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[Maria Fazzari]
CURRICULUM VITAE

PERSONAL DATA

Name: Maria Fazzari
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2020-2023
Expert in Biochemistry, Sector 05 / E1 - General Biochemistry (BIO/10)

POSITIONS AND EMPLOYMENT

August 2020 – to date
Type A Postdoctoral Fellow
Department of Medical Biotechnology and Translational Medicine, University of Milano, Segrate, Milano, Italy
Advisors: Laura Mauri, Professor

July 2018 - August 2020
Type B Postdoctoral Fellow
Department of Medical Biotechnology and Translational Medicine, University of Milano, Segrate, Milano, Italy
Advisors: Sandro Sonnino, Professor and Elena Chiricozzi, PhD

November 2017 - June 2018
Collaboration contract
Department of Medical Biotechnology and Translational Medicine, University of Milano, Segrate, Milano, Italy
Advisor: Nicoletta Landsberger, Professor

EDUCATION

2014-2017
PhD student in Experimental Medicine and Medical Biotechnology (Thesis Defence: February 19th, 2018)
Department of Medical Biotechnology and Translational Medicine, University of Milano
Thesis Title: "Novel approaches of "personalised medicine" as proof of principle for CDKL5-related pathologies"
Supervisor: Nicoletta Landsberger, Professor

2011-2014

Master of Science in Cellular and Molecular Biology

Department of Neuroscience, Neuroscience Institute Cavalieri Ottolenghi, University of Torino, Italy

Thesis Title: "HDAC1 and HDAC2 expression in the lineage of cerebellar interneurons"

Grade: 110/110 *cum laude*

Supervisors: Annalisa Buffo, Professor and Ferdinando Rossi, Professor

2008-2011

Bachelor's Degree in Biological Sciences

Division of Biochemistry, Department of Life Sciences and System Biology, University of Torino, Italy

Grade: 106/110

Thesis Title: "Hydrogen Bioproduction: optimization strategies through metabolic engineering"

Supervisor: Francesca Valetti, PhD

EDUCATIONAL EXPERTISE

Courses taught and tutorship activities

Academic year 2022/2023 – 6 hours

Professor of the Biochemistry module (SSD BIO/10), Course of Basic Sciences, Nursery Bsc program, Medical School, University of Milano (in quality of Biochemistry expert, course hold by Dr. Elena Chiricozzi)

Academic year 2021/2022 – 4 hours

Professor of the Biochemistry module (SSD BIO/10), Course of Basic Sciences, Nursery Bsc program, Medical School, University of Milano (in quality of Biochemistry expert, course hold by Dr. Elena Chiricozzi)

Academic year 2018/2019

Tutor of Biochemistry module (SSD BIO/10), Course of lab training, Course of Cell Molecules and Genes, International Medical School, University of Milano

Academic year 2017/2018

Tutor of Molecular biology (class I-2), Course of lab training, Medical biotechnology Bsc program, Medical School, University of Milano

Academic year 2016/2017

Tutor of Molecular biology (class 1-2) Course of lab training, Medical biotechnology Bsc program, Medical School, University of Milano

Internship and thesis supervisions

Academic year 2016/2017

Co-supervisor in the thesis of Anaïs Serati

Medical biotechnology Bsc program, University of Milano

Title: “Optimization of read-through strategies as a personalized therapeutic approach for CDKL5-related diseases”

Academic year 2016/2017
Co-supervisor in the thesis of Alice Fratton
Medical biotechnology Bsc program, University of Milano
Title: “First studies on the efficacy of nonsense mutation suppression as a therapeutic approach for personalized treatment of patients with mutations on CDKL5”

RESEARCH ACTIVITY

August 2020 – *present*
D*epartment of Medical Biotechnology and Translational Medicine, University of Milano, Italy (PostDoc)*

My research activity is currently focused on the neurotrophic and neuroprotective properties of GM1 oligosaccharide (OligoGM1) in relation to mitochondria dysfunction and neurodegeneration onset.
Several data suggest a specific role of GM1 ganglioside in neuronal differentiation and development, but the molecular mechanisms underlying these processes are largely unknown. Recently, my team found that only the GM1 oligosaccharide, rather than the ceramide portion, is directly involved in these processes (**Chiricozzi, Fazzari *et al* 2019 *Mol Neurobiol***). We found that GM1 modulates TrkA activity by stabilizing the TrkA-NGF complex with its oligosaccharide portion, inducing TrkA phosphorylation and MAPK-pathway activation which in turns triggers differentiation and protection signaling (**Chiricozzi, Fazzari et al 2019 *J Neurochem***). These findings provide a new view for the role of the oligosaccharide chain of gangliosides in plasma membrane signaling.
From here we developed the idea of impaired (Oligo)GM1–plasma membrane proteins interaction as main cause underlying neurodegeneration onset (i.e. Parkinson’s disease, PD) related to GM1 decline during aging and/or for epigenetic influences (**Chiricozzi, Fazzari *et al* 2019 *Sci Rep***). Accordingly, we found out that OligoGM1 systemically administered to PD mouse model completely rescues the physical impairment as well as the biochemical features reaching the healthy conditions. Proteomic and biochemical analyses revealed a broad spectrum of molecular events prompted by OligoGM1 in a Trk-dependent manner, involving calcium regulation, antioxidant mechanisms, mitochondrial bioenergetics, and anti-inflammatory response (**Chiricozzi, Fazzari *et al* 2019 *Mol Neurobiol*; Fazzari *et al* 2020 *Glycoconj J*; Lunghi, Fazzari *et al* 2020 *Glycoconj J***).
The specific key role of mitochondria in anti-inflammatory processes and in protection from oxidative stress has been reported by several studies and mitochondrial defects are often described as inducers of neurodegeneration. By mass spectrometry, I have identified that the administration of OligoGM1 determines an increased expression of proteins localized in mitochondria and involved in oxidative stress response (**Fazzari *et al* 2020 *Glycoconj J***). Accordingly, I showed that wild-type (wt) Neuro2a (N2a) neuroblastoma cells exposed to OligoGM1 display an increased mitochondrial density and an enhanced mitochondrial activity as testified by increased expression of electron transport flow complexes, oxygen consumption rate and ATP levels (**Fazzari *et al* 2020 *Glycoconj J***). Interestingly, using a N2a model of mitochondrial dysfunction, I found a recovery of impaired mitochondrial oxygen consumption as well as increased complex I and II activities upon OligoGM1 administration (**Fazzari *et al* 2020 *Glycoconj J***).
Considering the role of OligoGM1 as a regulator of mitochondria, I am currently evaluating whether OligoGM1-derived neuroprotection depends on a recovered mitochondria function. Specifically, I am exploring OligoGM1 activity in the following pathological contexts:

1. *Parkinson’s Disease (PD; unpublished data)*

I am examining the ability of OligoGM1 to stimulate biogenesis and mitochondrial activity, to protect against oxidative stress and to activate biochemical pathways responsible for neuroprotection using *in vitro* and *in vivo* models relevant for PD (MPTP and *B4galnt1*$^{+/-}$ model). Importantly, OligoGM1 administration to wt N2a cells (**Chiricozzi, Fazzari *et al* 2019 *Mol Neurobiol***) or dopaminergic neurons (unpublished data) was able to counteract the toxic effect of MPTP, a neurotoxin affecting mitochondria function. Interestingly,

OligoGM1 treatment reduced the level of mitochondrial ROS and, accordingly, p38 MAPK hyper-phosphorylation due to MPTP-derived oxidative stress (**Chiricozzi, Fazzari *et al* 2019 *Mol Neurobiol***; **Fazzari *et al* 2020 *Glycoconj J***).
Accordingly, the capability of OligoGM1 to recover mitochondria alteration in PD will be explored in the *B4galnt1*$^{+/-}$ model. Specifically, this project is performed in collaboration with Prof. Robert Ledeen, Rutgers University, Newark (USA), where I will be a researcher guest next April/May 2023 to conduct *in vivo* experiments. By completing this project, I intend to provide evidence linking the reduced level of GM1 to mitochondrial dysfunction and oxidative stress, important features of PD that have not been directly correlated so far.

2. *Amyotrophic Lateral Sclerosis (ALS; unpublished data)*

Using motor neurons (MNs) from SOD1G93A rats, a model of ALS in which the pathology is due to toxic gain-of-function of superoxide dismutase 1 (SOD1), I observed that OligoGM1 pre-treatment significantly increases neuronal survival and preserves the neuritic network in both wt and SOD1G93A MNs intoxicated with glutamate, triggering neuronal damage/death due to increased concentration of Na^+ and Ca^{2+} in the cell. Furthermore, OligoGM1 resulted in a significant reduction of mislocalisation from the nucleus to the cytoplasm of TDP-43, a protein that represents the major component of toxic ubiquitinated protein inclusions in the cytoplasm of MNs in ALS. Finally, administration of OligoGM1 led to a significant recovery of the mitochondrial network that was impaired by glutamate exposure and to the reduction of mitochondrial anion superoxide content. This project is carried out in collaboration with Dr. Michael Spedding of Spedding Research Solutions (France) and Prof. Vito de Pinto and Dr. Magrì of the University of Catania (Italy).

3. *Rett Syndrome (RTT, unpublished data)*

In collaboration with Prof. Landsberger's research group at Milano University, I am evaluating OligoGM1 ability to modulate antioxidant and mitochondrial status, both impaired in RTT. To this purpose, OligoGM1 was administered to primary *Mecp2*-knockout (ko) cortical neurons, a RTT model. The results showed that OligoGM1 leads to a significant reduction in mitochondrial ROS content, increased expression of proteins important for mitochondrial functioning and increased ATP production. Furthermore, immunofluorescence analyses showed a significant recovery in the expression of pre- and post-synaptic markers, typically down-regulated in RTT neurons. Finally, OligoGM1 led to the recovery of *in vivo* motor defects, which are one of the prominent features of RTT and are faithfully reproduced in the *Mecp2*-ko mouse model. By immunoblotting analyses of brain lysates, we observed a reduction of TrkA and TrkB receptor activation and a reduced expression of BDNF that were significantly recovered upon OligoGM1 administration. Additionally, the oxygen over-consumption due to mitochondrial complex II hyper-activity in *Mecp2*-ko cortices reached wt levels in mice treated with OligoGM1.This preliminary evidence suggests that the beneficial effect of OligoGM1 treatment can be attributed to the modulation of the BDNF/Trk cascade and of mitochondria function.

4. *GM3 synthase deficiency disease (unpublished data)*

Based on the efficacy of OligoGM1 treatment in *B4galnt1*$^{+/-}$ parkinsonian mice with reduced GM1 ganglioside content, GM3 synthase-deficient mice were daily treated with OligoGM1 and analyzed for seizure susceptibility (analysis in progress). In parallel, molecular defects and their possible recovery are currently studied in a HEK293T *GM3 synthase*-ko model. As expected, I found that GM3 synthase down-regulation determines alterations of lipidic profile (accumulation of LacCer), possibly leading to a lysosomal impairment.
Since GM3 synthase deficiency has been correlated with mitochondria dysfunction, the status of these organelles is currently under investigation. This project is carried out in collaboration with Prof. Jin-ichi Inokuchi, Tohoku Pharmaceutical University (Japan) and Prof. Goetzman of the University of Pittsburgh (USA).

July 2018 – August 2020
D*epartment of Medical Biotechnology and Translational Medicine, University of Milano, Italy (PostDoc)*

During this postdoctoral period, I started to carry out research in the field of Lipid Biochemistry and, in particular, of GM1 ganglioside and its functional oligosaccharide portion. Specifically, the aim of the

research project was to define the molecular mechanisms underlying the neurotrophic role of GM1 ganglioside, an essential lipid component of neuronal membranes. Indeed, while the neuroprotective and neurorestorative potential of this ganglioside is now known, its signaling was still poorly understood.
Moreover, low levels of GM1 ganglioside has been shown to be associated with Parkinson's disease (PD), and its deficiency is a condition that promotes PD. Indeed, GM1 content is essential for central nervous system functioning as is evident from the devastating consequences of its deletion in mouse models and its therapeutic effect in PD patients confirming its neurotrophic, protective and restorative roles. However, preclinical and clinical studies, while demonstrating a quite efficacy of exogenous administration of GM1 in PD patients, have been interrupted for two main reasons: its low permeability to the central nervous system level (blood-brain barrier passage - BBB) and ethical/bioavailability issues, due to animal origin of GM1.
The research project was aimed at *i)* understanding the role of the oligosaccharide portion in the neuroprotective-restorative activities of GM1; *ii)* identify the molecular mechanisms underlying the loss of neurotrophic signalling due to the reduction of plasma membrane levels of GM1, and *iii)* overcoming the limitations related to the use of GM1 as a potential drug. To this purpose, the research project developed on several fronts using different strategies:

1. *Study the neuroprotective role of OligoGM1 in vitro*

By using murine neuroblastoma cells (Neuro2a, N2a), we demonstrated that the exogenous administration the oligosaccharide portion of GM1 alone (OligoGM1) induces an increase in neuritic growth, assessed both by morphological parameters and by the expression of marker proteins of neuronal differentiation. The oligosaccharide, which is not internalized by the cells, directly activates the NGF TrkA receptor at plasma membrane, and downstream the MAPK differentiation cascade. Through bioinformatic analysis, we demonstrated that OligoGM1 directly interacts with TrkA and its NGF ligand, stabilizing the complex (**Chiricozzi, Fazzari *et al* 2019 *J Neurochem***). These results suggest that the differentiating role of GM1 is due to its saccharide portion rather than its ceramide moiety.
In order to verify whether exogenous administration of OligoGM1 is able to activate specific biochemical pathways, we performed a proteomic analysis (nLC-Esi-MS-MS) on N2a cells treated for 24h with OligoGM1. This analysis identified and quantified 744 proteins, of which 324 were significantly over-expressed in OligoGM1-treated samples (**Chiricozzi, Fazzari *et al* 2019 *Mol Neurobiol***). The bioinformatic analysis revealed that these proteins are involved in maintaining calcium homeostasis, modulating plasma membrane integrity, reducing oxidative stress and mitigating immune system signalling, thus reflecting the known neuroprotective properties of GM1. Some of these processes were investigated through biochemical assays that revealed the modulation of calcium flux in OligoGM1-induced differentiative and protective mechanisms in N2a cells (**Lunghi, Fazzari *et al* 2020 *Glycoconj J***).

2. *Evaluate the neurotrophic role of OligoGM1 in vitro*

In order to study the effect of OligoGM1 on neuronal differentiation *in vitro*, we decided to use mouse cerebellar granule cells, an accepted neuronal model (**Di Biase, Fazzari *et al* 2020 *Glycoconj J***). To this purpose, granule cells were plated in the presence of OligoGM1 and followed over time to assess both morphological parameters and biochemical signatures. From a morphological point of view, the neurons treated with OligoGM1 undergo an acceleration of the differentiation process. Biochemical analyses of protein markers typical of neuronal differentiation (MAP2, Synapsin, NeuC and PSD95) confirmed that OligoGM1-treated granule cells had an advanced stage of maturation compared to controls. This event is associated with increased activation of FAK and Src, the main regulators of the array of proteins involved in the turnover of focal adhesions, responsible in turn for neuronal motility and neuritic growth. Finally, we have shown that even in primary neurons the effect of OligoGM1 is due to a direct activation of TrkA at the plasma membrane.

3. *Characterize the OligoGM1 transport across the BBB*

Using a model of human blood-brain barrier (BBB) *in vitro*, in collaboration with Prof. Fabien Gosselet from Artois University (France), we demonstrated that OligoGM1 is able to efficiently cross an *in vitro* model of BBB in a time-concentration dependent paracellular way and with a 20-fold higher crossing rate if compared to GM1 (**Di Biase, Fazzari et al 2020 *Int J Mol Sci***). This study has improved the knowledge about OligoGM1 pharmacological potential, highlighting its promising therapeutic use.

4. *Study the pharmacokinetics of OligoGM1 in vivo*

Given the neurotrophic and neuroprotective potential of OligoGM1 *in vitro*, we went on to assess the ability of OligoGM1 to reach the central nervous system (CNS). For this, OligoGM1 was administered to C57BL6 wild type mice intraperitoneally and for different timings. Several central nervous system [brain (cortex, *substantia nigra*, hippocampus, striatum), cerebellum, brainstem)] and peripheral tissues (heart, blood, lungs, kidney, liver, spleen) were analysed for radioactivity distribution. We verified that OligoGM1 is able to pass the BBB and reach CNS (**Chiricozzi, Fazzari *et al* 2019 *Sci Rep***). To verify its stability, the oligosaccharide was extracted from the brain and separated using chromatography techniques. This last step allowed us to verify that the oligosaccharide reaching CNS is intact. Finally, using an *ex vivo* model (cultures of mouse embryos), we demonstrated that OligoGM1 owns no toxicity in this experimental model (unpublished data).

5. *Analyze of the effect of OligoGM1 in a PD mouse model*

Given the promising results of the *in vivo* and *in vitro* studies, we decided to investigate the effect of administering OligoGM1 in a PD mouse model. The chosen model (*B4galnt1*$^{+/-}$ mice) is represented by genetically modified C57BL6 mice with a heterozygous deletion for the *B4GALNT1* gene, which encodes for an enzyme crucial in the synthesis of GM1 (and all a-series gangliosides). This model, characterized by approximately half the GM1 content compared to a wt mouse, exhibits motor impairment, accumulation of alpha-synuclein in the *substantia nigra* and consequent death of dopaminergic neurons, recapitulating all the typical features of sporadic PD. Systemic treatment of these mice with OligoGM1 induced a rescue of motor impairment (measured by grip duration test/irritant removal test/pole climbing test), allowing the levels of wt mice to be reached. In addition, biochemical alterations were also rescued: reduction in alpha-synuclein accumulation, recovery of dopaminergic neurons in the *substantia nigra* and restoration of the dopamine level in the striatum (**Chiricozzi, Fazzari *et al* 2019 *Sci Rep***).

6. *Synthetize the oligosaccharide chain of the GM1 ganglioside by engineering an E. coli strain*

To avoid issues related to animal origin of OligoGM1, we worked on the engineering of an *E. coli* strain that allows its enzymatic production. The strain chosen was *E. coli* JM109 (DE3) positive for the lactose transporter (LAcY+) and the sialic acid transporter (NanT+), the two starting substrates, and negative for ß-galactosidase (LacZ-), the enzyme responsible for lactose degradation. Unfortunately, this strain is positive for aldolase (NanA+), the enzyme that degrades sialic acid to mannosamine. Therefore, using the technique known as the λ red recombinase system, the aldolase gene was removed from the genome of E. coli JM109 (DE3). The five genes specifically required for the enzymatic synthesis of OligoGM1 *i)* CMP-NeuAc-synthase, *ii)* Lst (α-2,3-sialyltranferase), *iii)* WbpP (UDP-GlcNAc C4 epimerase) *iv)* CgtA (ß-1,4-N-acetylgalactosaminyltransferase), *v.* CgtB (ß-1,3-galactosyltransferase)] were synthesized by PCR and successfully cloned into a single plasmid (BAC vector). In the near future, E.coli JM109 (DE3) NanA- will be engineered with the BAC plasmid containing the 5 genes with the aim of obtaining significant amounts of OligoGM1 (unpublished data).

March 2018 – July 2018

D*epartment of Medical Biotechnology and Translational Medicine, University of Milano, Italy (PostDoc)*

CDKL5 Deficiency Disorder (CDD), an X-linked atypical variant of Rett syndrome (RTT), is a childhood disorder characterized by altered in neuronal morphology and reduced BDNF mRNA levels. Interestingly, Fingolimod (an analogue of sphingosine-1 phosphate) has been demonstrated to increase BDNF mRNA levels and trigger BDNF protein release both *in vitro* and *in vivo* in an activity- and MAPK-dependent manner.

In this context, my research activity was aimed at studying the characteristics of *Cdkl5*-null neurons and at evaluating the possibility to rescue the pathological phenotype through treatment with pFTY720 (active form of Fingolimod). In particular, preliminary results showed a reduced total length of dendrites of immature *Cdkl5*-null neurons which was recovered in the presence of pFTY720 suggesting that the pharmacological treatment could ameliorate the pathological phenotype (collaboration with Dr. Marta Zagrebelsky, Technische Universität Braunschweig, Germany).

November 2014 – February 2018
Department of Medical Biotechnology and Translational Medicine, University of Milano, Italy (PhD Student)

As a PhD student, my research activity was related to Rett syndrome (RTT) and CDKL5 Deficiency Disorder, an atypical variant of RTT. RTT is a severe childhood neurological disorder representing one of the main causes of severe intellectual disability in girls.
Specifically, my research activity was focused on three main themes:

1. *Understand the mechanisms underlying synaptic defects of Mecp2-null mice (RTT model).*

My study was aimed at evaluating possible alterations of mRNAs localized at the level of dendritic spines. To this purpose, I optimized a fractionation protocol that allows to obtain RNA from cortical synaptoneurosomes. The RNA obtained from wt and *Mecp2*-null synaptoneurosomes was sequenced by RNAseq and analyzed by Gene Ontology, revealing an enrichment of genes involved in synaptic activity, thus validating the experimental approach.

2. *Evaluate the therapeutic potential of personalized medicine approaches on alterations affecting the CDKL5 gene.*

Specifically, I studied the possibility to suppress non-sense mutations through a process known as read-through, an event that occurs when a premature stop codon is recognized by a near-cognate tRNA and allows the protein elongation until the natural stop codon is reached. I mutagenized a vector codifying for CDKL5 fused to GFP in order to introduce different nonsense pathogenic mutations and evaluated the efficacy of aminoglycoside (gentamicin, genetics) and non-aminoglycoside (PTC124, GJ072) drugs. In parallel, I verified that the generated full-lenght proteins had a similar functionality if compared to the wt in terms of correct sub-cellular localization and kinase activity. The obtained results showed that the aminoglycoside drugs are able to induce an efficient read-through of all the tested premature stop codons giving rise to a full-length protein that is localized similarly to the wt protein although the activity catalytic is not completely restored (**Fazzari *et al* 2019 *RNA Biol***).
In the context of personalized medicine approaches in CDKL5-related pathologies, I collaborated with Dr. Domenico Giorgio for the characterization of a panel of 5' donor splice site splicing mutations that reduce complementarity with 5' of U1snRNA and the analysis of modified U1snRNAs capable of restoring defective splicing *in vitro* (**Balestra, Fazzari *et al* 2019 *Int J Mol Sci***).

3. *Study the role of CDKL5 protein in the cell cycle.*

In this study we found that CDKL5 silencing determines a significant increase of the number of cells with multipolar spindles together with defects in chromosomal segregation including micronucleation and the presence of binucleated cells. The re-expression of CDKL5 by siRNA-resistant vectors confirmed that these alterations depend on CDKL5. In particular, at the molecular level, we have demonstrated that the regulation of cell division by CDKL5 occurs through HIPK2-mediated phosphorylation of H2B (**Barbiero, Fazzari *et al*. 2017 *Sci Rep***).

January 2013 – April 2014
Department of Neuroscience, NICO (Neuroscience Institute Cavalieri Ottolenghi), University of Torino, Italy (Undergraduate Student)

My research activity has been started at Prof. Buffo's laboratory (University of Torino) working on the expression of HDAC1 and HDAC2 in the lineage of GABAergic cerebellar interneurons. The results indicated that HDAC1 and HDAC2 are expressed in different development phases and that their expression switch occurs in a specific moment. In particular, in the adult cerebellum they are extremely segregated in different cellular populations, astrocytes and mature GABAergic interneurons. Astrocytes, in particular the Bergmann glia, mainly express HDAC1, whereas mature GABAergic interneurons mainly express HDAC2. This clear segregation of HDACs suggests their role in the neural differentiation, where HDAC1 promotes differentiation into glia, whereas HDAC2 fosters neuronal differentiation.

Collaborations

The research activity described above is implemented in the context of national and international collaborations that allowed me to obtain a good degree of independence and large capability of discussion:

- Professor Nicoletta Landsberger, University of Milano, Milano, Italy
- Professor Nico Mitro, University of Milano, Milano, Italy
- Professor Gabriella Tedeschi, University of Milano, Milano, Italy
- Professor Vito De Pinto and Dr. Andrea Magrì, University of Catania, Catania, Italy
- Professor Jin-ichi Inokuchi, Tohoku Pharmaceutical University, Sendai, Japan
- Professor Robert Ledeen, Rutgers University, Newark, NJ, USA
- Professor Eric S. Goetzman, University of Pittsburgh, Pittsburgh, USA
- Dr. Tim Bartles, London College University, London, UK
- Dr. Michael Spedding, Hon FBPhS, Secretary General IUPHAR; President, Spedding Research Solutions SAS, Le Vesinet, France
- Professor Gosselet Fabien, Laboratoire de la Barrière Hémato-Encéphalique (LBHE), Université Artois, Lens Cedex, France
- Neuro_sys CNS/PNS service, 410 Chemin Dep, Gardanne, France

SCIENTIFIC PROJECTS: DIRECTION AND PARTECIPATION

Project: Dissecting mitochondrial dysfunction due to GM1 oligosaccharide deficiency in Parkinson's disease
Role: PI
Partners: Dr. Elena Chiricozzi, University of Milano, Italy; Prof. Robert Ledeen, Rutgers University, Newark, NJ, USA

Functional data and clinical studies suggest the existence of a positive loop between the age-dependent GM1 ganglioside deficiency and the neurodegeneration onset of sporadic Parkinson's Disease (sPD). Accordingly, PD patients display GM1 depletion in central and peripheral nervous system due to reduced activity of enzymes involved in its synthesis and the mouse model carrying heterozygous disruption of *B4galnt1* gene, encoding an enzyme required for GM1 biosynthesis (GM2/GD2 synthase), well recapitulate human sPD (aggregation of alpha-synuclein, loss of dopaminergic neurons, depletion of striatal dopamine, motor and non-motor dysfunctions). Recently, we demonstrated that GM1 neuroproperties are completely linked to the sole oligosaccharide (OligoGM1). Importantly, OligoGM1 systemically administered to PD *B4gant1*$^{+/-}$ mice was found to completely rescue PD phenotype (**Chiricozzi, Fazzari *et al* 2019 *Sci Rep***). Proteomic and biochemical analyses revealed several molecular events triggered by OligoGM1 including the increase of mitochondria bioenergetics (**Chiricozzi, Fazzari *et al* 2019 *Mol Neurobiol*; Fazzari *et al* 2020 *Glycoconj J***), whose impairment is undoubtedly proved in PD.
Based on this evidence, it is clear that GM1 is able at the same time to rescue PD phenotype and to stimulate and recover mitochondrial function thanks to its oligosaccharide portion. However, the molecular mechanism to functionally correlate the GM1 deficiency and mitochondria dysfunction in PD pathogenesis still remains to be elucidated. In this frame, the project is aimed at understanding mechanistically whether the progressive reduction of plasma membrane OligoGM1 triggers the impairment of mitochondria function in *B4gant1*$^{+/-}$ mice. In particular, we will pursue three main objectives: *i)* elucidate whether GM1 deficiency correlates with PD mitochondria impairment; *ii)* identify whether changes in the amount of PM-GM1 impair specific molecular pathways responsible for mitochondria integrity; *iii)* understand whether OligoGM1 replacement could rescue mitochondria dysfunctions.

Project: GM1 oligosaccharide activity against the pathognomonic biomarker of Parkinson's disease: aggregated alpha-synuclein
Role: CO-PI of Biochemistry unit
Partners: Dr. Elena Chiricozzi and Professor Giuliano Zanchetta, University of Milano, Italy; Professor Vito De Pinto and Dr. Andrea Magrì, University of Catania, Italy; Neuro_sys CNS/PNS service, 410 Chemin Dep, Gardanne, France; Dr. Tim Bartles, London College University, London, UK

Alpha-synuclein (αS) fibrillation and aggregation are considered crucial steps in PD pathophysiology and preventing its misfolding and/or promoting its clearance can represent the keystone for PD cure. In this scenario, GM1 ganglioside could represent the promising molecule able to directly interact with αS and inhibit its fibrillation, to promote its degradation via autophagy and finally avoid PD neurodegeneration. Unfortunately, the low GM1 capability to reach central nervous system hampers its clinical use. However, our newly discovery that the oligosaccharide chain alone is able to faithfully replicate the ganglioside functions opens new promising therapeutic perspective. In fact, losing the amphiphilicity of the ganglioside, OligoGM1 efficiently crosses the BBB via a passive paracellular route without being internalized and metabolized by cells, thus remaining active and capable to activate the Trk pathways (**Di Biase, Fazzari *et al* 2020 *Int J Mol Sci***). Such capability allowed its *in vivo* penetration in all brain regions and the complete recovery of a PD mouse model. The above evidence together with the capability of OligoGM1 to protect against αS toxicity *in vitro* and *in vivo* (unpublished results) suggest that soluble and hydrophilic GM1 oligosaccharide seems to be the long-awaited candidate to replace GM1 use in the clinic for the PD treatment. In this scenario, this project aims at understanding how OligoGM1 can protect from αS toxicity *in vitro*. Specifically the objectives of this project are *i)* to verify a direct OligoGM1:αS interaction preventing an αS amyloidogenic misfolding; *ii)* to analyse the molecular pathways modulated by OligoGM1 in presence of aggregated αS; *iii)* to address the involvement of glial cells and neuroinflammation due to aggregated αS. Positive results obtained *in vitro* will be further confirmed via *in vivo* studies.

Project: GM1-oligosaccharide as a new drug candidate for Rett syndrome
Role: PI
Partners: Dr. Elena Chiricozzi, University of Milano, Italy; Prof. Nicoletta Landsberger and Prof. Nico Mitro, University of Milano, Italy

Rett syndrome (RTT) is a severe neurodevelopmental disorder mainly caused by *MECP2* mutations. Due to poor understanding of the molecular consequences of MECP2 deficiency, currently no cure exists. Different studies led to the identification of some molecular pathways and cellular functions compromised in RTT, including neurotrophins' signaling and mitochondrial bioenergetics. Such impairments could be linked to the depletion of a specific plasma-membrane lipid, the ganglioside GM1. GM1 has a crucial role for neuronal homeostasis maintenance acting via modulation of neurotrophins' signaling and of mitochondrial function. It should be noted that a significant reduction in GD1a level, GM1 precursor at plasma membrane, was observed in both RTT patients and mice. It is therefore likely that GM1 deficiency at plasma membrane could positively correlate with neurotrophic and mitochondrial impairment observed in RTT. Given the recent *in vitro* and *in vivo* discovery that the soluble GM1-oligosaccharide is the bioactive portion of GM1 responsible for neurotrophic functions, we hypothesize that the plasma-membrane GM1 deficiency significantly contributes to RTT pathogenesis due to the alteration/loss of molecular interactions between the GM1-oligosaccharide and cell surface proteins that support neurotrophic signaling.
By performing MitoSOX-red staining, we found an enhanced ROS production in RTT neurons that was significantly recovered by OligoGM1 treatment. Additionally, OligoGM1 administration induced an increase of electron transport flow complexes expression which are downregulated in RTT neurons, possibly indicating a boosting of mitochondrial activity. By analysing confocal images, we found that *Mecp2*-ko neurons have a reduced expression of both synapsin1/2 and shank2 markers, while OligoGM1 administration was able to significantly increase their levels, meaning that OligoGM1 can reverts defects both at the pre- and the post-synaptic compartments.

Importantly, OligoGM1 systemically injected to RTT mice was able to counteract the worsening of the motor defect evaluated through rotarod test by recovering reduced TrkB/TrkA activation and increasing BDNF expression.
Our preliminary data support our idea that OligoGM1 can be a promising drug candidate for RTT, able to counteract the disease progression possibly acting through the stimulation of Trk signaling and the modulation of mitochondrial function.
By completing this project, we will provide information about the efficacy of OligoGM1 in RTT, assessing the *i)* mechanism of action, *ii)* the capability to modulate specific neuronal functions and *iii)* to recover neuronal defects and physical impairments typical of the syndrome.

Project: Unveiling GM1-oligosaccharide role in counteracting the mitochondria impairment in Amyotrophic Lateral Sclerosis
Role: PI
Partners: Dr. Elena Chiricozzi, University of Milano, Italy; Dr Michael Spedding, Spedding Research Solutions, France; Neuro-sys, Gardanne, France

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by accumulation of ubiquitylated proteinaceous inclusions and progressive degeneration of upper and lower motor neurons (MNs). Although its etiopathogenesis is still poorly understood, pathogenic proteins mislocalisation, misfolding and aggregation (i.e. SOD1 and TDP-43), glutamate toxicity, oxidative stress, and mitochondrial function failure have been described to play a crucial role. Additionally, a major change in glycosphingolipids metabolism and, in particular, a depletion of GM1 ganglioside has been reported in spinal cord and in neuromuscular junctions of SOD1 ASL mice. GM1 has been considered as a master regulator of the nervous system and accumulating evidence is pointing out its role in preventing neurodegeneration through its bioactive portion: the GM1 oligosaccharide (OligoGM1).
Exploiting proteomic and biochemical approaches, we demonstrated that OligoGM1 directly triggers TrkA-MAPK pathway activation inducing neuronal differentiation and protection by modulating mitochondrial biogenesis and function, calcium flux and redox balance in neuronal cells.
Based on these outcomes, we decided to test the OligoGM1 neuroprotective potential in MNs from SOD1G93A rat embryos, an ALS *in vitro* model.
We observed that the pre-treatment with OligoGM1, as well as GM1, significantly increased neuronal survival and preserved neurite networks both in wt and in SOD1G93A MNs injured with glutamate. Additionally, OligoGM1/GM1 reduced TDP-43 mislocalisation from nucleus to cytoplasm due to glutamate exposure and counteracted SOD1 aggregation. Finally, we found that glutamate-injured MNs have a reduced mitochondrial mass and increased anion superoxide levels. Interestingly, OligoGM1/GM1 administration was able to increase mitochondria network and to scavenge oxidative stress.
The aim of this project is to understand whether OligoGM1 neuroprotection in ALS *in vitro* models depends on its capability to counteract mitochondrial dysfunction. Specifically, we will analyse the capability of OligoGM1 *i)* to modulate mitochondrial dynamics (biogenesis, fusion, fission, mitophagy); *ii)* to counteract mitochondrial oxidative stress; *iii)* to modulate specific intracellular cascade that are responsible for rescuing impaired mitochondria.

Project: Understanding the cellular and molecular consequences of GM3 synthase deficiency
Role: PI
Partners: Prof. Elena Chiricozzi, University of Milano, Italy; Prof. Jin-ichi Inokuchi, Tohoku Pharmaceutical University, Sendai, Japan; Professor Eric S. Goetzman, University of Pittsburgh, Pittsburgh, USA

GM3 synthase enzyme catalyzes the formation of GM3 ganglioside starting from lactosylceramide, which represents the first step for the synthesis of complex gangliosides. Accordingly, the knock-out (ko) mouse model for GM3 synthase is devoid of gangliosides of series a and b (hence GM1) while it presents gangliosides of series o. Childrens with total GM3 synthase deficiency display neurodevelopmental impairment, while heterozygous mutations predispose to Parkinson's disease with high incidence.

The objective of this project is to understand the molecular mechanism underlying GM3 deficiency in GM3 synthase ko HEK293T cells and in GM3 synthase ko mice. Lipidic profile analyses of ko cells confirmed the absence of GM3 ganglioside and the consequent accumulation of LacCer substrate of GM3 synthase. Immunoblotting analyses revealed a significant increase of LAMP1 lysosomal marker, meaning possibly that LacCer accumulation may induce lysosomal biogenesis. By exploiting fluorigenic substrates, we found that ko cells have deregulated activity of different lysosomal enzymes meaning that an impairment of lysosomal function can occur. Additionally, considering that mitochondria alterations has been described to be correlated to GM3 synthase deficiency, mitochondria abundance and function and oxidative stress will be investigated.
By completing this project we aim to understand: *i)* how cell membrane composition can be affected in absence of GM3; *ii)* cellular and molecular events triggered by GM3 depletion and LacCer accumulation; *iii)* whether OligoGM1 administration can influence the pathological phenotype due to GM3 deficiency.
In parallel, considering the efficacy of OligoGM1 treatment in *B4galnt1*$^{+/-}$ mice, we are evaluating the oligosaccharide therapeutic potential in GM3 synthase-deficient mice.

Project: Assessing the toxicity and the pharmacokinetics properties of GM1 oligosaccharide
Role: CO-PI
Partners: Prof. Elena Chiricozzi, University of Milano, Italy; Neuro_sys CNS/PNS service, 410 Chemin Dep, Gardanne, France

This research project is aimed at analyzing in detail the fate of the GM1 oligosaccharide when *in vivo* administered. Specifically, we will administer OligoGM1 to wild-type rats in different doses and routes of administration to achieve the following objectives:
a. Study the pharmacokinetics of GM1 oligosaccharide
b. Perform toxicological screening
In vivo analyses will be preceded by *in vitro* studies aimed at understanding the metabolism and possible toxicity in a simpler system.
By concluding this project, we will identify: *i)* the best dose; *ii)* the best route of administration; *iii)* distribution in the body; *iv)* the presence of OligoGM1 derivatives as a result of its metabolism; *v)* excretion. Overall, the achievement of these objectives will allow us to obtain crucial information for completing the preclinical phase and the subsequent transition to the clinical phase. The data obtained will in fact be essential to estimate a safe starting dose for human studies and to identify the parameters for clinical monitoring of potential adverse effects.

PUBLICATIONS

My scientific research activity (2017-2022) is documented by 16 peer-reviewed full articles in international journals, with 201 citations and with an *h*-index of 10 (Scopus, September 2022). Moreover, my research has been reported in 39 congress communications, 11 of them published on the Special Issues of international scientific journals (https://publons.com/researcher/4712162/maria-fazzari/publications/). I have the first authorship for 9 of those publications.

1. Mauri L, Lunghi G, **Fazzari M**, Ciampa MG, Ricci C, Bartles T, Chiricozzi E, Vasile F, Civera M (2022) "Biophysical studies to understand GM1 α-synuclein interaction" J Neurochem 162 Suppl 1:14-165. doi: 10.1111/jnc.15668

2. **Fazzari M**, Henriques A, Lunghi G, Mauri L, Ciampa M G, Tedeschi G, Mitro N, Sonnino, Sandro, Spedding M, and Chiricozzi E (2022) "GM1-oligosaccharide as a novel neuroprotective agent for Amyotrophic Lateral Sclerosis" FEBS Open Bio 12:67-336 doi: 10.1002/2211-5463

3. **Fazzari M**, Lunghi, G, Di Biase E, Audano M, Fato P, Mauri L, Ciampa M G, Tedeschi G, Mitro N, Sonnino S and Chiricozzi E (2022) "The modulation of mitochondria function as a possible mechanism

for GM1 oligosaccharide-derived neuroprotection" Neurol Int 14(1), 109-157 doi: 10.3390/neurolint14010010

4. **Fazzari M**, Lunghi G, Chiricozzi E, Mauri L, Sonnino S (2022) "Gangliosides and the Treatment of Neurodegenerative Diseases: A Long Italian Tradition" Biomedicines 2;10(2):363 doi: 10.3390/biomedicines10020363

5. **Fazzari M**, Di Biase E, Lunghi G, Mauri L, Chiricozzi E, Sonnino S (2022) "Novel insights on GM1 and Parkinson's disease: A critical review" Glycoconj J. 39(1):27-38 doi: 10.1007/s10719-021-10019-7

6. Lunghi G, **Fazzari M**, Di Biase E, Mauri L, Chiricozzi E, Sonnino S (2021) "The structure of gangliosides hides a code for determining neuronal functions" FEBS Open Bio 11(12):3193-3200 doi: 10.1002/2211-5463.13197

7. Chiricozzi E, Aureli M, Mauri L, Di Biase E, Lunghi G, **Fazzari M**, Valsecchi M, Carsana EV, Loberto N, Prinetti A, Sonnino S (2021) "Glycosphingolipids" Adv Exp Med Biol 2021;1325:61-102 doi: 10.1007/978-3-030-70115-4_3

8. **Fazzari M**, Lunghi G, Di Biase E, Audano M, Fato P, Mauri L, Ciampa MG, Tedeschi G, Mitro N, Sonnino S and Chiricozzi E (2021) "Unravelling the mechanism of GM1-oligosaccharide neuroprotection: mitochondrial regulation" Febs Open Bio 11:472-472 doi: 10.1002/2211-5463.13205

9. Lunghi G, Di Biase E, **Fazzari M**, Ciampa MG, Fato P, Mauri L, Sonnino S and Chiricozzi C (2021) "GM1-oligosaccharide neuroprotective action in in vitro and in vivo models of Parkinson's Disease" Febs Open Bio 11:472-472 doi: 10.1002/2211-5463.13205

10. Chiricozzi E, Di Biase E, Lunghi G, Fazzari M, Loberto N, Aureli M, Mauri L, Sonnino S (2021) "Turning the spotlight on the oligosaccharide chain of GM1 ganglioside" Glycoconj J 38:101–117 doi: 10.1007/s10719-021-09974-y

11. Lunghi G, **Fazzari M**, Di Biase E, Mauri L, Sonnino S, Chiricozzi E (2020) "Modulation of calcium signaling depends on the oligosaccharide of GM1 in Neuro2a mouse neuroblastoma cells" Glycoconj J 37(6):713-727 doi: 10.1007/s10719-020-09963-7

12. **Fazzari M**, Audano M, Lunghi G, Di Biase E, Loberto N, Mauri L, Mitro N, Sonnino S, Chiricozzi E (2020) "The oligosaccharide portion of ganglioside GM1 regulates mitochondrial function in neuroblastoma cells" Glycoconj J 37:293-306 doi: 10.1007/s10719-020-09920-4

13. Di Biase E, Lunghi G, Maggioni M, **Fazzari M**, Pomè DY, Loberto N, Ciampa MG, Fato P, Mauri L, Sevin E, Gosselet F, Sonnino S, Chiricozzi E (2020) "GM1 Oligosaccharide Crosses the Human Blood-Brain Barrier In Vitro by a Paracellular Route" Int J Mol Sci doi: 10.3390/ijms21082858

14. Di Biase E, Lunghi G, **Fazzari M**, Maggioni M, Pomè D Y, Valsecchi M, Samarani M, Fato P, Ciampa MG, Simona Prioni, Mauri L, Sonnino S, Chiricozzi E (2020) "Gangliosides in the differentiation process of primary neurons: the specific role of GM1-oligosaccharide" Glycoconj J 37:329-343 doi: 10.1007/s10719-020-09919-x

15. Chiricozzi E, Lunghi G, Di Biase E, **Fazzari M**, Sonnino S, Mauri L (2020) "GM1 ganglioside is a key factor in maintaining the mammalian neuronal functions avoiding neurodegeneration Review for a Special issue: "Gangliosides: Modes of Action and Cell Fates" Int J Mol Sci *21*(3), 868 doi: 10.3390/ijms21030868

16. Chiricozzi E, Mauri L, Lunghi G, Di Biase E, **Fazzari M**, Maggioni M, Valsecchi M, Prioni S, Loberto N, Pomè DY, Ciampa MG, Fato P, Verlengia G, Cattaneo S, Assini R, Wu G, Alselehdar S, Ledeen RW, Sonnino S. (2019) "Parkinson's disease recovery by GM1 oligosaccharide treatment in the *B4galnt1*$^{+/-}$ mouse model" *Sci Rep* 18;9(1):19330 doi: 10.1038/s41598-019-55885-2

17. Balestra D, Giorgio D, Bizzotto M, **Fazzari M**, Ben Zeev B, Pinotti M, Landsberger N and Frasca A (2019) "Splicing mutations impairing CDKL5 expression and activity can be efficiently rescued by U1snRNA-based therapy" Int J Mol Sci 20(17), 4130 doi: 10.3390/ijms20174130

18. **Fazzari M**, Frasca A, Bifari F, and Landsberger N (2019) "Aminoglycoside drugs induce efficient read-through of CDKL5 nonsense mutations, slightly restoring its kinase activity" RNA biol 16:1414-1423 doi: 10.1080/15476286.2019.1632633

19. **Fazzari M**, Lunghi G, Di Biase E, Audano M, Maffioli E, Grassi Scalvini F, Tedeschi G, Mauri L, Mitro N, Chiricozzi E, Sonnino S (2019) "Mitochondrial modulation: a novel role for GM1 oligosaccharide" Glycoconj J 36:267 doi: 10.1007s10719-019-09880-4

20. Chiricozzi E, Lunghi G, Di Biase E, **Fazzari M**, Valsecchi M, Mauri L, Alselehdar S, Ledeen RW, S. Sonnino (2019) "The GM1 ganglioside oligosaccharide-TrkA interaction as starting biochemical information for the developing of a new therapy for the treatment of Parkinson's disease" Glycoconj J 36:267 doi: 10.1007s10719-019-09880-4

21. Lunghi G, **Fazzari M**, Di Biase E, Mauri L, Maffioli E, Grassi Scalvini F, Tedeschi G, Chiricozzi E, Sonnino S (2019) "GM1 oligosaccharide modulation of calcium signaling in neuronal functions" Glycoconj J 36:267 doi: 10.1007s10719-019-09880-4

22. Di Biase E, Lunghi G, **Fazzari M**, Prioni S, Chiricozzi E, Sonnino S (2019) "Neurotrophic properties of GM1 oligosaccharide: evidence on the development of primary neurons in culture" Glycoconj J 36:267 doi:10.1007s10719-019-09880-4

23. **Fazzari M**, Lunghi G, Di Biase E, Audano M, Mitro N, Sonnino S, Chiricozzi E (2019) "The oligosaccharide portion of ganglioside GM1 as mitochondrial regulator" J Neurochem 150(Suppl. 1), 73–161 doi: 10.1111/jnc.14776

24. Lunghi G, Maggioni M, Di Biase E, **Fazzari M**, Tedeschi G, Maffioli E, Grassi Scalvini F, Sonnino S, Chiricozzi E (2019) "GM1 oligosaccharide is the active portion responsible for GM1 neuroprotective properties" J Neurochem 150(Suppl. 1), 73–161 doi: 10.1111/jnc.14776

25. Chiricozzi E, Maggioni M, Di Biase E, Lunghi G, **Fazzari M**, Loberto N, Maffioli E, Grassi Scalvini F, Tedeschi G, Sonnino S (2019) "The neuroprotective role of the GM1 oligosaccharide, II^3Neu5Ac-Gg_4 in neuroblastoma cells" Mol Neurobiol 56:6673-6702 doi: 10.1007/s12035-019-1556-8

26. Chiricozzi E, Di Biase E, Lunghi G, **Fazzari M**, Maggioni M, Pomè DY, Loberto N, Casellato R, Mauri L, Sonnino S (2019) "GM1 promotes TrkA-mediated neuroblastoma cell differentiation by occupying a plasma membrane domain different from TrkA" J Neurochem 149:231-241 doi: 10.1111/jnc.14685

27. Barbiero I, Valente D, Chandola C, Magi F, Bergo A, Monteonofrio L, Tramarin M, **Fazzari M**, Soddu S, Landsberger N, Rinaldo C, Kilstrup-Nielsen C (2017) "CDKL5 localizes at the centrosome and midbody and is required for faithful cell division" Sci Rep 7(1):6228 doi: 10.1038/s41598-017-05875-z

EDITING AND REVIEWING

I have reviewed articles for the following journals:

2021 - to date

- Biomolecules
- International Journal of Molecular Science
- Toxins
- Glycoconjugate Journal

In addition, I was invited (2022) to join the Reviewer Board of Medicina journal (MDPI) and to be Guest Editor for the Special Issue "Sphingolipid Metabolism and Signaling in Health and Diseases" in Biomedicines journal (MDPI).

MEMBERSHIPS

- (BraYn) association, *2021 - to date*
- Italian Society of Biochemistry and Molecular Biology (SIB), *2021 - to date*
- Federation of European Biochemical Societies (FEBS), *2021 - to date*
- European Society for Neurochemistry (ESN), *2019 - to date*
- International Society for Neurochemistry (ISN), *2019 - to date*

MEETING ORGANIZATION

6th BioMeTra Workshop, September 20th, 2022
Department of Medical Biotechnology and Translational Medicine, University of Milano, Lita, Segrate, Milano (MI), Italia – *Local committee*

DISSEMINATION ACTIVITY

- Twitter Ambassador for ESN-ISN Advanced School, September *19th - 21st 2021*
- Seed4Innovation Acceleration meetings, *2021 - 2022*
- InnovaAgorà, Milano, Italy, *May 6th - 8th 2019*
- "Una settimana da bio": a project with the aim to allow students to immerse themselves for a week in scientific research, *September 2017*
- Third mission for University of Milano: activities in primary schools in Milano and neighbouring municipalities to describe with a lay language the principle of biochemistry, *2016 – to date*
- MeetMeTonight "Viaggio al centro della cellula", *2016 - 2018*

FUNDING

Funded projects

- Funding Agency: Jerome Lejeune Foundation – 2021/2023
  Title: "GM1 oligosaccharide as a new drug candidate for Rett syndrome"
  Amount of Money Allocated: EUR 40000
  Description: This project aims at analysing the OligoGM1 therapeutic potential in Rett Syndrome via *i)* modulating neurotrophins' pathways and promoting the recovery of neuronal defects associated with the lack of Mecp2 and *ii)* counteracting the redox imbalance and mitochondrial dysfunction observed in Rett Syndrome.
  Role: CO-PI of Biochemistry unit

- Funding Agency: Milano University, Seed4Innovation program – 2021/2022
  Title: "Oligosaccharides for the treatment of Parkinson's Disease: bacterial synthesis of GM1 oligosaccharide– OLIGOtoGO"
  Amount of Money Allocated: EUR 50000
  Description: This research project is aimed to develop a new procedure for GM1 oligosaccharide bacterial production.
  Role: CO-PI

- Funding Agency: Italian Ministry of Economic Development, Seed4Innovation_PoCe_MISE program – 2021/2022
  Title: "GM1 oligosaccharide: the new therapeutic drug for sporadic Parkinson's Disease"
  Amount of Money Allocated: EUR 40000
  Description: This research project is aimed at analyzing in detail the behaviour of the GM1 oligosaccharide once administered to a complex living organism. Specifically, we will test different doses and routes of administration in order to study OligoGM1 pharmacokinetics.
  Role: CO-PI

Projects under evaluation

- Funding Agency: Brainstorming Research Assembly for Young Neuroscientists association – BraYn starting grant 2022
  Title: Unveiling GM1-oligosaccharide role in counteracting ALS mitochondria impairment (GOmitALS)
  Start/Ending dates: January 1st 2023 / June 31st 2023
  Funding: EUR 5000
  Description: the aim of this project is to understand whether GM1 oligosaccharide neuroprotection in Amyotrophic Lateral Sclerosis *in vitro* models depends on its capability to counteract mitochondrial dysfunction.

- Funding Agency: International Society for Neurochemistry - ISN International Travel Support
  Title: Dissecting mitochondrial dysfunction due to GM1 oligosaccharide deficiency in Parkinson's disease
  Start/Ending dates: April 1st 2023 / June 31st 2023
  Funding: USD 4000
  Description: the aim of this project is to understand whether GM1 oligosaccharide deficiency is directly correlated to mitochondrial dysfunction. Specifically, the allocated funding will be utilized for joining Prof. Ledeen laboratory at Rutgers University (Newark, USA), a research group with a long-standing experience in Parkinson's disease related to GM1 deficiency in *B4galnt1* mouse model.

FELLOWSHIPS

1. Postdoctoral fellowship type-A, Department of Medical Biotechnology and Translational Medicine, University of Milano (years 2020-2023)

2. Postdoctoral fellowship type-B, Department of Medical Biotechnology and Translational Medicine, University of Milano (years 2018-2020)

3. Ministerial fellowship for PhD in Experimental Medicine and Medical Biotechnology, Department of Medical Biotechnology and Translational Medicine, University of Milano (years 2014-2017)

AWARDS

1. Best oral presentation - Glycolipid and Sphingolipid Biology "A Holistic Approach to Understanding Simple and Complex Sphingolipids" (Gordon Research Conference), March 27th - April 01st, 2022 Barga (Lucca), Italy

2. YSF award for attending Young Scientists' Forum, July 6th -9th 2022, Vimeiro, Portugal, and for attending IUBMB–FEBS–PABMB Congress, July 9th -14th 2022, Lisbon, Portugal

3. SIB (Italian Biochemical Society) award for participation to 45th online FEBS Congress , July 3rd -8th, 2021

4. ESN (European Society for Neurochemistry) award for participation in the 1st ESN-ISN Advanced School, September 19th -26th, 2021 Athens, Greece

5. ISN (International Society for Neurochemistry) travel award for participation to ISN-ASN meeting, August 4th -8th, 2019, Montréal, Canada

CONGRESS PARTICIPATION

1. 13th Targeting Mitochondria Congress to be held in October 26th – 28th, 2022, Berlin (Germany)

2. 5th BraYn rainstorming Research Assembly for Young Neuroscientists Congress to be held in September 28th – 30th, 2022, Roma (Italy)

3. 6th Workshop BIOMETRA to be held in September 20th, 2022, Segrate (Italy)

4. Young Scientists' Forum 2022, July 6th - 9th, 2022, Vimeiro (Portugal)

5. 25th IUBMB–46th FEBS–15th PABMB Congress, July 9th - 14th, 2022, Lisbon (Portugal)

6. Incontro dei Giovani Biochimici dell'Area Lombarda, June 20th, 2022, Milano (Italy)

7. Gordon Research Seminar on Glycolipid and Sphingolipid Biology, March 26th - 27th, 2022, Lucca (Italy)

8. Gordon Research Conference on Glycolipid and Sphingolipid Biology, March 27th - April 1st, 2022, Lucca (Italy)

9. 4th BraYn Brainstorming Research Assembly for Young Neuroscientists, October 20th - 22nd, 2021, online

10. 1st ESN-ISN advanced school, September 19th - 26th, 2021, Athens (Greece)

11. 45th FEBS virtual Congress, July 3rd - 8th, 2021

12. 1st ESN virtual Conference, May 25th - 26th, 2021

13. First online meeting of the "Protein" Group" of SIB, May 20th - 21st, 2021

14. 61° SIB MEETING, September 23rd - 24th, 2021, online

15. 5th Workshop BIOMETRA, September 27th, 2021, Segrate (Italy)

16. 4th Workshop BIOMETRA, September 23rd, 2019, Segrate (Italy)

17. 23rd ESN Biennial Conference, September 1st - 4th, 2019, Milano (Italy)

18. 25th International Symposium on Glycoconjugates, August 25th - 31st, 2019, Milano (Italy)

19. ISN-ASN Meeting, August 4th - 8th, 2019, Montréal (Canada)

20. Rett Syndrome Research, Towards The Future, September 27th - 29th, 2018, Roma (Italy)

21. 3rd Workshop BIOMETRA, September 24th, 2018, Segrate (Italy)

22. 2nd Workshop BIOMETRA, September 26th, 2017, Segrate (Italy)

23. PhD Students Meeting: Life Sciences for a Better Future, May 11st - 13rd, 2017, Santa Margherita Ligure (Italy)

24. Epigenetic mechanisms and their relevance for human pathology, February 20th - 21st, 2017, Segrate (Italy)

25. Neuroscience network at Statale, NEURO-NEST, February 2nd 2017, Milano (Italy)

26. EMBO conference: "The complex life of mRNA", October 5th - 8th, 2016, EMBL, Heidelberg (Germany)

27. FOCUS on CDKL5, November 4th, 2016, Torino (Italy)

28. 1st Workshop BIOMETRA, September 26th, 2016, Segrate (Italy)

29. EMBO conference: "RNA localization and local translation", June 28th, – July 3rd, 2015, Hersonissos (Greece)

COMMUNICATIONS TO SCIENTIFIC MEETINGS

1. **Fazzari M**, "GM1 oligosaccharide as mitochondrial modulator: implications in neurological diseases" Berlin, Germany **(2022)13th Targeting Mitochondria Congress**

2. **Fazzari M**, Lunghi G, Henriques A, Di Biase E, Mauri L, Ciampa MG, Maffioli E, Grassi Scalvini F, Audano M, Tedeschi G, Mitro N, Sonnino S, Spedding M, Chiricozzi E "GM1-oligosaccharide counteracts neurodegeneration of Amyotrophic Lateral Sclerosis SOD1G93A motor neurons" Segrate, Italia **(2022) Workshop BIOMETRA**

3. **Fazzari M**, Lunghi G, Di Biase E, Ciampa M G, Fato P, Mauri L, Zaccagnini L, Bartels T, Sonnino S and Chiricozzi E "Counteracting α-synuclein aggregation: a novel role for GM1 oligosaccharide" Roma, Italia **(2022) 4th Brainstorming Research Assembly for Young Neuroscientists**

4. Mauri L, Lunghi G, **Fazzari M**, Ciampa MG, Ricci C, Bartles T, Chiricozzi E, Vasile F, Civera M "Biophysical studies to understand GM1 α-synuclein interaction" published on J Neurochem 162 Suppl 1:14-165 **(2022) ISN-APSN meeting**

5. **Fazzari M**, Henriques A, Lunghi G, Mauri L, Ciampa M G, Tedeschi G, Mitro N, Sonnino, Sandro, Spedding M, and Chiricozzi E "GM1-oligosaccharide as a novel neuroprotective agent for Amyotrophic Lateral Sclerosis" Lisbon, Portugal **(2022) IUBMB, FEBS and PABMB Congress** doi:10.1002/2211-5463.13440

6. **Fazzari M**, Henriques A, Lunghi G, Mauri L, Ciampa M G, Tedeschi G, Mitro N, Sonnino, Sandro, Spedding M, and Chiricozzi E "GM1-oligosaccharide as a novel neuroprotective agent for Amyotrophic Lateral Sclerosis" Vimeiro, Portugal **(2022) Young Scientists' Forum 2022**

7. **Fazzari M**, Audano M, Di Biase E, Lunghi G, Maffioli E, Grassi Scalvini F, Mauri L, Ciampa MG, Sonnino S, Tedeschi G, Mitro N, and Chiricozzi E "GM1 oligosaccharide interaction at plasma membrane triggers a neuroprotective program acting via mitochondria modulation" Milano, Italy **(2022) Incontro Giovani Biochimici dell'area lombarda**

8. **Fazzari M**, Di Biase E, Lunghi G, Audano M, Mauri L, Ciampa MG, Tedeschi G, Mitro N, Sonnino S and Chiricozzi E "GM1 oligosaccharide membrane signalling: the switch to turn on a neuroprotective program acting via mitochondria modulation" Lucca (Barga), Italy **(2022) Glycolipid and Sphingolipid Biology "A Holistic Approach to Understanding Simple and Complex Sphingolipids" (Gordon Research Conference)**

9. Mauri L, Lunghi G, **Fazzari M**, Ciampa MG, Fato P, Ricci C, Bartels T, Sonnino S, Chiricozzi E, Vasile F, and Civera M "Biophysical studies to understand the role of membrane GM1 in avoiding α-synuclein aggregation" Lucca (Barga), Italy **(2022) Glycolipid and Sphingolipid Biology "A Holistic Approach to Understanding Simple and Complex Sphingolipids" (Gordon Research Conference)**

10. **Fazzari M**, Di Biase E, Lunghi G, Audano M, Mauri L, Ciampa MG, Tedeschi G, Mitro N, Sonnino S and Chiricozzi E "GM1 oligosaccharide membrane signalling: the switch to turn on a neuroprotective program acting via mitochondria modulation" Lucca (Barga), Italy **(2022) Glycolipid and Sphingolipid Biology "The Complex Biology and Pathobiology of Sphingolipids and Glycosphingolipids" (Gordon Research Seminar)**

11. **Fazzari M**, Di Biase E, Lunghi G, Fato P, Mauri L, Ciampa MG, Parravicini C, Palazzolo L, Maffioli E, Grassi Scalvini F, Eberini I, Tedeschi G, Sonnino S and Chiricozzi E. "Ganglioside-protein interaction at plasma membrane level: the role of GM1 oligosaccharide" **(2021) WEBPRO - Proteins on the Web 2021**

12. Lunghi G, **Fazzari M**, Di Biase E, Ciampa MG, Fato P, Maffioli E, Grassi Scalvini F, Tedeschi G, Sonnino S and Chiricozzi E. "The multitasking role of GM1 oligosaccharide in modulating neuronal intracellular signalling" **(2021) WEBPRO - Proteins on the Web 2021**

13. **Fazzari M**, Lunghi G, Di Biase E, Audano M, Fato P, Mauri L, Ciampa MG, Tedeschi G, Mitro N, Sonnino S and Chiricozzi E. "Unravelling the mechanism of GM1-oligosaccharide neuroprotection: mitochondrial regulation" published on Febs Open Bio, 2021. 11: p. 472-472. **(2021) 45th Virtual FEBS Congress**

14. Lunghi G, Di Biase E, **Fazzari M**, Ciampa MG, Fato P, Mauri L, Sonnino S and Chiricozzi C. "GM1-oligosaccharide neuroprotective action in in vitro and in vivo models of Parkinson's Disease" published on Febs Open Bio, 2021. 11: p. 472-472. **(2021) 45th Virtual FEBS Congress**

15. Lunghi G, Di Biase E, **Fazzari M**, Ciampa M G, Fato P, Mauri L, Zaccagnini L, Bartels T, Sonnino S and Chiricozzi E. "GM1 oligosaccharide role in preventing α-synuclein aggregation" **(2021) 61st SIB MEETING Virtual Edition**

16. **Fazzari M**, Lunghi G, Di Biase E, Audano M, Fato P, Mauri L, Ciampa M G, Tedeschi G, Mitro N, Sonnino S and Chiricozzi E. "Mitochondrial modulation: a novel mechanism underlying GM1-oligosaccharide neuroprotection" **(2021) 1st ESN Virtual Conference: Future perspectives for European neurochemistry – a young scientists conference**

17. Lunghi G, Di Biase E, **Fazzari M**, Ciampa M G, Fato P, Mauri L, Sonnino S and Chiricozzi E "The oligosaccharide portion of ganglioside GM1 displays neuroprotective properties in in vitro and in vivo models of Parkinson's Disease" **(2021) 1st ESN Virtual Conference: Future perspectives for European neurochemistry – a young scientists conference**

18. **Fazzari M**, Henriques A, Lunghi G, Di Biase E, Mauri L, Ciampa M G, Tedeschi G, Mitro N, Sonnino S, Spedding M and Chiricozzi E. "GM1-oligosaccharide protective effect in a glutamate-induced toxicity experimental model of Amyotrophic Lateral Sclerosis" Athens, Greece **(2021) 1st ESN-ISN Advanced School "From Neurodegeneration to Neural Carcinogenesis: Mechanisms and Common Biologies"**

19. Lunghi G, Di Biase E, **Fazzari M**, Ciampa M G, Fato P, Mauri L, Zaccagnini L, Bartels T, Sonnino S and Chiricozzi E. "GM1 oligosaccharide role in preventing α-synuclein aggregation" Athens, Greece **(2021) 1st ESN-ISN Advanced School "From Neurodegeneration to Neural Carcinogenesis: Mechanisms and Common Biologies"**

20. **Fazzari M**, Lunghi, G, Di Biase E, Audano M, Fato P, Mauri L, Ciampa M G, Tedeschi G, Mitro N, Sonnino S and Chiricozzi. "The modulation of mitochondria function as a possible mechanism for GM1 oligosaccharide-derived neuroprotection" Pisa, Italia published on Neurol. Int. 2022, 14(1), 109-157; https://doi.org/10.3390/neurolint14010010 **(2021) 4th Brainstorming Research Assembly for Young Neuroscientists**

21. **Fazzari M**, Frasca A, Lunghi G, Di Biase E, Casati S, Mauri L, Ciampa M G, Tedeschi G, Mitro N, Sonnino S, Landsberger N and Chiricozzi E. "GM1-oligosaccharide as a new drug candidate for Rett syndrome" Segrate, Italia **(2021) Workshop BIOMETRA**

22. **Fazzari M**, Lunghi G, Di Biase E, Audano M, Maffioli E, Grassi Scalvini F, Tedeschi G, Mauri L, Mitro N, Chiricozzi E, Sonnino S "Mitochondrial modulation: a novel role for GM1 oligosaccharide" Segrate, Italia **(2019) Workshop BIOMETRA**

23. Di Biase E, Lunghi G, **Fazzari M**, Maggioni M, Mauri M, Herrero Ezquerro MT, Chiricozzi E, Sonnino S "The neuroprotective role of GM1-oligosaccharide in MPTP models of Parkinson's Disease" Segrate, Italia **(2019) Workshop BIOMETRA**

24. **Fazzari M**, Lunghi G, Di Biase E, Audano M, Maffioli E, Grassi Scalvini F, Tedeschi G, Mitro N, Sonnino S and Chiricozzi E "GM1 oligosaccharide as mitochondrial regulator in neuronal cells" Milano, Italia **(2019) ESN Biennial Conference, Molecular Mechanism of Regulation of the Nervous System**

25. Lunghi G, Di Biase, **Fazzari M**, Maggioni M, Tedeschi G, Maffioli E, Grassi Scalvini F, Chiricozzi E, Sonnino S "GM1 oligosaccharide modulation of calcium signaling in neuronal function" Milano, Italia **(2019) ESN Biennial Conference, Molecular Mechanism of Regulation of the Nervous System**

26. Di Biase E, Lunghi G, **Fazzari M**, Prioni S, Sonnino S and Chiricozzi E "The oligosaccharide chain of GM1 ganglioside acts as a neurotrophic agent for neuronal development" Milano, Italia **(2019) ESN Biennial Conference, Molecular Mechanism of Regulation of the Nervous System**

27. **Fazzari M**, Lunghi G, Di Biase E, Audano M, Maffioli E, Grassi Scalvini F, Tedeschi G, Mauri L, Mitro N, Chiricozzi E, Sonnino S "Mitochondrial modulation: a novel role for GM1 oligosaccharide" published on Glycoconj J 36:267 doi:10.1007s10719-019-09880-4 Milano, Italia **(2019) 25th International Symposium on Glycoconjugates**

28. Chiricozzi E, Lunghi G, Di Biase E, **Fazzari M**, Valsecchi M, Mauri L, Alselehdar S, Ledeen RW, S. Sonnino "The GM1 ganglioside oligosaccharide-TrkA interaction as starting biochemical information for the developing of a new therapy for the treatment of Parkinson's disease" published on Glycoconj J 36:267 doi:10.1007s10719-019-09880-4 Milano, Italia **(2019) 25th International Symposium on Glycoconjugates**

29. Lunghi G, **Fazzari M**, Di Biase E, Mauri L, Maffioli E, Grassi Scalvini F, Tedeschi G, Chiricozzi E, Sonnino S "GM1 oligosaccharide modulation of calcium signaling in neuronal functions" published on Glycoconj J 36:267 doi:10.1007s10719-019-09880-4 Milano, Italia **(2019) 25th International Symposium on Glycoconjugates**

30. Di Biase E, Lunghi G, **Fazzari M**, Prioni S, Chiricozzi E, Sonnino S "Neurotrophic properties of GM1 oligosaccharide: evidence on the development of primary neurons in culture" published on Glycoconj J 36: 267 doi:10.1007s10719-019-09880-4 Milano, Italia **(2019) 25th International Symposium on Glycoconjugates**

31. **Fazzari M**, Lunghi G, Di Biase E, Audano M, Mitro N, Sonnino S, Chiricozzi E "The oligosaccharide portion of ganglioside GM1 as mitochondrial regulator" published on J Neurochem 150(Suppl. 1), 73–161 doi: 10.1111/jnc.14776, Montréal, Canada **(2019) ISN-ASN Meeting**

32. Lunghi G, Maggioni M, Di Biase E, **Fazzari M**, Tedeschi G, Maffioli E, Grassi Scalvini F, Sonnino S, Chiricozzi E "GM1 oligosaccharide is the active portion responsible for GM1 neuroprotective properties" published on J Neurochem 150(Suppl. 1) 73–161 doi: 10.1111/jnc.14776, Montréal, Canada **(2019) ISN-ASN Meeting**

33. Di Biase E, Lunghi G, **Fazzari M**, Maggioni M, Prioni S, Pomè DY, Mauri L, Valsecchi M, Loberto N, Chiricozzi E and Sonnino S "The OligoGM1 story: from the bench to the bed-side". Gargnano, Garda, Italia **(2019) Meeting of Young Biochemists of Lombardia region**

34. Chiricozzi E, Di Biase E, Lunghi G, Maggioni M, **Fazzari M**, Prioni S, Sevin E, Gosselet F, Ledeen R, Sonnino S "GM1 oligosaccharide as a new drug for sporadic parkinson's disease", Lisbon, Portugal **(2019) AD/PD 2019**

35. **Fazzari M**, Kilstrup-Nielsen C, Landsberger N "Translational read-through: proof of principle for a "personalised medicine approach" in CDKL5-related pathologies", Roma, Italy **(2018) Rett Syndrome Research, Towards The Future**

36. Frasca A, **Fazzari M**, Giorgio D, Tortora M, Brivio E, Palmieri M, Landsberger N "Basic and translational studies for the treatment of MECP2- and CDKL5-related disorders", Sala Napoleonica – via Sant'Antonio 12, Milano, Italia **(2017) 1st meeting traslazionale del gruppo di ricerca strategico in neuroscienze de "La Statale"**

37. **Fazzari M**, Kilstrup-Nielsen C, Landsberger N "PTC suppression strategy as "personalized medicine" approach for CDKL5 related pathologies", EMBL, Heidelberg, Germania **(2016) The complex life of mRNA**

38. Stefanelli G, Gandaglia A, Giorgio D, **Fazzari M**, Di Marino D, Ausió J, Costa M, Kilstrup-Nielsen C, Landsberger N "Brain phosphorylation of MeCP2 at serine 164 is developmentally regulated and globally alters its chromatin association", Vienna, Austria **(2016) RTT 50.1**

39. Stefanelli G, Gandaglia A, Giorgio D, **Fazzari M**, Di Marino D, Ausió J, Costa M, Kilstrup-Nielsen C, Landsberger N "Brain phosphorylation of MeCP2 at serine 164 is developmentally regulated and alters its chromatin association globally", Itasca, USA **(2016) Rett Syndrome Symposium**

ORAL PRESENTATIONS

1. **Fazzari M** – *invited speaker*
   "GM1 oligosaccharide as mitochondrial modulator: implications in neurological diseases" 13th Targeting Mitochondria Congress, October 27th, 2022, Berlin, Germany

2. **Fazzari M**, Di Biase E, Lunghi G, Audano M, Mauri L, Ciampa MG, Tedeschi G, Mitro N, Sonnino S and Chiricozzi E "GM1 oligosaccharide membrane signalling: the switch to turn on a neuroprotective program acting via mitochondria modulation" Glycolipid and Sphingolipid Biology "A Holistic Approach to Understanding Simple and Complex Sphingolipids" (Gordon Research Conference), March 31st, 2022, Lucca (Barga), Italy

3. **Fazzari M**, Di Biase E, Lunghi G, Audano M, Mauri L, Ciampa MG, Tedeschi G, Mitro N, Sonnino S and Chiricozzi E "GM1 oligosaccharide membrane signalling: the switch to turn on a neuroprotective program acting via mitochondria modulation" Glycolipid and Sphingolipid Biology "The Complex Biology and Pathobiology of Sphingolipids and Glycosphingolipids" (Gordon Research Seminar), March 27th, 2022, Lucca (Barga), Italy

4. **Fazzari M** – *invited speaker*
   "Glycosphingolipids-protein interaction at plasma membrane: the switch to turn on specific intracellular signalings" BIOMETRA Seminars, March 22th, 2022, L.I.T.A., Segrate, Italy

5. **Fazzari M**, Frasca A, Lunghi G, Di Biase E, Casati S, Mauri L, Ciampa M G, Tedeschi G, Mitro N, Sonnino S, Landsberger N and Chiricozzi E "GM1-oligosaccharide therapeutic potential for treatment of Rett syndrome" 61° SIB MEETING Virtual Edition, September 24th, 2021

6. **Fazzari M**, Kilstrup-Nielsen C and Landsberger N "PTC suppression strategy in *CDKL5*-related disorders: analysing the feasibility of a "personalised" medicine approach" Workshop BIOMETRA", September 26th, 2017, L.I.T.A., Segrate, Italy

7. **Fazzari M**, Kilstrup-Nielsen C and Landsberger N "Stop codon read-through strategy in *CDKL5*-related pathologies" PhD Students Meeting: Life Sciences for a Better Future, May 11-13th, 2017, Santa Margherita Ligure, Italy

8. **Fazzari M**, Tramarin M, La Montanara P and Landsberger N "Analysis of synaptoneurosomes: may alteration of stored mRNAs have a role in Rett syndrome pathophysiology?" Fall School in Neuroscience, September 29th - October 2nd, 2015, Baveno, Italy

COURSES

December 3rd, 2021
Course on radiation protection
L.I.T.A. Segrate (Milan), Italy

September 3rd and 17th, 2021
Intensive course in experimental design and biostatistics
Online course of the Guido Bernardini Foundation

September 19th-21st, 2021
ESN-ISN Advanced School "From Neurodegeneration to Neural Carcinogenesis: Mechanisms and Common Biologies"
Biomedical Research Foundation of the Academy of Athens, Athens, Greece

November 22nd-27th, 2018
XL ALEMBIC Theoretical and Practical Course of Optical and Electron Microscopy
Advanced Light and Electron Microscopy Center (ALEMBIC), IRCCS San Raffaele Hospital, Milano, Italy

March 29th-31st, 2017
Introductory course to animal experimentation
IRCCS-Pharmacological Research Institute "M. Negri", Milano, Italy

September 29th - October 2nd, 2015
Fall School in Neuroscience
Baveno (Stresa), Italy

TECHNICAL SKILLS AND COMPETENCES

Thanks to research in the laboratory, managing the work of trainees and tutoring activities, I acquired over the years excellent communication, teamwork and management skills.

TECHNICAL SKILLS During my scientific training I acquired a great experience in the field of biochemistry and molecular/cellular biology and, in particular, in the following methodologies: *i)* **Biochemistry:** SDS-PAGE and Western Blotting, subcellular fractionation and preparation of synaptoneurosomes from murine cortex, immunoprecipitation, methods for analyzing the ganglioside content of cells in culture; metabolic radiolabeling techniques of lipids, methods for the characterization of lipid and protein content by thin layer chromatography, enzymatic assays with fluorigenic and natural substrates; *ii)* **Cell biology:** maintenance and manipulation (transient and stable transfection, pharmacological treatment) of cell cultures (HeLa, HEK293, N2a, NIH3T3, COS7, human fibroblasts, preparation of cortical primary neurons from mouse embryos); *iii)* **Molecular biology:** DNA/RNA extraction, primers design, PCR, qPCR, bacterial transformation/electroporation, preparation of competent bacteria, Mini-Mini-Maxi Prep, plasmid vector mutagenesis, cloning; *iv)* **Imaging experiments:** sectioning of fixed cerebellar tissue using cryostat, immunohistochemistry and immunocytochemistry with qualitative and quantitative analysis of wide-field and confocal microscopy fluorescence images; *v)* ***In vivo*****:** manipulation of C57BL/6 and CD1 strains (wild-type and transgenic), behavioral analysis (Rotarod test, Pole test, NOR), subcutaneous and intraperitoneal injection of substances, sacrifice via perfusion or cervical dislocation, brain dissection, isolation of different organs and embryos.

INFORMATIC SKILLS Excellent knowledge of Microsoft Office, LateX, Adobe Photoshop, Inkscape, statistical analysis software (Prism), image analysis software (ImageJ).

Languages

- Italian (native)
- English (B2, fluent)

Data	September 8th 2022	Luogo	Segrate, Milano, Italy