CREATION AND CHARACTERIZATION OF *IN VITRO* AND *IN VIVO* MODELS TO INVESTIGATE THE PATHOGENIC MECHANISM OF MUTANT FUS/TLS-INDUCED ALS

Ludo Van Den Bosch, Belgium
Grant: € 150 000

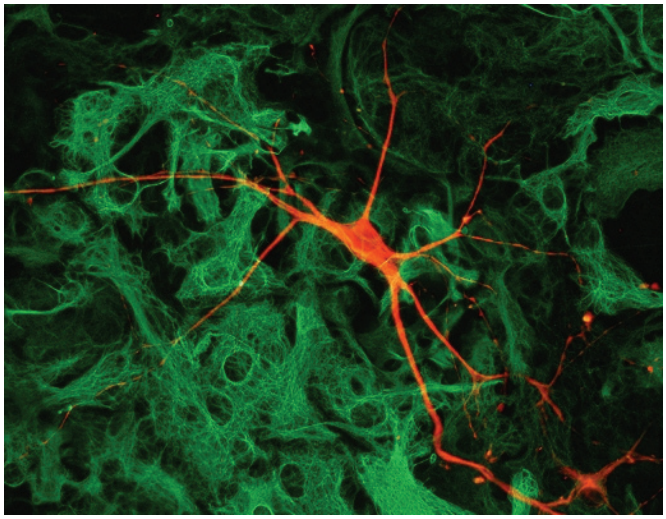
Loss of motor neurons in the motor cortex, brain stem and spinal cord is the hallmark of amyotrophic lateral sclerosis (ALS). This results in progressive muscle weakness, atrophy and ultimately death of the patient on average 2 to 5 years after the diagnosis. In the vast majority of patients (90 %) there is no familial history (sporadic ALS: SALS), while in a subset of patients (10 %) an underlying genetic cause is present (familial ALS: FALS). Clinically, both forms of ALS are indistinguishable and it is generally accepted that similar pathways and mechanisms causing selective motor neuron death are involved in both types of ALS. As a consequence, all ALS patients will benefit from a better insight into the pathogenesis of genetically determined forms of ALS. In addition, therapeutic strategies that work in these FALS models will most likely also make a difference for SALS patients.

For about 15 years, the major known genetic cause of familial ALS were mutations in the Cu/Zn superoxide dismutase 1 (SOD1) gene and most of our knowledge on ALS is based on models generated by (over)expressing the mutant SOD1 gene. Recently, dominant mutations in the gene encoding FUS/TLS were discovered as a new cause of familial ALS.

No information is available on the disease mechanism underlying mutant FUS/TLS-induced ALS.

As this research is currently severely hampered by the absence of model systems to study mutant FUS/TLS-induced motor neuron degeneration, the first aim of this project is to create new FUS/TLS-related ALS models. We will create *in vitro* and *in vivo* models to gain insights into mutant FUS/TLS-induced ALS.

We have fibroblasts available from ALS patients with the Flemish FUS/TLS mutation. We will also create stably transfected neuronal cell lines (over)expressing wild type and mutant FUS/TLS as well as primary motor neuron cultures.



Cultured primary motor neuron (red) on a feeder layer of astrocytes (green)

In addition, we will create transgenic mice (over)expressing wild type or mutant FUS/TLS and these mouse model(s) will be extensively phenotyped.
Using these different models, we will try to get insights into the pathogenic mechanism. We want to find out whether FUS/TLS mutations cause motor neuron pathology through a 'loss-of-function' and/or a 'gain-of-function'. In addition, we will try to unravel the molecular mechanism(s) involved in FUS/TLS-related motor neuron death. We will focus on aggregation, axonal transport and excitotoxicity. We are convinced that studying the pathogenic mechanism of mutant FUS/TLS in particular and of disturbances of the RNA metabolism in general could give insights into the pathogenic mechanism of both FALS and SALS.
By making use of the combination of cell cultures, small animal and mouse models, we hope to gain insights into the pathogenic mechanism of mutant FUS/TLS-induced motor neuron loss underlying the ALS pathology. We will compare these pathways with those described for mutant SOD1-induced ALS. Common pathways will be highly relevant for sporadic ALS, as those are independent of the disease causing gene. These new insights will hopefully lead to the identification of new drug targets that can hopefully stop and/or cure this dreadful and fatal disease.

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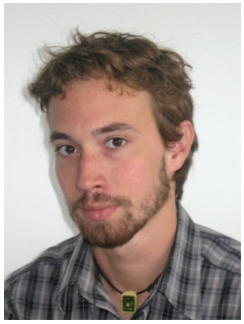

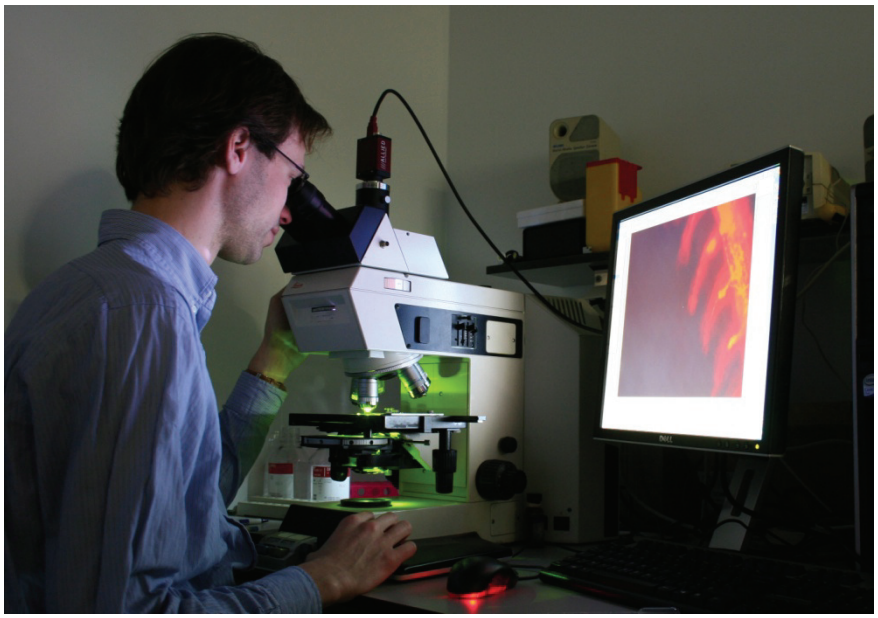

Overview central lab

Creation and characterization of *in vitro* and *in vivo* models to investigate the pathogenic mechanism of mutant FUS/TLS induced ALS.

Call for projects 2010
Grant: 150 000 €
Project Duration: 3 years

Investigator: Ludo van den Bosch, University Hospital Leuven, Belgium

Updated results - December 2012

The goal of this research project is to investigate whether similar mechanisms are responsible for familial ALS caused by defects in the FUS/TLS gene, a genetic cause of ALS.

We have created new model systems to get a better insight into the disease process of ALS. We have reprogrammed skin cells from patients and can generate different cell types from these cells by supplying them with specific growth factors. By doing so, we can grow motor neurons from patients in culture dishes and we can study the characteristics of these cells and the effects of the gene defect. In addition, we expressed the genetic material from patients carrying the FUS/TLS gene defect in cultured cells. The genetic defect results in the mislocalization of the protein to the cytoplasm and the accumulation of this protein in stress granules. Strategies that counteract these defects could be helpful to cure the disease.

In a first step to extrapolate the results obtained in culture dishes to patients, we also expressed the defective human FUS/TLS gene in the fruit fly. This results in flies with severe motor problems. We are now screening for factors that 'cure' these motor problems. The advantage of the fruit fly is that it is possible to perform these experiments in a very short time frame (due to the short generation time of the fly) and at a reasonable cost. Once we have more information on the pathways that are important to counteract the adverse effects of the defective FUS/TLS gene, we will confirm this in vertebrate models. Apart from different rodent models that we are developing, we are also using zebrafish. Injecting the human, mutant FUS/TLS gene in zebrafish embryos results in shorter motor axons and we are testing strategies to counteract this problem. Last but not least, we will test the strategies that are successful in flies and fish in rodent models that overexpress the defective human FUS/TLS gene. As this apparently leads to general toxicity, we are currently constructing transgenic mouse models in which we can switch on the mutant gene at different time points and/or in selected tissues. Once these mouse models are available, drugs can be screened on them.

In conclusion, we have created and are creating new model systems that will allow us to get new insights into the pathological mechanism of the complicated disease that ALS is. In the future, we will use these models to screen for drugs that stop and/or cure ALS.