DEVELOPMENT OF SPECIFIC BIOMARKERS FOR ALS CLINICAL PROGRESSION: VALIDATION AND POTENTIAL ROLES IN PATHOGENESIS.

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Using SELDI mass spectrometry proteomics, we previously identified down regulation of a "3 protein" profile in the cerebrospinal fluid (CSF) of patients with ALS, including VGF fragment and cystatin C that is 95% accurate, 91% sensitive and 97% specific. One of these proteins is VGF, which is a member of granin family of proteins (chromogranin A and B) that form neurosecretory granules.
We developed a quantitative immunoassay for VGF, Cystatin C (CysC) and Chromogranin A (CgA) and determined if the content of VGF, CysC or CgA is reduced in patients with ALS compared to normal and disease controls and relationship with clinical progression. We found that CSF levels of VGF, CysC and CgA by ELISA immunoassays identified patients with ALS at levels below as 0.02U/L, 4µg/ml, and 17U/L respectively with 77% sensitivity and 97% specificity, from healthy controls. Validation studies showed that weaker ALS patients showed lower levels of VGF, CgA and CysC in the CSF. The concentration of VGF and CgA in the patients with clinical weakness in two segments was significantly lower than those with weakness in only one segment (p=0.038 and 0.046 respectively).
To further explore the potential roles of VGF in ALS disease progression, we found that VGF content in CSF was lower in G93A SOD-1 transgenic mice compared to normal littermates. Treatment of the spinal cord motorneurons cultures from G93A SOD-1 transgenic mice with VGF peptide (aa588-617) provided neuroprotection against glutamate receptor mediated excitotoxic death. Furthermore, we also found that the life expectancy of the ALS transgenic G93A SOD-1 mouse line is significantly shortened by targeted deletion of one VGF allele in G93A SOD-1 transgenic, *Vgf+/Vgf-* mice.
Our findings suggest that the quantitative immunoassays of VGF, Cystatin C and Chromogranin A in the CSF seem to correlate with human and animal disease progression. Moreover we found that reduced VGF expression as reflected by decreased VGF content in CSF could contribute to ALS pathogenesis due to loss of its potential neuroprotective effect. These studies further support the potential diagnostic and therapeutic significance of VGF, CysC and CgA as biomarkers of ALS clinical progression.