A new transgenic reporter mouse model for monitoring by optical imaging, in a spatial-temporal dimension, the oxidative stress in Parkinson's disease (PD)

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PD is neurodegenerative disorder associated with loss of the dopaminergic neurons (DA) in the substantia nigra and the degeneration of projecting nerve fibers in striatum. A majority of PD cases is idiopathic (90-95%). Aging is a factor associated with the onset of PD, and failure of normal cellular processes that occurs with aging is believed to cause increased vulnerability of DAergic neurons. Familial forms of PD involving mutations in a number of genes have also been described [1]. In both idiopathic and genetic cases of PD, oxidative stress is thought to be the common underlying mechanism that leads to cellular dysfunction and demise: the substantia nigra of PD patients exhibit increased levels of oxidized lipids and decreased levels of reduced glutathione. Several antioxidant enzymes, whose gene expression are commonly under the regulation of the transcription factor Nrf2, can serve as target proteins utilized toward development of disease-modifying therapy for PD. Transcription factor Nrf2 binds to the antioxidant responsive elements (ARE): activation of this pathway protects cells from oxidative stress-induced cell death. Studies on oxidative stress *in vivo* should yield crucial information, from both scientific and medical aspects. The need for investigational tool to decipher the role of oxidative stress in PD allows the generation of a novel reporter mouse.

After an extensive bibliographic research and a consequent bioinformatics analysis, we have designed a promoter sequence composed by 8 minimal ARE derived from four genes optimized to have only binding sites for the Nrf2 factor. This promoter was tested in transfected cell lines treated with different oxidative stress inducers and with unrelated stimuli as negative controls. The selectivity and the magnitude of the reporter response were evaluated by luciferase enzymatic assay and univocally allowed us to choose the appropriate promoter arrangement for ARE reporter mouse. This reporter system (see scheme below) is suitable for multimodality imaging, because it allows *in vivo* detection via both fluorescent (tdTomato) and bioluminescent imaging (Luc2). Moreover, the reporter system has a conditional expression by the presence of loxP-stop-loxP cassette allowing restricting the expression of the reporter genes to any specific cell lineage after breeding with transgenic Cre mice. The reporter system was inserted by homologous recombination in a locus of the mouse genome engineered with insulators (EP 1298988B1) allowing the expression of reporter in every cell of the genetically modified mouse.

The validation of the reporter system *in vivo* reveals that the presence of STOP sequence blocks the expression of reporter gene, while after deletion of STOP sequence by breeding with CMW-cre mouse reporter gene is ubiquitous expressed. As a proof of principle to the faculties of ARE reporter mouse to monitor *in vivo* the oxidative stress dynamics we tested the response to a well know antioxidant agent t-BHQ. As expected, this test demonstrated that tBHQ increases the luminescent emission from the ARE-Luc2 reporter mouse and, interestingly, the system is also able to monitor the decrease of photon emission that corresponds to the recovery of the damage.

PD-like neurochemical and pathological features can be reproduced by neurotoxicants such as MPTP [2] and we showed that bioluminescent ARE reporter mouse can be used as a tool to study the Nrf2 pathway *in vivo*: we measured the increased of photon emission in the head area in the MPTP treated mice *vs* sham animals and our imaging data indicates that Nrf2 pathway is early activated by the MPTP treatment. These preliminary data suggest us to proceed using ARE mouse model as a very performant tool to evaluate a therapeutic strategy to reduce oxidative stress in PD model.

1. Schapira AH et al Etiology and pathogenesis of PD.Mov Disord.2011 26.
2. Jackson-Lewis V et al Protocol for the MPTP mouse model of PD. Nature Prot 2007 2(1).