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Progresses in the development of *in vivo* redox measurements: new tools for longitudinal studies in Rett syndrome

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Mutations in the X-linked *MECP2* gene are associated with a wide spectrum of neurological disorders, of which Rett syndrome represents the first identified and surely the best characterize. Although MeCP2 has been originally described as an epigenetic transcriptional repressor, it is now evident that it actually behaves as a multifunctional protein. Indeed, in addition to its role in inhibiting gene transcription, it can serve as a chromatin remodeling factor, a transcriptional activator and a modulator of mRNA splicing (1). As consequence of its involvement in all such molecular processes, lack or mutations of *MECP2* generate a plethora of symptoms, as those displayed in Rett syndrome. Importantly, several mouse models of *Mecp2* recapitulate many of the typical RTT phenotypes, thus helping in the characterization of the molecular, functional and behavioral features of the pathology. Remarkably, they also served to establish that Rett syndrome is a potentially reversible neurological condition, thus encouraging many preclinical studies that in some cases advanced to clinical trials in patients (2). However, both clinical and preclinical studies have clearly demonstrated to suffer from the lack of measurable outcomes that could be used to longitudinally describe the progression of both the symptoms and the pathological mechanisms occurring in Rett syndrome. Based on these measurements, which are here defined as "biomarkers", researchers could easily test the efficacy of a treatment in ameliorating or stalling the progression of the disorder. To date, however, to the best of our knowledge, such biomarkers are unavailable to the Rett syndrome scientific community and a spatial and temporal map of the genesis and occurrence of phenotypes is still lacking.

Currently, the approaches proposed in the field of *Mecp2* studies are mostly based on assessments that produce only steady state data taken at a singular and defined time

point. This is clearly incompatible with longitudinal studies, highlighting the urgency of identifying biomarkers of the disease progression. Ideally, measurable outcomes should satisfy the following criteria: i) they should be detectable both in animal models and humans, thus ensuring the clinical relevance of the analysis; ii) they should be measurable at different time points in the same subject; iii) they should provide quantitative and not only qualitative data. By considering a measurable outcome that possess all these features, researchers could reach a more detailed comprehension of the genesis of RTT pathological mechanisms and their progression in time, thus contributing to the development of sound rescue strategies. This obviously represents the roadmap to obtain efficient therapeutic approaches for Rett syndrome.

In light of these considerations, the review recently proposed by Mueller on disturbed redox homeostasis and oxidative stress in Rett syndrome, and published in the Special Issue on Regression in Developmental Disorders, fits well with the current needs of Rett research. In fact, the author not only comprehensively reviews the state of knowledge on redox imbalance and oxidative stress in Rett syndrome, but also suggests a novel genetic tool to analyze redox alterations along the disease progression. We find this an interesting and promising approach for the study of RTT.

Alterations in redox have been reported both in patients and in animal models (3,4), implying the possible importance of such imbalance in the pathogenesis of RTT. Further, although it is not currently possible to clearly establish a molecular mechanism through which *Mecp2* deficiency generates redox imbalance, data from *Mecp2* re-expression experiments support a link between them (3). This association could depend on either mitochondrial dysfunction or impaired detoxification or both. Further, preclinical and clinical reports showed the benefic impact of interventions aimed at balancing redox impairments. However, we still have to understand when oxidative insults appear and whether different tissues are differently susceptible. Once again, this stresses the importance of longitudinal studies for the comprehension of the timing through which redox imbalance is associated with the pathology.

So far measurements of redox were based on histological or biochemical assessments; however; the review by Mueller highlights the development of genetic tools, already used in *in vitro* experiments (5) that now allow longitudinal studies in mice. These systems exploit transgenic mice expressing optical sensors of redox, such as a redox sensitive fluorescent protein (roGFP). The possibility of using different promoters driving the expression of the transgene enables to image redox changes selectively

within specific cell compartments, cells types and tissues. Redox can be quantitatively measured in a specific tissue of the same animal along the disease progression. Indeed, an interest aspect of redox-based impairments highlighted in the review is the cyclic succession of three temporal stages: redox alterations, followed by oxidant burden that eventually leads to oxidative tissue damage. Together these stages set in motion a vicious cycle, in which oxidative tissue damages enhance the production of free oxygen radicals that maintain and amplify the damage. It is intriguing to imagine that initial redox alterations arise during the so-called pre-symptomatic phase of the disorder characterized by subclinical manifestations, while oxidative stress and tissue damage concur to the overt phase of the pathology. If this is the case, the use of these animals could shed new light on the molecular basis of the pre-symptomatic and symptomatic phase of the pathology. Moreover, these probes could potentially enable to measure the benefits of any therapeutic treatment, including those that not necessarily directly modulate the redox status. Indeed, we foresee that the use of conditional Mecp2 animals in combination with redox sensors could generate novel tools to clarify how *Mecp2* deficiency impacts redox balance in specific brain areas as secondary affect. Similarly, this tool would offer a new measurable outcome to test the rescue potential of drugs targeting the effects of conditional *Mecp2* ablation, an undeniable advantage for the purpose of preclinical studies.

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