FUNCTIONAL CHARACTERIZATION OF TWO NOVEL SUSCEPTIBILITY LOCI IN SPORADIC ALS

Jan H. Veldink, The Netherlands
Grant: € 200 000

The etiology of ALS is largely unknown, but there is evidence that several distinct molecular mechanisms (e.g. glutamate-mediated excitotoxicity, mitochondrial dysfunction, protein aggregation) may play a role in the onset and progression of ALS. However, compelling evidence of a causative role for any of these mechanisms is still lacking. Approximately 5–10% of patients with ALS have a family history (FALS), and these patients most frequently inherit the disease in an autosomal dominant manner. The majority of ALS cases, however, has no obvious family history and is referred to as sporadic ALS. Sporadic ALS is considered to be a complex disease with modifying genes and environmental factors affecting its clinical expression. The identification of genetic variants associated with sporadic ALS may be a first step to unravel the complex nature of the pathogenesis of this disease. In order to identify associated genetic risk factors for ALS, we performed through a large international collaboration a two-stage Genome Wide Association (GWA) study encompassing 20,540 subjects. A GWA study in 2,345 ALS patients and 9,106 controls from The Netherlands, Belgium, Sweden, Ireland, and USA was followed up in a second, independent cohort of 2,871 cases and 6,218 controls from The Netherlands, Ireland, Germany, France, UK, Poland, and USA. We were able to identify two new parts in the DNA, the molecule in our body that carries all hereditary information: *19p13.3* and *9p21.2* as novel susceptibility loci for sporadic ALS. However, the role and significance of these genes in the process of motor neuron degeneration during ALS still remain to be elucidated.

Therefore the **overall objective** of this project is to establish the functional role of *19p13.3* and *9p21.2* in motor neuron degeneration and disease progression during ALS.

The overall objective has been subdivided into **two key objectives**: **1**) Functional characterization of *UNC13A* (protein coded by 19p13.3 locus) in motor neuron degeneration during ALS, and **2**) Identification and characterization of causal variants in the *9p21.2* locus related to ALS pathogenesis.

To reach this goal we will use a unique multidisciplinary approach of human postmortem studies, human and mouse genetics, cell culture systems, and shRNA-mediated gene knockdown.

This study is the first to systematically assess the functional role of this recently discovered ALS related genetic variation in the pathogenesis of sporadic ALS. The results from this work will generate **a unique platform for the study of sporadic ALS** and will provide an enhanced understanding of ALS disease mechanisms and may thereby ultimately allow the **development of novel therapies.**

TEAM

The present research proposal is part of an ongoing collaboration between the University Médical Center Utrecht research lines on motor neuron disease (Pr Van den Berg/ Dr Veldink) and neuronal connectivity/mouse genetics (Dr Pasterkamp). The patient-oriented research line on motor neuron disease of the Department of Neurology involves the whole spectrum of motor neuron diseases including *amyotrophic lateral sclerosis* (quality of life and care; end-of-life decisions; treatment strategies; pathogenesis; environmental and genetic risk factors). The basic research line at the Department of Neuroscience & Pharmacology involves studies on neuronal morphology and function in human and mouse tissue, tissue culture models for several brain regions and disorders, and genetic mouse models (including phenotypic and behavioral analyses). Only the combined expertise and research results of the Departments of Neuroscience & Pharmacology and Neurology will allow for a multidisciplinary and translational approach as the one outlined in this proposal.

R.J. Pasterkamp, PhD is leading the research group. His research focuses on the molecular mechanisms underlying nervous system wiring and on the question how these mechanisms are disrupted during neural disease (ALS). The multidisciplinary approach of his research group includes cutting-edge technologies at the molecular level (e.g. proteomics), cellular level (e.g. high-throughput and live cell imaging), and in living animals (e.g. genetic mouse models, in utero electroporation). Dr. Pasterkamp has published in journals of the highest repute (e.g. J Neurosci, Nature, Cell, PNAS, Nature Genetics, Mol Psychiatry).

J.H. Veldink, MD/PhD as a clinical neurologist and senior researcher, is part of the group, headed by Prof Leonard van den Berg and internationally recognized for the expertise in research aiming at the identification of genetic and environmental/life style factors that increase susceptibility or modify disease course in ALS. In 2005, a large nation-wide prospective population-based study aiming at a complete ascertainment of incident ALS patients in the Netherlands (Prospective ALS study in the Netherlands (PAN)) was initiated, for which he made the design. As a result of these efforts a detailed ALS database and biobank of >2,500 patients is now available. He has acquired the necessary computer programming skills to deal with large datasets that typically result from genome-wide SNP and microarray studies, which formed the basis of the result in our recent publication on UNC13A and 9p21.2 in Nature Genetics. Through his international collaborations and collaboration with prof. Ritsert Jansen of the Groningen Bioinformatics Centre, he is currently working on a meta-analysis of all available genome-wide genotyping and gene-expression data in ALS.

Characterization of the role of UNC13a and C9ORF72 in amyotrophic lateral sclerosis

Call for projects 2010
Grant: 200 000 €
Project Duration: 3 years

Investigators: Jan H. Veldink, UMC Utrecht, The Netherlands

Updated results - December 2012

The aim of our project is to understand the role/function of UNC13A and C9ORF72, two recently discovered genes that contribute to ALS.

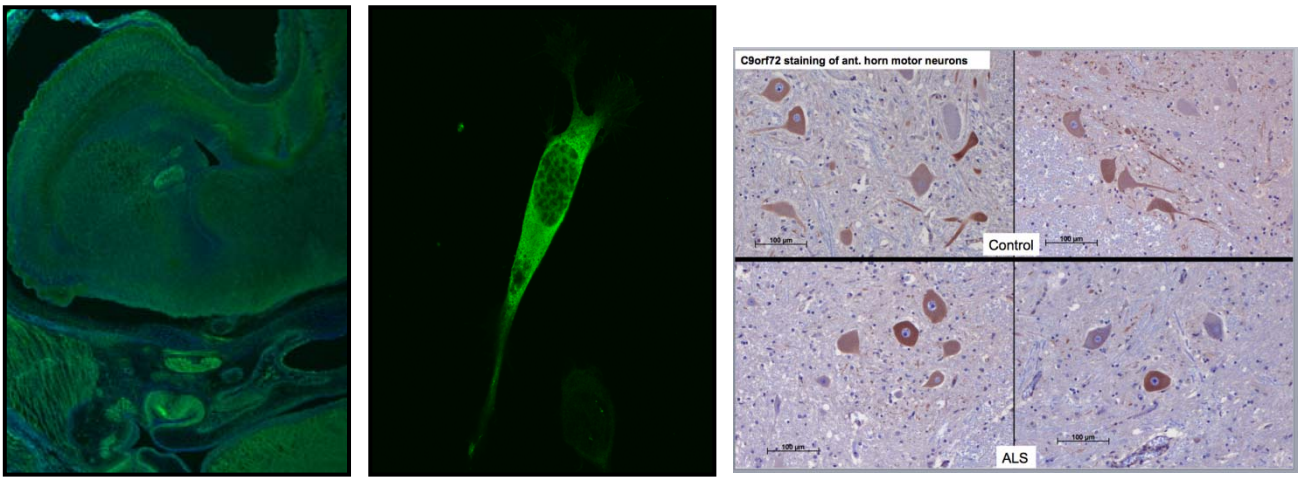
We are investigating the distribution of these proteins in the brain and spinal cord, but also on the level of a single cell (Figure 1). We have identified a list of proteins that possibly interact with C9ORF72. We are currently confirming these binding partners and are studying their function.

Furthermore we are developing mouse models for C9ORF72.

We also performed a large study to find rare genetic mutations in UNC13a and C9ORF72 that possibly play a role in ALS. We investigated 1019 sporadic ALS patients and 1103 healthy controls and identified 14 mutations, 11 in UNC13a and 3 in C9ORF72.

The role of UNC13A in ALS is being investigated using genetically engineered mice. We are cross-breeding mice that have a lower amount of UNC13A protein than normal mice with mice that have an ALS-causing mutation (SOD1) to look at the effect of this on ALS development and survival.

FIGURE 1:



From left to right: Expression of the C9ORF72 protein in mice brain, a single cultured cell and in the spinal cord of an ALS patient and a healthy control individual.