

¹H NMR metabolomics study of cerebrospinal fluid (CSF) in amyotrophic lateral sclerosis (ALS) patients

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Introduction:

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative motor neuron disorder. Pathophysiological mechanisms involved in this disease are complex but remain for the most part unknown. This lack of knowledge might explain the absence of reliable biological marker. CSF is an attractive potential source for ALS biomarkers. High resolution ¹H NMR metabolic profiling provides a new approach and opportunity to explore CSF metabolic fingerprint of ALS patients. In this study, we aimed to evaluate the ability of CSF metabolomic analysis to differentiate ALS patients from a non-ALS population. The biochemical variations in CSF using multivariate statistical analysis provide information on the global profile in order to find disease-unique biomarkers.

Material and Methods:

CSF samples were collected by lumbar puncture at the time of diagnosis from patients with ALS (n=14) and from patients without neurodegenerative diseases (n=16). 100 μ L of deuterium oxide were added to 500 μ L of CSF prior to analysis by ¹H NMR spectroscopy. ¹H NMR spectra were performed on a Bruker DRX-500 spectrometer (Bruker SADIS, Wissembourg, France), using a standard spin-echo pulse sequence. ¹H spectra were collected with 128 transients (and 8 dummy scans) in 32K data points with a spectral width of 7500 Hz, a recycling time of 30s. Spectra were processed using WinNMR version 3.5 software (Bruker Daltonik, Karlsruhe, Germany). Prior to Fourier transformation (FT), the FIDs were zero-filled to 64K data points which provided sufficient data points for each resonance and a linebroadening factor of 0.3Hz was applied. All spectra were corrected for phase distortion and baseline was manually corrected for each spectrum. Each spectrum was integrated using WinNMR software integral function. The obtained concentrations of 15 metabolites were analyzed by PCA based on the correlation matrix.

Results:

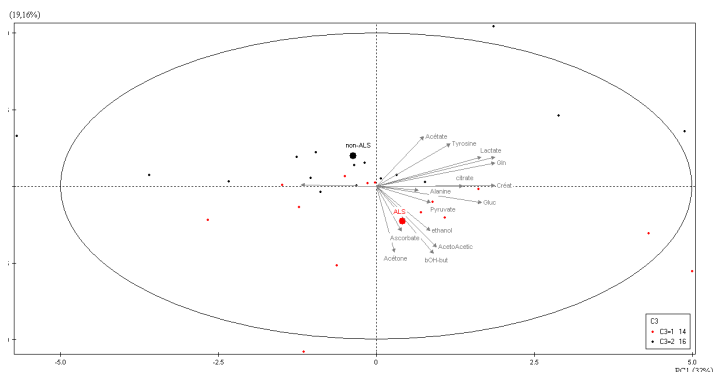
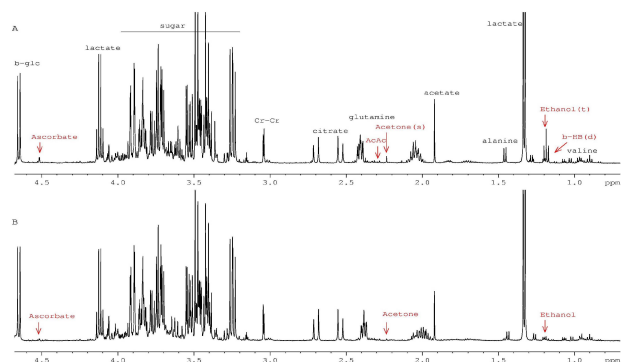


Figure 1: Typical ¹H NMR spectra of CSF samples from: A) ALS B) non-ALS patients.

Figure 2: PCA score plot of 30 CSF samples. Red circles: ALS patients, black circles: non-ALS patients.

A separation with respect to pathology is seen. The two principal components PC1 and PC2 explain 51.2% of variance in the NMR data. Higher concentrations of metabolites such as acetone, β -hydroxybutyric acid (b-HB), acetoacetic acid (AcAc), ascorbate and ethanol contribute to the separation.

Discussion:

CSF screening by NMR spectroscopy could be a good, rapid and easy tool to improve early diagnosis of ALS. Our results led us to suspect a perturbation in the ketone bodies may be in relation with a perturbation of glucose metabolism as already proposed. A more important number of samples should validate these results and allow improving the discrimination between the two populations.