

BIOGRAPHICAL SKETCH

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NAME: Marco Onorati, Ph.D.

POSITION TITLE: Associate Professor

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
University of Pisa, Italy	M.S.	10/2003	Biological Sciences
Scuola Normale Superiore of Pisa, Italy	Ph.D.	10/2007	Neurobiology
University of Milan, Italy	Post Doc	07/2013	Neural Stem Cells
Yale School of Medicine, USA	Associate Research Scientist	11/2016	Neuroscience

A. Personal Statement

My research focuses on understanding the cellular and molecular events orchestrating neural stem cell differentiation to generate distinct types of neurons in the central nervous system. An important element of this research is the integration of complementary approaches that combines: 1) human pluripotent and neural stem cell models to investigate the mechanisms of neurodevelopment and disease; 2) comparative genomic and cellular analyses to identify human-specific features of brain development and evolution; and 3) genetic and molecular analyses of human neurodevelopmental and neurodegenerative disorders. I have a solid background in stem cell biology and neuroscience as attested by the last 16 years of work. During my Postdoctoral experience in Prof. Elena Cattaneo's laboratory at the University of Milan, my work was focused on the elucidation of the developmental processes surrounding the generation of medium-spiny neurons (MSNs). MSNs are the most vulnerable neurons in Huntington's disease, a devastating neurodegenerative disorder that causes progressive motor, psychiatric, and cognitive dysfunctions (Onorati et al., 2014 Nature Neuroscience; Delli Carri, Onorati et al., 2013 Development; HD Consortium, 2013 Cell Stem Cell). Then, at the Yale School of Medicine, with the mentorship of Prof. Nenad Sestan, I have set an advanced system of human neuroepithelial stem (NES) cells to model early human neurodevelopment, Zika virus-induced microcephaly and to validate a potential treatment with a drug already approved by the FDA and EMA (Onorati et al., 2016 Cell Reports). In 2017 I moved to the University of Pisa and, since then, the laboratory has been devoted to investigating the elective integration of human NES cells into a spinal cord injury model and regenerative approaches (Dell'Anno et al., 2018, Nature Communications; De Vincentiis et al., 2023 Small). On-going projects are centered on neurodevelopmental disorders with genetic basis, including *WDR62* mutations in primary microcephaly. We found a novel *WDR62* subcellular pattern from Golgi apparatus to mitotic spindle poles and demonstrated the impact of

WDR62 mutations on neural progenitors using patient-derived iPSCs (Dell'Amico et al., 2023 eLife). Onorati Lab is also exploring Zika virus and TORCH-pathogens effects on the telencephalic transcription factor *FOXP1*, which mutations are associated with *FOXP1* syndrome (Lottini et al., 2022 Stem Cell Reports). My lab is also actively involved in deciphering cellular and genetic mechanisms at the basis of schizophrenia. By analyzing a cohort of patients with sleep spindle endophenotype, we are delineating the neuronal impairment observed in cultured cells (Vecchi et al., submitted). Lastly, we are elucidating the underlying molecular and cellular mechanisms implicated in neurodegenerative conditions, such as amyotrophic lateral sclerosis. Employing human cortical and spinal organoids, our investigations seek to delineate the impact of the *C9ORF72* and *TDP43* mutations in motor neuron vulnerability.

B. Positions, Scientific Appointments, and Honors

Current positions

Nov 2019- Associate Professor, Department of Biology, University of Pisa
Dec 2016- Member of the Committee of the Ph.D. Program in Biology, University of Pisa
Jul 2024- National Scientific Habilitation as Full Professor in Cytology and Comparative Anatomy

Previous positions

2013-16 Associate Research Scientist at the Department of Neuroscience, Yale School of Medicine, New Haven (CT), USA (Advisor: Prof. Nenad Sestan)
2008-13 Postdoctoral Associate at the Department of Biosciences, University of Milan, Italy (Advisor: Prof. Elena Cattaneo)

Honors

2003 Summa cum laude and honor of "Abbraccio accademico" (top 1% of students in this course), University of Pisa
2007 Summa cum laude for Ph.D. dissertation, Scuola Normale Superiore of Pisa
2008 Best Ph.D. thesis in 2007 (Prize XIII SIBS)
2012 FIRB 2010 - Futuro in Ricerca grant (Italian Ministry of University and Research)
2016-2018 Visiting Research Scientist, Department of Neuroscience, Yale School of Medicine
2017 NARSAD young investigator Grant, Brain & Behavior Research Foundation

Institutional responsibilities

Dec. 2016- Member of the Committee of the Ph.D. Program in Biology, University of Pisa
Nov. 2016- Faculty member at Department of Biology, University of Pisa
Jun. 2024- Member of the Committee of Bioethics, University of Pisa

Scientific society membership

Member of Italian Society of Developmental and Cell Biology (GEI-SIBSC) since 2016
Member of International Society for Stem Cell Research (ISSCR) 2013
Member of the Directive Board of Italian Society for Neuroscience (SINS) since 2022

Editorial activity

Reviewer for peer review international journals: "Cell Reports", "Nature Communications", "Neuroscience", "Infection, Genetics and Evolution", "BMC Neuroscience", "Frontiers Cellular Neuroscience".

Guest Editor for Cells https://www.mdpi.com/journal/cells/special_issues/Neural_Stem

Review Editor for Frontiers in Neuroanatomy <https://loop.frontiersin.org/people/863867/overview>

C. Contributions to Science

Researcher unique identifiers: ORCID: 0000-0001-8517-930X, Scopus Author ID: 15052280000
Author H-index: 16 (Scopus), 18 (Scholar); Citations: 2144 (Scopus), 2980 (Scholar); Articles: 35

1. Early publications during my PhD, under the supervision of Prof. Robert Vignali at University of Pisa and Prof. Federico Cremisi at Scuola Normale Superiore, directly addressed the molecular mechanisms and genetic control of neural cell fate during eye development and retinogenesis. I performed a molecular dissection of OTX transcription factors to understand their role during the specification of photoreceptors and bipolar cells in the retina. I also worked on the role of chromatin architectural HMGA proteins, during the development of the nervous system.

a. Onorati M, Cremisi F, Liu Y, He RQ, Barsacchi G, Vignali R. A specific box switches the cell fate determining activity of XOTX2 and XOTX5b in the *Xenopus* retina. *Neural Dev.* 2007 Jun 27;22:12.

b. Benini F*, Onorati M*, Altamura S, Manfioletti G, Vignali R. Identification and developmental expression of *Xenopus* hmga2beta. *Biochem Biophys Res Commun.* 2006 Dec 15;351(2):392-7. *co-first authors.

c. Macrì S, Sgarra R, Ros G, Maurizio E, Zammitti S, Milani O, Onorati M, Vignali R, Manfioletti G. Expression and functional characterization of Xhmg-at-hook genes in *Xenopus laevis*. *PLoS One.* 2013 Jul 25;8(7):e69866.

d. Macrì S, Simula L, Pellarin I, Pegoraro S, Onorati M, Sgarra R, Manfioletti G, Vignali R. Hmga2 is required for neural crest cell specification in *Xenopus laevis*. Macrì S, Simula L, Pellarin I, Pegoraro S, Onorati M, Sgarra R, Manfioletti G, Vignali R. *Dev Biol.* 2016 Mar 1;411(1):25-37.

2. During my Post-doc in the Prof. Elena Cattaneo's laboratory at the University of Milan, my work was focused on the elucidation of the developmental processes surrounding the generation of medium-spiny neurons (MSNs), these being the GABAergic projection neurons of the striatum. MSNs are the most vulnerable neurons in Huntington's disease (HD), a devastating autosomal dominant neurodegenerative disorder that causes progressive motor, psychiatric and cognitive dysfunctions. A polyglutamine repeat expansion within the huntingtin protein causes widespread brain neurodegeneration although MSNs (and neocortical neurons) are the most affected. My effort was focused on human embryonic stem (hES) cells for the optimization of an ontogeny-recapitulating MSN differentiation protocol. The study was complemented by a transcriptional and functional analysis of the developing human striatum and neocortex.

a. Delli Carri A, Onorati M*, Lelos MJ, Castiglioni V, Faedo A, Menon R, Camnasio S, Vuono R, Spaiardi P, Talpo F, Toselli M, Martino G, Barker RA, Dunnett SB, Biella G, Cattaneo E. Developmentally coordinated extrinsic signals drive human pluripotent stem cell differentiation towards fully functional DARPP-32+ medium-sized spiny neurons. *Development.* 2013 Jan 15;140(2):301-12.

*co-first authors

b. Delli Carri A*, Onorati M*, Castiglioni V, Faedo A, Camnasio S, Toselli M, Biella G, Cattaneo E. Human pluripotent stem cell differentiation into authentic striatal projection neurons. *Stem Cell Rev.* 2013 Aug;9(4):461-74. *co-first authors

c. Onorati M, Castiglioni V, Biasci D, Cesana E, Menon R, Vuono R, Talpo F, Goya RL, Lyons PA, Bulfamante GP, Muzio L, Martino G, Toselli M, Farina C, Barker RA, Biella G, Cattaneo E. Molecular and Functional Definition of the Developing Human Striatum. *Nature Neurosci.* 2014 Nov 10.

d. Castiglioni V, Faedo A, Onorati M, Vuono R, Bulfamante GP, Muzio L, Martino G, Barker RA and Cattaneo E. DACH1 expression during human neocortical and striatal development. Submitted revised.

3. Furthermore, pluripotent stem cells, and in particular iPS cells, offer the unprecedented opportunity to in vitro model neurodegenerative diseases, such as HD. Work on HD iPS cells showed peculiar phenotypes related to the pathophysiology of this devastating disease, offering a new platform for further investigations of the molecular and cellular dysfunctions or screenings for target molecules.

a. Castiglioni V, Onorati M, Rochon C, Cattaneo E. Induced pluripotent stem cell lines from Huntington's disease mice undergo neuronal differentiation while showing alterations in the lysosomal pathway. *Neurobiol Dis.* 2012 Apr;46(1):30-40.

b. The HD iPSC Consortium: Mattis VB, Ebert A, Svendsen SP, Svendsen CN, King A, Casale M, Winokur ST, Batugedara G, Vawter M, Donovan PJ, Lock LF, Thompson LM, Zhu Y, Fossale E, Singh

R, Gillis T, Mysore J, Li J, Seong I, Shen Y, Chen X, Wheeler V, MacDonald M, Gusella JF, Akimov S, Arbez N, Juoppen T, Ratoviski T, Chiang JH, Kim WR, Chighladze E, Watkin E, Zhong C, Makri G, Cole RN, Margolis RL, Song H, Ming G, Ross CA, Kaye JA, Daub A, Sharma P, Mason AR, Finkbeiner S, Yu J, Thomson JA, Rushton D, Brazier SP, Battersby AA, Redfern A, Tseng H, Harrison AW, Kemp PJ, Allen ND, Onorati M, Castiglioni V, Cattaneo E, Arjomand J, Svendsen C. Induced Pluripotent Stem Cells from Patients with Huntington's Disease Show CAG Repeat-Expansion-Associated Phenotypes. *Cell Stem Cell*. 2012 Aug 3;11(2):264-78

c. Wray S, Self M, NINDS Parkinson's Disease iPSC Consortium, NINDS Huntington's Disease iPSC Consortium*, NINDS ALS iPSC Consortium, et al. (*: Gusella JF, MacDonald ME, Wheeler VC, Ross CA, Akimov S, Arjomand J, Thompson LM, King A, Hermanowicz N, Winokur S, Svendsen CN, Mattis VB, Onorati M, Cattaneo E, Allen ND, Kemp PJ, Kim KS, Finkbeiner S). Creation of an Open-Access, Mutation-Defined Fibroblast Resource for Neurological Disease Research. *PLoS ONE* 2012 August 27; 7(8)

d. Laterza C, Merlini A, De Feo D, Ruffini F, Menon R, Onorati M, Fredrickx E, Muzio L, Lombardo A, Comi G, Quattrini A, Taveggia C, Farina C, Cattaneo E, Martino G. iPSC-derived neural precursors exert a neuroprotective role in immune-mediated demyelination via the secretion of LIF. *Nature Commun*. 2013 Oct 29;4:2597.

4. Neural stem (NS) cells are an ad infinitum self-renewing population of multipotent cells, with the properties of neurogenic radial glia. NS cells were efficiently derived from ES cells and from fetal and adult mouse brain, as well as from iPS cells. They offer a promising system for dissecting the mechanisms of neural differentiation, are suitable for genetic and drug high-throughput screening and represent a potential source for cell replacement therapies. During my stay in Prof. Nenad Sestan's laboratory I have set an advanced system of human neuroepithelial stem (NES) cells to model early human neurodevelopment, Zika virus-induced microcephaly and to validate a potential drug treatment.

a. Onorati M, Camnasio S, Binetti M, Jung CB, Moretti A, Cattaneo E. Neuropotent self-renewing neural stem (NS) cells derived from mouse induced pluripotent stem (iPS) cells. *Mol Cell Neurosci*. 2010 Mar;43(3):287-95.

b. Albieri I*, Onorati M*, Calabrese G, Moiana A, Biasci D, Badaloni A, Camnasio S, Spiliotopoulos D, Ivics Z, Cattaneo E, Consalez GG. A DNA transposon-based approach to functional screening in neural stem cells. *J Biotechnol*. 2010 Oct 1;150(1):11-21. *co-first authors

c. Onorati M, Binetti M, Conti L, Camnasio S, Calabrese G, Albieri I, Di Febo F, Toselli M, Biella G, Martynoga B, Guillemot F, Consalez GG, Cattaneo E. Preservation of positional identity in fetus-derived neural stem (NS) cells from different mouse central nervous system compartments. *Cell Mol Life Sci*. 2011 May;68(10):1769-83.

d. Ebert AD, Shelley BC*, Hurley AM, Onorati M*, Castiglioni V*, Patitucci TN*, Svendsen SP, Mattis VB, McGivern JV, Schwab AJ, Sareen D, Kim HW, Cattaneo E, Svendsen CN. EZ spheres: a stable and expandable culture system for the generation of pre-rosette multipotent stem cells from human ESCs and iPSCs. *Stem Cell Res*. 2013 May;10(3):417-27. *co-second authors.

e. Onorati M, Li Z, Liu F, Sousa AM, Nakagawa N, Li M, Dell'Anno MT, Gulden FO, Pochareddy S, Tebbenkamp ATN, Han W, Pletikos M, Gao T, Zhu Y, Bichsel C, Varela L, Szigeti-Buck K, Lisgo S, Zhang Y, Testen A, Gao XB, Mlakar J, Popovic M, Flamand M, Strittmatter SM, Kaczmarek LK, Anton ES, Horvath TL, Lindenbach BD, Sestan N. Zika Virus Disrupts Phospho-TBK1 Localization and Mitosis in Human Neuroepithelial Stem Cells and Radial Glia. *Cell Reports*. 2016 16, 2576–2592.

5. As an independent principal investigator (now Associate Professor from 2019) at the University of Pisa, the research interests are focused on neurodevelopmental disorders with genetic and environmental bases. *WDR62* is a spindle pole-associated protein which mutations represent the second most common cause of microcephaly. We revealed a novel *WDR62* subcellular pattern from Golgi apparatus to mitotic spindle poles and demonstrated the impact of *WDR62* mutations on neural progenitors from patient iPSCs. Viral infections by teratogenic pathogens (such as ZIKV, CMV, etc.) represent a further challenge to normal brain development. We have recently demonstrated the impairment of the telencephalic transcription factor *FOXP1*, which mutations are associated with *FOXP1* syndrome, a spectrum that includes microcephaly, autism, and developmental delay. We are also devoting our effort on regenerative approaches to lesions of the central nervous system, such as spinal cord injury, by cell therapy and nanomedicine.

- a. Dell'Amico C, Angulo Salavarria MM, Takeo Y, Saotome I, Dell'Anno MT, Galimberti M, Pellegrino E, Cattaneo E, Louvi A, Onorati M. Microcephaly-associated protein WDR62 shuttles from the Golgi apparatus to the spindle poles in human neural progenitors. *Elife*. 2023 Jun 5;12:e81716.
- b. Lottini G, Baggiani M, Chesi G, D'Orsi B, Quaranta P, Lai M, Pancrazi L, Onorati M*, Pistello M, Freer G, Costa M. Zika virus induces FOXG1 nuclear displacement and downregulation in human neural progenitors. *Stem Cell Reports*. 2022 Jul 12;17(7):1683-1698. *co-last authors
- c. Dell'Anno MT*, Wang X*, Onorati M*, Li M, Talpo F, Sekine Y, Ma S, Liu F, Cafferty WBJ, Sestan N, Strittmatter SM. Human neuroepithelial stem cell regional specificity enables spinal cord repair through a relay circuit. *Nat Commun*. 2018 Aug 24;9(1):3419 *co-first authors
- d. De Vincentiis S, Baggiani M, Merighi F, Cappello V, Lopane J, Di Caprio M, Costa M, Mainardi M, Onorati M, Raffa V. Low Forces Push the Maturation of Neural Precursors into Neurons. *Small*. 2023 Apr 14:e2205871.
- e. Merighi F, De Vincentiis S, Onorati M, Raffa V. Long-Term Mouse Spinal Cord Organotypic Slice Culture as a Platform for Validating Cell Transplantation in Spinal Cord Injury. *J Vis Exp*. 2024 Apr 12;(206)

6. The lab is also actively involved in deciphering the cellular and genetic mechanisms at the basis of schizophrenia. By analyzing a cohort of patients with sleep spindle endophenotype, we are delineating the neuronal impairment observed in cultured cells. Lastly, we are elucidating the underlying molecular and cellular mechanisms implicated in neurodegenerative conditions, such as amyotrophic lateral sclerosis. Employing human cortical and spinal organoids, our investigations seek to delineate the impact of the C9ORF72 and TDP43 mutations in motor neuron vulnerability.

- a. Angulo Salavarria MM, Dell'Amico C, D'Agostino A, Conti L, Onorati M. Cortico-thalamic development and disease: From cells, to circuits, to schizophrenia. *Front Neuroanat*. 2023 Mar 2;17:1130797.
- b. Elena Rita Vecchi, Claudia Vittoria Olmeda, Daniele Bottai, Palma Finelli, Cristina Gervasini, Laura Mangiaterra, Francesco Lombardi, Claudio Sanguineti, MM Angulo Salavarria, Marco Onorati, Luciano Conti, Armando D'Agostino Generation and benchmarking of a collection of hiPSC lines from Schizophrenia Patients with Diverse Clinical Profiles. *BioRxiv*, doi: <https://doi.org/10.1101/2024.01.14.575590>