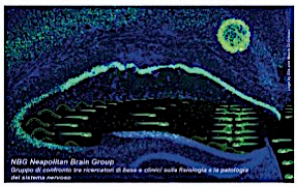
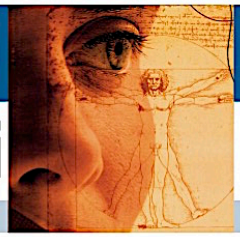


15 dicembre | ore 9.00 2016



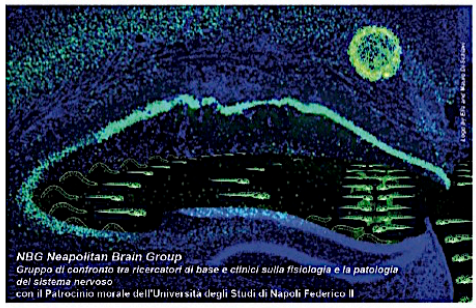
Seminari - Convegni

5° meeting del Neapolitan Brain Group

(Coordinatore: prof. Ennio Del Giudice)

LE NEUROSCIENZE NELL'AREA NAPOLETANA: PRIMA GIORNATA D'INCONTRO

Neapolitan Brain Group
Gruppo di confronto tra ricercatori di base e clinici sulla fisiologia e la patologia del sistema nervoso
con il Patrocinio morale dell'Università degli Studi di Napoli Federico II



ce in ge - BIOTECNOLOGIE AVANZATE
listening to biomolecules to silence disease

Venue:
Sala Convegni - CEINGE-Biotecnologie Avanzate, Via Gaetano Salvatore 486 - 80145, Napoli, Italy
(ingresso anche dal policlinico AOU Federico II di Napoli: via Pansini 5 o Via De Amicis)

Listening to biomolecules to silence disease

Il Neapolitan Brain Group (NBG)

L'NBG è un gruppo di confronto tra ricercatori di base e clinici dell'area napoletana (e, più in generale, campana), interessati allo studio della fisiologia e delle patologie del sistema nervoso. Il gruppo nasce nel 2015 su idea del Professor Ennio Del Giudice dell'Università degli Studi di Napoli Federico II, Dipartimento di Scienze Mediche Traslazionali (Sezione di Pediatria).
Il gruppo vuole quindi essere un'occasione di incontro, in un'atmosfera informale, per tutti gli appassionati di ricerca clinica e di base che intendano migliorare la reciproca conoscenza e, per quanto possibile, esplorare momenti di collaborazione proficua.
L'NBG è aperto a tutti coloro che siano interessati a queste tematiche, in particolare ai giovani in formazione, quali dottorandi, postdoc, specializzandi, studenti e tirocinanti delle Università e degli Enti di Ricerca.
Il 4 giugno 2015, il 4 febbraio ed il 28 aprile 2016 sono stati organizzati i primi incontri con brevi comunicazioni su invito, mentre il 9 giugno 2016 è stata organizzata una riunione monotematica di interesse generale su "Meccanismi molecolari, fisiopatologici e clinici nella neuroprotezione dell'ipossia neonatale". In queste riunioni hanno parlato più di 50 tra Ricercatori, Medici, Dottorandi, Postdoc e Specializzandi delle diverse Università napoletane, dell'Università di Salerno, del CNR, del TIGEM, dell'SZN e del CEINGE.
Per questa quinta riunione si è scelto di organizzare una giornata di brevi comunicazioni sulla base di proposte dei partecipanti al gruppo.

Comitato scientifico

Ennio Del Giudice (UNINA), L. Annunziato (UNINA), A. Usiello (CEINGE, SUN), Carla Lucini (UNINA), Elia Di Schiavi (IBBR, CNR), Mauro Cataldi (UNINA)

Per maggiori informazioni e per essere aggiornati sul gruppo visitate:
http://www.neapolitanbraingroup.it/
https://www.facebook.com/NBG2000/

Per essere inseriti alla mailing list scrivete a:
Elia Di Schiavi (elia.dischiavi@ibbr.cnr.it).

Gli organizzatori del gruppo NBG: Ennio Del Giudice (endelgiu@unina.it), Carla Lucini (lucini@unina.it), Mauro Cataldi (mauro.cataldi@unina.it), Elia Di Schiavi (elia.dischiavi@ibbr.cnr.it).

PROGRAMMA

Introduzione: presentazione dell'NBG 09.00 – 09.15

PRIMA SESSIONE Chair: F. Salvatore (CEINGE, UNINA), E. Del Giudice (UNINA)

MOLECULAR NEUROBIOLOGY 09.15 – 10.30
Imperatore R. (ICB,CNR)
Orexin, endocannabinoid and leptin interaction affects hypothalamic Tau phosphorilation.
Indrieri A. (TIGEM)
Synthetic long non-coding RNAs [SINEUPs] rescue defective gene expression in vivo.
Giuditta A. (UNINA)
Brain metabolic DNA in memory processing and genome turnover.
De Biase D. (UNINA)
Lipofuscin storage and autophagy dysregulation in aged bovine brain.
D'Angelo L. (UNINA)
Nothobranchius furzeri: a model for studying neurobiology of ageing in the fast lane.

NEURODEVELOPMENT/NEUROPHYSIOLOGY 10.30 – 11.45
D'Aniello S. (SZN)
Novel implications in Neurotrophins during the development of the nervous system
Dubbioso R. (UNINA)
Center-surround organization of the human sensorimotor system.
Esposito M. (SUN)
Developmental coordination disorder in children and sleep architecture: a case-control study.
Intartaglia D. (TIGEM)
MIR-204/211 in eye development and disease: an intricate relationship
Vitiello G. (UNINA)
An integrated clinical and genetic approach to corpus callosum abnormalities.

EPILEPSY AND EPILEPTIC DISORDERS 11.45 – 13.15
Miceli F. (UNINA)
Pathogenetic mechanisms for early-onset epileptic encephalopathy caused by mutations in Kv7.2 voltage-gated K+ channels.
Di Giaimo R. (UNINA)
Molecular and cellular role of cystatin B in cerebral cortex development and in the etiopathogenesis of EPM1.
Poeta L. (IGB, CNR)
Histone methylation-demethylation defects in forms of Intellectual Disability and Refractory Epilepsy: Analysis of disease-related pathways in in vitro and in vivo models.
Terrone G. (UNINA)
A novel combination of drugs with antioxidant effects prevents progression of epilepsy in a rat model of acquired epileptogenesis.
Cataldi M. (UNINA)
Carbachol-induced network oscillations in an in vitro limbic system brain slice.
Viggiano A. (UNISA)
Ketogenic diet prevents neuronal firing increase within the substantia nigra during pentylenetetrazole-induced seizure in rats.

BREAK: 13.15 – 14.00

SECONDA SESSIONE Chair: L. Annunziato (UNINA), A. Usiello (CEINGE, SUN)

PSYCHIATRIC DISORDERS 14.00 – 15.30
Buonaguro E.F. (UNINA)
Genetic-driven partial reduction of dopamine transporter function recapitulates ADHD- but not schizophrenia-related phenotypes.
Catone G. (SUN)
Psychotic like experiences in help seeking adolescents and relationship with bullying victimization.
De Risi M. (TIGEM)
Autism-like behavioral symptoms are associated to striatal dopamine system dysregulation in mucopolysaccharidosis type III-A
Cappuccio G. (UNINA)
A pseudogene increasing LRFN5 expression in a patient with 14q21.2 deletion and autism
Errico F. (CEINGE, UNINA)
Potential involvement of the NMDA receptor agonist D-aspartate in schizophrenia.
Guida F. (SUN)
Lactobacillus casei DG restores dysbiosis-induced gut inflammation, depressive behaviour and recognition memory deficit associated with physiological alterations in the hippocampus

NEURODEGENERATIVE DISORDERS 15.30 – 17.15
Colarusso A. (UNINA)
Towards the exploitation of CNF1 toxin as a potential treatment of some central nervous system diseases.
Penna E. (UNINA)
Local synthesis of APP in synaptosomal fraction from brain of Alzheimer's disease animal model
Di Schiavi E. (IBBR, CNR)
Identification of neuroprotective molecules using a C. elegans model of Spinal Muscular Atrophy
Pulcrano S. (IGB, CNR)
Dopaminergic differentiation using microRNAs.
Sorrentino N.C. (TIGEM)
New therapeutic approaches to treat CNS pathology in lysosomal storage disorders
Saracino D. (SUN)
A cluster of progranulin C157KfsX97 mutations in Southern Italy: clinical characterisation and genetic correlations.
Gallo V. (UNINA)
Neurological abnormalities in a SCID patient carrying mutation of ARTEMIS, ADA and ERCC6 genes identified through Next Generation Sequencing

NEUROIMAGING/BIOMARKERS 17.15 – 18.15
Tarallo A. (UNINA)
Micro-RNA as biomarkers in Pompe Disease.
Mazio F. (SUN)
Encephalic volumetric alterations in long-term survivor Acute Lymphoblastic Leukemia patients identified with VBM analysis.
Tedeschi E. (UNINA)
A model of possible applications of advanced MRI techniques in the field of inherited metabolic disorders: Fabry disease.
Vallelunga A. (UNISA)
Serum MIR-148b as a potential biomarker for Multiple System Atrophy:a pilot study.

Concluding remarks: E. Del Giudice 18.15 – 18.30

ABSTRACTS

MOLECULAR NEUROBIOLOGY

Orexin, endocannabinoid and leptin interaction affects hypothalamic Tau phosphorilation.

Imperatore R.[1], Palomba L.[2], Morello G.[1], Piscitelli F.[1], Forte N.[1], Di Costanzo A.[3], Cristino L.[1], Di Marzo V.[1]

1. Endocannabinoid Research Group -Institute of Biomolecular Chemistry, CNR, Pozzuoli, Naples, Italy;
2. Department of Biomolecular Sciences, University of Urbino "Carlo Bo", Italy;
3. Department of Medicine and Health Sciences, Località tappino, University of Molise, Campobasso, Italy

The hypothalamus is the most extensively interconnected area of the brain supporting energy homeostasis. Because of their intrinsic functional activities and the necessity to adapt to drastic changes in the nutritional status, neural feeding circuitries are endowed with synaptic plasticity modulated by neurotransmitters and hormones among which the leptin, ECs and OXA are the master regulators of energy homeostasis. Synaptic plasticity is strictly regulated by the fine balance between phosphorylated/unphosphorylated Tau protein mainly controlled by the GSK-3β activity. GSK-3β is constitutively active under resting conditions and is inactivated by extracellular signals like leptin through phosphorylation of Ser-9 residue. In opposition GSK-3β is activated by LPA-mediates Tyr-216 phosphorylation with subsequent phosphorylation of Tau. LPA is a bioactive lipid precursor of, or produced by, 2-AG. We found that leptin deficiency leads to an increase of OX-A-induced biosynthesis of 2-AG accompanied by a strong increase of pTau/Tau ratio, a condition which was reverted after acute i.p. leptin injection. On this basis we hypothesized a functional orexin/endocannabinoid/leptin interaction as upstream signaling for the regulation of Tau phosphorylation in the hypothalamus. In order to test this hypothesis we examined, in an *in vitro* model of hypothalamic cells and in samples of human obese subjects and patients affected by Alzheimer disease, whether a correlation exists between OX-A, 2-AG and LPA levels and how these molecole affect Tau phosphorylation. The understanding of the functional interaction between ECs and OX system in Tau phosphorylation could offer novel approaches for the study of tauopathies.

Synthetic long non-coding RNAs [SINEUPs] rescue defective gene expression *in vivo.*

Indrieri A.[1], Grimaldi C.[1], Zucchelli S.[2,3], Tammaro R.[1], Gustincich S.[2,4,5], Franco B.[1,6,*].

1. Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli (NA), Italy.
2. Area of Neuroscience, SISSA, Trieste, Italy.
3. Dipartimento di Scienze della Salute, Universita' del Piemonte Orientale, Novara, Italy.
4. Department of Neuroscience and Brain Technologies, Italian Institute of Technology, Genova, Italy
5. TransSINE Technologies, Yokohama, Japan.
6. Medical Genetics Services, Department of Translational Medicine, Federico II University, Naples, Italy.

Non-coding RNAs provide additional regulatory layers to gene expression as well as the potential to being exploited as therapeutic tools. Non-coding RNA-based therapeutic approaches have been attempted in dominant diseases, however their use for treatment of genetic diseases caused by insufficient gene dosage is currently more challenging. SINEUPs are long antisense non-coding RNAs that up-regulate translation in mammalian cells in a gene-specific manner, although, so far evidence of SINEUP efficacy has only been demonstrated in *in vitro* systems. We now show that synthetic SINEUPs effectively and specifically increase protein levels of a gene of interest *in vivo*. To address the potential use of this technology in increasing expression of an endogenous protein we tested SINEUPs in a medakafish model of microphthalmia with linear skin defects (MLS) syndrome, a rare neurodevelopmental mitochondrial disorder characterized by microphthalmia and brain abnormalities caused by increased cell death in the central nervous system (CNS). We demonstrated that SINEUPs rescue haploinsufficient gene dosage in the MLS medakafish model restoring the function of the mitochondrial respiratory chain and blocking the increased cell death in CNS thus consequently leading to amelioration of the disease phenotype.
Our results demonstrate that SINEUPs act through mechanisms conserved among vertebrates and that SINEUP technology can be successfully applied *in vivo* as a new research and therapeutic tool for gene-specific up-regulation of endogenous functional proteins.

Brain metabolic DNA in memory processing and genome turnover.

Prisco M., Casalino J. and Giuditta A.

Biology Dept. Federico II University, Naples

Brain metabolic DNA (BMD) was investigated in adult rats receiving [3H]thymidine and exposed to a variety of experimental conditions. Data indicated that BMD synthesis was not elicited by DNA damage or cell division but resulted from modifications of brain activity. Indeed, BMD markedly decreased after learning an appetitive task or a spatial habituation task and markedly increased after learning a passive avoidance task and a two-way active avoidance task. Comparable effects were reported by other laboratories (1,2). In addition, newly synthesized BMD i) suffered a large selective loss in non-learning rats allowed post-trial sleep; ii) was strongly inhibited by the brain noradrenergic bundle; and iii) was modulated by circadian oscillations with acrophase in the waking period. The circadian pattern became sharper in rats living in an enriched environment but disappeared in rats exposed to an impoverished environment. Interestingly, the content of brain DNA also manifested a circadian rhythm with acrophase delayed one hour with respect to the BMD acrophase. Recent data on CD1 mice receiving BrdU suggest that BMD synthesis is localized in astroglial processes as result of reverse transcription. The data demonstrate that BMD is directly involved in memory acquisition and processing and in genome turnover (3).

1. Reinis S. and Lamble R.W. (1972) *Physiol. Chem. Phys.*4: 335-338;
2 Ashapkin V. et al. (1983) *Biokhimija* 48: 355-362;
3. Giuditta A. et al., *Rev. Neurosci*.in press.

Lipofuscin storage and autophagy dysregulation in aged bovine brain.

De Biase D.[1], Costagliola A.[1], Pagano T.B.[1], Piegari G.[1], Wojcik S.[2], Dziewiątkowski J.[2], Grieco E.[3], Mattace Raso G.[4], Russo V.[1], Papparella S.[1], Paciello O.[1]

1. Department of Veterinary Medicine and Animal Production, University of Naples Federico II via Delpino, 1 80137 , Naples, Italy
2. Department of Anatomy and Neurobiology, Medical University of Gdansk, Debinki 1 80-211Gdansk, Poland
3. Azienda Sanitaria Locale, Salerno, Italy
4. Department of Pharmacy, University of Naples Federico II, Via Montesano 49, 80131 Naples, Italy

Aging is a natural process that affects most biological functions. Age-related lipofuscin accumulation in cerebral cells has been implicated in autophagy impairment leading to the persistence of cytotoxic damaged proteins and organelles. The aim of our study was to investigate if lipofuscin accumulation can be associated with the impairment of autophagy leading to pathologic proteins accumulation and apoptotic cell death in bovine brains. For our study, we collected samples from the brain of old (aged 11-20 years) and young (aged 1-5 years) Podolic dairy cows. Formalin-fixed and paraffin embedded sections were stained with routine and special staining techniques. Primary antibodies for Amyloid precursor protein (APP) and autophagy markers such as Beclin-1 and LC3 were used to perform immunofluorescence and Western blot analysis. Immunofluorescence for cleaved caspase-3 and β−amyloid1-42 was also performed. Histologically, the most consistent morphological finding was the age-related accumulation of intraneuronal lipofuscin. Furthermore, in aged bovine brains, immunofluorescence detected a strongly positive immunoreaction to APP, β-amyloid1-42, LC3 and Cleaved Caspase 3. Beclin-1 immunoreaction was weak or absent. In young controls, the immunoreaction for Beclin-1 and LC3 was mild while the immunoreaction for APP, β-amyloid1-42 and Cleaved Caspase 3 was absent. Western blot analysis confirmed an increased APP expression and LC3-II/LC3-I ratio and a decreased expression of Beclin-1 in aged cows. These data suggest that the age-related formation and accumulation of lipofuscin in aged bovine brains may be related to autophagy impairment and promote Aβ accumulation and the activation of the caspase cascade, ensuing apoptosis.

***Nothobranchius furzeri*: a model for studying neurobiology of ageing in the fast lane.**

D'Angelo L., Castaldo L., Lucini C., de Girolamo P.

Department of Veterinary Medicine and Animal Productions, University of Naples Federico II

Nothobranchius furzeri has emerged as an exciting vertebrate model system for ageing, due to its naturally compressed lifespan and short generation time. These features are a result of an adaptation to its seasonal habitat, characterized by ephemeral water pools in southeast Africa, where water is present only during the monsoon season. This fish maintains its compressed life span and short generation time in the laboratory, when water is in constant supply. Aged *N. furzeri* exhibits a comprehensive range of phenotypes, typical of vertebrate ageing, such as cognitive impairment, decline in mitochondrial function, and increased incidences of neoplastic lesions. Moreover, similar to other ageing model systems, the life span of this organism can be experimentally manipulated by environmental interventions such as dietary restriction, temperature and drug treatments. The *N. furzeri* brain has been studied with reference to its morphology and gene expression patterns. In course of ageing, the following cellular phenotypes have been observed: 1) dramatic reduction of stem cell activity; 2) glial hypertrophy (gliosis), visualized as over-expression of glial fibrillary acidic protein (GFAP); 3) neuronal degeneration, as measured by Fluoro-Jade B staining; and 4) accumulation of lipofuscin. The genome sequencing revealed that 7 genes in the region associated with lifespan had already been linked to the regulation of ageing or lifespan in humans or model organisms. For example, these include the gene encoding progranulin, which has been implicated in neurodegenerative diseases. It represents therefore a valuable model for addressing studies in the neurobiology of ageing.

NEURODEVELOPMENT/NEUROPHYSIOLOGY

Novel implications in Neurotrophins during the development of the nervous system

D'Agostino Y., Locascio A., Spagnuolo A., Ristoratore F., Sordino P. and D'Aniello S.

Biology and Evolution of Marine Organisms - Stazione Zoologica Anton Dohrn Napoli

Our research project is focused on the discovery of new roles of Neurotrophins (NT) and their receptors (TRK) during the development and physiology of the vertebrate nervous system. Neurotrophins (BDNF, NGF, NT3, NT4/5 and NT6/7) are growth factors that control development, differentiation, synaptic plasticity and survival of several types of neuronal and glial cells in the embryonic and adult central nervous system.
The project consists of a multidisciplinary study based on molecular, genetic, behavioural and bioinformatic approaches with the goal to acquire novel insights on the NT's regulatory networks. To reach this aim we generated a BDNF *knock-out* line in zebrafish using the CRISPR/Cas9 technology, and at the moment we are in the process of characterizing the fish phenotype as well as in analysing several transcriptomes that we have generated, with a particular attention to the transcriptional and translational regulatory roles of non-coding genes.
We expect that the spectrum of approaches used in this study will be instrumental to the development of a new animal model system that will be useful to improve our understanding of the pivotal role played by NTs in the evolution and function of the brain. Moreover, we will devote special commitment in trying to predict the biomedical impact of our discoveries in terms of diagnosis and treatment of human neurodegenerative diseases.

Center-surround organization of the human sensorimotor system.

Dubbioso R.[1,2]*, Raffin E.[1,3], Karabanov A.[1], Thielscher A.[1,4], Santoro L. [2], Manganelli F.[2], Siebner H.R.[1,5].

1. Danish Research Center for Magnetic Resonance, Center for Functional and Diagnostic Imaging and Research, Copenhagen University Hospital Hvidovre, Denmark.
2. Department of Neurosciences, Reproductive Sciences and Odontostomatology, University Federico II of Naples, Italy.
3. INSERM, U1216, Grenoble Institut des Neurosciences, GIN, F-38000 Grenoble, France
4. Department of Electrical Engineering, Technical University of Denmark, Kongens Lyngby, Denmark.
5. Department of Neurology, Copenhagen University Hospital Bispebjerg, Copenhagen, Denmark.

Surround inhibition (SI) is the capacity of an excited neuron to reduce the activity of its neighbours. This biological process is well known in the motor system to selectively activate a single muscle by inhibition of neural populations controlling neighbouring muscles. SI has also been demonstrated in the somatosensory cortex to help sharpen sensory perceptions. However, detailed studies about surround modulation mechanisms in the interaction between motor and sensory system are missing. Herein we evaluated in vivo surround modulation in the sensorimotor system by using a transcranial magnetic (TMS) stimulation technique, called short latency afferent inhibition (SAI). In SAI the cutaneous peripheral sensory stimulation at fingers precedes few milliseconds the TMS stimulation applied to the primary motor cortex (M1). We demonstrated that if the peripheral stimulation was applied to the II finger, the motor evoked potential (MEP) recorded from the first dorsal interosseous (FDI) muscle was inhibited (homotopic stimulation), whereas a facilitatory effect was observed for the surrounding abductor digiti minimi (ADM) muscle (heterotopic stimulation). Likewise, if we stimulated the V finger, the MEP recorded from the ADM muscle was inhibited (homotopic stimulation) with a facilitation for the surrounding FDI muscle (heterotopic stimulation). These center-inhibitory and surrounding-facilitatory mechanisms of the sensorimotor system had a precise cortical representation along M1, with the FDI muscle located more laterally respect to the ADM muscle. We showed for the first time the existence of a center-surround organization in the human sensorimotor system, somatotopically organized within M1.

Developmental coordination disorder in children and sleep architecture: a case-control study.

Esposito M., Precenzano F., Carotenuto M.

Department of Mental and Physical Health and Preventive Medicine, Child and Adolescents Neuropsychiatry Unit, Second University of Naples

Background: Developmental coordination disorder (DCD) is a discrete motor disorder recognized when acquisition and execution of coordinated motor skills are below what would be expected at a given chronologic age and opportunity for skill learning and use; difficulties are manifested as clumsiness and as slowness and inaccuracy of performance of motor skills. In 2012 by Barnett et al. explored the relationship between DCD and sleep behaviour using a questionnaire-based study. To the best our knowledge, there are no PSG study and/or NREM sleep instability (CAP) analysis studies in DCD children. Aims of the present study are the following: assessing the sleep macrostructure and CAP and their relationship with severity of movement alteration in a sample of DCD children Methods: 42 DCD children (26 M, 10.12 ± 1.98 years) and 79 typical developing children (TD) (49 M, 9.94 ± 2.84 years). All children underwent a motor-coordination, visual-motor integration and PSG evaluation. Results: DCD children showed significant reduction in sleep time parameters (TIB, p=.003; SPT p=.003; TST; p=.001) and in REM% representation (p<0.001) than TD. Moreover, DCD children showed significant differences in NREM instability organization than TD in particular regarding CAP fast components distribution that resulted significantly related with motor coordination performance. Conclusions: Motor coordination and visual-motor integration seem to be closely linked to the sleep architecture. Our findings may be interpreted as pilot study for further, more detailed studies on the link between night-time sleep mode and motor impairment in children.

MIR-204/211 in eye development and disease: an intricate relationship

Intartaglia D., Naso F., Falanga D., Salierno F.G., Bhat R., Barbato S., Pizzo M., Banfi S., Conte I.

Telethon Institute of Genetics and Medicine (TIGEM)ù

In recent years the role of small non-coding RNAs in the control of vertebrate eye development started to be explored. We demonstrated that miR-204 and miR-211, two paralogs that share the same seed sequence, play a crucial role for the development and function of the nervous system and the retina.
Particularly, we showed that miR-204 is required in the nervous system of Medaka fish for the control of axon guidance; moreover, we demonstrated that its inactivation results in microphtalmia, abnormal lens formation and altered dorsoventral patterning of the retina.
While the role of miR-204/211 in eye development has been well characterized, the relationship occurring between their expression and function and the onset of retinal diseases is still not completely characterized.

We performed loss of function studies in two *in vivo* models. We generated a miR-211 homozygous mutant mouse line and we are exploiting Crispr/Cas9 genome editing approach in order to create a Medaka double knockout for miR-204.

The functional analysis of miR-211 homozygous mutant mice revealed a progressive retinal degeneration characterized by a strong downregulation of photoreceptors markers together with an impaired electroretinogram response to light stimuli. The resulting phenotype mimics some of the pathological signs characteristic of retinal degenerative diseases, such as retinitis pigmentosa and age-related macular degeneration.

These preliminary results suggest a possible link between miR-211 loss of function and pathological processes in the retina. Thus, further studies will be needed to shed light on the therapeutic potential of miR-211 in the treatment of retinal degenerative disease.

An integrated clinical and genetic approach to corpus callosum abnormalities.

Vitiello G.[1], Borgatti R.[2], Boccia M.[1], Nitsch L.[3], Genesio R.[3], Zuffardi O.[4], D'Amico A.[5], Terrone G[1], The Italian CCA Study Group, Del Giudice E.[1].

1. Department of Translational Medicine, Section of Pediatrics, Federico II University, Naples, Italy.
2. Neuropsychiatry and Neurorehabilitation Unit, Scientific Institute, IRCCS Eugenio Medea, Bosisio Parini, Lecco, Italy.
3. Department of Molecular Medicine and Medical Biotechnologies, Federico II University, Naples, Italy.
4. Department of Molecular Medicine, University of Pavia, Pavia, Italy.
5. Department of Advanced Biomedical Sciences, Federico II University, Naples, Italy.

Corpus callosum abnormalities (CCAs) have an estimated prevalence ranging from 0.3% up to 1.0% in patients undergoing brain imaging. CCAs can be identified incidentally, or can be part of a developmental disease. Italian CCA Study Group has the aim to better define the genetic basis of CCAs through a multidisciplinary approach combining clinical, neuroimaging, and molecular studies.
We performed a retrospective study of 631 patients with radiologically identified isolated and syndromic CCAs, reviewing clinical features, neuroradiological aspects, genetic etiology, and chromosomal microarray (CMA) results.
Syndromic CCAs subjects were prevalent (62%) and they showed the most severe clinical features.
Cortical malformation due to neuron migration defect was 11% of cerebral malformation associated to CCAs, followed by posterior fossa anomalies. 27% of syndromic CCAS have clinical genetic diagnosis, including chromosomal rearrangements on high resolution karyotype (21%), microdeletion/microduplication syndrome (36%) and monogenic disease (43 %).
Isolated CCAs anomalies have mildest clinical features, although intellectual disability was present in 48%of cases and epilepsy in 12%. CMA study only in 6% of isolated CCAs showed pathogenetic copy number variants (CNVs), encompassing causative genes associated to neuropsychiatric problems.
A high percentage of patients remains without a diagnosis, Next-generation sequencing (NGS) strategies will increase the probability to identify new causative genes of CCAs and to redefine genotype-phenotype correlation.

EPILEPSY AND EPILEPTIC DISORDERS

Pathogenetic mechanisms for early-onset epileptic encephalopathy caused by mutations in Kv7.2 voltage-gated K+ channels.

Miceli F.[1], Soldovieri M.V.[2], Ambrosino P.[2], Mosca I.[2], De Maria M.[2], Manocchio L.[2], Medoro A.[2], Cimino M.[1], Onore M.E.[1], Millichap J.J.[3], Cooper E.C.[4], and Taglialatela M.[1,2].

1. Department of Neuroscience, University of Naples Federico II, Naples, Italy;
2. Department of Medicine and Health Science, University of Molise, Campobasso, Italy;
3. Epilepsy Center and Division of Neurology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA;
4. Departments of Neurology and Neuroscience and Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA.

Changes in gating of voltage-dependent ion channels are often responsible for genetic channelopathies. Voltage-gated K channels of the Kv7.2 subfamily have a crucial pathogenetic role in neonatal-onset epilepsies; Kv7.2 mutations are responsible for heterogeneous forms of rare neonatal epilepsies, ranging from benign neonatal convulsions to severe early-onset encephalopathy (EOEE). In our studies, by means of an international expert-curated registry (www.rikee.org), we have explored the pathogenetic disease mechanisms in patients carrying *de novo* missense Kv7.2 variants causing EOEE; in this work, we have focused on two recurrent variants leading to amino acid changes in distinct and functionally-relevant regions of the protein, namely the R198Q in the voltage-sensing S4 transmembrane region, and the R325G in the proximal C-terminus immediately past the core transmembrane domain, each found independently in four affected individuals. The functional consequences of these mutations have been addressed by a combination of electrophysiological, biochemical, modeling, and immunocytochemical approaches. The results obtained suggest that, Kv7.2 R198Q channels are functional and exhibit an enhanced voltage sensitivity, possibly due to a stabilization of the activated configuration of the voltage sensor itself (1). On the other hand, Kv7.2 R325G channels are non-functional, and this appears as the consequence of an impaired regulation by PIP2, a critical phosholipid controlling electromechanical coupling (2). These novel results highlight novel and distinct pathogenetic mechanisms for Kv7.2-EOEE, which provide important criteria for patient stratification and, possibly, personalized treatment.

References
1. Millichap J.J. et al., *Epilepsia* (2016, in press)
2. Soldovieri M.V. et al., *Scientific Report* (2016, in press)

Molecular and cellular role of cystatin B in cerebral cortex development and in the etiopathogenesis of EPM1.

Di Giaimo R.[1,2,3], Cappello S.[2], Goetz M.[3,4].

1. Department of Biology, University of Napoli, Complesso Universitario Monte S.Angelo, via Cinthia 80126 Napoli, Italy
2. Max Planck Institute of Psychiatry, Kraepelinstraße 2-10, 80804 München, Germany
3. Institute of Stem Cell Research, Helmholtz Center Munich, German Research Center for Environmental Health, D-85764 Neuherberg, Germany.
4. Physiological Genomics, Biomedical Center, Ludwig-Maximilians University Munich, D-82152 Planegg, Germany.

Progressive myoclonus epilepsy of the Unverricht–Lundborg-type (EPM1) is an autosomal recessive neurodegenerative disorder that has the highest incidence among the progressive myoclonus epilepsies worldwide. Loss of-function mutations in the gene encoding CYSTATIN-B (CSTB) are the primary genetic cause of EPM1. CSTB-KO mice show neurological disorder in mice similar to EPM1-patients. We studied the expression of CSTB in the mouse developing cortex and found CSTB enriched in the neural-stem-cells where it co-localizes with a microtubular marker. The physiological function of CSTB in the CNS and the dysfunction caused by the mutants are still unknown. Our results show for the first time that CSTB is secreted and found in the embryonic cerebrospinal-fluid. Interestingly, cystatin-C, another protein of the Cystatin-family, is a secreted protein and its overexpression can rescue the CSTB-KO phenotype. We also identified CSTB in the conditioned-medium of human cerebral organoids. Wt-CSTB is secreted when overexpressed in mouse primary cells from embryonal cortex and this is not the case for a pathological mutant. Overexpression of CSTB and a pathological mutant by in-utero electroporation in mouse developing cortex, show opposite effect on recruitment of inhibitory interneurons and NG2+oligodendrocyte progenitors suggesting that CSTB acts as attractive cue for these two populations of ventrally derived cells. Interestingly, wt-CSTB or the EPM1-mutant induce formation of an additional band of intermediate progenitors, absent in the contra-lateral hemisphere. Our results clearly indicate a neurogenic role of CSTB during cortical development, which is altered by overexpression of the protein or by the expression of an EPM1-mutant form.

Histone methylation-demethylation defects in forms of Intellectual Disability and Refractory Epilepsy: Analysis of disease-related pathways in *in vitro* and *in vivo* models.

Poeta L.[1], Padula A.[1], Ranieri A.[1], Attianese B.[1], Valentino M.[1], Shoubridge C.[2], Helin K.[3], Gecz J.[2], Di Schiavi E.[4], Filosa S.[4], Schwartz C.[5], Altucci L.[1,6], vanBokhoven H.[7], Miano M.G.[1]

1. Institute of Genetics and Biophysics "Adriano Buzzati Traverso", CNR, Naples 80131, Italy
2. Department of Paediatrics, University of Adelaide, South Australia 5006, Australia
3. Centre for Epigenetics, University of Copenhagen, Copenhagen DK-2200, Denmark
4. Institute of Bioscience and Bioresources, CNR, Naples 80131, Italy
5. Greenwood Genetic Center, Greenwood, South Carolina. 29646, USA
6. Second University of Naples, Naples 80100, Italy
7. Radboud University Nijmegen Medical Centre, 6500 HB, Nijmegen, The Netherlands

Mistakes in histone methylation-demethylation rounds have been directly involved in several forms of Intellectual Disability (ID) with Epilepsy and/or Refractory Epilepsy (RE). Lysine-specific demethylase 5C (*KDM5C*) is an X-linked gene, which encodes a chromatin JmjC eraser with H3K4me2/3 demethylase activity. *KDM5C* is frequently mutated in a spectrum of X-linked ID (XLID) and/or RE. It functions as a transcriptional repressor that is critical for transition of neural progenitors to neurons. We identified a disease path, linking functionally *KDM5C* to another XLID/Epilepsy gene, encoding the homeotic transcription factor ARX, whose mutations impair severely *KDM5C* transcript regulation. Furthermore, we analysed two additional XLID proteins that also bind *KDM5C* promoter. They are PHD Finger Protein 8 (PHF8), a H3K9 demethylase; and Zinc Finger Protein 711 (ZNF711), a transcriptional factor, which role is almost unknown. We observed that PHF8 and ZNF711, which co-occupy the target promoter, induce cooperatively the *KDM5C* stimulation. We propose that the transcriptional induction by ARX does not synergize with the action of the PHF8/ZNF711 complex. We screened a number of compounds targeting chromatin enzymes. We used as cell disease model neuronally-differentiated *Arx* KO/Kdm5C-depleted ES cells that show GABAergic abnormalities in association with a global increase of H3K4me3 signal. We are testing *in vivo* epi-treatments in several *ARX* disease models, in worm and mouse, presenting specific defects in neuronal structures and functions. Ongoing efforts will allow us to identify druggable hallmarks that could open up towards the exploitation of potential strategies to treat the growing group of ID and RE diseases caused by defects in chromatin and/or transcriptional regulators.

A novel combination of drugs with antioxidant effects prevents progression of epilepsy in a rat model of acquired epileptogenesis.

Terrone G.[1,2], Pauletti A.[1], Pastore A.[3], Antoine D.J.[4], Balosso S.[1], Pascente R.[1], Salamone A.[1], Villa B.R.[1], Ravizza T.[1], Del Giudice E.[2], Vezzani A.[1]

1. Dipartimento di Neuroscience, IRCCS-Istituto di Ricerche Farmacologiche "Mario Negri";
2. Dipartimento di Medicina Translazionale, Sezione di Pediatria Università Federico II di Napoli;
3. Unità di Metabolomica e Protemica, IRCCS-Ospedale "Bambino Gesù", Roma,
4. MRC Centre for Drug Safety Science, Department of Molecular & Clinical Pharmacology, University of Liverpool, Liverpool, UK

Aim: We investigated whether oxidative stress generated during epileptogenesis can be efficiently resolved by a transient treatment with N-acetyl-cysteine (NAC) and sulforaphane (SFN), two drugs known to raise the levels of the antioxidant glutathione (GSH). The main scope was testing their therapeutic effects on spontaneous seizures, cell loss and comorbidites.
Methods: Using a rat model of acquired epilepsy induced by electrically-provoked status epilepticus (SE), we studied oxidative stress generation during epileptogenesis in SE-exposed rats by measuring brain and plasma disulfide HMGB1 and GSSG/GSH levels by LC-MS/MS and HPLC, respectively, and by analyzing oxidative stress markers (Nrf2, iNOS, Xct) by immunohistochemistry. We tested the effects of anti-oxidant drugs (NAC and SFN, administered intraperitonally 1 h post-SE for 2-weeks) on blocking oxidative stress and HMGB1 oxidation, as well as on seizure onset and their progression by EEG analysis. Cognitive deficits and neuronal death were also analyzed.
Results: The combined treatment decreased oxidative stress more efficiently than either drug alone, and decreased disulfide HMGB1 in brain and blood. As compared to vehicle-injected rats, treatment significantly delayed the onset of the first spontaneous seizure, blocked the disease progression and rescued the cognitive deficits. Drugs prevented the loss of calretinin-positive hilar interneurons and CA1 pyramidal cells.
Conclusion: A transient post-injury intervention with antioxidant drugs mediates clinical relevant therapeutic effects in a rat model of SE-induced epilepsy. Amelioration of oxidative stress during epileptogenesis interferes with disease onset and its progression and improves comorbidities. These drugs may have disease-modifying effects in patients exposed to potential epileptogenic injuries.

Carbachol-induced network oscillations in an *in vitro* limbic system brain slice.

Cataldi M.[1], Lévesque M. [2], Vigliotti C.[1], Chen L.Y.[2], Hamidi S.[2], Avoli M.[2].

1. Division of Pharmacology, Department of Neuroscience, School of Medicine, "Federico II" University of Naples, Naples, Italy
2. Montreal Neurological Institute and Department of Neurology & Neurosurgery, McGill University, 3801 University Street, Montréal, PQ, Canada H3A 2B4

We employed simultaneous field potential recordings from CA3, subiculum and entorhinal cortex in an *in vitro* brain slice preparation to understand the involvement of these limbic areas in the generation of the field potential oscillations that are induced by bath application of the muscarinic receptor agonist carbachol. Regularly spaced oscillations that mainly presented at theta frequency range (5-12 Hz) occurred synchronously in all three structures in the presence of carbachol. These oscillations, which disappeared when slices were perfused with pirenzepine or with glutamatergic receptor antagonists, were categorized as short (< 4 s) and long (> 4s) with short events oscillating at higher frequencies than long events. Field oscillations were highly synchronized between regions and latency analysis revealed that they often initiated in the entorhinal cortex later than in the other two structures. Blocking $GABA_A$ receptors modified the activity patterns of both short and long oscillations and decreased their coherence in the theta frequency range. Finally, blocking KCC2 activity disclosed a pattern of recurrent short oscillations. Our results suggest that in the presence of carbachol both subiculum and CA3 most often drive theta generators in the entorhinal cortex and that these oscillations are influenced but not abolished by altering $GABA_A$ receptor signaling.

Ketogenic diet prevents neuronal firing increase within the substantia nigra during pentylenetetrazole-induced seizure in rats.

Viggiano A.[1], Stoddard M.[2], Pisano S.[3], Operto F.F.[3], Iovane V.[4], Monda M.[5], Coppola G.[6].

1. Dept. Medicine and Surgery, University of Salerno, Baronissi, SA, Italy. Electronic address: aviggiano@unisa.it.
2. Dept. Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA.
3. UO Child and Adolescent Neuropsychiatry, Medical School, University of Salerno, Salerno, Italy.
4. Dept. Medicine and Surgery, University of Salerno, Baronissi, SA, Italy.
5. Dept. Experimental Medicine, Second University of Naples, Naples, Italy.
6. Dept. Medicine and Surgery, University of Salerno, Baronissi, SA, Italy; UO Child and Adolescent Neuropsychiatry, Medical School, University of Salerno, Salerno, Italy.

The mechanism responsible for the anti-seizure effect of ketogenic diets is poorly understood. Because the substantia nigra pars reticulata (SNr) is a "gate" center for seizures, the aim of the present experiment was to evaluate if a ketogenic diet modifies the neuronal response of this nucleus when a seizure-inducing drug is administered in rats. Two groups of rats were given a standard diet (group 1) or a ketogenic diet (group 2) for four weeks, then the threshold for seizure induction and the firing rate of putative GABAergic neurons within the SNr were evaluated with progressive infusion of pentylenetetrazole under general anesthesia. The results demonstrated that the ketogenic diet abolished the correlation between the firing rate response of SNr-neurons and the seizure-threshold. This result suggests that the anti-seizure effect of ketogenic diets can be due to a decrease in reactivity of GABAergic SNr-neurons.

PSYCHIATRIC DISORDERS

Genetic-driven partial reduction of dopamine transporter function recapitulates ADHD- but not schizophrenia-related phenotypes.

Buonaguro E.F.[1], Mereu M.[2], Contarini G.[2,3], Avagliano C.[1], Latte G.[1], Manago' F.[3], Iasevoli F.[1], Papaleo F.[3], de Bartolomeis A.[1].

1. Department of Neuroscience, Reproductive and Odontostomatological Science, University School of Medicine "Federico II", Naples, Italy
2. Department of Pharmaceutical Science, University of Padova; Padova, Italy
3. Department of Neuroscience and Brain Technologies, Istituto Italiano di Tecnologia, via Morego 30, 16163 Genova, Italy

Attention deficit hyperactivity disorder (ADHD) and schizophrenia (SZ) are neuropsychiatric diseases with a strong genetic component. These diseases share alterations in the dopaminergic system, but if and how dopamine-related genetic variations might differentially lead to ADHD or SZ is not yet clear. Variations in the dopamine transporter (DAT) were suggested to be potentially implicated in all of them, and DAT null mutant mice (DAT-/-) have been extensively studied. However, these mice exhibit extreme phenotypes more relevant to the dystonia-parkinsonism syndrome. Here, we studied DAT hypo-functional mice (DAT+/-) to investigate the selective DAT-dependent alterations in psychiatric-relevant developmental trajectories. Attentional and impulsivity deficits were evident in DAT+/- male mice. At the molecular level, DAT+/- mice showed a selectively reduced expression of *Homer1a* in the PFC, while other brain regions were mostly unaffected. Similarly, no DAT-dependent effect was evident for both Arc and *Homer1b* expression throughout the brain. Low doses of amphetamine reverted DAT+/- hyperactive phenotype. Notably, amphetamine shifted DAT-dependent *Homer1a* altered expression from PFC to striatal regions. Both behavioral and molecular phenotypes observed appear to be more closely reminiscent of an ADHD phenotype rather than other dopamine-mediated psychiatric diseases, such as SZ. These findings suggest that a genetic-driven condition of DAT hypo-function might cause aberrations of the neurodevelopmental trajectory consistent with ADHD.

Psychotic like experiences in help seeking adolescents and relationship with bullying victimization.

Catone G., Marotta R., Pisano S., Lennox B., Carotenuto M., Gritti A., Pascotto A., Broome M.R.

1. Department of Mental and Physical Health and Preventive Medicine, Second University of Naples – SUN - Italy
2. Faculty of Educational Science, Suor Orsola Benicasa University – Naples – Italy
3. Department of Psychiatry, University of Oxford – Oxford - UK

Background: Psychotic like experiences (PLEs) are common in the general population and increase the risk of psychotic disorders. Adolescent are at high risk group for this condition. Stressful events, such as bullying, have a role in the onset of PLEs.
Aims: to assess PLEs in an adolescent help-seeking population from a child and adolescent mental health service, and to assess the association among them and bullying victimization.
Methods: Participants were help seeking (HS) adolescents, referring to a Child and Adolescents Neuropsychiatric clinic, initially screened for PLEs. They completed an assessment including characteristics of PLEs and bullying victimization. We paid particular attention to different kind of PLEs and victimization.
Results: 50 PLEs positive adolescents screened from 324 HS (15,4%) constituted the sample. Paranoia and verbal bullying were the most frequent PLEs and form of victimization represented, respectively. Verbal bullying was strongly associated with paranoia (O.R: 4.40, C.I: 2.8-5.9, $p<0.001$). Results remained significant after controlling for confounders (socio-demographics, measures of anxiety and depression). Furthermore, social manipulation showed a good association with paranoia, and physical bullying with grandiosity. Verbal bullying was also associated with psychotic negative symptoms, but on controlling for emotional symptoms and other forms of victimization, this led to a reduction of the effect.
Conclusion: PLEs are relevant in HS adolescent. Bullying victimization was associated with these phenomena. In particular, verbal bullying and social manipulation predicted paranoia score significantly.

Autism-like behavioral symptoms are associated to striatal dopamine system dysregulation in mucopolysaccharidosis type III-A

De Risi M.[1,2], Tufano M.[3], Carboncino A.[3], Sorrentino N.C.[1], Sambri I.[1], Ballabio A.[1], Fraldi A.[1] and De Leonibus E.[1,3]

1. Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli (Naples), Italy
2. PhD School in Behavioral Neuroscience, University of Rome "La Sapienza", Rome, Italy
3. Institute of Genetics and Biophysics, CNR, Naples, Italy

Mucopolysaccharidosis III-A (MPS-IIIA) is neurodegenerative lysosomal storage disorders caused by deficiency of enzyme sulfamidase. As consequence, the heparan sulfate (HS) accumulate inside cells, finally leading to neuronal death. The pathology early manifests with autism-like behavioural symptoms (ALBSs), including self-injury, stereotypic behaviours, social behaviour dysfunctions and then show dementia and motor impairment in paediatric age. ALBSs in MPS-IIIA have dramatic impact on children and parents life and are resistant to behavioural and antipsychotic therapies. Although this the disease mechanisms leading to them remain unexplored. In this study we explored the hypothesis that ALBSs in MPS-IIIA precede neurodegeneration, and are due to HS-mediated changes in dopamine (DA) metabolism occurring in the first stage of the pathology.
We first identified ALBSs endophenotypes in young male MPS-IIIA KO mice, such as reduced pre-pulse inhibition (PPI) and behavioural response to cumulative doses of the classical antipsychotics haloperidol. Interestingly MPS-IIIA KO systematically retract from social contact when confronted with WT littermates in the social interaction tube test. ALBSs progresses toward generalized behavioural dysfunction in old male mice, thus fully recapitulating the human clinical progression. ALBSs are associated to striatal increase in tyrosine hydroxylase (TH), the DA synthesis-rating enzyme, in the absence of neurodegeneration and lysosomal dysfunction makers.
These findings identify in MPS-IIIA mice, for the first time, ALBSs and associated striatal DA dysregulation preceding lysosomal dysfunction, which is fundamental for designing appropriate antipsychotic therapy.

A pseudogene increasing LRFN5 expression in a patient with 14q21.2 deletion and autism

Cappuccio G.[1,2], Alagia M.[1], Borzone R.[2], Genesio R.[2], Mormile A.[2], Nitsch L.[2], Granese B.[1,2], Del Giudice E.[1], and Brunetti-Pierri N.[1]

1. Department of Translational Medicine, Section of Pediatrics, Federico II University, Naples, Italy.
2. Telethon Institute of Genetics and Medicine, Pozzuoli, Naples, Italy.
3. Department of Molecular Medicine and Medical Biotechnology, Federico II University, Naples, Italy.

Autism spectrum disorder (ASD) is a disorder with impaired social relationships, language and communication that are frequently associated with intellectual disability (ID). Underlying molecular defects can be identified in 30–40% of ASD patients using chromosomal microarray analysis and whole exome sequencing. We report a 16 year-old boy with ASD bearing a microdeletion at chromosome 14q21.2 inherited from the father who has borderline cognitive impairment. The deletion affects a '*gene desert*' and *LRFN5* is the closest gene in the non-deleted interval. *LRFN5* encodes a protein involved in synaptic plasticity that has been heavily implicated in neurobehavioral phenotypes. We hypothesized a positional effect of the pseudogene chr14.232.a included within the deleted region that is predicted to bind the miRNAs miR-3689a-5p and miR-718 targeting the 3'-UTR of the *LRFN5* gene. In agreement with this hypothesis, we found decreased expression of both *LRFN5* gene and chr14.232.a pseudogene in the proband's fibroblasts compared to controls. Interestingly, following transfection of the chr14.232.a pseudogene in the patient's fibroblasts, *LRFN5* expression was increased. Based on the data generated so far, we speculate that the chr14.232.a pseudogene functions as a miRNA decoy to regulate *LRFN5* expression through sequestration of *LRFN5* specific miRNAs. In conclusion, this study may unravel a novel mechanism of gene regulation involved in neurodevelopmental disorders.

Potential involvement of the NMDA receptor agonist D-aspartate in schizophrenia.

Errico F.[1,2], Nuzzo T.[1,3], Napolitano F.[1,3], Punzo D.[1,3], Di Maio A.[1], De Rosa A.[1], Usiello A.[1,3]

1. Laboratory of Behavioural Neuroscience, Ceinge Biotecnologie Avanzate, Naples.
2. Department of Molecular Medicine and Medical Biotechnology, University of Naples "Federico II".
3. Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, Second University of Naples (SUN), Caserta.

Increasing evidence points to hypofunction of N-methyl D-aspartate receptors (NMDARs) in schizophrenia. D-aspartate is an atypical amino acid that activates NMDARs through the binding to their glutamate site. D-aspartate occurs abundantly in the embryonic brain of mammals and rapidly decreases after birth, due to the postnatal onset of D-Aspartate Oxidase (DDO) catabolic activity. The agonistic role of D-aspartate on NMDARs and its neurodevelopmental occurrence make this D-amino acid a potential mediator for some of the NMDAR-related alterations observed in schizophrenia. Accordingly, we found that non-physiological, increased D-aspartate levels in knockout mice for *Ddo* gene (*Ddo-/-*) are associated to enhancement of dendritic length and spine density in cortical and hippocampal pyramidal neurons, improvement of memory and brain connectivity, and protection against sensorimotor gating deficits and abnormal circuits activation induced by the psychotomimetic drug, phencyclidine. Our detection of D-aspartate content in *post-mortem* brains has shown a significant 40% reduction of this D-amino acid in the prefrontal cortex of patients with schizophrenia, compared to non-psychiatric subjects. We have recently replicated these results in a new and larger cohort of *post-mortem* cortical samples since we found a significant 30% decrease in D-aspartate levels selectively in the dorsolateral prefrontal cortex, but not in the hippocampus, of schizophrenia-affected patients. Interestingly, such reduction is associated to increased enzymatic activity of DDO in patients with schizophrenia. Overall, our results in mice and humans suggest the potential involvement of altered D-aspartate metabolism as a factor contributing to dysfunctional NMDAR-mediated transmission in schizophrenia.

***Lactobacillus casei* DG restores dysbiosis-induced gut inflammation, depressive behaviour and recognition memory deficit associated with physiological alterations in the hippocampus**

Guida F.[1], Turco F.[2], Iannotta M.[1], Furiano A.[1], De Gregorio D.[1], Palumbo I.[2], Sarnelli G.[2], Mazzitelli M.[1], Napolitano F.[3,5], Boccella S.[1], Luongo L.[1], Palazzo E.[1], Usiello A.[3,4] de Novellis V.[1], Cuomo R.[2] and Maione S.[1]

1. Department of Experimental Medicine, Section of Pharmacology, Second University of Naples, Naples, Italy
2. Department of Clinical Medicine and Surgery, Federico II University of Naples, Naples, Italy
3. Ceinge Biotecnologie Avanzate, Naples, Italy
4. Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, Second University of Naples, Caserta, Italy.
5. Department of Molecular Medicine and Medical Biotechnology, University of Naples "Federico II", Naples, Italy.

Background and aims: The microbiota-gut-brain axis (MGBA) represents the main substrate where the reciprocal interaction between the chronic inflammatory bowel and psychiatric disorders takes place. Such communication involves multiple pathways that are highly debated. Here, we have investigated the supraspinal biomolecular mechanisms altering affective behavior in dysbiosis condition.
Methods We examined the behavioral, the biochemical and electrophysiological alterations in an antibiotic-induced experimental dysbiosis. Inflammation of small intestine has also been assessed. Mice were exposed to a mixture of antimicrobials for 2 weeks. Afterwards, they received *Lactobacillus casei* DG or vehicle up to 7 days via oral gavage.
Results: Perturbation in microbiota induced an overall gut inflammatory state, accompanied by sickness behaviors, including increased immobility in the tail suspension test and reduced social recognition in the social recognition paradigm. Altered behavior was associated with changes in the BDNF/TrkB signaling and neuronal firing activity in the hippocampus. Moreover, morphological rearrangements of non-neuronal cells in brain areas controlling emotional behavior were observed. *LCDG* counteracted the gut inflammation and restored the behavioural as compared with the control mice receiving vehicle. Same treatment also normalized the biochemical and functional changes occurring in certain brain areas.
Conclusions: Our findings clarify some of the supraspinal biomolecular modifications leading to behavioural alterations associated with gut dysbiosis. Probiotic treatment restored intestinal immune environment and normalized mice behaviour. This study suggests that intestinal microbiota

perturbation might contribute to the affective disorder development in patients with inflammatory bowel disorders.

NEURODEGENERATIVE DISORDERS

Towards the exploitation of CNF1 toxin as a potential treatment of some central nervous system diseases.

Colarusso A.[1], Parrilli E.[1], Fabbri A.[2], Fiorentini C.[2], and Tutino M.L.[1].

1. Dipartimento di Scienze Chimiche, Università di Napoli Federico II, Complesso Universitario M. S. Angelo, via Cintia Napoli.
2. Istituto Superiore di Sanità, Viale Regina Elena, Roma

The CNF1 toxin constitutively activates Rho GTPases in eukaryotic cells and induces their proteasomal degradation, a process exploited by *Escherichia coli* pathogenic strains to increase their invasion into endothelial cells1. Nevertheless, as Rho GTPases regulate actin cytoskeleton remodeling, they directly influence neuronal growth and their fine-tuning could be exploited in the treatment of severe CNS disorders. Following this rationale, CNF1 injection in the brain has been demonstrated to rescue the motor and cognitive deficits displayed by mice models of Rett syndrome, Alzheimer's disease and Parkinson's disease2-5. CNF1 treatment effects result (amongst the other) in counteracting abnormal IL-6 cytokine levels, bioenergetics and mitochondria dysfunctions observed in these pathologies.
Considering CNF1 promising applications, the definition of the most suitable route of administration of this toxin is an important issue that should be taken into account for its development as a safe therapeutic agent. In particular, the employment of CNF1 BBB crossing engineered constructs may allow the replacement of the invasive icv injection with a simple intravenous administration. In this context, our research group is developing a series of CNF1 protein variants enriched of functional and structural peptides which might promote the fulfillment of the abovementioned aim. So far, we managed to recombinantly produce some promising toxin variants and demonstrate the preservation of their catalytic and biological activity on cultured cells. Currently, we are looking for collaborators who can deepen the functional characterization of our CNF1 variants both in terms of therapeutic efficacy and toxicity.

1. Doye A, et al. (2002). CNF1 exploits the ubiquitin-proteasome machinery to restrict Rho GTPase activation for bacterial host cell invasion. Cell. 2002 Nov 15;111(4):553-64.
2. De Filippis B, et al. (2012). Modulation of RhoGTPases improves the behavioral phenotype and reverses astrocytic deficits in a mouse model of Rett syndrome. Neuropsychopharmacology. 37(5):1152-63.
3. Loizzo S, et al. (2013). CNF1 increases brain energy level, counteracts neuroinflammatory markers and rescues cognitive deficits in a murine model of Alzheimer's disease. PLoS One. 8(5):e65898.
4. Musilli M, et al. (2013). Behavioral effects of Rho GTPase modulation in a model of Alzheimer's disease. Behav Brain Res. 2013 Jan 15;237:223-9.

5. Musilli M, et al. (2016) Therapeutic effects of the Rho GTPase modulator CNF1 in a model of Parkinson's disease. Neuropharmacology. 109:357-65.

Local synthesis of APP in synaptosomal fraction from brain of Alzheimer's disease animal model

Cefaliello C.[1], Penna E.[1], Barbato C.[1], Lippiello P.[2], Perrone-Capano C.[2], Miniaci M.C.[2], Crispino M.[1]

1. Department of Biology,
2. Department of Pharmacy, University of Naples Federico II, Naples, Italy

It is now well accepted that the presynaptic domains of axons are endowed with a strategic local system of protein synthesis, independent of the cell body, that makes key contributions to synaptic plasticity. Nonetheless, it was not much explored whether its deregulation leads to the neurodegerative diseases. In this study, we used transgenic mice overexpressing APP as an animal model (TG) for Alzheimer's disease (AD), and examined alteration of the capability of local protein synthesis in the synaptosomes. The synaptosomal fraction of brain homogenate is enriched with presynaptic microdomains, and thus has served as a very useful tool for studying synaptic protein synthesis. Our data showed that the synaptic synthesis of about 90 kDa protein is strongly enhanced in the synaptosomal fraction of 2 months old TG mice's cerebral cortex and cerebellum compared to control mice. This 90 kDa protein was finally identified as APP by use of click-it metabolic labeling and reaction. In addition, few other newly synthesized proteins were found to be differentially expressed in the synaptosomes of TG. These results suggests that the modulation of synaptic protein synthesis may be part of the molecular mechanism leading to synaptic degeneration in AD.

Identification of neuroprotective molecules using a *C. elegans* model of Spinal Muscular Atrophy

Gallotta I., Mazzarella N., Camera A., Visone S., Zampi G., Di Schiavi E.

Institute of Bioscience and BioResources (IBBR), CNR, Via P. Castellino 111, Naples, Italy

Neuronal degeneration underlies serious pathologies that cause extreme personal discomfort and social costs. Molecules and pathways that prevent neuronal death are largely unknown. Uncovering them will help design strategies aimed at preventing neurodegeneration and shed new light on the molecular mechanisms underlying neuron survival. We took advantage of a Spinal Muscular Atrophy (SMA) model, generated using cell-specific RNAi, as a tool to discover natural compounds, synthetic drugs and genes presenting a neuroprotective function. Spinal muscular atrophy is a neuromuscular disorder characterized by the selective degeneration of lower spinal cord motor neurons, which leads to progressive muscle atrophy and death. SMA is caused by mutations of the Survival of Motor Neuron gene, *Smn1*, and although the genetic bases of SMA have been extensively studied, no effective treatment is available yet. We developed an innovative genetic model which enabled us to efficiently reduce the function of *smn-1* gene specifically in motor neurons. Transgenic strains in which *smn-1* is knocked down, present an age-dependent and progressive degeneration of motor neurons that results in altered backward movement associated to neuronal cell degeneration and death. Using this genetic model we screened a panel of chemicals, natural compounds and natural extracts and we found a number of conditions that can rescue cell death but not the onset of the degenerative process, while others fully protect neuronal integrity and survival. By candidate gene approach and by random mutagenesis we also identified some of the genetic interactors that, when mutated, are able to completely prevent neuronal death.

Dopaminergic differentiation using microRNAs.

Pulcrano S.*[1], De Gregorio R.*[1], De Sanctis C.[2], Volpicelli F.[3], Perrone-Capano C.[3], di Porzio U.[1], Caiazzo M.[1], Bellenchi G.C.[+,1,2].

1. Institute of Genetics and Biophysics "Adriano Buzzati-Traverso," CNR, Naples, Italy;
2. IRCCS Neuromed, Pozzilli (IS), Italy;
3. Department of Pharmacy, University of Naples "Federico II", Naples, Italy.

Dysfunctions in the midbrain dopaminergic circuits (mDA) affect motor and motivational functions and are related to serious neurological and psychiatric conditions like Parkinson's disease (PD), schizophrenia, attention deficit hyperactivity disorders (AHDH) and drug addiction.
The *in vitro* development of patient-derived mDA neurons could unveil the pathological processes involved in the specific loss of mDA cells and represent a powerful model to screen drugs for regenerative and repair medicine.
To improve the *in vitro* generation of DA neurons, we screened transcriptions factors and miRNAs upregulated during the development of mDA neurons and evaluate their ability to modulate dopaminergic differentiation.
By using mouse midbrain primary cultures (mE12.5-PCs) and mouse embryonic fibroblasts (MEFs) we revealed that the combined overexpression of Nurr1 with specific microRNAs increased the number of TH+ cell. Two of the most efficient miRNAs are bioinformatically predicted as regulators of wnt's pathway but only one, the miR-34b/c, really affects the Wnt1 gene and promotes cell cycle exit facilitating the maturation of DA-differentiating cells. The combination of this miR-34b/c with the Nurr1 and Ascl1 double the percentage of TH+ neurons obtained from reprogrammed MEF.
These data describe a role for miR-34b/c during the DA development and suggest a new universal cocktail to boost the *in vitro* generation of DA cells useful to improve reprogramming efficiency on patient-derived fibroblasts.

New therapeutic approaches to treat CNS pathology in lysosomal storage disorders.

Sorrentino N.C.[1], Cacace V.[1], Maffia V.[1], Strollo S.[1] and Fraldi A.[1]

1. Telethon Institute of Genetics and Medicine, Via Campi Flegrei 34, 80078 Pozzuoli, Naples, Italy

Mucopolysaccharidosis type IIIA (MPS-IIIA) is one of the most common and severe forms of neurodegenerative lysosomal storage disorders (LSDs). MPS-IIIA is caused by inherited defect of a lysosomal hydrolase sulfamidase (SGSH) that leads to the accumulation of toxic material into the cells. The brain represents the most affected tissue in MPS-IIIA but to date there is no cure that can treat neuropathology in this disorder. In a previous study, we have provided a proof-of-principle in MPS-IIIA mice demonstrating the therapeutic efficacy of engineered forms of SGSH capable to be highly secreted from the liver (through the addition of alternative signal peptides belonging to highly secreted enzymes) and to efficiently cross the blood-brain-barrier –BBB– (through the addition of a BBB-binding domain) upon systemic administration of adeno-associated viruses (AAV) bearing the modified SGSH. We are now moving towards a clinic trial by testing the efficacy of this approach in a large MPS-IIIA animal model (Huntaway dogs). A drawback of this approach is that the presence of the BBB (even in the case of enzyme modified to cross the BBB) allowed only a portion of the enzyme loaded in the blood to reach the brain. Therefore, we are also developing an alternative approach based on intra-cerebrospinal delivery of AAV serotypes with high glia tropism to convert glia cells into a brain-proximal factory for highly secreted forms of SGSH, thus avoiding the BBB crossing. Overall, our studies are instrumental to develop novel and minimally invasive therapeutic strategies for MPS-IIIA and likely for other LSDs.

A cluster of progranulin C157KfsX97 mutations in Southern Italy: clinical characterisation and genetic correlations.

Saracino D.[1], Coppola C.[1], Oliva M.[1], Puoti G.[1], Lus G.[1], Le Ber I.[2], Caroppo P.[3], Piccoli E.[3], Tagliavini F.[3], Di Iorio G.[1], Rossi G.[3]

1. Second Division of Neurology, Second University of Naples & InterUniversity Center of Research in Neurosciences (CIRN) – Naples, Italy
2. ICM, Institut du Cerveau et de la Moelle épinière – Paris, France
3. Division of Neurology V - Neuropathology, Neurological Institute Foundation "Carlo Besta" – Milan, Italy

Frontotemporal lobar degeneration (FTLD) is a group of neurodegenerative diseases displaying high clinical, pathological and genetic heterogeneity. The main genes involved are the genes coding for microtubule-associated protein tau (*MAPT*), progranulin (*GRN*) and chromosome 9 open reading frame 72 *(C9ORF72*). Several autosomal dominant *GRN* mutations have been reported, accounting for 5-10% of FTLD cases worldwide. In this study we described the clinical characteristics of seven Italian patients carrying the *GRN* C157KfsX97 *null* mutation and demonstrated the existence of a founder effect by means of haplotype sharing analysis. All the patients underwent a complete clinical, neuropsychological and instrumental assessment, including morphological and functional brain imaging and CSF biomarkers assay. Clinical diagnoses were Frontotemporal dementia behavioural variant (bvFTD) in 5 cases and Corticobasal syndrome (CBS) in 2 cases. Five cases were familial. We performed plasma progranulin dosage, *GRN* gene sequencing and haplotype sharing study, analysing 10 short tandem repeat (STR) markers, spanning a region of 11.08 Mb flanking *GRN* on chromosome 17q21. The deletion g.101349_101355delCTGCTGT, resulting in the C157KfsX97 *null* mutation, was found in all patients. We observed shared alleles among 6 patients for 8 consecutive STR markers spanning a 7.29 Mb region. This common haplotype strongly supports a founder effect. Our observations confirm the elevated clinical variability described among *GRN*-mutated FTLD cases also with this particular mutation. Moreover this is the first study reporting the likely existence of a founder effect for C157KfsX97 mutation in Southern Italy.

Neurological abnormalities in a SCID patient carrying mutation of *ARTEMIS*, *ADA* and *ERCC6* genes identified through Next Generation Sequencing

Gallo V., Cirillo E., Giardino G., Grasso F., Galasso G., Cocule M., Pignata C.

Department of Traslational Medical Sciences, Section of Pediatrics, University Federico II of Naples

Severe combined immunodeficiencies (SCIDs) represent a group of distinct congenital disorders responsible for severe dysfunctions of the immune system. Affected patients often die during the first two years of life if appropriate treatments to reconstitute their immune system are not undertaken. In addition, different forms of SCID are also associated with extraimmunological features, predominantly involving the central nervous system, leading to complex and unusual clinical phenotypes.We described a female SCID patient with a T-B-NK+ immunological phenotype, which after BMT developed a series of neurological complications. She was born to consanguineous parents and was admitted to our Institution at 2 month of life due to chronic suppurative otitis media, failure to thrive, severe pancytopenia, interstitial pneumonia caused by P. ijroveci and erythrodermia. The immunological evaluation showed a severe lymphopenia with persistent absence of the proliferative response to mitogenes. Thus, a diagnosis of SCID was made and, at 7 month of life, the patient was treated with a succesfull bone marrow transplantation from matched unrelated donor. Unexpectedly, during the follow up post-BMT, the patient developed a series of extraimmunological feature involving neurological abnormalities (macrocephaly, cerebral and cerebellar atrophy, global developmental delay with hyperactivity disorder), sensorineural deafness, dysmorphic features and skeletal abnormalities. Genetic investigations revealed a double heterozygous for mutations of the ADA and ERCC6 genes and a deletion of ARTEMIS gene, which may have a role in the pathogenesis of the extraimmunological features. Particularly our finding highlight a possible role for SCID genes during neuronal development and maintenance in humans.

NEUROIMAGING/BIOMARKERS

Micro-RNA as biomarkers in Pompe Disease.

Tarallo A.[1,2], Gatto F.[2], Coletta M.[1,2], Karali M.[2], Banfi S.[2], Acampora E.[1], Fecarotta S.[1], Della Casa R.[1], Porto C.[1,2], Toscano A.[3], Musumeci O.[3], Mongini T.[4], Vercelli L.[4], Santoro L.[5], Ruggiero L.[5], Deodato F.[6], Taurisano R.[6], Bembi B.[7], Dardis A.[7], Pijnappel W.P.[8], van der Ploeg A.T.[8], Parenti G.[1,2]

1. Department of Translational Medical Sciences, Federico II University, Naples, Italy
2. Telethon Institute of Genetics and Medicine, Pozzuoli, Italy
3. Department of Neurosciences, University of Messina, Italy
4. Department of Neurosciences, University of Torino, Italy
5. Department of Neurosciences, Federico II University, Naples, Italy
6. Ospedale Pediatrico Bambino Gesu', Rome, Italy
7. Azienda Ospedaliero Universitaria "Santa Maria della Misericordia", Udine, Italy
8. Erasmus Medical Center, Rotterdam, the Netherlands

Pompe Disease (PD) is a metabolic myopathy caused by deficiency of acid-alpha-glucosidase (GAA) that results in generalized tissue glycogen accumulation and secondary cardiac and skeletal muscle destruction.
Despite progress in the treatment (based on enzyme replacement therapy, ERT), PD remains associated with unmet medical needs: variable response to ERT; need for reliable biochemical markers of disease progression and of ERT efficacy; need for novel therapeutic targets.
We have evaluated micro-RNAs (miRNAs) as potential biomarkers for PD.
MiRNAs profiles were studied by using Next Generation Sequencing (NGS) in tissues from PD mouse. We identified 198 miRNA that were differentially expressed with statistical significance (FDR< 0.05) in muscle (gastrocnemius), and 66 in heart.
We also analyzed miRNAs profiles by NGS in plasma samples from 10 PD patients and 10 age matched controls. We found 55 miRNAs that were significantly dysregulated in PD samples with respect to controls (28 down-regulated and 27 up-regulated)
A comparison between mice and patients results showed that 55 miRNAs were differentially expressed in mouse gastrocnemius and plasma, 3 in heart and plasma, one was differentially expressed in all samples examined.
Some of these miRNAs are already known to modulate the expression of genes involved in pathways such as autophagy, muscle regeneration, inflammation that may be relevant for PD pathophysiology.

These results suggest that circulating and tissue-specific miRNAs can represent novel biomarkers for PD. Future research, on larger cohorts of patients, should be aimed at defining the correlations of miRNA levels with phenotype, genotype, disease progression, response to therapies.

Encephalic volumetric alterations in long-term survivor Acute Lymphoblastic Leukemia patients identified with VBM analysis.

Mazio F.[3], D' Amico A.[3], Di Paolo N.[3], Riccio M.P.[1], Aloj G.[2], Pastorino G.[4], Parasole R.[2], Bravaccio C.[1], Brunetti A.[3]

1. Mental and Fisical Health and Preventive Medicine Department, Second University of Naples
2. Pediatric Hemato-Oncology Department, Santobono-Pausilipon Children's Hospital, Naples
3. Neuroradiology Unit, Advanced Biomedical Science Department, University Federico II of Naples
4. Traslational Medical Science Department, Child Psychiatry Unit, University Federico II of Naples

Aims and objectives
New chemotherapic (CT) and radiotherapic (RT) treatments have led to the existence of a large group of long-term survivor Acute Lymphoblastic Leukemia (LLA-LTS) patients, but may cause neurocognitive impairment. Magnetic resonance imaging (MRI) and Voxel Based Morphometry (VBM) may help in the identification of underlying anatomical alterations.
Methods and materials
We prospectively selected 26 LLA patients, 13 treated with CT+RT (group A) and 13 with CT (group B). All underwent MRI at our institution on a 3t scanner. Acquired volumetric sequences were imported on a dedicated software (SPM8), used for segmentation and VBM analysis. We quantified regional and total values of white matter (WM), gray matter (GM) and CSF of both groups. Neuropsychometric tests (WAIS-R, WISC-IV, d2-R, WCST) were used for clinical evaluation. A two-sample T-test was used for group comparisons.
Results
Group A had a significantly ($p<0,05$) larger CSF volume and significantly ($p<0,01$) smaller GM volumes of the superior frontal gyrus, posterior medial frontal gyrus, paracentral lobule, inferior parietal lobule, precuneus, temporal medial gyrus, parahippocampal gyrus, medial and posterior cingulate compared to B. Significant ($p<0,01$) differences were found for intelligence, performance, attention and memory measures of WAIS-R. No significant inter-group differences were observed for WISC-4th, d2-R and WCST, but both showed worse working memory, processing speed, concentration and attention than population norm.
Conclusion
Our findings, while limited by a relatively small population size, could be related to specific neurocognitive alterations observed in these two groups of patients, and represent an initial attempt in elucidating their anatomical basis.

A model of possible applications of advanced MRI techniques in the field of inherited metabolic disorders: Fabry disease.

Tedeschi E.[1], Russo C.[1], Cocozza S.[1], Olivo G.[1], Pontillo G.[1], Saccà F.[2], Pisani A.[3], Brunetti A.[1]

1. Department of Advanced Biomedical Sciences, University "Federico II", Naples, Italy
2. Department of Neurosciences and Reproductive and Odontostomatological Sciences, University "Federico II", Naples, Italy
3. Department of Public Health, Nephrology Unit, University "Federico II", Naples, Italy

Fabry disease (FD) is a metabolic disorder caused by insufficient lysosomal α-galactosidase A activity. This enzymatic defect leads to pathological storage of glycosphingolipids, occurring in all tissues and causing multi-organ progressive dysfunction. Neurological involvement is denounced on brain MRI by the presence of white matter hyperintensities and increased basilar artery diameter. Aim of this work is to provide an overview of potential applications of advanced MRI techniques to the comprehension of cerebral involvement pattern and possible mechanisms underlying neurological phenomenology observed in FD.
Recent evidence suggests the presence of subtle motor symptoms in FD caused by abnormal functional connectivity of motor cortex. Using Resting State functional MRI (RS-fMRI) analysis, we demonstrated presence of major alterations of the cortico-striatal pathway and reduced activation in basal ganglia.
Similarly, we used advanced MRI techniques to clarify the exact incidence of T1-pulvinar sign (PS), long be considered a distinguishing feature of FD, and to determine whether relaxometry changes could be detected in this region independently from the presence of T1w-hyperintensity. We found lower incidence of PS compared to what previously described, coupled with no significant differences in these regions between FD and healthy controls when analyzing relaxometry and QSM maps. Our results allow to hypothesize that PS, although easy-to-identify, is a rare pictorial neuroradiological sign not specific of FD.
In conclusion, in our experience, the application of advanced MRI techniques may be used to shed new light on the physiopathological mechanisms and on the pattern of cerebral involvement in FD, and in many other rare genetic disorders as well.

Serum MIR-148b as a potential biomarker for Multiple System Atrophy:a pilot study.

Vallelunga A., Dati G., Barone P., Valente E.M., Pellecchia M.T.

Center for Neurodegenerative Diseases, Neuroscience Section, Department of Medicine and Surgery, University of Salerno

Early diagnosis of Multiple System Atrophy (MSA) may be difficult due to overlapping clinical features with other parkinsonisms. Early differentiation between MSA and PD has clinical, therapeutic and prognostic consequences and may be difficult, if based solely on clinical examination. Despite growing research efforts, no reliable biomarker currently exists for the diagnosis of MSA. miRNAs are small noncoding RNAs with a key role in post-transcriptional gene regulation. Recent studies have revealed that some miRNAs are differentially expressed in human brain and regulate the expression of genes associated with specific neurodegenerative disorders. In this study we aimed to assess if mir-148b expression could distinguish MSA from PD patients. Moreover, we aimed to evaluate if mir-148b expression is preserved in serum samples stored at -20°C. We enrolled 25 patients affected by PD and 22 patients with MSA. miRNAs were extracted from 200 µl of serum samples stored at -20°C using total RNA purification kit and mir-148b was quantified using miRcury LNA assay. We confirmed that mir-148b was upregulated in MSA compared to PD patients with a fold change of 3.3. Moreover, we showed that levels of mir-148b in sera stored at 20°C were highly similar to the profile of <1 year-old sera stored at 80°C. Our results suggest that serum mir-148b can discriminate MSA from PD patients and deserves to be further assessed as a specific, non-invasive biomarker for differential diagnosis of MSA. Moreover, our pilot study suggests that circulating miRNAs retain their integrity under long-term suboptimal storage temperatures.

INDICE DEI RELATORI (in ordine alfabetico)