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Confronting ALS: understanding multicellular contribution to neurodegeneration : computational analysis and hiPSCs in vitro modelling as a multidisciplinary approach

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I want to end with a quote:

“Il peggio non è il dolore ma la consapevolezza di tutto. ‘Sta malattia è ‘na bestiaccia.”

“The worst isn't the pain but the awareness of it all. This disease is an ugly beast.”

-*Mattia Limone*

Traveller, sambuca drinker, ALS patient, but mostly my uncle

(Massafra, 17 giugno 1956 – Latina, 30 Maggio 2022)

A reminder that when talking about ALS “the best possible scenario” is shorter than a five-year PhD, which felt impossibly long to me but definitely felt impossibly long and excruciating to you, ciao zio.

Endless thanks to the people that stand besides ALS patients, especially my father, *Angelo*. Thank you for showing me that love can be stronger than any pain if you are taking care of somebody close to your heart. I would have preferred not to learn it this way but an invaluable life lesson, nonetheless.

“Insurmountable” has definitely acquired a new meaning to me...

Curriculum Vitae

Francesco Limone was born in Latina, Italy, on the 12th of May 1992. He pursued his four year bachelor's degree in Molecular and Cell Biology at the University of York (United Kingdom) where he got interested in stem cell and developmental biology. During his undergraduate studies, he spent a year working in Prof. Thomas Graf's lab, at Center for Genomic Regulation (Barcelona, Spain), where he started working with induced Pluripotent Stem Cells (iPSCs) and got interested in their use for modelling biological processes in vitro. After graduating with honours on the 7th of July 2015, he moved to Paris to start a one-year Master in Developmental and Stem Cell Biology at Université Pierre et Marie Curie (Paris 6, Sorbonne Université, France). For his master internship he moved to Cambridge (Massachusetts, United States) and worked on his thesis in Dr. Kevin Eggan's lab at the Harvard Stem Cell and Regenerative Biology Department (HSCRB). Here he started using human pluripotent stem cells to generate human neurons in vitro to model brain-specific biology and neurodegeneration, with a focus on Amyotrophic Lateral Sclerosis (ALS). After graduating second in his class on 30th of June 2016, he kept on working at HSCRB as a technician to further dissect the role of multiple cell types in disruptions in ALS.

In January 2018, Francesco embarked in his doctoral studies in a shared PhD mentorship between Dr. Kevin Eggan and Prof. Niels Geijsen at Leiden University Medical Center (LUMC, formerly at Hubrecht Institute, University of Utrecht, Netherlands). In his doctoral work, in collaboration with Prof. Beth Stevens's group (Children's Hospital, Boston, United States), he focused on projects using single-cell RNA-sequencing and human pluripotent stem cell models for the understanding of disease mechanisms in ALS with a focus on the contribution of glial cells. Francesco also worked on the development of new in vitro differentiation schemes for motor neurons from human pluripotent cells with Prof. Steven McCarroll's and Dr. Ralda Nehme's group at the Stanley Center for Psychiatric Research (Broad Institute, Cambridge, USA) as well as the characterization and optimization of differentiation protocols for microglia and Oligodendrocytes Progenitor Cells from hiPSCs. He also contributed to studying immune system's involvement in ALS and the role of cytoskeletal proteins and ion channels in motor neuron's function. Since 2023 he is started as a postdoctoral fellow in Dr. Shane Liddelow's lab at the Neuroscience Institute, NYU Grossman School of Medicine (New York University Langone Health, New York, USA).

List of publications

In press, pre-prints and manuscripts in preparation

Limone F^{*}, Couto A^{*}, Wang JY, Zhang Y, McCourt B, Huang C, Jani M, Paladino M, Li QZ, Keaney J, Gillet G, Goodman WA, Kadiu I, Eggan K, Burberry A. “C9orf72 promotes tolerance via restriction of co-stimulation”. Under revision, *Science Translational Medicine*, 2023.

Joseph BJ, Marshall KA, Mann JR, Prudencio M, **Limone F**, Vanoye CG, Simkin D, Leyton M, Nash L, Guerra San Juan I, Gold J, Zhang C, Wichterle H, George AL Jr, Petrucelli L, Eggan K, Kiskinis E. “TDP-43 Dysfunction Triggers Exon Skipping and Aggregation of the Epilepsy Gene KCNQ2 in ALS/FTD”. Under revision, *Nature Neuroscience*, 2023.

Limone F^{*}, Mordes D^{*}, Couto A, Burberry A, Pietiläinen O, Joseph B, Ghosh S, Meyer D, Goldman M, Bortolin L, Kadiu I, McCarroll S, Eggan K. “Single-nucleus sequencing reveals enriched expression of genetic risk factors sensitises Motor Neuron to degeneration in ALS”. Under revision, *Nature Aging*, 2022. *bioRxiv*, <https://doi.org/10.1101/2021.07.12.452054>

Hao J, Wells M, Niu G, Guerra San Juan, **Limone F**, Fukuda A, Leyton-Jaimes M, Joseph B, Qian M, Mordes D, Budnik B, Dou Z, Eggan K. “Loss of TBK1 activity leads to TDP-43 proteinopathy through lysosomal dysfunction in human motor neurons”. Under revision, *Nature Communications*, 2021. *bioRxiv* <https://doi.org/10.1101/2021.10.11.464011>

Publications

Donal MJ, Therrien M, Jereb S, Kamath T, Atkeson T, Marsh SE, Goeva A, Lojek N, Murphy S, Joung J, Liu B, **Limone F**, Eggan K, Hacohen N, Bernstein BE, Glass KC, Leinonen V, Blurton-Jones M, Zhang F, Epstein CB, Macosko EZ, Stevens B. “A resource for generating and manipulating human microglial states *in vitro*”. Accepted, *Nature Immunology*, 2023. *bioRxiv* <https://doi.org/10.1101/2022.05.02.490100>

Limone F^{corresp.}, Mitchell JM, Guerra San Juan I, Ghosh S, Smith JLM, Raghunathan K, Couto A, Meyer D, Mello C, Nemesh J, Smith BM, McCarroll S, Pietiläinen O, Nehme R, Eggan K^{corresp.}. “Efficient generation of lower induced Motor Neurons by coupling Ngn2 expression with developmental cues”. *Cell Reports*, 2022, <https://doi.org/10.1016/j.celrep.2022.111896>

Rapino F, Natoli T, **Limone F**, O'Connor E, Black J, Tegtmeyer M, Chen W, Norabuena E, Narula J, Hazelbaker D, Angelini G, Barrett L, O'Neil A, Beattie UK, Thanos JM, de Rivera H, Sheridan SD, Perlis RH, McCarroll SA, Stevens B, Subramanian A, Nehme R, Rubin LL. “Small molecules screen reveals pathways that regulate C4 secretion in stem-cell derived astrocytes”. *Stem Cell Reports*, 2022, <https://doi.org/10.1016/j.stemcr.2022.11.018>

Limone F, Klim JR, Mordes D. “Pluripotent Stem Cell Strategies to Rebuild the Human Brain”. *Frontiers in Aging Neuroscience*, 2022. [10.3389/fnagi.2022.1017299](https://doi.org/10.3389/fnagi.2022.1017299)

Guerra San Juan I, Nash L, Smith K, Qian M, Burberry A, Dorr A, Couto A, Klim J, Pintacuda G, **Limone F**, Whisenant E, Noble C, Melnik V, Potter D, Holmes A, Verhage M, Eggan K. “Loss of STMN2 Function Causes Motor Neuropathy”. *Neuron*, 2022, [10.1016/j.neuron.2022.02.011](https://doi.org/10.1016/j.neuron.2022.02.011).

Ashoti A^{*}, **Limone F**^{*}, van Kranenburg M, Alemany A, Baak M, Vivié J, Piccioni F, Creighton M, Eggan K, Geijsen N. “Considerations and practical implications of performing a phenotypic CRISPR/Cas survival screen”. *PLoS ONE*, 2022, [10.1371/journal.pone.0263262](https://doi.org/10.1371/journal.pone.0263262)

Fukuda A, Hazelbaker D, Motosugi N, Hao J, **Limone F**, Beccard A, Mazzucato P, Messina A, Okada C, Guerra San Juan I, Qian M, Umezawa A, Akutsu H, Barrett L, Eggan K. "The de novo DNA methyltransferases DNMT3A and DNMT3B initiate erosion of dosage compensation in human pluripotent stem cells". *Stem Cell Reports*, 2021, [10.1016/j.stemcr.2021.07.015](https://doi.org/10.1016/j.stemcr.2021.07.015)

Burberry A, Wells M, **Limone F**, Couto A, Smith K, Keaney J, Gillet G, van Gastel N, Wang J, Pietiläinen O, Qian M, Eggan P, Cantrell C, Mok J, Kadiu I, Scadden D, Eggan K. "C9orf72 suppresses systemic and neural inflammation induced by gut bacteria". *Nature*, 2020 [10.1038/s41586-020-2288-7](https://doi.org/10.1038/s41586-020-2288-7)

Klim J*, Williams L*, **Limone F**, Guerra San Juan I, Davis-Dusenbery B, Mordes D, Burberry A, Steinbaugh MJ, Gamage K, Kirchner R, Moccia R, Cassel S, Chen K, Wainger B, Woolf C, Eggan K. "ALS-implicated protein TDP-43 sustains levels of STMN2, a mediator of motor neuron growth and repair". *Nature Neuroscience*, 2019 [10.1038/s41593-018-0300-4](https://doi.org/10.1038/s41593-018-0300-4)

Mordes D, Prudencio M, Goodman L, Klim J, Moccia R, **Limone F**, Pietiläinen O, Chowdhary K, Dickson D, Rademakers R, Bonini N, Petrucelli L, Eggan K. "Dipeptide repeat proteins activate a heat shock response found in C9orf72-ALS/FTLD patients", *Acta Neuropathological Comm.*, 2018 [10.1186/s40478-018-0555-8](https://doi.org/10.1186/s40478-018-0555-8)

Nehme R*, Zuccaro E*, Ghosh S, Li C, Sherwood J, Pietiläinen O, Barrett L, **Limone F**, Worringer K, Kommineni S, Zang Y, Cacchiarelli D, Meissner A, Adolfsson R, Haggarty S, Madison J, Muller M, Arlotta P, Fu Z, Feng G, Eggan K. "Combining NGN2 programming with developmental patterning generates human excitatory neurons with NMDAR-mediated synaptic transmission". *Cell Reports*, 2018 [10.1016/j.celrep.2018.04.066](https://doi.org/10.1016/j.celrep.2018.04.066)

Kriiger PHL*, Di Stefano B*, de Wit E*, **Limone F**, van Oevelen C, de Laat W, Graf T. "Cell-of-origin specific 3D genome structure acquired during somatic cell reprogramming". *Cell Stem Cell*, 2016 [10.1016/j.stem.2016.01.007](https://doi.org/10.1016/j.stem.2016.01.007)

Di Stefano B, Collombet S, Jakobsen J, Wierer M, Lackner A, Sardina JL, Segura Morales C, Stadhouders R, **Limone F**, Mann M, Porse B, Thieffry D, Graf T. "C/EBP α creates elite cells for iPSC reprogramming by upregulating Klf4 and increasing levels of Lsd1 and Brd4", *Nature Cell Biology*, 2016 [10.1038/ncb3326](https://doi.org/10.1038/ncb3326)