Gene expression analysis in motor neurons in a mouse model for ALS using laser microdissection and quantitative PCR

Janssen LTF, Van Vught PWJ, Van den Berg LH, Van Muiswinkel FL
Department of Neurology, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Utrecht

Amyotrophic lateral sclerosis (ALS) is progressive neurological disorder characterized by a loss of upper and lower motoneurons beginning in mid-adult life. So far, only one gene has been identified in which mutations cause familial ALS. Mutations within this gene, i.e. Cu, Zn superoxide dismutase (SOD1), are found in only 1-2% of all patients. Transgenic mice carrying mutated human SOD1 (mut hSOD) develop a disease phenotype that mimics ALS. Although this model has provided many new insights, as yet the pathogenesis of ALS remains unknown. Since the motor neurons are specifically affected, analysis of changes in gene expression in these neurons is of particular interest and may help to elucidate the molecular mechanisms underlying ALS.
So far, gene expression analysis has been performed on cell homogenates of total spinal cord, consisting of both motor neurons and non-neuronal cells, thus making it difficult to distinguish between motor neuron- or glial specific changes. Therefore, to determine motor neuron and glial specific gene expression profiles as function of disease progression, in the present study we have exploited laser microdissection to isolate specific populations of either motor neurons or surrounding non-neuronal cells from control mice, carrying wild type hSOD1, and ALS mice carrying mut hSOD1 at different stages of disease.
Spinal cords from hSOD mice and mut hSOD mice were rapidly removed and motor neurons and surrounding non-neuronal cells were isolated from cryosections by laser microdissection. Total RNA of 100 cells was isolated and processed to cDNA. To validate our method, besides a conventional PCR for choline acetyltransferase (ChAT) and glial fibrillary acidic protein (GFAP) as markers for motor neurons and glial cells, respectively, a caspase-I quantitative PCR was performed to confirm and extend previously published data. Currently, quantitative PCR experiments of a selection of novel candidate genes are in progress.

Linnea T.F. Janssen, Department of Neurology, Rudolf Magnus Institute of Neuroscience, University medical center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, t 030 2507600, e-mail T.F.Janssen@students.uu.nl

Poster session Neuroscience 1