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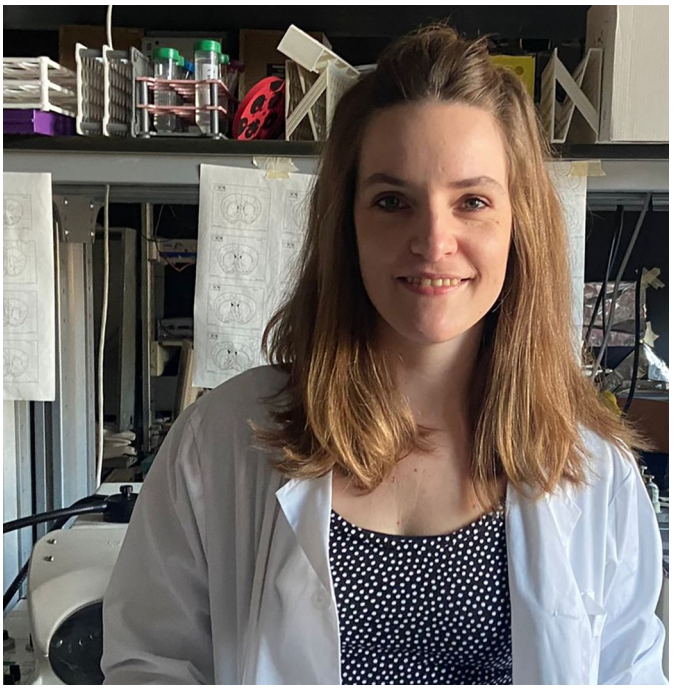
FIRST PERSON

First person – Sara Bonzano

First Person is a series of interviews with the first authors of a selection of papers published in Disease Models & Mechanisms, helping researchers promote themselves alongside their papers. Sara Bonzano is first author on 'NR2F1 shapes mitochondria in the mouse brain, providing new insights into Bosch-Boonstra-Schaaf optic atrophy syndrome', published in DMM. Sara is a postdoc in the lab of Silvia De Marchis at the University of Turin, Turin, Italy, investigating the cellular and molecular mechanisms by which NR2F1 regulates postnatal brain plasticity.



Sara Bonzano

How would you explain the main findings of your paper to non-scientific family and friends?

Intellectual disability (ID) is a condition that affects over 150 million people worldwide. ID can arise from multiple causes, but genetic factors stand out as one of the leading culprits. The prevalent genetic conditions for ID include Down syndrome and Fragile X syndrome, but ID is also associated with several different rare genetic conditions. Among them, Bosch-Boonstra-Schaaf optic atrophy syndrome (BBSOAS) is an emerging rare neurodevelopmental disorder caused by altered function of a gene called *NR2F1*, and characterized by mild-to-severe ID, visual impairment, seizures and autistic traits. Notably, the mechanisms causing this pathology are still largely unknown. Recently, alterations in the function of mitochondria, which are small cell organelles fundamental to energy production, have been reported in muscles of two BBSOAS patients, suggesting that altered NR2F1 activity might affect mitochondrial function. Furthermore, several neurological symptoms described in BBSOAS patients, common to other disorders, are often associated with mitochondrial dysfunction in the brain. We have thus formulated the hypothesis that NR2F1 can regulate mitochondria in neural cells. By exploiting the mouse as an animal model, we have first shown that many key mitochondrial proteins can be influenced by the regulatory activity of NR2F1. Afterwards, through genetic manipulation of NR2F1 function in brain cells, we have been able to demonstrate that complete loss of NR2F1 in a population of neurons crucial for learning and memory (i.e. adult-born hippocampal neurons) leads to typical signs of mitochondrial dysfunction, including a reduced abundance of mitochondria, associated with changes in their morphology and with altered composition of mitochondrial proteins. In addition, the loss of NR2F1 in these neurons negatively affects their integration into the hippocampal neuronal circuitry, which can lead to cognitive deficits. Furthermore, we have shown a mitochondrial phenotype in the whole brain of mice carrying half the dose of NR2F1, a validated BBSOAS model. Thus, our data provide the first demonstration of a direct role for NR2F1 in shaping neuronal mitochondria in the adult brain that could explain some of the symptoms found in BBSOAS patients.

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What are the potential implications of these results for your field of research?

Studies over the past 20 years clearly demonstrated NR2F1 pleiotropic and crucial function during the ontogenesis of the nervous system, but NR2F1 is also highly expressed in multiple brain areas and neural cell types in the postnatal brain, in which its function is still largely unknown. What stands out in our study is the demonstration of novel cellular/molecular mechanisms controlled by NR2F1 in the postnatal brain that can be instrumental to future studies on the basic mechanisms for fundamental research in neuroscience. On the other hand, our data open up a promising avenue for identifying new cellular mechanisms involving mitochondrial dysfunction as a contributing factor in the pathophysiology of BBSOAS, a rare genetic disease caused by NR2F1 mutations. By providing strong evidence of the involvement of mitochondrial dysfunction in BBSOAS pathogenesis, I envisage that our study paves the way for further research on the mechanisms and role of mitochondrial dysfunction in this rare neurological disease, also opening up new possibilities for therapeutic intervention. What I also think is interesting is that, beyond BBSOAS, our data open up novel

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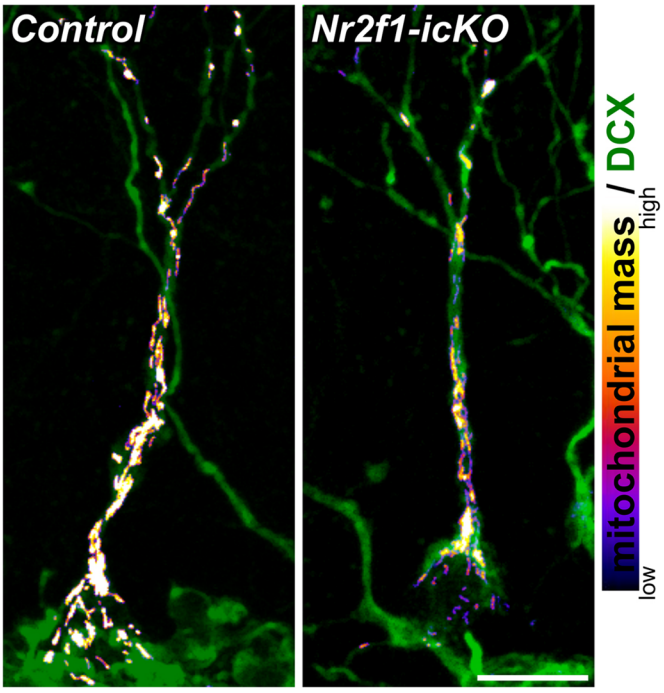

Representative immunofluorescence images showing reduction in mitochondrial mass in adult-born doublecortin (DCX)$^+$ hippocampal neurons lacking the nuclear receptor NR2F1 (*Nr2f1*-icKO) *in vivo*. Scale bar: 10 μm.

perspectives for further research on other neurological disorders – such as Down syndrome, Alzheimer's disease and Parkinson's disease – in which altered expression and function of NR2F1 have recently been reported.

What are the main advantages and drawbacks of the experimental system you have used as it relates to the disease you are investigating?

Mouse models offer a unique opportunity to further understand the role of altered function of target proteins in the pathogenesis of human diseases. However, we are also aware that all these models come with their intrinsic caveats and limitations. In this study, we took advantage of the conditional knockout mouse line to study mitochondrial alteration in a subpopulation of neurons crucial for cognition for which genesis is maintained in the adult mouse brain. Thanks to the induction of NR2F1 loss selectively in those cells, we efficiently revealed the mitochondrial phenotype and the functional consequences of NR2F1 ablation in a cell-autonomous manner. Nevertheless, full NR2F1 ablation is never observed in human patients.

In addition, we revealed that this alteration is not limited to knockout (KO) cells, but also presents in the whole brain of constitutive *Nr2f1*-heterozygous mice (i.e. the most widely used mouse model for BBSOAS). However, the aforementioned model still does not encompass the entire population of patients, as it fails to represent the wide range of pathogenic mutations that have been identified so far. In this context, the emergence of recently developed mouse models that carry a specific mutation found in individual patients appears to be a highly promising method to dissect BBSOAS pathophysiology. For our next studies, it thus becomes imperative to compare these 'humanized' mice at the molecular and functional levels with *Nr2f1* haploinsufficient/conditional KO mice, aiming at better characterizing genotype–phenotype correlation.

What has surprised you the most while conducting your research?

Perhaps one of the aspects that amazed me the most was that once the central hypothesis had been made (i.e. that alteration in NR2F1 function had a direct effect on mitochondria), everything went practically smoothly in every experiment and strategic approach, obviously having set up the optimal conditions for the strategy itself. This is something that has rarely happened to me in the past and has surprised me greatly!

What do you think is the most significant challenge impacting your research at this time and how will this be addressed over the next 10 years?

I think that one of the hardest challenges in my research topic, and in general in the context of neurobiology, is that we are facing one of the most complex biological systems and that, despite significant advancements in the research field, our understanding of the brain and its complexities are still limited. I believe that adopting a multidisciplinary approach is necessary to seek to understand the complexity of the structure, function, development and disorders of the nervous system. As there is much research underway dedicated to unravelling its mysteries, only exploring the nervous system from multiple angles will lead to breakthroughs and advancements that may not be feasible within the confines of a single discipline.

In this study, we propose that the nuclear receptor NR2F1 acts among the mitochondrial gene expression regulatory network in neurons, and we provide strong evidence of the possible involvement of mitochondrial dysfunction in BBSOAS. Of course, I am aware that we have only scratched the surface of the mitochondrial dysfunction underlying the tangled phenotype of NR2F1 loss/haploinsufficiency in the brain. In the future, I would like to be able to clarify whether the restoration of mitochondrial function can be exploited for therapeutic purposes for BBSOAS in mice carrying human NR2F1 mutations, with the hope of launching projects, also including clinicians and, eventually, BBSOAS patient associations.

"Funding plays a crucial role in the career of a scientist, particularly for aspiring young researchers aiming to establish themselves as independent investigators."

What changes do you think could improve the professional lives of scientists?

Funding plays a crucial role in the career of a scientist, particularly for aspiring young researchers aiming to establish themselves as independent investigators. Here in Italy, there is a general lack of financial resources allocated to basic scientific research and development, making the budget allocated (especially for fundamental or basic research) often insufficient to meet the growing demands and needs of the scientific community. Another critical issue connected to this is the lack of long-term funding stability, making it difficult for us to plan and execute long-term projects. Thus, I strongly hope that something will change, and I'm convinced that the government's foresight on research is commendable as it recognizes the significance of funding and supporting scientific endeavours. In the long term, this foresight could foster an environment conducive to nurturing young scientists as I am, enabling us to pursue our research goals, which ultimately contribute to societal progress and development.

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What's next for you?

I have recently been recruited as a research associate in tenure track for the next 3 years at the University of Turin, and I am very happy and excited to pursue my research activities and look forward to starting my own research projects here…of course without overshadowing my family (which is currently in expansion!).

Reference

Bonzano, S., Dallorto, E., Molineris, I., Michelon, F., Crisci, I., Gambarotta, G., Neri, F., Oliviero, S., Beckervordersandforth, R., Lie, D. C. et al. (2023). NR2F1 shapes mitochondria in the mouse brain, providing new insights into Bosch-Boonstra-Schaaf optic atrophy syndrome. *Dis. Model. Mech.* **16**, dmm049854. doi:10.1242/dmm.049854