Metals concentrations in Amyotrophic Lateral Sclerosis patients originating from a restricted geographical area

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Introduction:

Amyotrophic Lateral Sclerosis (ALS) is a rare neurodegenerative disorder with an incidence of about 1/100.000 case per year. It is characterized by a selective degeneration of both upper and lower motor neurons in the brain, brainstem, and spinal cord (Fig.1), resulting in paralysis due to muscle weakness and atrophy, leading to death in 3-5 years since the first manifestations of symptoms[1]. Neurodegenerative disorders such as ALS have been linked to iron and metals metabolism in different studies through the years[2-4]. Transition metal induced toxicity has been proposed to be involved in ALS[5] and higher concentrations of metals and proteins that regulate metal homeostasis have been described in ALS patients[6]. This poster reports the preliminary results of the analyses performed on a cohort of subject with defined ALS all originating from a restricted geographical area (7 patients and 5 controls). We applied a change in the approach to the study of ALS, by choosing to focus on a restricted cohort of subjects, in order to analyze different aspects of this multifactorial disease: the same environmental exposure could help to minimize the differences among the subjects under investigation.

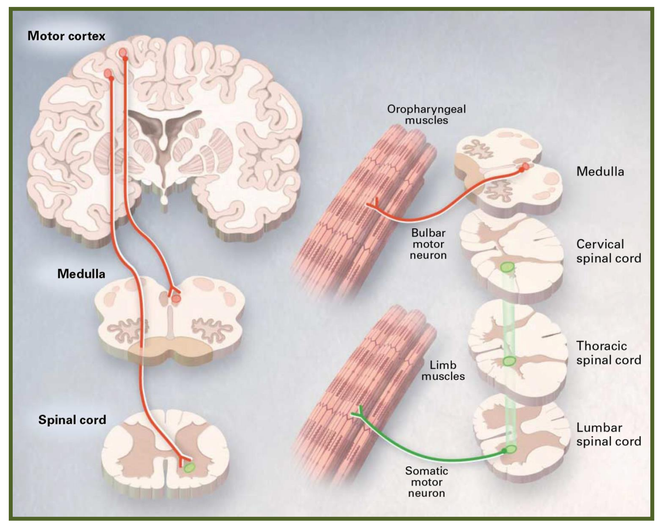

Fig.1 *Neuronal pathways affected in ALS*

Materials and Methods:

All subjects gave informed consent after genetic counselling and blood was collected. ALS diagnosis was according El Escorial criteria; genomic DNA was extracted according to standard procedures and all patients were genotyped for the main ALS genes: SOD1 whole gene, FUS exons 5-6-13-14-15, TARDBP exon 6 through direct sequencing and C9ORF72 G_4C_2 hexanucleotide repeat through RP-PCR[7]. Serum was obtained after centrifugation and stored at -80°C. Samples of serum and urine were diluted (1:20 and 1:10, respectively) with 0.05% Triton X-100 in MilliQ water and analyzed by ICP-MS (Bruker AURORA M90 ICP-MS). Statistical analyses on the results have been carried out both with classical statistical elaborations (t-test and Principal Component Analysis) and with Auto CM algorithm, a special kind of Artificial Neural Network able to define the strength of the associations of each variable with all the others and to visually show the map of the main connections, that has already been used in ALS studies[8].

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 highlighted elevated levels of Cr, Ni and Pb both in controls and in patients' group, if compared to literature data for the general population[8]. Higher concentrations of Ni and Pb were found in the patients' group, compared to the control's group (p-value = 0.0001 and 0.01). Surprisingly significant higher concentrations of As were found in the control's group (p-value = 0.05) (Tab. 1). Principal Component Analysis (PCA) confirmed these observations, and was able to discriminate between the two groups. The most important feature of the control group was the high concentration of As and a low concentrations of all the other metals analyzed. This observation was confirmed by Auto-CM analysis, that discriminated the two groups, clustering the control group with high levels of As (Fig. 2).

Element	Average Patients ± SD (µg/L)	Average Controls ± SD (µg/L)	Reference Values (µg/L)[9]
Cr	1.57 ± 0.12	1.54 ±0.06	0.07-0.28
Fe	1261 ± 429	1225 ± 160	648-1301
Ni	9.44 ± 1.02*	2.10 ± 0.92*	0.26-0.75
Cu	1130 ± 157	1141 ± 108	648-1301
Zn	811 ± 114	835 ± 72	597-1028
As	0.51 ± 0.14*	0.73 ± 0.18*	NA
Se	97 ± 10	89 ± 6	56-105
Sr	39 ± 12	34 ± 5	23-61.5
Cd	0.08 ± 0.03	0.06 ± 0.01	0.03-0.2
Pb	2.16 ± 0.72*	1.26 ± 0.29*	0.2-0.98

Tab 1. *Averages of the measures of metals concentrations in sera. *: p-value ≤ 0.05, NA: Not Available.*

Among the three metals that were significantly different, no one emerged as more relevant than the others in the discrimination of the control's group from the patient's group. Regarding urine analysis, all metals analyzed but Ni and Sr showed higher concentration than general population[9]. Intriguingly, Pb had low concentration both in patients' and in controls' urine, at contrast with the high levels of this metal found in serum analysis. Differences between patients and controls were significant for Fe (p = 0.01), Ni (p = 0.01), Zn (p = 0.008), As (p = 0.04), Sr (p = 0.02), Cd (p = 0.05). However, it must be noticed that the control group consisted only of two subjects, different for age and geographical origin from the patients' group. PCA analysis discriminated the two groups: the control's group had low levels of metals, and the patient's group had higher metal levels, with only a patients' sub-group was showing high levels of As.

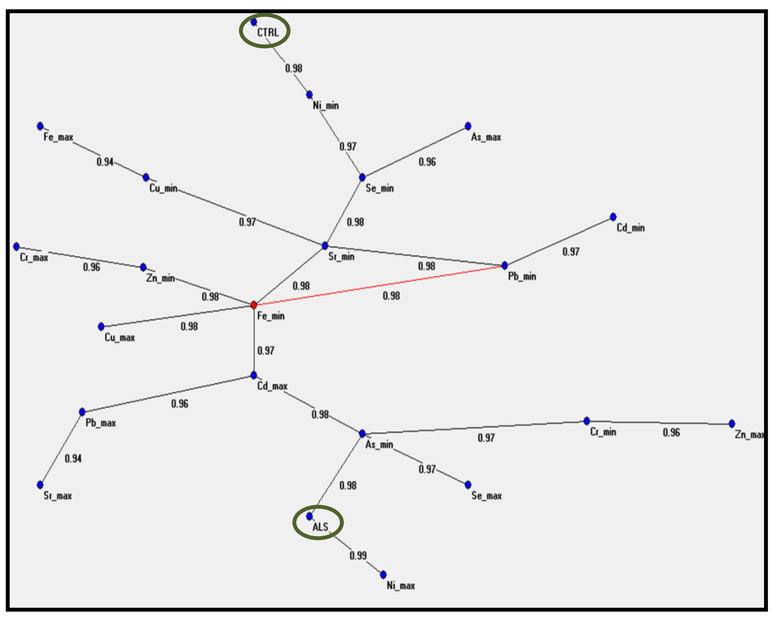

Fig. 2 *Auto-CM analysis of metals' concentrations in serum: Semantic connectivity map showing the connections between the variables. Values on the arches refer to the strength of the association between two adjacent nodes, the range is from 0 to 1.*

Discussion:

Despite much research, the etiology of Amyotrophic Lateral Sclerosis still has to be clarified. Only a small percentage of the cases is attributable to genetic defects[10], and environmental factors could play a crucial role. Here we report the preliminary results of the analysis of metals' status in a small cohort of subjects originating from a restricted area, thus sharing environmental exposure. Our results confirm the hypothesis of a possible association between Pb exposure and ALS[11, 12] and provides further suggestions. The first one regards Ni, higher in ALS patients, and a second one, probably more unusual, is that high As levels have been found in the control's group. A great part of the lead damage in cellular physiology is caused by its ability to substitute for diverse polyvalent cations in their binding sites[13]. Pb and Ni have substantial affinity for protein thiols. Direct substitution of iron in various types of FeS clusters by thiophylic metals has been demonstrated[14]. Arsenic is known to interact with thiol-rich proteins, such as glutaredoxin, involved in FeS proteins biosynthesis, making it plausible that dysregulation of homeostasis of these metals could affect FeS-protein dependent (or related) events, opening a new and still unexplored area in ALS research.

Further studies will be aimed at evaluating a panel of rarer metals (Mn, Al, Co, V, U, Mo, Ag, Sn). We have also planned to perform an evaluation of serum proteins through a proteomic approach in order to expand the knowledge on the effect of dysregulated metals homeostasis on circulating proteins.

We believe that the study of affected subjects in such geographic isolates would provide a representative model for the evaluation of environmental influences on Amyotrophic Lateral Sclerosis.

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