METAL AND PROTEOMIC ANALYSIS OF SPORADIC ALS PATIENTS WITH COMMON GEOGRAPHICAL ORIGIN

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Introduction:

Neurodegenerative disorders such as Amyotrophic Lateral Sclerosis (ALS) have been linked to iron and metals metabolism in different studies through the years[1,2]. Transition metal induced toxicity has been proposed to be involved in ALS[3]. Several researchers have analyzed different groups of patients with non similar environmental exposure by investigating metals in different tissues, but these studies have produced contrasting results[4-7]. Proteomic studies are currently being performed to search for possible biomarkers[8,9]. At present, few studies on gel-based proteomics in ALS are reported, performed on different tissues[10-13,] but none on serum. This poster reports the preliminary results of a study performed on a cohort of subjects with defined sporadic ALS, all originating from a restricted geographical area (7 patients and 5 controls), so that the same environmental exposure could help to minimize the differences among the subjects under investigation.

Materials and Methods:

Blood was collected from all subjects. ALS diagnosis was according El Escorial criteria with clinically defined sporadic cases; all patients were genotyped for the main ALS genes (SOD1, FUS, TARDBP, C9ORF72). Samples of serum were analyzed by ICP-MS for metal quantification and results have been evaluated through classical statistical methods and with Auto CM algorithm[14]. For proteomic analyses, immobilized pH gradient strips for the 1std were prepared, proteins were reduced with 1% 2-mercaptoethanol. The 2nd dimension was run on a gradient polyacrylamide gel. Selected spots were identified by Mass Spectrometry (MS). Classical statistical analyses have been applied on the results.

Results:

Genetic analyses gave negative results in all the patients, allowing us to rule out at least the most frequently mutated genes as disease causes. Analyses performed on serum samples (Tab. 1) highlighted elevated levels of Al, Ni, Cr, Ba and V both in controls and in patients if compared to the reference values for the Italian population[15]. Only the As concentrations were significantly different between the two groups and, quite surprisingly, As resulted lower in patients (p = 0,01), as did Mn (p = 0,10) and Hg, two well known neurotoxic elements. Auto-CM analysis linked closely high concentrations of Al and Se to the ALS group (Fig.1). However, according to the t-test, the differences among the two groups were not statistically significant (p = 0,13 and p=0,12).

To assess the results from 2DE experiments, the integrated volumes of the spots were compared between the controls' group and the patients' group. The statistical significance of the differences was evaluated with Student's t-test. Results are shown in Table 2. In the first set of experiments (NL pH gradient 4-10) **APOA2** protein resulted decreased by 30% in patients with respect to controls. **SAMP** showed a significant decrease only in the group of patients with late onset. When we focused on the acid portion of IEF (NL pH gradient 3-7) **APOA1** and **TTHY** were decreased, the former particularly in late-onset patients. Only **ANT3** resulted increased in patients, particularly in the early-onset group. Finally, **RET4** was decreased only in the early-onset group (Fig 2). The trend in concentrations of proteins according to the years from disease onset is reported in Fig. 3. It is remarkable that some proteins undergo a quite gradual decrease (TTHY, RBP4, ZA2G), whereas others show a drastic reduction in the first 5 years of disease (APOA1, APOA2, HPTα, FETUA).

Element	Average Patients ± SD (µg/L) [n = 6]	Average controls ± SD (µg/L) [n = 5]	p-value	Reference Values (µg/L) [15]
As	0.34 ± 0.03	0.44 ± 0.07	0.01	NA
Al	23.22 ± 5.37	17.15 ± 6.88	0.13	0.4-5.3
Mn	1.33 ± 0.69	2.36 ± 1.12[a]	0.10	0.31-1.02
Se	100.2 ± 11.9	89.5 ± 8.2	0.12	56-105
Ni	2.82 ± 0.35	3.00 ± 0.42	0.45	0.26-0.75
Pb	1.09 ± 0.41	0.83 ±0.40	0.29	0.20-0.98
Hg	0.90 ± 0.80	1.62 ± 0.90	0.20	0.32-2.75
Cu	1140 ± 216	1142 ± 136	0.99	648-1301
Fe	1165 ± 521	1225 ± 202	0.81	886-2455
Zn	846 ± 151	835 ± 105	0.90	597-1028
Co	0.49 ± 0.09	0.51 ± 0.03	0.65	0.06-0.42
Cr	1.56 ± 0.18	1.54 ± 0.08	0.84	0.07-0.28
Ba	13.26 ± 4.87	12.75 ± 2.14	0.83	0.32-1.37
Sn	0.14 ± 0.03	0.17 ± 0.07	0.45	0.27-1.69
U	0.03 ± 0.01	0.02 ± 0.01	0.75	NA
V	0.96 ± 0.11	0.94 ± 0.12	0.79	0.0.-0.11
Sr	38.4 ± 19.7	34.5 ± 7.2	0.69	23-61.5

Tab 1. Averages of the measures of metals concentrations in serum.
NA: Not Available. [a]One subject from controls not analyzed.

Protein	All	Onset ≤ 60 years (n = 4)	Onset > 60 years (n = 3)
Apolipoprotein A-I	-17 % *	=	-22 % **
Transthyretin	-30 % *	-28 % **	-32 % **
Antithrombin-III	+71 % *	+71 % **	=
Retinol-binding protein 4	=	-25 % **	=
Serum amyloid P-component	=	=	-77 % *
Apolipoprotein A-II	-30 % *	-29 % **	-33 % **

Tab. 2 Different expression of the proteins identified with 2D-E in the comparison between patients and controls, showed as percentage variation. *p ≤ 0.05, **p ≤ 0.1

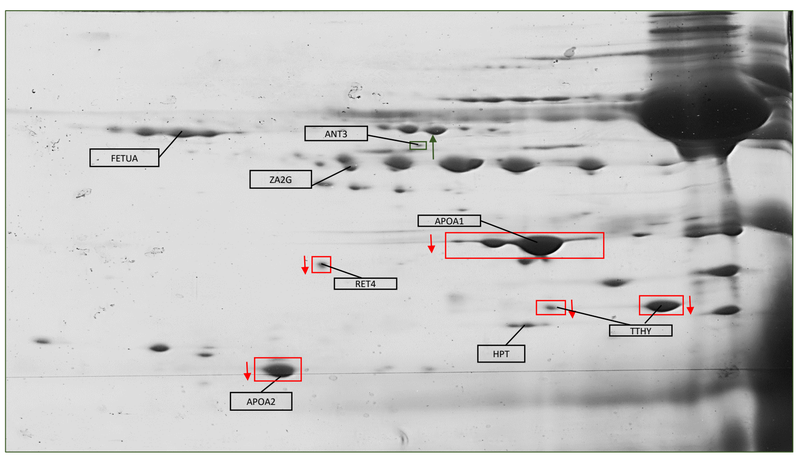

Fig.1 2D-E gel showing the significant spots identified. Red shapes: proteins decreased in patients; Green shapes: proteins increased in patients; No shape: other relevant proteins.

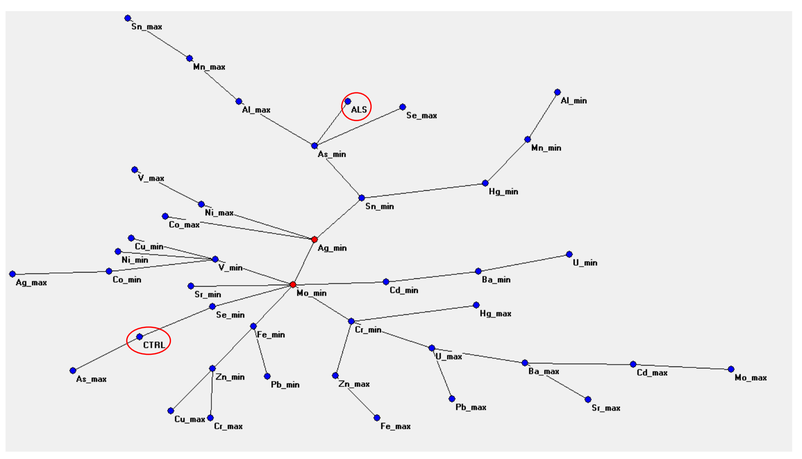

Fig.2 Semantic connectivity map showing the connections between metals concentrations, ALS group and Control group. Adjacent nodes have the strongest association.

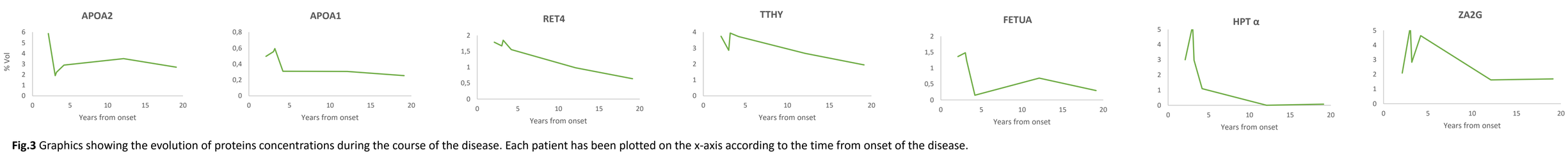

Fig.3 Graphics showing the evolution of proteins concentrations during the course of the disease. Each patient has been plotted on the x-axis according to the time from onset of the disease.

Conclusions:

Altered metals' concentrations could be possibly related to environmental exposure, due to the presence in the area, where subjects involved in this study originate, of waters reported to be strongly polluted due to Acid Mine Drainage[16]. The lower levels of As found in patients is of particular interest since it is known that its metabolism in cells elicits the generation of oxidative stress. Metals found in lower concentration in patients' sera could reflect their accumulation in some body districts/tissues, where they exert toxic effects. Besides this, metals can compete for the binding sites of metal-containing proteins, such as those containing iron-sulfur clusters[17].

Regarding proteomics data, the proteins in which we registered an alteration, are involved in the Acute Phase Response. Indeed the different expression with respect to controls could be referred to the disease status of the subject analyzed. We also noticed an alteration in some proteins related to lipid homeostasis, that is consistent with the proposed metabolic shift towards an increased peripheral use of lipids[18]. However, we would like to highlight the fact that all the proteins found differentially expressed in this study have already been described in other studies.

In this context, despite the small group analyzed here, we found our data comparable to studies involving much a much higher number of patients, strengthening our approach, based on a small number of patients but with a common environmental exposure.

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