 \pm 0.02$  vs  $0.41 \pm 0.02$ ,  $p=0.001$ ), coupled to increased MD ( $0.67 \cdot 10^{-3} \pm 0.02 \cdot 10^{-3} \text{mm}^2/\text{s}$  vs  $0.68 \cdot 10^{-3} \pm 0.03 \cdot 10^{-3} \text{mm}^2/\text{s}$ ,  $p=0.001$ ) and RD ( $0.50 \cdot 10^{-3} \pm 0.02 \cdot 10^{-3} \text{mm}^2/\text{s}$  vs  $0.52 \cdot 10^{-3} \pm 0.03 \cdot 10^{-3} \text{mm}^2/\text{s}$ ,  $p<0.001$ ) values, was observed; no difference emerged in AD maps.

**Conclusion**

We observed the presence of alterations in extrapyramidal system of FD patients, in line with recent evidences suggesting the presence of brain changes as a possible consequence of subtle motor symptoms observed in this condition. Our results confirmed that in FD patients, along with functional changes, a microstructural damage of this pathway is also present.

**Modulation of CSP $\alpha$ /DNJ-14 ameliorates SMA related neurodegeneration in *C. elegans***

Santonicola P.<sup>1</sup>, Giuliano T.<sup>2</sup>, Zampi G.<sup>1</sup>, Fraldi A.<sup>2</sup>, Di Schiavi E.<sup>1</sup>

<sup>1</sup>Institute of Biosciences and BioResources, CNR, Naples,

<sup>2</sup>Telethon Institute of Genetics and Medicine, Pozzuoli

Invited lecture

**The role of the child neurologist**

Striano P.

'G. Gaslini' Institute, University of Genova, Genova, Italy

The term 'precision medicine' describes a rational treatment strategy that reverses or modifies the pathophysiological effects leading to the disease. In epilepsy, single case and small cohort reported document early precision medicine strategies in particular genetic epilepsies. The large number of different syndrome and seizure types together with an interindividual variable response to AEDs makes the treatment of epilepsy challenging. Luckily, in the last few years there has been a large increase in the knowledge of epilepsy genetics, genome-wide analyses and next generation sequencing methods have given the possibility to write a new chapter in the book of treatment of epilepsy, the chapter of precision medicine. Epilepsy offers a good opportunity to personalization of therapy if we consider that at least one third of epileptic patients do not achieve complete seizure control with currently available pharmacological treatments. In addition drug discovery is not targeted and instead relies on development using in vivo seizure models. Epilepsy therapy is still essentially limited to treating the symptoms, the seizures rather than the preceding epileptogenic events. In this review we summarize the established evidence regarding pharmacogenomics in epilepsy and discuss the basis of precision medicine is still largely empirical.

Invited lecture

**Personalized therapies for developmental epileptic encephalopathies**

Taglialatela M.

Section of Pharmacology, Dept. of Neuroscience, University of Naples Federico II, Naples, Italy

Developmental epileptic encephalopathies are epileptic conditions characterized by progressive cognitive impairment beyond the expected for the epilepsy activity. Their main features include severe pharmaco-resistant epilepsy, severely abnormal electroencephalograms, early-age onset, neurocognitive impairment, variable phenotype and usually normal brain MRI. Next-generation sequencing technologies have revolutionized DEEs diagnosis, allowing early identification of the molecular defects in an ever-growing number of genes. Early genetic diagnosis, by providing information about transmission modalities and pathogenetic mechanisms, is critical for prognostic assessment, genetic counselling, and, in some cases, personalised treatment attempts. Among genetic targets for DEEs, potassium channels have been identified as critical players, particularly in severe early-onset epilepsies, allowing to translate decades of knowledge of their pathophysiological and pharmacological roles in innovative and personalized treatment attempts. However, efficacy and safety of some of these etiology-based approaches has often proven to be suboptimal, reinforcing the need for a more in-depth understanding of the pathophysiological mechanisms by which specific gene variants lead to distinct phenotypes amenable to therapeutic intervention. In the present lecture, I will review available literature data and our current efforts in personalized approaches in DEEs caused by mutations in potassium channel genes, focusing in particular on KCNQ2- and KCNT1-related epilepsies. Emphasis will be on the ongoing pharmacological attempts, highlighting specific problems associated to the treatment of these early-onset and severe DEEs, with regard to both efficacy and toxicity of available compounds. I will also summarize the many advancements from both academic and industrial groups toward the identification of compounds with improved pharmacokinetic and pharmacodynamics properties over existing molecules. The goal of the presentation will be to update the audience on the status of the field, highlighting how finding a solution to the challenges currently being faced in DEE field might also benefit other forms of early-onset and, possibly, more common later-onset epilepsies.

## Thick corpus callosum: neuroradiological, clinical and genetic characterization of an Italian series

Vitiello G.<sup>1,2</sup>, D'Amico A.<sup>3</sup>, Romaniello R.<sup>4</sup>, Severino M.<sup>5</sup>, Arrigoni F.<sup>6</sup>, Genesio R.<sup>2</sup>, Imperati F.<sup>1</sup>, Zuffardi O.<sup>7</sup>, Iolascon A.<sup>2,8</sup>, The Italian CCA Study Group, Nitsch L.<sup>2</sup>, Borgatti R.<sup>4</sup>, Del Giudice E.<sup>1</sup>

<sup>1</sup>Department of Translational Medicine, Federico II University, Naples, Italy

<sup>2</sup>Department of Molecular Medicine and Medical Biotechnology, Federico II University, Naples, Italy

<sup>3</sup>Department of Advanced Biomedical Sciences, Federico II University, Naples, Italy

<sup>4</sup>Neuropsychiatry and Neurorehabilitation Unit, Scientific Institute, IRCCS Eugenio Medea, Bosisio Parini, Lecco, Italy

<sup>5</sup>Neuroradiology Unit, Istituto Giannina Gaslini, Genoa, Italy

<sup>6</sup>Neuroimaging Laboratory, E. Medea Research Institute, Bosisio Parini, Italy

<sup>7</sup>Unit of Medical Genetics, Department of Molecular Medicine, University of Pavia, Pavia, Italy

<sup>8</sup>CEINGE-Biotecnologie Avanzate, Naples, Italy

Corpus callosum abnormalities (CCA) has an estimated prevalence ranging from 0.3% up to 0.7% in patients undergoing brain imaging. Thick Corpus callosum (TCC) entails a group of rare anomalies: moreover, etiologies and prognostic data are very scanty. Recent ultrasound and fetal MRI diagnostic techniques have improved the antenatal detection of TCC giving rise to a prognostic dilemma in genetic counseling.

We performed a retrospective study of 79 patients from The Italian CCA Database, classifying non-syndromic (NS n=25) and syndromic (S, n=54) CCA, reviewing clinical features, neuroradiological aspects and genetic etiologies. All patient performed Diffusion Tensor Imaging (DTI) to study either aberrant supracallosal bundle or callosal thickening. NS-TCC subjects were 31% and showed mild-moderate intellectual disability in 80% of cases (20/25). Autism was reported in 32% and epilepsy in 28%. Genetic and molecular diagnosis was obtained in only 2/25 (8%) of NS hyperplasia, including microdeletion syndrome (1p36 deletion) and monogenic condition (DCX mutation). S-TCC patients showed high rate of intellectual disability (93%) and behavior problems (30%). Molecular diagnosis was possible in 33% of patients and we were able to describe for the first time TCC in the following: Williams-Beuren syndrome, Phelan-McDermid syndrome, Turner syndrome, PIK3R2 and MAST1 mutations.

A high percentage of patients (75%) remained without a diagnosis. Combined high resolution CMA studies and next-generation sequencing (NGS) strategies will increase the probability to identify new causative genes of TCC. DTI study will improve knowledge about TCC and human connectome.



## **Phytocannabinoid treatment in a mouse model of West syndrome with spontaneous seizures**

Verrillo L.<sup>1,2</sup>, Drongitis D.<sup>1</sup>, Bingham S.<sup>3</sup>, Barra A.<sup>1</sup>, Terrone G.<sup>4</sup>, Del Giudice E.<sup>4</sup>, Iannotti F.<sup>5</sup>, Poeta L.<sup>1</sup>, Di Marzo V.<sup>5</sup>, Miano M.G.<sup>1</sup>

<sup>1</sup>Institute of Genetics and Biophysics “Adriano Buzzati-Traverso”, CNR, Naples, Italy

<sup>2</sup>University “Luigi Vanvitelli”, Caserta, Italy

<sup>3</sup>GW Research Ltd, Salisbury, UK

<sup>4</sup>Department of Translational Medical Sciences, Section of Pediatrics, University of Naples Federico II, Naples, Italy

<sup>5</sup>Institute of Biomolecular Chemistry, CNR, Pozzuoli, Italy.

Keynote lecture

**Understanding progression of neurodegenerative diseases: the role of lysosomes and tunneling nanotubes**

Zurzolo C.

Institut Pasteur, Paris France

Neurodegenerative diseases like Prion disease, Alzheimer's (AD), Parkinson's (PD) and Huntington's (HD) disease are part of a larger group of protein misfolding disorders characterized by the progressive accumulation and spreading of different protein aggregates. Like Prions, misfolded forms of  $\alpha$ -syn, tau, A $\beta$  and Htt proteins associated with AD, PD and HD can be transmitted experimentally in cellular and in animal models where act as 'seeds' to recruit endogenous protein into aggregates. However, the mechanism of intercellular transfer is still debated. We have shown that different misfolded protein aggregates involved in neurodegenerative diseases propagate between cells in a prion-like manner using physical connections called tunneling nanotubes. Thus, we propose that TNTs contribute to the progression of the pathology of neurodegenerative diseases by spreading in the brain misfolded protein assemblies. Tunneling nanotubes (TNTs) are F-actin containing channels between remote cells, which have recently been recognized as a novel mechanism for long-range intercellular communication in vitro and in vivo. Unlike other cellular protrusions (e.g. filopodia and cytonemes), TNTs directly connect cells, creating temporary cytoplasmic continuity between distant cells of different types. In particular, we have shown that propagation of  $\alpha$ -synuclein fibrils between neuronal cells through TNTs occurs inside lysosomes and that astrocytes have a role in degrading  $\alpha$ -syn fibrils rather than in transfer. In my presentation I will show unpublished data on the lysosomal trafficking in TNTs and their possible involvement in the intercellular propagation of misfolded  $\alpha$ -synuclein. I will also present structural data revealing that TNTs are novel organelles very different from filopodia. Finally, I will discuss preliminary evidence showing the presence of TNT structures in brain.

**Poster Presentations**  
(in alphabetical order of the presenting author)

**gH-625liposome as delivery of PACAP in central nervous system and as neuroprotective agent of neuronal disorders**

Barra T.<sup>1</sup>, Falanga A.<sup>2,3</sup>, Del Genio V.<sup>4</sup>, Galdiero S.<sup>2,4</sup>, Valiante S.<sup>1,5</sup>

<sup>1</sup>Department of Biology, University of Naples "Federico II", Naples, Italy

<sup>2</sup>Department of Agricultural Sciences, University of Naples "Federico II", Portici, Italy

<sup>3</sup>CiRPEB- University of Naples "Federico II", Via Mezzocannone 16, 80134, Napoli, Italy

<sup>4</sup>Department of Pharmacy - University of Naples "Federico II", Naples, Italy

<sup>5</sup>National Institute of Biostructures and Biosystems (INBB), Rome, Italy

Pituitary adenylate cyclase-activating polypeptide (PACAP) exerts many effects in central nervous system (CNS) as neuroprotection. However, as therapeutic agent, it has a rapid degradation, so it has been needed to develop nano-system to deliver functional PACAP in CNS; our system involves a peptide, gH-625 which is a domain perturbing membrane derived from glycoprotein H of the Herpes simplex virus 1: we functionalized liposomes with gH625 and then evaluated the ability of gH625-liposome loaded with rhodaminated PACAP to reach and cross an in vitro dynamic flow BBB model. This model is realized in a bioreactor, with a double flow chamber separated by a porous membrane; bEnd3 cells, were seeded on the porous membrane in the upper chamber. An anti-ZO-1 immunofluorescence confirmed that tight-junction of BBB were formed. For 2 hours we followed the delivery of gH625-liposomes loaded with PACAP: we showed an increase of gH625 liposome-PACAP in the lower chamber compared to non-functionalized liposomes-PACAP. Furthermore, we evaluated PACAP neuroprotection, treating dopaminergic neurons, seeded in the lower chamber with MPP+, a neurodegenerative agent. After 24h of exposure to MPP+, PACAP exerts neuroprotection at 10<sup>-8</sup>M. Finally, we demonstrated, by ELISA, an increase of PACAP concentration both in upper and lower chambers during time. Dopaminergic neurons were exposed to the full concentration (i.e. about 10<sup>-8</sup>M) of PACAP within 60 minutes. Our nanodelivery system results effective to expose dopaminergic neurons to suitable concentration of neuroprotective PACAP. Hence, this nanodelivery tool can be a suitable approach to protect neurons from injuries in neurodegenerative disease.

## **Alteration of excitation-inhibition balance and synaptic plasticity in the primary motor cortex of Rett syndrome**

Bernardo P., Bravaccio C., Coppola A., Manganelli F., Dubbioso R.

Dipartimento di Scienze Mediche Traslazionali, University of Naples "Federico II", Naples, Italy

Rett syndrome (RTT) is an X-linked dominant neurodevelopmental disorder due to pathogenic mutations in the MECP2 gene. Motor impairment constitutes the core diagnostic feature of RTT. Preclinical studies have consistently demonstrated alteration of excitation/inhibition (E/I) balance and aberrant synaptic plasticity at cortical level. Herein we aimed at understanding neurobiological mechanisms underlying motor deficit by assessing in "vivo" synaptic plasticity and E/I balance in the primary motor cortex (M1).

On 14 patients with typical RTT, 9 epilepsy controls patients and 11 healthy controls we applied paired-pulse TMS protocols to evaluate the excitation index, a biomarker reflecting the contribution of inhibitory and facilitatory circuit activity in M1. In addition, TMS-theta burst stimulation was used to probe LTP-like plasticity in M1. Motor impairment, assessed by means of ad hoc clinical scales, was correlated with neurophysiological measures.

RTT patient with severe motor phenotype displayed a significant increase of the excitation index ( $p < 0.001$ ), as demonstrated by the reduction of short-interval intracortical inhibition (SICI) and increase of intra-cortical facilitation (ICF), suggesting a shift toward cortical excitation likely due to GABAergic dysfunction. LTP-like plasticity in M1 was also abolished in the same subgroup ( $p = 0.01$ ) and correlated with greater motor disability (all  $p < 0.003$ ).

TMS is a method that can be used to assess cortical motor function in RTT patients with severe disability. Our findings support the introduction of TMS measures in clinical and research settings to probe the neurobiological mechanism underlying motor impairment and to longitudinally monitor the progression of the disorder and response to treatment.

## **Zebrafish as model for neurobehavioral toxicology**

Capriello T., Donizetti A., Cofone R., Aliperti V., D'Aniello S., Ferrandino I.

Department of Biology, University of Naples "Federico II", Naples, Italy

Zebrafish is increasingly accepted in the scientific community like a suitable model organism for the screening of ecotoxicological effects induced by chemicals and environmental pollutant on vertebrates. The present study is conducted both on embryos, being transparent and allowing to follow embryogenesis, and adults, classified as the second most used animal model in the field of neurodegenerative disorders research like Parkinson's and Alzheimer's diseases. In addition, zebrafish is recently become crucial for neurobehavioral studies because its neuropathological behaviours comparable to that shown in human. The objective of this study was to find out more about effects due to aluminium (Al) by studying zebrafish behaviour. Al, indeed, is recognized as very harmful for the nervous system influencing neurodegenerative and neuromuscular diseases. In this light, embryos were exposed to 50, 100 and 200  $\mu\text{M}$  AlCl<sub>3</sub> for 72 h, and their swimming abilities were analysed by DanioVision behavioural equipment. mRNA expression level during embryogenesis of different marker genes of neural development and function as c-fos, appa and appb was also analysed by qPCR. Adults, instead, were treated with 400  $\mu\text{M}$  AlCl<sub>3</sub> for 20 days and the effects of Al on behaviour with L/D choice assay, mirror and social test were assayed at 10, 15 and 20 days. Our data showed a clear toxic effect of AlCl<sub>3</sub> on the behavioural activities and on gene expression taken into consideration. This prove once more that zebrafish as model organism may speed up neurobehavioral studies and highlight unknown correlations existing between common and rare environmental pollutants and human neurological disorders.

## **Glutaric Aciduria Type I: an OMIC study of cerebral ammonium accumulation mechanism**

Caterino M.<sup>1,2</sup>, Costanzo M.<sup>1,2</sup>, Fedele R.<sup>2</sup>, Capasso M.<sup>1,2</sup>, Lasorsa V.A.<sup>1,2</sup>, Ballhausen D.<sup>3</sup>, Gonzalez-Melo M.<sup>3</sup>, Ruoppolo M.<sup>1,2</sup>

<sup>1</sup>Department of Molecular Medicine and Medical Biotechnology, Federico II University, Naples, Italy

<sup>2</sup>CEINGE - Biotecnologie Avanzate, Naples, Italy

<sup>3</sup>Center of Molecular Diseases, Lausanne University Hospital, Lausanne, Switzerland

Glutaric acidemia type I (GA-I) is a rare metabolic disease in which glutaryl-CoA dehydrogenase (gcdh) enzyme is deficient. This autosomal recessive disorder has an incidence of 1:100,000 and is characterized by accumulation of glutarate, 3-hydroxy-glutarate and glutarylcarnitine in plasma, urine and tissues. Affected patients show severe dystonia, bilateral striatal degeneration, subdural hemorrhages and spongiform vacuolization of white matter, with neuronal loss and astrogliosis in the striatum. Low-protein diets, anti-catabolic treatments and liver or kidney transplants are the actual therapeutic strategies adopted. Despite the efforts made for the treatment and the follow up, no really effective therapy exists for GA-I patients. For this study, a GA-I mouse model was developed using CRISPR-CAS9 genome editing technology by introducing the R411W mutation in the gcdh gene of Sprague-Dawley mice (gcdhki/ki).

A combined mass spectrometry-based proteomic and metabolomic approach was used to study the alterations in the brain proteome and metabolome of 3, 6 and 9 week-old mice. Within the metabolomic dataset, the accumulation of the markers of disease was found, as expected. Proteomics data revealed alteration of proteins involved in synaptic signal transmission and synapse organization. Furthermore, glutaminase, involved in the production of cerebral NH<sub>4</sub><sup>+</sup>, typical of GA-I neuropathogenesis, was found to be significantly increased in the mouse model, compared to wild type mice. These results will aim at studying in deep the mechanism of cerebral ammonium accumulation in GA-I with the future goal of its prevention as therapeutic strategy.

## **lin-45/B-RAF neuroprotective role in a *C.elegans* model of Spinal Muscular Atrophy**

Cieri E.<sup>1</sup>, Santonicola P.<sup>1</sup>, Zampi G.<sup>1</sup>, Hensel N.<sup>2,3</sup>, Claus P.<sup>2,3</sup>, and Di Schiavi E.<sup>1</sup>

<sup>1</sup>Institute of Biosciences and BioResources, IBBR, CNR, Naples, Italy

<sup>2</sup>Institute of Neuroanatomy and Cell Biology, Hannover Medical School, Hannover, Germany

<sup>3</sup>Center for System Neurosciences (ZSN), Hannover, Germany



## **Brain structural plasticity in CMT1A explored via a combined VBM and TBSS approach**

Cocozza S.<sup>1</sup>, Pontillo G.<sup>1</sup>, Russo C.<sup>1</sup>, Vola E.A.<sup>1</sup>, Dubbioso R.<sup>2</sup>, Tozza S.<sup>2</sup>, Manganelli F.<sup>2</sup>, Quarantelli M.<sup>3</sup>, Brunetti A.<sup>1</sup>

<sup>1</sup>Department of Advanced Biomedical Sciences, University "Federico II", Naples, Italy

<sup>2</sup>Department of Neurosciences and Reproductive and Odontostomatological Sciences, University "Federico II", Naples, Italy

<sup>3</sup>Institute of Biostructure and Bioimaging, National Research Council, Naples, Italy

### Introduction

Although being eminently a peripheral nervous system disease, several reports of central nervous system (CNS) involvement have been described in different forms of Charcot-Marie-Tooth disease, including its most common subtype 1A (CMT1A). Aim of this study was to investigate the presence of structural brain damage of CMT1A patients.

### Methods

20 patients with genetically confirmed CMT1A were enrolled along with 20 age- and sex-comparable healthy controls (HC). Brain MRI exams included a 3D-GE-T1w for the Voxel-Based Morphometry Analysis (VBM), and Diffusion Tensor Imaging (DTI) data for the Tract-Based Spatial Statistics (TBSS) analysis. CMT1A patients also underwent clinical and electrophysiological examinations including the determination of the CMT Neuropathy Score (CMTNS) as a global measure of disease severity, and the Compound Motor Action Potential (CMAP) as an index of distal arm axonal damage.

### Results

The VBM analysis revealed a cluster of significantly increased GM density in CMT1A patients compared to HC encompassing the right paravermian portions of the cerebellar lobules III, IV and V. No between-group difference emerged at TBSS analysis when considering DTI metrics. A significant negative correlation ( $r=-0.738$ ,  $p=0.003$ ) was found between the CMAP values and the age, sex and TIV-adjusted z-scores of the first eigenvariate extracted from the cluster of significant between-group difference at VBM analysis.

### Conclusion

CMT1A patients showed cerebellar gray matter modifications occurring independently from central white matter microstructural alterations, possibly representing a structural plasticity mechanism which compensates the primary peripheral nerve damage.

**The recombinant production of full-length human CDKL5\_1 in an Antarctic marine bacterium**Colarusso A., Calvanese M., Lauro C., Parrilli E., Tutino M.L.

Dept. of Chemical Sciences, University of Naples "Federico II" Naples, Italy

CDKL5 deficiency disease (CDD) is a rare and severe neurodevelopmental disorder caused by mutations in the X-linked cdkl5 gene, and no cure for this devastating condition is available. CDKL5 is an unusually big (about 1000 aa) cyclin-dependent like kinase abundantly expressed in the brain, which exerts its function in neurons both in the nucleus and in the cytoplasm and synaptosome. Besides the kinase catalytic domain, which accounts for the first 300 aa residues, the remaining 2/3 of the protein sequence is predicted as "disordered", making CDKL5 a large IDPR, i.e. a protein containing an Intrinsically Disordered Protein Region [1]. This feature allows CDKL5 to exert several different functions in the neuron, by interacting transiently with proteins/complexes and modulating the signal transmission by its protein kinase function. Furthermore, the IDPR nature of CDKL5 makes it impossible to produce the full-length enzyme in conventional recombinant systems. Therefore, an emerging unconventional cell factory was challenged, i.e. the Antarctic marine bacterium *Pseudoalteromonas haloplanktis* TAC125 [2-3]. This communication describes the main issues encountered and the strategies which allowed us to achieve the successful production of CDKL5 by the psychrophilic cell factory. This novel protein production platform can be used for other IDPR production, opening the way to their use either in basic science or in possible protein replacement therapy approaches.

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## **Functional brain connectome in drug-naïve Parkinson's disease patients**

De Micco R.<sup>1</sup>, Agosta F.<sup>2</sup>, Basaia S.<sup>2</sup>, Siciliano M.<sup>1</sup>, Cividini C.<sup>2</sup>, Tedeschi G.<sup>1</sup>, Tessitore A.<sup>1</sup>, Filippi M.<sup>2</sup>

<sup>1</sup>Department of Advanced Medical and Surgical Sciences, University of Campania "L. Vanvitelli", Naples, Italy;

<sup>2</sup>Neuroimaging Research Unit, Institute of Experimental Neurology, Division of Neuroscience, San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milan, Italy

**Objective.** To investigate whole-brain network topologic organization in a large cohort of drug-naïve Parkinson's disease (PD) patients using resting-state functional MRI (rs-fMRI); and to determine whether early functional connectivity measures may predict disease progression overtime.

**Materials and methods.** 147 drug-naïve PD patients were consecutively enrolled. Motor, non-motor and neuropsychological assessments as well as rs-fMRI were performed at baseline. 38 age- and sex-matched controls were also enrolled in the study. Non-hierarchical cluster analysis using motor, non-motor and neuropsychological data were applied to stratify PD patients in two subtypes: 77 patients were grouped as "early/mild" and 70 as "early/severe". After the baseline assessments, all patients started a dopaminergic replacement therapy accordingly to current international guidelines and were followed for an observation period, lasting a maximum of 24 months, with a clinical follow-up every 12-months. Graph analysis and connectomics were used to assess global and local topological network properties and regional functional connectivity (FC) at baseline in both PD patients and controls. **Results.** Decreased FC involving mainly striato-frontal, striato-temporal and limbic connections differentiated "early-mild" from "early-severe" PD patients. FC abnormalities at baseline were found to be an independent predictor of levodopa requirement over 2-years.

**Conclusions.** Our findings revealed that a specific subtype of PD patients, characterized by severe motor and non-motor burden as well as widespread FC abnormalities, may be identified at the time of diagnosis. This FC pattern may reflect the presence of more diffuse neuropathological changes. Combined clinical and neuroimaging tools are promising to stratify risk of PD progression overtime.

## **Anticipated expression of D-aspartate oxidase since embryonic stage drastically reduces D-aspartate levels in the mouse brain and influences spatial memory at adulthood**

De Rosa A.<sup>1,2</sup>, Usiello A.<sup>1,3</sup>

<sup>1</sup>CEINGE Biotechnologie Avanzate, Naples, Italy

<sup>2</sup>Department of Experimental Medicine, Sapienza University of Rome, Italy

<sup>3</sup>Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, Second University of Campania, L. Vanvitelli, Caserta, Italy

Together with D-serine (D-Ser), D-aspartate (D-Asp) is the only free D-amino acid present in substantial amount in the mammalian brain. Several studies revealed that D-Asp has a transient emergence in the brain, being abundant in the embryonic phase and in the first post-natal days before significantly decreasing thereafter. It has long been established that D-aspartate oxidase (DDO) is the enzyme responsible for D-Asp catabolism. Accordingly, the post-natal decrease of D-Asp content is associated with a concomitant, progressive increase in Ddo expression and DDO activity in the rodent's brain. D-Asp exists at extracellular level and acts as an agonist at NMDA and mGlu5 receptors (NMDAR and mGluR5, respectively). To clarify the still enigmatic biological meaning of D-Asp during brain development, we have generated a knockin mouse model in which the expression of DDO is anticipated since the zygotic stage. As a proof of the successful achievement of our gene targeting strategy, we found an increased transcription of cerebral Ddo in knockin mice during ontogenesis. As a result of this, we observed a selective depletion of cerebral D-Asp content in the embryonic and postnatal brain. Then, we performed a morphological and behavioural characterization of Ddo knockin mice. Histological analysis revealed no gross differences in brain size or structural organization. On the other hand, we observed a significant increase of parvalbumin-positive interneurons in the medial prefrontal cortex of mutant mice, which was accompanied by increased spatial memory in the Morris water maze task.

### **D-Aspartate treatment attenuates myelin damage and stimulates myelin repair**

De Rosa V.<sup>1</sup>, Secondo A.<sup>1</sup>, Pannaccione A.<sup>1</sup>, Ciccone R.<sup>1</sup>, Formisano L.<sup>1</sup>, Guida N.<sup>1</sup>, Crispino R.<sup>2</sup>, Fico A.<sup>3</sup>, Polishchuk R.<sup>2</sup>, D'Aniello A.<sup>1</sup>, Annunziato L.<sup>1</sup>, Boscia F.<sup>1</sup>

<sup>1</sup>Department of Neuroscience, Reproductive and Dentistry Sciences, Division of Pharmacology, School of Medicine, University "Federico II" of Naples, Italy

<sup>2</sup>Telethon Institute of Genetics and Medicine (TIGEM), Naples, Italy

<sup>3</sup>Institute of Genetics and Biophysics "A. Buzzati-Traverso", Consiglio Nazionale delle Ricerche, Naples, Italy

Glutamate signaling may orchestrate oligodendrocyte precursor cell (OPC) development and myelin regeneration through the activation of glutamate receptors at OPC-neuron synapses. Recently, D-aminoacids are emerging as molecules with important roles in brain cells. Among them, D-Aspartate is a D-aminoacid exerting modulatory actions at glutamatergic synapses. Chronic administration of D-Aspartate has been proposed as therapeutic treatment in diseases related to myelin dysfunction and NMDA receptors hypofunction, including schizophrenia and cognitive deficits. Here, we investigated the effects of D-Asp both in vitro, during OPC differentiation and myelination, and in vivo, in mice fed with the copper chelator cuprizone, a model of myelin damage and repair. We found that 100µM D-Aspartate exposure accelerated developmental myelination in cerebellar organotypic slices and stimulated progenitor differentiation into myelin-producing oligodendrocytes. Behavioural testing, confocal and electron microscopy analyses demonstrated that oral administration of 20mM D-Aspartate solution during in vivo remyelination improved motor coordination, accelerated myelin recovery, and significantly increased the number of small-diameter myelinated axons. Chronically administered during demyelination, D-Aspartate also attenuated myelin loss and inflammation. Functional studies demonstrated that D-Aspartate boosting effects on OPC differentiation involved an orchestrated stimulation of calcium signaling pathways that are consequent to a cooperative activation of glutamate transporters, AMPA and NMDA receptors and NCX3 exchanger. In fact, while blocking NMDA or NCX3 significantly prevented D-Aspartate-induced  $[Ca^{2+}]_i$  oscillations, blocking AMPA receptors and glutamate transporters prevented both the initial and oscillatory  $[Ca^{2+}]_i$  response as well as D-Aspartate-induced inward currents in OPC. Our findings suggest that exogenous D-Aspartate treatment might produce beneficial effects during demyelination and remyelination processes.

## **Mitochondrial dysfunctions trigger the onset of neuroinflammation in animal models of Parkinson's disease**

Di Martino R.<sup>1</sup>, Sirabella R.<sup>1</sup>, Sgobio C.<sup>2</sup>, Annunziato L.<sup>1</sup>, Scorziello A.<sup>1</sup>

<sup>1</sup>Division of Pharmacology, Department of Neuroscience, Federico II University of Naples, Italy

<sup>2</sup>DZNE (Center for Neurodegenerative Disease) Munich, Germany

Recent findings demonstrated that mitochondria might have a role in neuroinflammation occurring in Parkinson's Disease. The present study was addressed to explore the role of mitochondrial function during ageing, in the tissue of Striatum and Midbrain, and in primary culture of neurons and astrocytes, from mice expressing the human A53T variant of  $\alpha$ -synuclein (A53T mice). Mitochondrial function monitored with confocal microscopy shown an overload of  $\text{Ca}^{2+}$  ions and mitochondrial membrane depolarization in mesencephalic neuron. By Western Blotting we showed a reduction in NCX3 in neurons and an increase of NCX1 in astrocytes from A53T compared with WT. Moreover, we demonstrated that in the Midbrain, already at 4 months, there is a rise up of Cyt-C but decrease of MnSOD, correlated to an increase of nNOS, while at 16 months in Striatum we observed a rise up of GFAP and IBA-1, supporting an inflammatory status. In conclusion, these results suggest that the mitochondrial dysfunction in mesencephalic neurons could occur before the onset of inflammation in A53T animal model of PD, while in the Striatum the neuronal damage is translated into neuroinflammation. To confirm the role of mitochondria in the neuroinflammation also in glial cells, we performed experiments of proteomic in isolated microglia from PDGF- $\alpha$ -synuclein WT animal model. Preliminary results at 2 months shown a changement many proteins correlated to mitochondrial dynamics and metabolism. These preliminary results let suggest that mitochondrial damage occurs already in the asymptomatic stage of PD and that might be responsible for the onset of neuroinflammation.

## **Quantitative proteomic analysis of mouse brain in models of neurodevelopmental disorders caused by mutations in *Aristaless*-related homeobox gene**

Drongitis D.<sup>1</sup>, Costanzo M.<sup>2,3</sup>, Caterino M.<sup>2,3</sup>, Verrillo L.<sup>1</sup>, D'Agostino G.<sup>4</sup>, Poeta L.<sup>1</sup>, Mallardo M.<sup>1</sup>, Di Schiavi E.<sup>5</sup>, Sabatino L.<sup>4</sup>, Ruoppolo M.<sup>2,3</sup>, Miano M.G.<sup>1</sup>.

<sup>1</sup>Institute of Genetics and Biophysics "Adriano Buzzati-Traverso", CNR, Naples, Italy

<sup>2</sup>Department of Molecular Medicine and Medical Biotechnology, University of Naples "Federico II", Naples, Italy

<sup>3</sup>CEINGE, Biotechnologie Avanzate scarl, Naples, Italy

<sup>4</sup>University of Sannio, Benevento, Italy

<sup>5</sup>Institute of Biosciences and Bioresources, CNR, Naples, Italy

*Aristaless*-related homeobox is an X-linked gene encoding a bi-functional transcription factor with a key role in neuronal migration and brain morphogenesis. Mutations in *ARX* have been identified in a wide spectrum of neurodevelopmental disorders (NDDs) that includes X-linked Lissencephaly with Abnormal Genitalia (XLAG), Epileptic Encephalopathy (EE), Intellectual disability (ID) and autism. Given the pleiotropic activity of *ARX*, the molecular consequences of its mutations remain unclear, as well as the pathogenic determinants of each endo-phenotype. Here we report on the proteomic analysis performed in two distinct *Arx*-disease mouse models: *Arx*KO/Y, which recapitulate the cortical malformation associated to loss-of-function (LoF) mutations found in XLAG patients and *Arx*(GCG)7/Y, which represents the best surrogate to study the refractory epilepsy associated to polyalanine expansions found in EE patients.

Our goal is to generate for the first time the "proteomic profiles" caused by two different *Arx* disease alleles and to figure out which are the common and distinct disease-determinants at birth. Specifically, we have applied a label-free comparative proteomic strategy to whole neonatal brains isolated from *Arx*KO/Y, *Arx*(GCG)7/Y and WT male mice. A high-resolution liquid chromatography coupled to tandem mass spectrometry was carried out in each experimental group. To investigate the potential cellular processes affected by each *Arx* mutant allele, the identified proteomic datasets were clusterized according to PANTHER and STRING bioinformatic tools. Preliminary data on the alterations in the levels of proteins involved in neuronal homeostasis and cytoskeleton structures will be discussed. Further studies in *C. elegans* *Arx/alr-1*(KO) mutants are planned to validate conserved protein signatures.

## **Deficiency of lysosomal acid alpha-glucosidase in a patient with Dystroglycanopathy due to GMPPB deficiency: a link between congenital glycosylation defects and lysosomal diseases**

Gragnaniello V.<sup>1</sup>, Fecarotta S.<sup>1</sup>, Tarallo A.<sup>1,2</sup>, Damiano C.<sup>1,2</sup>, Strollo S.<sup>2</sup>, Minopoli N.<sup>1,2</sup>, Baldi R.<sup>1</sup>, Tuzzi R.<sup>1</sup>, Romano A.<sup>1</sup>, Strisciuglio P.<sup>1</sup>, Schoser B.<sup>3</sup>, Parenti G.<sup>1,2</sup>

<sup>1</sup>Department of Translational Medical Sciences, Federico II University, Naples, Italy

<sup>2</sup>Telethon Inst of Genetics and Medicine, Pozzuoli, Italy

<sup>3</sup>Department of Neurology, Ludwig-Maximilians-University, Munich, Germany

### Background

GDP mannose pyrophosphorylase B (GMPPB) deficiency is one of several dystroglycanopathies, a heterogeneous group of neuromuscular disorders characterized by aberrant glycosylation of alpha-dystroglycan (ADG). GMPPB catalyzes the formation of GDP-mannose, required for mannosylation and glycosylation of dystroglycan, and probably of other proteins. The aim of this study was to evaluate the effects of GMPPB deficiency on lysosomal function.

### Methods and results

We studied six GMPPB cell lines (1 fibroblast, 5 myoblasts) by imaging techniques, enzymatic activity assays and western blot to analyze protein maturation.

Electron microscopy showed intracellular storage (glycogen, confirmed with PAS staining; lipids; multilamellar / multi-vesicular bodies). The activity of several lysosomal enzymes, particularly acid alpha-glucosidase (GAA) was reduced; GAA processing was impaired, with a relative deficiency of the active isoforms. GAA lysosomal targeting was also affected, with reduced co-localization with the lysosomal marker LAMP2 on immunofluorescence analysis. These abnormalities were corrected in vitro by recombinant GAA (rhGAA), which showed normal maturation and lysosomal targeting.

### Discussion

Our data show a deficiency of several lysosomal enzymes, in particular GAA, in a group of patients with dystroglycanopathy. These deficiencies may play a role in the pathogenesis of the disease. Our results also suggest a surprising and previously unreported link between congenital glycosylation defects and lysosomal diseases, and may contribute to a better understanding of the pathogenesis and to new therapeutic opportunities.



## **Pharmacological enhancement of human acid alpha-glucosidase by allosteric chaperones**

Iacono R.<sup>1,2</sup>, Cobucci-Ponzano B.<sup>2</sup>, Ferrara M.C.<sup>2</sup>, Porto C.<sup>3</sup>, Rossi B.<sup>3</sup>, Roig-Zamboni V.<sup>4</sup>, Germany S.<sup>4</sup>, Bourne Y.<sup>4</sup>, Parenti G.<sup>3,5</sup>, Sulzenbacher G.<sup>4</sup>, Moracci M.<sup>1,2</sup>

<sup>1</sup>Department of Biology, University of Naples Federico II, Naples, Italy

<sup>2</sup>Institute of Biosciences and Bioresources CNR, Naples, Italy

<sup>3</sup>Telethon Institute of Genetics and Medicine, Pozzuoli, Italy

<sup>4</sup>Centre National de la Recherche Scientifique CNRS, Marseille, France

<sup>5</sup>Department of Translational Medical Sciences, University of Naples Federico II, Naples, Italy

Pompe disease (PD) is a rare lysosomal storage disease caused by deficiency of the lysosomal enzyme acid  $\alpha$ -glucosidase (GAA). The disease is characterized by glycogen accumulation in lysosomes triggering severe secondary cellular damage and resulting in progressive motor handicap and premature death. To date, enzyme replacement therapy (ERT) with recombinant human GAA (rhGAA) is the only approved treatment for PD. The use of pharmacological chaperones (PC) acting as enzyme enhancers when co-administered with rhGAA is recognized as an interesting therapeutic alternative (1). However, the chaperones identified so far are also active site-directed molecules and potential inhibitors of target enzymes. We demonstrated that N-acetylcysteine (NAC) is a novel allosteric chaperone that improved the stability of rhGAA as a function of pH and temperature without affecting its catalytic activity. The thorough biochemical characterization, complemented by studies in patients fibroblasts and in a PD mouse model, demonstrated that the combination of NAC and rhGAA resulted in better correction of enzymatic activity, compared to rhGAA alone (2). The high-resolution crystal structure of rhGAA in complex with NAC, explained at the molecular level the stabilizing effect of this chaperone (3). Recent enzymatic screening allowed the identification of novel and unexpected compounds, already approved for the human use, that increase the stability of rhGAA and enhance its activity on cultured PD fibroblasts, thus representing potential allosteric chaperones for the treatment of Pompe disease.

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**The games of genes: the role of SMN and hnRNPQ in neurodegeneration**Santonicola P.<sup>1</sup>, La Rocca E.<sup>1</sup>, Cieri F.<sup>1</sup>, Zampi G.<sup>1</sup>, Rizzo F.<sup>2</sup>, Nizzardo M.<sup>2,3</sup>, Corti S.<sup>2,3</sup>, Di Schiavi E.<sup>1</sup><sup>1</sup>Institute of Biosciences and Bioresources, CNR, Naples, Italy<sup>2</sup>Dino Ferrari Centre, University of Milan, Italy<sup>3</sup>Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

The heterogeneous ribonuclear proteins (hnRNPs) are a family of RNA-binding proteins that post-transcriptionally regulate gene expression, by controlling mRNA stability, localization, transport and alternative splicing. Many RNA-binding proteins implicated in mRNA trafficking in motor neurons (MNs) have shown to interact with Survival Motor Neuron (SMN) protein, which is part of the SMN complex. Mutations in Smn cause Spinal Muscular Atrophy (SMA), a motor neuron specific disorder. hnRNPQ is one of the major components of the SMN complex. hrp-2 is the *C. elegans* homolog of hnRNPQ. To dissect its relationship to smn-1, we determined that hrp-2 null animals arrest as larvae, have a severe reduction in the lifespan and show locomotion defects, all phenotypes very similar to smn-1 null mutants. The overexpression of hrp-2 in all neurons in a SMA model where smn-1 is specifically silenced in D-type MNs (Gallotta et al. HMG, 2016) rescues the degeneration of MNs, reduce the number of apoptotic MNs and the defect in backward locomotion (Rizzo et al. Brain 2019). Furthermore, by motif enrichment analysis of differentially expressed/spliced genes in SMA patient and healthy people, we identified the RNA motif 7, which is specifically recognized by hnRNPQ. We found new neuron-specific genes altered only in SMA MNs possessing motif 7, suggesting their possible involvement in MNs survival and function. Therefore, we are investigating in vivo, using *C. elegans* as model system, the role of hrp-2 and of its downstream targets in the neurodegeneration and in smn-1 pathway.

## **Inspecting tau pathology in AD retina, a window to brain neurodegeneration: implications for neuroprotective intervention(s)**

Latina V.<sup>1</sup>, Giacomazzo G.<sup>2</sup>, Balzamino B.O.<sup>3</sup>, Micera A.<sup>3</sup>, Coccurello R.<sup>2,4</sup>, Calissano P.<sup>1</sup>, Amadoro G.<sup>1,5</sup>

<sup>1</sup>European Brain Research Institute (EBRI), Rome, Italy

<sup>2</sup>IRCSS Santa Lucia Foundation, Rome, Italy

<sup>3</sup>Research Laboratories in Ophthalmology, IRCCS-G.B. Bietti Foundation, Rome, Italy

<sup>4</sup>Institute for Complex System (ISC)-CNR, Rome, Italy

<sup>5</sup>Institute of Translational Pharmacology (IFT)-CNR, Rome, Italy

### **Objectives**

Retina is site of early, extra-cerebral manifestations of Alzheimer's Disease (AD). Amyloid- $\beta$  (A $\beta$ ) plaques and neurofibrillary tangles (NFT) of hyperphosphorylated tau protein are reported in eyes from AD patients and transgenic animals. However, the pathological relevance of other post-translational modifications of tau in both AD retinal and cerebral neurodegeneration has not yet been investigated.

We addressed the causal role of the neurotoxic 20-22kDa NH<sub>2</sub>-derived tau peptide (aka NH<sub>2</sub>htau) in AD retina deterioration and the potential therapeutic action of a novel cleavage-specific anti-NH<sub>2</sub> monoclonal tau antibody (12A12mAb). The 12A12mAb targets the 20-22kDa NH<sub>2</sub>-derived peptide from pathological truncation on the N-term domain of tau without any cross-reaction with the normal full-length protein. This mAb is endowed with potent neuroprotective action in vivo when intravenously administrated into 6-month-old Tg2576 AD animal model, by prospecting its therapeutic use in human beings.

### **Methods**

Western blotting, immunofluorescence.

### **Results**

By biochemical and morphological analyses carried out on retinal specimens from 6-month-old Tg2576 mice, we show that: (i) the expression level of toxic NH<sub>2</sub>htau peptide is significantly increased in transgenic AD group when compared to wild-type littermate controls; (ii) antibody-mediated neutralization of the NH<sub>2</sub>htau peptide is beneficial to ocular AD phenotype by exerting statistically-significant anti-amyloidogenic and anti-inflammatory effects.

### **Conclusions**

This in vivo study has potential translational implications by helping to: 1) develop harmless tau-directed immunotherapy to treat, minimize or reverse the visual impairment due to AD-related retinal injury; 2) identify the retinal AD-related tau changes in order to monitor the corresponding cerebral changes by early non-invasive diagnosis.

## **Ex vivo characterization of iPSCs-derived neurons in a patient diagnosed with non-syndromic intellectual disability and neonatal-onset epilepsy**

Longobardi E.<sup>1</sup>, Moutton S.<sup>2,3</sup>, Laudati G.<sup>1</sup>, Cicatiello R.<sup>4</sup>, Nitsch L.<sup>4</sup>, Miceli F.<sup>1</sup>, Vitobello A.<sup>3,5</sup>, Taglialatela M.<sup>1</sup>

<sup>1</sup>Division of Pharmacology, Department of Neuroscience, University of Naples "Federico II", Naples, Italy

<sup>2</sup>Reference Center for Developmental Anomalies, Department of Medical Genetics, Dijon University Hospital, Dijon, France

<sup>3</sup>INSERM U1231, LNC UMR1231 GAD, Burgundy University, Dijon, France

<sup>4</sup>Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italy;

<sup>5</sup>Laboratoire de génétique, Innovation en diagnostic génomique des maladies rares UF6254, Plateau Technique de Biologie, CHU Dijon, Dijon, France

**Key words:** KCNQ potassium channels, iPSC, epilepsy

**Background:** Induced Pluripotent Stem Cells (iPSCs) are powerful investigational tools to dissect the molecular pathogenesis of human diseases affecting brain function where no bioptic material is available. In the present study we have derived and used in neural induction experiments iPSCs from a proband carrying a homozygous KCNQ3 frameshift mutation (c.1599dup; p.Phe534Ile Fs\*15) and affected with neonatal-onset epilepsy and non-syndromic intellectual disability (Lauritano et al., 2019), together with those from a non-mutation carrier brother.

**Methods:** Fibroblasts obtained from punch skin biopsies were used for iPSC generation by a non-integrated, episomal reprogramming system. Neural precursor cells were obtained from iPSC and then differentiated into neurons. Cell subtypes were characterized by immunofluorescence and RT-PCR; G-banding assessed chromosomal morphology and integrity. Patch clamp recordings were also performed.

**Results:** iPSCs clones from proband and control displayed a normal karyotype and typical morphological features such as growth in tightly packed colonies with defined borders and a high nucleus to cytoplasm ratio. Major stemness markers were also expressed. Both control and proband iPSCs differentiated into NPCs and mature neurons, as revealed by the expression of Nestin/Pax6 and NeuN/ $\beta$ 3tubulin, respectively. In iPSC-derived neurons, patch-clamp recordings revealed a large density of voltage-gated sodium and potassium currents, confirming the acquisition of a differentiated neuronal phenotype.

**Conclusions:** Our preliminary data indicate the possibility to generate iPSCs from fibroblasts of a patient carrying an epileptogenic mutation in KCNQ3, and their differentiation into mature neurons. Further functional, morphological and molecular characterization is ongoing to identify pathogenetically-relevant differences between proband and control cells.

## **Expression of TrkA receptor in adult zebrafish brain**

Petrovici A., Solcan C., Castaldo L., D'Angelo L., De Girolamo P., Lucini C.

<sup>1</sup>USAMV Iasi, Department of Veterinary Medicine, Romania

<sup>2</sup>Università degli Studi Federico II, Department of Veterinary Medicine and Animal Productions, Naples, Italy

In our previous study, the presence and distribution of nerve growth factor (NGF) was described in zebrafish brain [Cacialli et al 2019]. In mammals, signaling of mature NGF is transduced by TrkA receptor and acts as mediator in many essential functions in the central nervous system. Since TrkA receptor was described in developing zebrafish [Nittoli], the aim of the present study is to describe the pattern of distribution of TrkA mRNA in the brain of adult zebrafish.

The investigation was conducted on brain fixed in paraformaldehyde. Paraffin sections were treated by In situ hybridization using digoxigenin-labeled riboprobes for TrkA [Nittoli et al 2018].

In the telencephalon, TrkA mRNA was found in the olfactory bulbs, specifically in the glomerular layer and in the external cellular layer. In addition, some positive round small neurons were seen in the regions of the dorsal area and rare positive neurons in the ventral area.

In the diencephalon, TrkA mRNA was seen in some neurons of the hypothalamus, in few neurons of mammillary body and synencephalon.

In the mesencephalon, TrkA mRNA was detected in neurons of the optic tect, specifically in the periventricular gray zone and superficial gray and white zone, and in neurons of the semicircular tori.

Concerning rhombencephalon, TrkA mRNA was highly present in neurons of all regions of the cerebellum, and in neurons of cerebellar crest.

These findings demonstrated the wide expression of TrkA receptor throughout all brain regions of adult zebrafish.

### **Initial Brain Aging and High Fat-High Fructose Diet: Effect on Mitochondrial Bioenergetics, Oxidative Status and Cholesterol Homeostasis in Rat Brain**

Mazzoli A.<sup>1</sup>, Crescenzo R.<sup>1</sup>, Spagnuolo M.S.<sup>2</sup>, Iannotta L.<sup>1</sup>, Cancelliere R.<sup>1</sup>, Gatto C.<sup>1</sup>, Canè M.<sup>1</sup>, Nazzaro M.<sup>1</sup>, Iossa S.<sup>1</sup>, Cigliano L.<sup>1</sup>

<sup>1</sup>Dept of Biology, University of Naples Federico II, Naples, Italy

<sup>2</sup>ISPAAM, CNR, Naples, Italy

Middle age is an early stage of the aging process, during which the consumption of diets rich in saturated fats and/or simple sugars might influence brain function, but only few data are available on this issue. Our aim was to investigate the impact of a diet rich in saturated fat and fructose (HFF) on mitochondrial physiology and cholesterol homeostasis in brain, where this lipid is involved in the maintenance of several neuronal processes. We focused on critical areas for learning and memory, i.e. hippocampus and frontal cortex of middle-aged rats (11 months). Middle-aged rats were fed HFF or control diet for 4 weeks. Mitochondrial function was analyzed by high-resolution respirometry and by assessing respiratory complexes levels. A decrease in the activity of complex I was detected in both brain areas of middle-aged rats. In hippocampus, an age-decrease in mitochondrial respiratory capacity and complex IV content, partly reversed by HFF diet, was evident. Higher oxidative protein damage, decreased antioxidant defenses, and increased UCP2 and PGC-1 alpha were found in hippocampus of middle-aged rats. HFF feeding induced a significant reduction in the amount of UCP2, PGC-1 alpha and PPAR alpha, together with higher protein oxidative damage, in both brain areas. HFF feeding also induced the alteration in key proteins of the regulatory network of brain cholesterol levels that could predispose to neurodegenerative diseases.

Our results point to middle age as a condition of early brain aging for mitochondrial function, with hippocampus being an area more susceptible to metabolic impairment than frontal cortex.

## **Synthesis and pharmacological characterization of conformationally restricted retigabine analogues as novel neuronal Kv7 channels activators**

Miceli E.<sup>1</sup>, Ostacolo C.<sup>2</sup>, Nappi P.<sup>1</sup>, Lauritano A.<sup>1</sup>, Carotenuto L.<sup>1</sup>, Soldovieri M.V.<sup>3</sup>, Ambrosino P.<sup>4</sup>, Campiglia P.<sup>5</sup>, Tagliatela M.<sup>1</sup>

<sup>1</sup>Dept. Neuroscience, University of Naples Federico II, Naples, Italy

<sup>2</sup>Dept. of Pharmacy, University of Naples Federico II, Naples, Italy

<sup>3</sup>Dept. of Medicine and Health Science, University of Molise, Campobasso, Italy

<sup>4</sup>Dept. of Science and Technology (DST), University of Sannio, Benevento, Italy

<sup>5</sup>Dept. of Pharmacy, University of Salerno, Salerno, Italy

Kv7 channels are attractive targets for antiepileptic treatment because of their critical involvement in controlling neuronal excitability; indeed, retigabine, a Kv7 activator, is the only compound approved for human use.

Unfortunately, its major limitations (scarce selectivity among Kv7 channels of different subunit composition, poor pharmacokinetics, side-effects related to chemical instability) have recently led to retigabine market withdrawal.

To possibly overcome some of these limitations, we have designed and synthesized a small library of 42 novel conformationally-restricted retigabine derivatives, and characterized some of these compounds with respect to: selectivity, chemical stability, and in vitro pharmacokinetics.

The pharmacological properties of the novel retigabine derivatives were evaluated in CHO cells transiently expressing Kv7.2, Kv7.3, Kv7.2+Kv7.3, Kv7.4 or Kv7.5 channels using the whole-cell configuration of the patch-clamp technique.

All synthesized compounds were investigated for their ability to enhance Kv7.2 currents. When tested on homomeric Kv7.2 channels, two indole-based derivatives (defined as 23a and 24a) showed efficacy higher than retigabine. When compared to retigabine ( $0.93 \pm 0.43 \mu\text{M}$ ), the EC<sub>50</sub>s for Kv7.2 current enhancement by 23a ( $0.08 \pm 0.04 \mu\text{M}$ ) was lower, whereas no change in potency was observed for 24a ( $0.63 \pm 0.07 \mu\text{M}$ ). When compared to retigabine, 23a and 24a also showed higher potency in activating heteromeric Kv7.2/Kv7.3 and homomeric Kv7.4 channels. Substitution of a tryptophan at position 236, located within the pore region of the channel, with a leucine largely prevents the ability of retigabine, 23a and 24a to activate Kv7.2 channels, suggesting that the indole-based derivatives and retigabine recognize the same hydrophobic pocket. Finally, 23a and 24a displayed an improved chemical stability over retigabine after 6 hours of exposure to UV/visible light.

## **Lysosomal amyloid deposition impairs autophagy and is a druggable target for the neurodegeneration in Lysosomal storage diseases**

Monaco A.<sup>1</sup>, Maffia V.<sup>1</sup>, Sorrentino N.C.<sup>1</sup>, Sambri I.<sup>1</sup>, Ezhova Y.<sup>1</sup>, Giuliano T.<sup>1</sup>, Cacace V.<sup>1</sup>, Nusco E.<sup>1</sup>, De Risi M.<sup>1</sup>, De Leonibus E.<sup>1</sup>, Schrader T.<sup>2</sup>, Klärner F.-G.<sup>2</sup>, Bitan G.<sup>3</sup>, Fraldi A.<sup>1,4</sup>

<sup>1</sup>Telethon Institute of Genetics and Medicine, Pozzuoli, Italy

<sup>2</sup>Department of Chemistry, University of Duisburg-Essen, Essen, Germany

<sup>3</sup>Department of Neurology, David Geffen School of Medicine, Brain Research Institute, and Molecular Biology Institute, University of California, Los Angeles, California

<sup>4</sup>Department of Translational Medicine, University of Naples "Federico II", Naples, Italy

The aggregation of amyloidogenic proteins characterizes the onset of several neurodegenerative conditions. How amyloid aggregation causes neurotoxicity is not completely understood. Lysosomal storage diseases (LSDs) are metabolic disorders caused by inherited lysosomal deficiencies and characterized by lysosomal storage and autophagy dysfunction often associated with a neurodegenerative course. Here we found that several amyloid proteins, including  $\alpha$ -synuclein, PrP, Tau and amyloid  $\beta$ -protein progressively build up in the brain of a mouse model of mucopolysaccharidosis (MPS) type IIIA, one of the most common and severe types of LSDs. A major fraction of the amyloid deposit forms in perikaryal-localized lysosomes affecting lysosomal distribution and, therefore impairing autophagosome-lysosome encountering and autophagosome clearance. Treating MPS-IIIa mice with CLR01, a "molecular tweezer" that acts as a broad-spectrum inhibitor of self-assembly of multiple amyloidogenic proteins, restored lysosomal-autophagic flux and significantly ameliorated neuropathological signs. Together, these data provide a new neuropathogenic link between amyloid deposition and autophagy-lysosomal pathway and also identify CLR01 as a potent drug candidate for the treatment of MPS-IIIa and likely of other LSDs.



## **A novel gain-of-function variant in *kcnq5* in a patient with neurodevelopmental delay and drug-resistant epilepsy**

Nappi M.<sup>1</sup>, Rivier-Ringenbach C.<sup>2</sup>, Lauritano A.<sup>1</sup>, Miceli F.<sup>1</sup>, Lesca G.<sup>3</sup>, Taglialatela M.<sup>1</sup>

<sup>1</sup>Section of Pharmacology, Department of Neuroscience, University of Naples "Federico II", Naples, Italy

<sup>2</sup>Department of Pediatrics, L' Hopital Nord-Ouest, Villefranche-Sur-Saône, France

<sup>3</sup>Service de Génétique, Centre de Référence Anomalies du Développement, Hospices Civils de Lyon, Bron, France

The M current ( $I_{KM}$ ) is a potassium current which plays a fundamental role in controlling neuronal excitability.  $I_{KM}$  is mainly composed by heteromeric KCNQ2 and KCNQ3 subunits, although KCNQ5 subunits, may also contribute to its molecular heterogeneity. In the present work, we report the identification of a novel variant in KCNQ5 in a proband with neurodevelopmental delay and drug-resistant focal hypertonic seizures with onset at 15 months and we describe the changes triggered by the mutation on channel function in vitro. This variant leads to the substitution of the Glycine 347, located at the bottom of the S6 transmembrane domain, with a Serine (G347S).

Patch-clamp recordings revealed that, when compared to KCNQ5, homomeric KCNQ5 G347S channels carried currents >10 times larger in size, also showing a marked (>15 mV) hyperpolarization shift in  $V_{1/2}$ , the midpoint potential of activation, and slower deactivation kinetics; all these biophysical properties indicate a strong gain-of-function in vitro phenotype. Qualitatively similar results were obtained when KCNQ5 G347S mutant subunits were co-expressed together with KCNQ3 subunits; in fact, when compared to KCNQ3+KCNQ5 channels, KCNQ3+KCNQ5 G347S channels displayed a >2-fold increase in current size and a hyperpolarizing shift in  $V_{1/2}$  of about 8 mV.

In the present work we have demonstrated the crucial pathogenic role of the novel G347S variant located in the pore of the KCNQ5 subunit, and highlighted this position as critical for regulating channel function.

### **Sex-specific neurodegeneration in *C. elegans***

Onorato G.<sup>1</sup>, Guardascione G.<sup>1</sup>, Zampi G.<sup>1</sup>, Paglione M.<sup>1</sup>, Santonicola P.<sup>1</sup>, Sola F.<sup>1</sup>, Maggi A.<sup>2</sup>, Di Schiavi E.<sup>1</sup>

<sup>1</sup>Institute of Biosciences and Bioresources, National Research Council (CNR), Naples, Italy

<sup>2</sup>Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy.

## **Social cognition in children and adolescents with Specific Learning Disorders**

Pastorino G.M.G., Stellato M., Amadori E., D'Onofrio F., Coppola G., Operto F.F.

University of Salerno

Background: Social Cognition (SC) has been defined as cognitive cleverness that allows people to acquire information deriving from social and emotional external world just to better understand themselves and organize smartly their behaviours in order to better interact with external world. Objectives: The purpose of our study is to evaluate SC, in particular facial emotion recognition (ER), in children and adolescents diagnosed with Specific Learning Disorder, and correlate them with intelligence and executive functions.

Methods: Our work is a cross-sectional observational study. Fortyseven children and adolescents aged between 7 and 18 years with a diagnosis of SLD and 32 sex/age-matched controls were recruited. All participants were administered a standardized battery tests to evaluating social cognition (NEPSY-II), executive functions (EpiTrack Junior) and cognitive levels (WISC-IV).

Results: Emotion recognition mean score was significantly lower in the SLD group than in the controls to t-student test for unpaired samples ( $p < 0.05$ ). SLD group performed significantly lower than control in their abilities to identify neutral, happiness, sadness, anger and fear compared to controls ( $p < 0.05$ ). Deficits in ER weren't related to the patients' age and sex, but were related to the subtype of diagnosis of Specific Learning Disorder and to a deficit of executive functions (ER-EpiTrack Junior; ER-IML). There was no correlation with the cognitive profile.

Conclusion: Our results show that children and adolescents with Specific Learning Disorders had social cognition deficit independently of intelligence, and this deficit could be potentially related to some aspects of SLD it-self as well as to an executive dysfunction.

**Cystatin B deficiency influences synaptic plasticity and secretion mechanisms in organoids from EPM1 patients**

Penna E.<sup>1</sup>, Chambery A.<sup>2</sup>, Russo R.<sup>2</sup>, Ricciardi L.<sup>1</sup>, Stella F.<sup>1</sup>, Canafoglia L.<sup>3</sup>, Cappello S.<sup>4</sup>, Crispino M.<sup>1</sup>, Di Giaimo R.<sup>1,2</sup>

<sup>1</sup>Department of Biology, University Federico II, Naples, Italy

<sup>2</sup>Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania "Luigi Vanvitelli", Caserta, Italy

<sup>3</sup>Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy

<sup>4</sup>Max Planck Institute of Psychiatry, Munich, Germany

Progressive Myoclonus Epilepsy (EPM1) is a rare neurodegenerative disease of the central nervous system, caused by mutation of the gene encoding Cystatin B (CSTB). The molecular mechanisms are unknown, although a common denominator of many neurodegenerative diseases is deregulation of synaptic plasticity. Interestingly, we recently showed involvement of CSTB in synaptic physiology (Penna et al. 2019). We demonstrated that CSTB is present and locally synthesized in the synaptic regions of rodent's cerebral cortex and human cerebral organoids (hCOs, used as a model system of human cortical early neurogenesis), and it is secreted by synaptosomes under depolarizing conditions. Here, we generated hCOs from EPM1 patients and control individuals. We analysed the CSTB expression level in the homogenate from hCOs and in the corresponding synaptosomal fractions, confirming a downregulation of CSTB protein in both fractions from patients' organoids compared to controls. We performed proteomic analysis of these samples in order to investigate the molecular pathways altered in the pathology. Among the 172 proteins up-regulated in patients hCOs, several of them were related to redox process, in line with previous papers reporting oxidative damage in CSTB KO mice and in neurons of EPM1 patients. Out of 350 proteins down-regulated in patients hCOs, more than 100 proteins were involved in vesicular transport and secretion, strongly supporting our hypothesis of an impairment of the secretion mechanisms in EPM1. Our findings are shedding a new light on the CSTB role in the synaptic plasticity and on the molecular mechanism altered in EPM1.

## **Metabolism of Polysialic acid (polySia) is defective in multiple pre-clinical models of Huntington's disease**

Pepe G.<sup>1</sup>, Amico E.<sup>1</sup>, Capocci L.<sup>1</sup>, Sönmez A.<sup>2</sup>, Boltje T.J.<sup>3</sup>, Parlato R.<sup>2</sup>, di Pardo A.<sup>1</sup>, Maglione V.<sup>1</sup>

<sup>1</sup>IRCCS Neuromed, Pozzilli (IS) Italy

<sup>2</sup>Institute of Applied Physiology, University of Ulm, Ulm, Germany

<sup>3</sup>Radboud University Nijmegen, The Netherlands

Defective metabolism of sialic acid-containing glycosphingolipids (gangliosides) has been described as important determinant of disease pathogenesis in Huntington's disease (HD), a rare hereditary neurodegenerative condition with complex pathogenic profile and with no cure available. Reduced levels of gangliosides in HD mice and in human cells was associated with perturbed expression of the biosynthetic enzymes, sialyltransferases, which are commonly involved also in the synthesis of glycoproteins.

Thus, the hypothesis is that alteration of ganglioside metabolism in HD may represent a phenomenon of a more general global defective metabolism of sialoconjugates which may likely interfere also with glycoprotein homeostasis. In this study, we explored for the first time whether or not the metabolism of sialic acid-containing glycoproteins is actually compromised in HD. Particular attention was paid to the analysis of the Polysialic acid (polySia), the sugar polymer commonly linked to the neural cell adhesion molecule (NCAM) that is well known to be essential for brain development and plasticity.

Interestingly, our findings demonstrate that metabolism of polySia becomes precociously perturbed in HD, even at the early stages of mouse embryonic development. Although only speculative by now, the early aberrant levels of polySia in HD may provide evidence to elaborate a different concept of the disease and eventually investigate still unexplored aspect of any associated neurodevelopmental impairment.

## **A CNV positional effect analysis implicates enhancer-mediated SHH dysregulation in a patient with multiple congenital anomalies and malformations.**

Pinelli M.<sup>1,2</sup>, Pignataro P.<sup>3</sup>, Bianco S.<sup>4</sup>, Genesio R.<sup>3</sup>, Cappuccio G.<sup>1,2</sup>, Chiariello A.M.<sup>4</sup>, Nicodemi M.<sup>4</sup>, Nitsch L.<sup>3</sup>, Brunetti-Pierri N.<sup>1,2</sup>

<sup>1</sup>Telethon Institute of Genetics and Medicine (TIGME), Pozzuoli, Italy

<sup>2</sup>Dipartimento di Scienze Mediche Traslazionali, Università degli Studi di Napoli "Federico II", Naples, Italy

<sup>3</sup>Dipartimento di Medicina Molecolare e Biotecnologie mediche, Università degli Studi di Napoli "Federico II", Naples, Italy

<sup>4</sup>Dipartimento di Fisica, Università di Napoli Federico II, and INFN Napoli Complesso Universitario di Monte Sant'Angelo, Naples, Italy

### Introduction

Despite application of genome-wide diagnostics, such as chromosomal microarrays and whole exome sequencing, a significant proportion of patients with suspected genetic disease continue to lack a definitive diagnosis. The underlying genetic defects in a subgroup of these cases may be altered expression of disease genes due to copy-number variants (CNV) involving regulatory non-coding elements. Therefore, in a cohort of cases with suspected genetic diseases who underwent chromosomal microarray analysis, we searched for CNV interposed between relevant disease genes and their corresponding enhancers.

### Methods

Out of a dataset of 1,176 CNV from 682 patients, we selected CNVs that were: (1) smaller than 1 Mb, (2) devoid of disease genes, and (3) mapped between disease genes and their enhancers. Then, we manually analyzed these gene-CNV-enhancers groups for concordance between involved genes and corresponding phenotypes. Finally, we predicted effect of CNV on chromatin folding by the strings-and-binders method.

### Results

235 CNV (34%) mapped between a disease gene and one of its enhancers and 26 gene-phenotype relationships were consistent after manual evaluation. Among these cases, the best CNV candidate was a 50 kb de novo deletion encompassing a region between sonic hedgehog gene (SHH) and two of its enhancers, in a patient with limb abnormalities, aortic malformation, cardiac arrhythmia, and facial dysmorphisms.

### Conclusion

Systematic analysis of potential positional effect of CNV has potential for identifying the genetic defects in undiagnosed cases and, in our analysis, it detected a CNV affecting contacts of SHH, an important transcription factor involved in development, with its enhancers.

## **The case of a young patient with drug-resistant epilepsy partialis continua**

Quatrini M., Parisi P., Ferretti A., Striano. P.

Sant'Andrea Hospital, Pediatrics UOC, Sapienza University of Rome, Rome, Italy

Epilepsia partialis continua (EPC) is a particular type of epilepsy which is distinguished from "common epilepsy" by its characteristic semiological features. In this case report we describe a 14-year-old-boy with EPC with jerks of the right corner of the mouth. The patient's abnormal symptoms occurred continuously and disturbed his normal daily life. Sleep EEG was performed, which revealed the presence of focal EEG abnormalities that were fairly active during sleep. We detected serologies and PCR for the viruses that interest the central nervous system, that resulted negative. The biochemical blood tests were negative. We didn't observe structural abnormalities in cerebral RM scans. Antineuronal antibodies in CSF were negative. We detected anti-PELO antibodies, we are waiting for the results. genome sequencing was performed. We didn't find significant mutations. However, at date, the boy has the same symptoms without responding to drug therapy.

## **Inhibition of the histone demethylase LSD1 leads cells to senescence**

Saccà C.D.<sup>1</sup>, Gorini F.<sup>2</sup>, Amente S.<sup>2</sup>, Majello B.<sup>1</sup>

<sup>1</sup>Division of Genetics, Department of Biology, University of Naples, 'Federico II', Naples, Italy

<sup>2</sup>Department of Molecular Medicine and Medical Biotechnologies, University of 'Federico II', Naples, Italy

Gliomas comprise about 30 percent of all brain tumors and central nervous system tumors, and 80 percent of all malignant brain tumors. Gliomas are classified according to the type of glial cell involved in the tumor, as well as the tumor's genetic features, which can help to predict how the tumor will behave over time and the treatments most likely to work. Glioblastoma, also known as glioblastoma multiforme (GBM), is one of the most aggressive and highly proliferating brain tumor. Induction of senescence, a stress response that leads cells to exit from the cell cycle is one of the mechanisms that prevents proliferation. Senescent cells may have a deleterious effects on the tissue microenvironment, with the acquisition of a senescence-associated secretory phenotype (SASP) of the cells. This phenotype converts senescent cells into proinflammatory cells which have the ability to promote tumor progression. We investigated the role of the Lysine-specific demethylase 1 (LSD1) in Glioblastoma tumor cells (GBM) finding that it functions as a regulatory hub controlling different aspects of cellular senescence as DNA damage, proliferation and cellular migration. In our recent studies we clarify a mechanism whereby LSD1 controls GBM cells senescence through the regulation of HIF-1 $\alpha$ .

Moreover we also demonstrate that LSD1 inhibition doesn't result in interleukin (IL6 and IL8) upregulation suggesting that LSD1 inhibition activates Senescence in a SASP-independent manner in GBM cells. The mechanism underlying this behavior is under investigation.

Overall our results have implications for the use of epigenetic regulators drugs for GBM cancer therapy, in particular the inhibition of LSD1 can be exploited in the future as adjuvant for GBM therapy.



### **Amyloid precursor protein (APP) maturation and intracellular localization are controlled by a specific 37/67kDa laminin receptor inhibitor in neuronal cells**

Bhattacharya A.<sup>1</sup>, Limone A.<sup>1</sup>, Minopoli G.<sup>1</sup>, Napolitano F.<sup>2</sup>, Montuori N.<sup>2</sup>, Lavecchia A.<sup>3</sup>, Sarnataro D.<sup>1</sup>

<sup>1</sup>Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università di Napoli Federico II

<sup>2</sup>Dipartimento di Scienze mediche Traslazionali, Università di Napoli Federico II,

<sup>3</sup>Dipartimento di Farmacia, Università di Napoli Federico II

Amyloid precursor protein (APP) is processed along both the non-amyloidogenic pathway preventing amyloid beta peptide (Abeta) production and the amyloidogenic pathway, generating Abeta, whose accumulation characterizes Alzheimer's disease. Items of evidence report that the intracellular trafficking plays a key role in the generation of Abeta and that the 37/67kDa LR, acting as a receptor for Abeta, may mediate Abeta-pathogenicity. We show herein that the specific 37/67kDa LR inhibitor, NSC48478, is able to reversibly affect the maturation of APP and production of C-terminal fragments, in a NH<sub>4</sub>Cl-dependent manner, resulting in the partial accumulation of the immature APP isoform in the endoplasmic reticulum and in Transferrin-positive recycling endosomes, suggesting alteration of the APP intracellular trafficking pathway. These effects were accompanied by an inactivation of the MAPK-ERK1/2 axis, revealing NSC48478 inhibitor as a novel small compound to be tested in disease conditions.

**The activation of Mucolipin TRP channel 1 (TRPML1) protects motor neurons exposed to the cycad neurotoxin L-BMAA by promoting autophagic clearance**Tedeschi V.<sup>1</sup>, Petrozziello T.<sup>1</sup>, Sisalli MJ.<sup>1</sup>, Boscia F.<sup>1</sup>, Canzoniero L.M.T.<sup>2</sup>, Secondo A.<sup>1</sup><sup>1</sup>Department of Neuroscience, Reproductive and Odontostomatological Sciences, School of Medicine, "Federico II" University of Naples, Naples, Italy<sup>2</sup>Division of Pharmacology, Department of Science and Technology-DST, University of Sannio, Benevento, Italy

Cellular clearance mechanisms including the autophagy-lysosome pathway are impaired in the central nervous system (CNS) of amyotrophic lateral sclerosis (ALS) patients. However, how the defects in lysosomal function contribute to the pathogenesis of ALS is unclear. Therefore, we investigated the role of lysosomal function in an in vitro model of amyotrophic lateral sclerosis/Parkinson-dementia complex (i.e. motor neurons exposed to the cycad neurotoxin beta-methylamino-L-alanine named L-BMAA). Considering that the lysosomal Ca<sup>2+</sup> channel Mucolipin TRP channel 1 (TRPML1) plays a crucial role in lysosomal function, we studied the role of the channel in this neurotoxic model. Under physiological conditions, TRPML1 colocalized with the endoplasmic reticulum (ER), that drives calcium refilling of lysosomes. In fact, the specific and irreversible SERCA inhibitor thapsigargin reduced lysosomal Ca<sup>2+</sup> refilling measured by the genetically-encoded Ca<sup>2+</sup> indicator GCaMP3 attached to the lysosomal channel (GCaMP3-ML1). However, TRPML1 expression was dramatically reduced in motor neurons exposed to the L-BMAA, a condition characterized by the impairment of ER Ca<sup>2+</sup> store. Therefore, lysosomal Ca<sup>2+</sup> release and ER Ca<sup>2+</sup> content were both dysregulated by L-BMAA. Very interestingly, the specific membrane-permeable synthetic agonist of TRPML1, ML-SA1, rescued motor neurons from death and ER stress induced by chronic exposure to L-BMAA. Furthermore, under the same conditions, the pre-incubation of ML-SA1 prevented the elevation of the ER stress marker GRP78 and of the autophagy-related proteins p62/SQSTM1 and LC3-II. Notably, ML-SA1 per se induced the activation of the autophagy initiators p-AMPK and beclin 1. Collectively, we propose that the pharmacological stimulation of lysosomal Ca<sup>2+</sup> channel TRPML1 can efficiently rescue motor neurons from L-BMAA toxicity by improving autophagy-dependent clearance mechanisms.

## **Towards a therapy for phosphomannomutase 2 deficiency: the prodrug approach for the delivery of alpha-glucose-1,6-bisphosphate**

Sodano E.<sup>1</sup>, Rolando B.<sup>1</sup>, Rimoli M.G.<sup>2</sup>, Cubellis M.V.<sup>3,4</sup>, Monticelli M.<sup>4</sup>, Allocca M.<sup>3,5</sup>, Liguori L.<sup>3,5</sup>, Andreotti G.<sup>3</sup>, Lazzarato L.<sup>1</sup>

<sup>1</sup>Department of Drug Science and Technology, University of Turin, Turin, Italy

<sup>2</sup>Department of Pharmacy, "Federico II" University of Naples, Naples, Italy

<sup>3</sup>Institute of Biomolecular Chemistry, National Research Council, Pozzuoli, Italy

<sup>4</sup>Department of Biology, "Federico II" University of Naples, Naples, Italy

<sup>5</sup>Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania "Luigi Vanvitelli", Caserta, Italy

Phosphomannomutase2 deficit, or PMM2-CDG, is the most common congenital disorder of glycosylation affecting over 1,000 patients globally and it is still without a cure. The majority of the mutations causing the disease destabilize PMM2, which, under physiological conditions, catalyses the conversion of mannose-6-phosphate into mannose-1-phosphate. Mannose-1-phosphate is in turn converted into guanosine diphosphate-mannose, a fundamental precursor for N-glycosylation process of proteins. All PMM2-CDG patients have some residual PMM2 enzymatic activity since complete absence of this activity is incompatible with life. A promising strategy to cure PMM2-CDG is based on the use of pharmacological chaperones (PCs), which are low molecular weight compounds able to stabilize the mutated enzyme and increase its intracellular concentration. In-vitro tests have demonstrated that alpha-glucose-1,6-bisphosphate binds, stabilizes and increases the activity of PMM2 (both wild type and mutants). Regrettably, it cannot be directly administered to cell cultures for its inability to cross cell membranes. In this framework, prodrug strategy plays a central role in drug development allowing the improvement of a specific parent drug. Consistently, we designed and synthesized a promising prodrug of alpha-glucose-1,6-bisphosphate, 2,3,4-tri-O-acetylglucose-1,6-bis(diphenylphosphate) (LipoG-1,6P). LipoG-1,6P has shown great chemical stability at physiological pH while its half-life in human serum was 10.5 hours. Moreover, its cytotoxicity in HEK293 cells was evaluated by MTT test. The next step will be evaluating the capability of LipoG-1,6P to cross the cell membrane, to release PC (alpha-glucose-1,6-bisphosphate) and consequently enhance PMM2 enzymatic activity.

## **Impairment of BDNF signaling, modulation of synaptic markers and inflammation are involved in the early response to a short term western diet in middle-aged rats**

Nazzaro M.<sup>1</sup>, Spagnuolo M.S.<sup>2</sup>, D'Ambrosio F.<sup>1</sup>, Mazzoli A.<sup>1</sup>, Iossa Susanna<sup>1</sup>, Cigliano L.<sup>1</sup>

<sup>1</sup>Department of Biology, University of Naples Federico II, Naples, Italy

<sup>2</sup>Department of Bio-Agrofood Science, ISPAAM, CNR, Naples, Italy

In the last decades obesity has become an epidemic problem with a devastating impact on health of individuals on the long-term. Although excessive energy intake is known to negatively affect cerebral physiology, the early effect of western diets on brain function, particularly at middle-age, is still unknown. Since middle-age is a critical stage in the life course, our aim was to investigate whether a short-term diet, rich in fat and fructose, affects inflammatory status, BDNF levels and signaling, and synaptic function. To this aim, middle aged rats (11 months old) were fed a high-fat high-fructose (HFF) or a control diet for 4 weeks. A significant increase of inflammatory markers was observed in hippocampus, but not in frontal cortex, of HFF rats. Notably, a significant diet-dependent reduction in BDNF amount was detected in both cerebral region, while the amount of its receptor, TrkB, was reduced only in hippocampus of treated rats. Furthermore, the level of the presynaptic protein synaptotagmin I was found significantly reduced in both cortex and hippocampus, while no change in the level of synapsin I and synaptophysin were detected. Intriguingly, we observed a significant diet dependent increase of the post synaptic protein PSD 95, likely representing a compensative response to the decrease of synaptotagmin I.

Overall, our findings highlight that a short term HFF diet induces hippocampal inflammation and impairs synaptic function as well as BDNF signaling in middle aged rats. These alterations of brain physiology might prime molecular events involved in the onset of neurodegenerative diseases.

## **The functional coupling between endoplasmic reticulum and lysosomes for intracellular Ca<sup>2+</sup> handling is disrupted in brain ischemia**

Tedeschi V., Petrozziello T., Sisalli M.J., Vinciguerra A., Pignataro G., Di Renzo G., Annunziato L., Secondo A.

Department of Neuroscience, Reproductive and Odontostomatological Sciences, School of Medicine, "Federico II" University of Naples, Naples, Italy

A growing interest has been recently devoted to the role of intracellular Ca<sup>2+</sup> stores in brain ischemia. For instance, disturbances of Ca<sup>2+</sup> content in the endoplasmic reticulum (ER) have been reported as one of the main mechanisms underlying the neurological disease. Interestingly, lysosomes are emerging as other important Ca<sup>2+</sup>-storing organelles, cooperating with ER in the handling of intracellular Ca<sup>2+</sup> concentration ([Ca<sup>2+</sup>]<sub>i</sub>). One of the main regulators of lysosomal Ca<sup>2+</sup> levels is represented by Mucolipin TRP channel 1 (TRPML1), a non-selective cation channel releasing lysosomal Ca<sup>2+</sup> into the cytosol.

Here we investigated the role of ER/lysosome Ca<sup>2+</sup> coupling and the contribution of TRPML1 in brain ischemia. Our results showed that under physiological conditions TRPML1 activation induced by its specific agonist ML-SA1 or by lysosomal v-ATPase inhibitor bafilomycin A1 significantly increased [Ca<sup>2+</sup>]<sub>i</sub> in cortical neurons. ML-SA1-induced Ca<sup>2+</sup> leak from lysosomes strongly reduced ER Ca<sup>2+</sup> content, whereas TRPML1 inhibitor trans-Ned19 or channel knocking-down increased ER Ca<sup>2+</sup> levels. However, this interplay was disrupted under hypoxic conditions produced by exposing cortical neurons to oxygen and glucose deprivation (OGD) followed by reoxygenation (Rx). Indeed, during OGD/Rx both ER and lysosomal Ca<sup>2+</sup> levels were significantly impaired. Interestingly, trans-Ned19 administration during the reoxygenation phase prevented dysfunctional lysosomal Ca<sup>2+</sup> homeostasis and neuronal death. In consideration of the role played by lysosomes in autophagy regulation, we showed that trans-Ned19 hampered the autophagic flux during hypoxia thus protecting neurons. Collectively, our data demonstrate that a disruption in the functional interplay between ER and lysosomes underlies neuronal loss occurring in brain ischemia.

**Characterisation of an infantile rat model of de novo status epilepticus: long-term outcomes**

Terrone G.<sup>1,2</sup>, Di Sapia R.<sup>1</sup>, Salamone A.<sup>1</sup>, Craparotta I.<sup>3</sup>, Zaniani N.R.<sup>1</sup>, Tolomeo D.<sup>1</sup>, Micotti E.<sup>1</sup>, Marchini S.<sup>3</sup>, Ravizza T.<sup>1</sup>, Del Giudice E.<sup>2</sup>, Vezzani A.<sup>1</sup>

<sup>1</sup>Department of Neuroscience, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milano, Italy

<sup>2</sup>Department of Translational Medicine, Federico II University, Naples, Italy

<sup>3</sup>Department of Oncology, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milano, Italy

Background: Paediatric status epilepticus (SE) may result from acquired, metabolic, immune, genetic or unknown causes. We characterized an infantile rat model of de novo SE to study its long-term pathologic consequences.

Methods: SE was induced by unilateral intra-amygdala injection of 2 µg kainic acid (KA) in cortical electrode-implanted postnatal day (P)13 male rat pups. Controls were injected with saline. Glial activation and Fluoro-Jade-positive degenerating neurons were analysed by immunohistochemistry; neuroinflammation and oxidative stress markers were measured by RTqPCR. Different cohorts of SE-exposed P13 rats were longitudinally video-EEG monitored, exposed to the Morris Water Maze, and to brain magnetic resonance imaging (MRI).

Results: SE was defined by the appearance of continuous spikes with a frequency >1.0 Hz and an amplitude at least 2.5-fold higher than the baseline. SE occurred  $31.0 \pm 2.3$  min after KA injection and lasted for  $3.5 \pm 0.5$  h (mean  $\pm$  SEM, n=9). Glia activation, induction of the iktogenic cytokines IL-1 $\beta$  and TNF- $\alpha$  and HMGB1, oxidative stress markers were measured in rats (n=6-7 rats each group) from 2h to 1 week post-SE. Degenerating neurons were detected in cortex, hippocampus, amygdala, striatum and reticular thalamic nucleus. Spontaneous recurrent seizures (3-5/week) developed around 1 month after SE in about 60% of rats (n=19). MRI showed progressive atrophy in cortical and subcortical regions starting before epilepsy onset. Rats displayed cognitive impairment after epilepsy onset.

Conclusions: This infantile SE rat model can be exploited for mechanistic studies, to test novel drugs and for developing biomarkers of disease onset and progression.

## **Hydra vulgaris as a versatile model for neuroscience: from neural networks to nanostructured devices to modulate neuronal activity**

Tommasini G., Fardella F., Fergola E., Amenta M.L., Marchesano V., Tino A., Tortiglione C.

Istituto di Scienze Applicate e Sistemi Intelligenti "E.Caianiello" Consiglio Nazionale delle Ricerche, Pozzuoli, Italy

Comparative approaches aimed at identifying cardinal shared features of neurons and neural circuits may greatly help to understand how the nervous systems function. At the base of metazoan evolution Cnidarians, which include jellyfishes and polyps, have long been utilized in laboratories as experimental organisms to address development and regeneration issues. In recent years extensive genomic and transcriptomic resources, genetic engineering and gene-editing technologies draw the attention of neuroscientists to the simple nerve net of *Hydra vulgaris*, focussing on nervous system circuitry, with the final goal to decipher the whole brain activity map. By calcium imaging three anatomical non overlapping neural networks were identified in *Hydra* and associated with specific behaviours providing useful clues to understand how neural circuits relate to behaviour or internal brain states in bilaterians. An alternative approach to elucidate the links between neuronal activity, anatomy, and behaviour is offered by nanostructured materials and nanodevices that, acting at the same scale of target neurons, offer an unprecedented tool to probe, monitor, record and control neural activity. We will show recent advances achieved in our laboratory interfacing *Hydra* with different types of nanoparticles. Starting from metal-based semiconducting colloidal nanoparticles, eliciting a precise animal behaviour by acting on tentacle neurons, we will show the effect of two photovoltaic materials to induce animal behaviour and opsin gene transcription, up to novel approaches to activate intracellular pathways through controlled drug delivery. The results show the feasibility to use *Hydra* not only for basic understanding of neuronal function but also for the design and validation of new devices to interface with the nervous system for diagnosis and therapeutics.

**Levels of Cystatin B are essential for interneuron migration in individuals with EPM1 epilepsy**Tovecci I.<sup>1,2</sup>, Di Matteo F.<sup>2</sup>, Pipicelli F.<sup>2</sup>, Ayo Martin A.C.<sup>2</sup>, Hoffmann A.<sup>2</sup>, Canafoglia L.<sup>3</sup>, Cappello S.<sup>2</sup>, Di Giaimo R.<sup>1,2</sup><sup>1</sup>Department of Biology, University Federico II, Naples, Italy<sup>2</sup>Max Planck Institute of Psychiatry, Munich, Germany<sup>3</sup>Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy

Progressive myoclonus epilepsy (PME) of Unverricht-Lundborg-type (EPM1) is an autosomal recessive neurodegenerative disorder that has the highest incidence of PME worldwide. CystatinB (CSTB) gene is a small ubiquitous protein, responsible for the primary defects in patients with EPM1. Our previous results showed that levels of functional CSTB during mouse embryonic neurogenesis influence proliferation of progenitors and modulate neuronal migration. Here, we analyzed the possible role of CSTB during human neurogenic development by using 3D models, named human cerebral organoids (hCOs). To this aim, we generated in parallel hCOs from 2 controls and 2 EPM1 patients (UL1 and UL4) with different genetic background and we showed that patients-derived-hCOs were significantly smaller than controls. In agreement with the smaller size, we detected a decrease in proliferating cells and premature differentiation. Interestingly, CSTB is secreted into the medium of hCOs, suggesting a novel role in the extracellular space. We then took advantage of a recently developed protocol that reproduce in vitro the dorso-ventral forebrain axis and then the tangential migration of ventral interneurons occurring during development (Bagley et al. 2017). We generated fused organoids composed of ventrally-derived control neurons and dorsally-derived control or patients' cells. We found that the number of ventrally derived interneurons was significantly decreased in the dorsally patterned UL1 organoids compared to the dorsally patterned CTRL organoids, indicating that levels of CSTB are critical for interneuron recruitment.

Overall our data suggest a role of CSTB in the regulation of early stages of human brain development.



## **FIG4 regulates the homeostasis of endosomal compartments in different cell types**

Valente V., Zerillo L., Fasano D., Sarnataro D., Pierantoni G.M., Paladino S.

Dep. Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples

Mutations in the inositol phosphatase FIG4 were firstly associated with Charcot-Marie-Tooth 4J (CMT4J) neuropathy, a rare recessive demyelinating form of CMT with highly variable onset characterized by severe motor dysfunction and involvement of motor and sensory neurons. Interestingly, frameshift and other missense mutations have been reported to be responsible of Yunis-Varon syndrome and familial epilepsy with polymicrogyria extending the spectrum of phenotypes associated with FIG4 mutations.

FIG4 dephosphorylates the endolysosome-enriched PI(3,5)P<sub>2</sub> to generate PI(3)P. Enlarged LAMP2 positive vacuoles with watery appearance or filled with electron dense material (depending on cell type) are found in neurons, muscle and cartilage of FIG4 null mice, suggesting a dysfunction of these compartments. However, the pathogenic mechanism(s) remain still elusive.

We show that the FIG4 knockdown, by using specific short hairpin RNAs, drastically alters the whole endo-lysosome axis: not only lysosomes appear as large dots, but also late and early endosomes are numerous and enlarged. In addition, the levels of endocytic resistant proteins are increased, suggesting an alteration of their dynamics. In contrast, the morphology of exocytic organelles is comparable to wild-type cells. In addition, the trafficking of transferrin and EGF receptors, which follow different routes, is altered in FIG4-depleted cells. In both cases, progressive accumulation of proteins inside the cells is observed, indicating that protein trafficking through endosomal compartments is altered.

Overall, these data indicate that FIG4 activity is crucial for the homeostasis and function of endosomal compartments in different cells types. The dysfunction of these pathways might underlie the pathogenesis of FIG4-associated diseases.

**Antipsychotic effects on the synaptic plasticity-related gene Homer1a are dependent on dose, receptor profile, timing of administration, and brain regions**

Vellucci L., Avagliano C., Buonaguro E.F., Iasevoli F., de Bartolomeis A.

Laboratory of Molecular and Translational Psychiatry and Unit of Treatment Resistant Psychosis, Section of Psychiatry, Department of Neurosciences, University of Naples Federico II, Italy

Antipsychotics modulate key molecules of glutamate-dopamine-mediated synaptic plasticity, i.e. Homer1. Studying the patterns of Homer1a modulation may allow making inferences on how antipsychotics elicit plastic synaptic changes. Here, we tested the hypothesis that antipsychotics effects on Homer1a expression may be mediated by a combination of receptor profile, dose, timing of administration, and brain regions of interest (ROI).

In situ hybridization histochemistry was used to measure Homer1a mRNA expression in discrete cortical and subcortical ROI by different doses of short and long-term aripiprazole (step 1), different doses of short and long-term haloperidol (step 2), and behaviorally comparable doses of short and long-term haloperidol, aripiprazole, and olanzapine (step 3). A mixed 3way ANOVA was used to contemporarily account for all the independent effects and their combination. We found no significant interactions between topography of gene expression, timing of antipsychotic administration and dose/receptor profile. A consistent interaction between topography of gene expression and timing of antipsychotic administration was found, while no interactions between timing of antipsychotic administration and dose/receptor profile were demonstrated. At the univariate analysis, consistent topography and timing of administration effects were found. A dose effect was found for short-term haloperidol in both the striatum and the cortex. A receptor profile effect was found for short-term antipsychotics in striatum. Notably, dose and receptor profile effects were lost in long-term treatments.

These data may help making inferences on short and long-term antipsychotic-mediated modulation of glutamatergic activity and synaptic plasticity processes, which may putatively reflect their profile of clinical action and side effects.

### **Dysregulation of autophagy in Synj1-associated early-onset Parkinsonism**

Zerillo L.<sup>1</sup>, Valente V.<sup>1</sup>, Fasano D.<sup>1</sup>, Natale G.<sup>2</sup>, De Rosa A.<sup>2</sup>, Picillo M.<sup>3</sup>, Amodio G.<sup>4</sup>, Pellecchia M.T.<sup>3</sup>, Barone P.<sup>3</sup>, De Michele G.<sup>2</sup>, Nitsch L.<sup>1</sup>, Pierantoni G.M.<sup>1</sup>, Renna M.<sup>1</sup>, Remondelli P.<sup>3</sup>, Criscuolo C.<sup>2</sup>, Paladino S.<sup>1</sup>

<sup>1</sup>Dept Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italy

<sup>2</sup>Dept of Neuroscience, Reproductive, and Odontostomatological Sciences University of Naples Federico II, Naples, Italy

<sup>3</sup>Dept of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Salerno, Italy

Several genes responsible for some of the hereditary forms of Parkinson's disease are implicated in regulating membrane trafficking. We have recently highlighted that alteration of homeostasis and functions of early endosomal compartments is associated with early-onset parkinsonism caused by SYNJ1 mutations, emphasizing the role of endosomal trafficking in the pathogenesis of Parkinson's disease.

Alteration of lysosome structure was observed in Synj1-deficient cells, despite any substantial difference in the levels of two lysosomal markers, Lamp-1 and cathepsin D. Because trafficking toward lysosomes is unaffected upon Synj1 silencing, we hypothesized that the alteration of lysosomes could be due to changes in the autophagic pathway, whose activity is critical in many neurodegenerative diseases.

Higher levels of the autophagic markers, LC3-II and p62/SQSTM1, were observed in Synj1 silenced cells with respect to control interfered cells as well as the increase of the number of autophagosomes. In addition, the autophagic flux results perturbed as observed by using the tandem eGFP-mCherry-LC3 probe. Moreover, we also observed the alteration of signalling modulators of autophagy. Nevertheless, the clearance of autophagy substrates results reduced in Synj1 depleted cells.

All these data indicate a role of Synj1 in the autophagy pathway from one side and highlights a potential role of autophagy dysregulation in PARK20 pathogenesis from the other one.

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