BIOGRAPHICAL SKETCH

NAME: Alessandro Fraldi

POSITION TITLE: Assistant Professor (tenure-track) of Medical Genetics, University of Naples "Federico II'

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Faculty of Science, University of Naples "Federico II", Italy	BSc DEGREE (cum laude)	10/2000	MOLECULAR BIOLOGY
Department of Genetics, Faculty of Science, University of Naples "Federico II", Italy	PhD	03/2004	HUMAN GENETICS
Telethon Institute of Genetics and Medicine (TIGEM), Naples, Italy		2004-2011	HUMAN GENETICS/ MOLECULAR THERAPY
Lysosomal Disease Research Unit" Woman's and Children's Hospital, Adelaide, Australia		2005-2006	HUMAN GENETICS

A. PERSONAL STATEMENT

My laboratory mainly focuses on (I) the study of lysosomal dysfunction and protein aggregation in neurodegenerative lysosomal diseases (LSDs) and (II) the development of innovative therapeutic strategies for the treatment of brain pathology in lysosomal storage diseases (LSDs).
Over the years I have accumulated experience in the study of LSDs. I have worked on both characterization of LSD phenotype, particularly the neurological aspects, and testing new therapeutic approaches for LSD treatment using mice and pigs as model systems. In particular, my lab developed novel brain directed AAV-mediated gene therapy approaches for Sanfilippo syndrome, one of the most common and severe form of LSD. One of these studies constituted the basis for designing the first gene therapy clinical trial for Sanfilippo A patients that is now under the phase II/III. I have also built a very solid expertise in cell biology and biochemistry, which I applied to study the molecular mechanisms underlying LSD neuropathogenesis using both cellular and mouse models of LSDs with the final aim to identify new druggable therapeutic targets. Ongoing projects in my lab are focused on developing therapies for the treatment of the CNS in Sanfilippo syndrome based on the use of anti-amyloids drugs to counteract lysosomal-autophagy pathway dysfunction that we identified as a major factor driving neurodegeneration in these severe neuropathologies.

1. Monaco A, Maffia V, Sorrentino NC, Sambri I, Ezhova Y, Giuliano T, Cacace V, Nusco E, De Risi M, De Leonibus E, Schrader, T, Klärner FG, Bitan G, **Fraldi A*.** *The amyloid inhibitor CLR01 relieves autophagy and ameliorates neuropathology in a severe lysosomal storage disease.* **Molecular Therapy 2020** *Apr 8;28(4):1167-1176. doi:10.1016/j.ymthe.2020.02.005* ***corresponding author** PubMed PMID: 32087148; PubMed Central PMCID: PMC7132627
2. Sambri I, D'Alessio R, Ezhova Y, Giuliano T, Sorrentino NC, Cacace V, De Risi M, Cataldi M, Annunziato L, De Leonibus E and **Fraldi, A***. *Lysosomal dysfunction disrupts presynaptic maintenance and restoration of presynaptic function prevents neurodegeneration in lysosomal storage diseases.* **EMBO Molecular Medicine. 2017** 9, 112-132 ***corresponding author -cover highlight-** PubMed PMID: 27881461; PubMed Central PMCID: PMC5210158.
3. Sorrentino NC, Cacace V, De Risi M, Maffia V, Strollo S, Tedesco N, Nusco E, Romagnoli N, Ventrella D, Huang Y, Liu N, Kalled SL, Choi VW, De Leonibus E, **Fraldi A*.** *Enhancing the therapeutic potential of sulfamidase for the treatment of Mucopolysaccharidosis IIIA* **Molecular Therapy- *Methods & Clinical***

***Development,* 2019** Oct 28;15:333-342 ***corresponding author** PubMed PMID: 31788497; PubMed Central PMCID: PMC6881609.

4. Sorrentino NC, D'Orsi L, Monaco C, Nusco E, Sambri I, Spampanato C, Polishhuck EV, Saccone P, De Leonibus E, Ballabio A, **Fraldi A***. *A highly secreted sulfamidase engineered to cross the blood-brain barrier corrects the CNS pathology of mice with mucopolysaccharidoses type IIIA.* **EMBO Mol Med. 2013** May; 5(5):675-90 ***corresponding author** PubMed PMID: 23568409; PubMed Central PMCID: PMC3662312.

B. POSITIONS AND HONORS

Positions and Employment

2017-present Assistant Professor (tenure track), Department of Translational Medicine University of Naples "Federico II'

2013-present Faculty Member of the European School of Molecular Medicine (SEMM)

2011-2020 Faculty and Investigator, Molecular Therapy Program, Telethon Institute of Genetics and Medicine (TIGEM)

Other Experience and Professional Activities

-Journal Reviewer: "Ad hoc" reviewer for Science Translational Medicine, EMBO Mol. Med., Human Molecular Genetics, Autophagy, Human Gene Therapy, PLOS One, Neuroscience and Scientific Reports

-Reviewer panel member: European Research Council (ERC), Medical Research Council (MRC), Sanfilippo Children's Foundation, Agency for Health Quality and Assessment of Catalonia.

2017- Meeting Organization: Co-organizer and Scientific committee member of XVII National Congress of Italian Neuroscience Society (SINS), (Ischia, Italy, 2017)

2005-present Member, American Society of Gene & Cell Therapy

2020-present Scientific Advisory Board Member, Sanfilippo Fighters Foundation

Invited lectures to internationally established conferences/advances schools

2009- FEBS advanced course, Ortona, 2009
2011- Sanfilippo research and treatment workshop Northampton, 2011
2011- Brains for Brain conference Frankfort, 2011
2013- XIX Annual Meeting German Society for Gene Therapy (DG-GT), 2013
2013- Brains for Brain conference Frankfort, 2013
2015- John van Geest Centre for Brain Repair, University of Cambridge, 2015
2017- Telethon Convention, Riva del Garda, 2017
2017- II evento nazionale sulle mucopolisaccaridosi, Milan, 2017
2017- International Workshop on "Inhibition of Protein-Protein Interactions in Alzheimer's Disease and Related Proteinopathies" Lille University, 2017
2019- International Workshop on "Strategies and tools for modulating pathologic protein self-assembly, Porto (Portugal) March 21-22, 2019
2019- ESGCT Spring School 2019, Tigem, Pozzuoli, April 3-5, 2019
2019- 23rd ESN biennial meeting on molecular mechanisms of regulation of the nervous system, Milan, September 1-4, 2019
2019- EGSLD course on Lysosomes and Lysosomal Diseases, Catalonia (Spain),10-13th October 2019

Honors

2000- B.Sc. Cum Laude, University of Naples "Federico II"
2002- International Mobility Program Award, University of Naples "Federico II"
2003- EMBO fellowship, program 2003
2004- Ph.D. Summa cum Laude, University of Naples "Federico II"
2005- EMBO fellowship, program 2005

C. CONTRIBUTIONS TO SCIENCE

Selected peer-reviewed publications

1. As a postdoctoral fellow I studied the mechanisms of action of SUMF1, a master regulator of the activities of sulfatases, a group of lysosomal enzymes whose deficiency causes different forms of LSDs. Moreover, during my postdoc at the Lysosomal Disease Unit in Adelaide (Australia) I contributed to characterize the first mouse model of Sanfilippo A (MPS-IIIA) and provided the first proof-of-principle demonstrating the therapeutic efficacy of AAV-mediated gene therapy approach in Sanfilippo A using the characterized mouse model.

a. **Fraldi A**, Biffi A, Lombardi A, Visigalli I, Pepe S, Settembre C, Nusco E, Auricchio A, Naldini L, Ballabio A, Cosma MP. *SUMF1 enhances sulfatase activities in vivo in five sulfatase deficiencies*. **Biochem J 2007**;403:305-12. PubMed PMID: 17206939; PubMed Central PMCID: PMC1874239.
b. **Fraldi A***, Hemsley K, Crawley A, Lombardi A, Lau A, Sutherland L, Auricchio A, Ballabio A, Hopwood J. *Functional correction of CNS lesions in an MPS-IIIA mouse model by intracerebral AAV-mediated delivery of sulfamidase and SUMF1 genes*. **Hum Mol Genet 2007**;16:2693-702. ***corresponding author.** PubMed PMID: 17725987
c. **Fraldi A**, Zito E, Annunziata F, Lombardi A, Cozzolino M, Monti M, Spampanato C, Ballabio A, Pucci P, Sitia R, Cosma MP. *Multistep, sequential control of the trafficking and function of the multiple sulfatase deficiency gene product, SUMF1 by PDI, ERGIC-53 and ERp44*. **Hum Mol Genet 2008**;17:2610-21. PubMed PMID: 18508857
d. Tardieu M., Zerah M., Husson B., de Bournonville S., Deiva K., Adamsbaum C., Vincent F., Hocquemiller M., Broissand C., Furlan V., Ballabio A., **Fraldi A**., Crystal R., Baugnon T., Roujeau T., Heard, J.M. and Danos O. *Intracerebral administration of AAV rh.10 carrying human SGSH and SUMF1 cDNAs in children with MPSIIIA disease: results of a phase I/II trial*. **Human Gene Therapy. 2014**; June 25(6):506-16. PubMed PMID: 24524415.

2. I contributed to the elucidation of mechanisms underlying lysosomal dysfunction in LSDs. In particular, we have provided the first demonstration of a block of autophagy in a severe form of LSDs. Our HMG 2007 paper describing these results is considered a landmark in the field (cited over 400 times). Many subsequent papers, inspired by our work showed similar results in several types of LSDs

a. Settembre C, **Fraldi A***, Jahreiss L, Spampanato C, Venturi C, Medina D, de Pablo R, Tacchetti C, Rubinsztein DC, Ballabio A. *A block of autophagy in lysosomal storage disorders*. **Hum Mol Genet 2008**;17:119-29. ***co-first author.** PubMed PMID: 17913701
b. **Fraldi A**, Annunziata F, Lombardi A, Kaiser HJ, Medina DL, Spampanato C, Fedele AO, Polishchuk R, Sorrentino NC, Simons K, Ballabio A. *Lysosomal fusion and SNARE function are impaired by cholesterol accumulation in lysosomal storage disorders*. **EMBO J 2010**; Nov 29:3607-20. PubMed PMID: 20871593; PubMed Central PMCID: PMC2982760.
c. Medina DL, **Fraldi A***, Bouchè V, Annunziata F, Mansueto G, Spampanato C, Puri C, Pignata A, Martina JA, Sardiello M, Polischuk R, Puertollano R and Ballabio A. *Transcriptional activation of lysosomal exocytosis promotes cellular clearance.* **Dev. Cell. 2011;** 21(3):421-430. ***co-first author.** PubMed PMID: 21889421; PubMed Central PMCID: PMC3173716
d. **Fraldi A*,** Klein AD, Medina DL, Settembre C. *Brain Disorders Due to Lysosomal Dysfunction*. **Annu Rev Neurosci. 2016** Jul 8;39:277-95. ***corresponding author** PubMed PMID: 27090953

3. A research line in my lab is focused on developing new gene therapy approaches to treat the CNS in LSDs (particularly in the Sanfilippo syndrome) based on the use of AAV as viral vectors to transfer the therapeutic gene to the CNS. We have pioneered the use of engineered variants of therapeutic genes to improve the therapeutic potential of these approaches

a. Sorrentino NC, D'Orsi L, Monaco C, Nusco E, Sambri I, Spampanato C, Polishhuck EV, Saccone P, De Leonibus E, Ballabio A, **Fraldi A***. *A highly secreted sulfamidase engineered to cross the blood-brain barrier corrects the CNS pathology of mice with mucopolysaccharidoses type IIIA.* **EMBO Mol Med. 2013** May; 5(5):675-90 ***corresponding author** PubMed PMID: 23568409; PubMed Central PMCID: PMC3662312
b. Sorrentino NC, Maffia V, Strollo S, Cacace V, Romagnoli N, Manfredi A, Ventrella D, Dondi F, Barone F, Giunti M, Graham AR, Huang Y, Kalled S, Auricchio A, Bacci ML, Surace EM and **Fraldi A***. *A comprehensive map of CNS transduction by eight recombinant adeno-associated virus serotypes upon cerebrospinal fluid administration in pigs* **Molecular Therapy. 2015** Feb;24(2):276-86 ***corresponding author** PubMed PMID: 26639405; PubMed Central PMCID: PMC4817820

c. Sorrentino NC, Cacace V, De Risi M, Maffia V, Strollo S, Tedesco N, Nusco E, Romagnoli N, Ventrella D, Huang Y, Liu N, Kalled SL, Choi VW, De Leonibus E, **Fraldi A*.** *Enhancing the therapeutic potential of sulfamidase for the treatment of Mucopolysaccharidosis IIIA* **Molecular Therapy- *Methods & Clinical Development,* 2019** Oct 28;15:333-342 ***corresponding author** PubMed PMID: 31788497; PubMed Central PMCID: PMC6881609
d. **Fraldi A***, Serafini M, Sorrentino NC, Gentner B, Aiuti A, Bernardo ME. *Gene therapy for mucopolysaccharidoses: in vivo and ex vivo approaches.* **. IJ of Pediatr. 2018; 44 (Suppl 2): 130. *co-corresponding author** PubMed PMID: 30442177; PubMed Central PMCID: PMC6238250

4. More recently, I discovered a new mechanism driving neurodegeneration in Sanfilippo syndrome. By studying the mouse model of Sanfilippo A, one of the most and severe forms of neurodegenerative LSDs, we demonstrated that neuronal cell bodies provide a major site for progressive deposition of multiple amyloid proteins including α-synuclein, PrP, Tau and amyloid ß-protein. Importantly, we provided evidence that preventing amyloid deposition by using CLR01, a potent broad-spectrum inhibitor of amyloid self-assembly effectively protects against neurodegenerative processes in Sanfilippo A mice by relieving autophagy-lysosomal dysfunction. Our data put new insights in the processes triggering neurodegeneration in LSDs and show for the first time that targeting amyloid aggregation is an effective strategy to treat the CNS pathology in Sanfilippo A and likely in other LSDs.

a. Sambri I, D'Alessio R, Ezhova Y, Giuliano T, Sorrentino NC, Cacace V, De Risi M, Cataldi M, Annunziato L, De Leonibus E and **Fraldi, A***. *Lysosomal dysfunction disrupts presynaptic maintenance and restoration of presynaptic function prevents neurodegeneration in lysosomal storage diseases.* **EMBO Molecular Medicine. 2017** 9, 112-132 ***corresponding author -cover highlight-** PubMed PMID: 27881461; PubMed Central PMCID: PMC5210158
b. Monaco A, Maffia V, Sorrentino NC, Sambri I, Ezhova Y, Giuliano T, Cacace V, Nusco E, De Risi M, De Leonibus E, Schrader, T, Klärner FG, Bitan G, **Fraldi A*.** *The amyloid inhibitor CLR01 relieves autophagy and ameliorates neuropathology in a severe lysosomal storage disease.* **Molecular Therapy 2020** *Apr 8;28(4):1167-1176.* ***corresponding author** PubMed PMID: 32087148; PubMed Central PMCID: PMC7132627
c. Monaco A & **Fraldi A*.** *Protein Aggregation and Lysosomal Dysfunction: A vicious cycle in Lysosomal Storage Diseases.* **Front. Cell. Neurosci. 2020***.* Mar 11;13:37*.* ***corresponding author** PubMed PMID: 32218723; PubMed Central PMCID: PMC7079699

Book chapters and meeting abstracts in peer-reviewed Journals

1. Parenti, G and **Fraldi A*.** *Pathogenesis of Mucopolysaccharidoses: Dysfunction of Lysosomes*; Chapter 5 in **Mucopolysaccharidoses Update 2018**; Edited by Shunji Tomatsu: ISBN: 978-1-53613-986-0 ***co-corresponding author**
2. Monaco, A; Ezhova, Y; Giuliano, T; Sorrentino, NC; and **Fraldi, A*.** *Targeting neuronal proteostasis to treat the CNS in lysosomal storage diseases.* Meeting abstract of the 6th Meeting of the Neapolitan Brain Group. **BMC Neuroscience 2018**, 19 (suppl 3):67 ***corresponding author**
3. Sorrentino, NC.; Cacace, V; Maffia, V; Strollo, S; Tedesco, N; Nusco, E; Romagnoli, N; Ventrella, D; Barone, F ; Bacci, M; Huang, Y; Liu, N; Kalled, S; Choi, V and **Fraldi, A***. *Enhanced Version of Human Sulfamidase Significantly Ameliorates CNS Pathology When Delivered to the MPS-IIIA Mice by AAV-Mediated Intra-CSF Injection*. Meeting Abstract of 21st Annual Meeting of the American-Society-of-Gene-and-Cell-Therapy (ASGCT). **Molecular Therapy 2018** 26(5)(suppl 1): 132-132 ***corresponding author**

Complete List of Publications: H-index = 23 (Web of Science)

D. ADDITIONAL INFORMATION

Patents

Granted

WO 2012085622 A1: Therapeutic strategies to treat CNS pathology in mucopolysaccharidoses

Applications

"Inhibition of lipofuscin aggregation by molecular tweezers" Application No.: 62/663,948

"Treatment of lysosomal storage disorders" Application No.: 62/663,964

"Therapy of sulfatase deficiencies". Application No.: EP 18169924.0

Funding

- COMPLETED

-2010 National MPS Society grants - role - PI
-2011 Telethon grant - role: PI
-2011 Mariani Foundation grant - role: PI
-2013 Jonah's Just Begun grant - role: PI –
-2015 National MPS Society grant - role – PI

Industrial support
-2009-2011 Sanofi Genzyme
-2012-2017 SHIRE

- RECENTLY COMPLETED RESEARCH SUPPORT (last three years)

Grant (Fraldi)
Cure Sanfilippo foundation 04/2018-12/2020
Targeting Amyloid Aggregation as a New Therapeutic Approach to Treat the CNS in Sanfilippo Syndrome
Role: PI

Grant (Fraldi)
Sanfilippo Children's foundation 01//2019-02/2020
Generation of "super active" variants of lysosomal enzymes to treat the CNS in Sanfilippo syndrome
Role: PI

MDBR Grant (Fraldi)
Orphan Disease Center 03/2019-07/2020
Generation of lysosomal enzyme variants with enhanced therapeutic potential for the treatment of MPS
Role: PI

- ACTIVE FUNDING

Grant (Fraldi)
Cure Sanfilippo Foundation & Sanfilippo Children's Foundation joint Grant (Fraldi) 03/2021-02/2023
Strengthening the rationale for the use of CLR01 in the treatment of Sanfilippo syndrome
Role: PI

Core Grant (Fraldi)
Telethon Foundation 01/2016-03/2021
Mechanisms of Neurodegeneration and CNS therapy in LSDs
Role: PI

University of Pennsylvania Pilot Grant (Fraldi) 02/2021-01/2022
Orphan Disease Center
The "molecular tweezer" CLR01 as a new potent drug candidate to slow CNS pathology progression in MPS
Role: PI

- PENDING

R01 - PAR-18-689 (PI: Gal Bitan) 09/2020 - 08/2026
NIH
Funding title: Innovative Therapies and Tools for Screenable Disorders in Newborns
Role: Co-PI