A MULTIDISCIPLINARY APPROACH TO STUDY SPORADIC AMYOTROPHIC LATERAL SCLEROSIS IN PATIENTS WITH COMMON GEOGRAPHICAL ORIGIN.

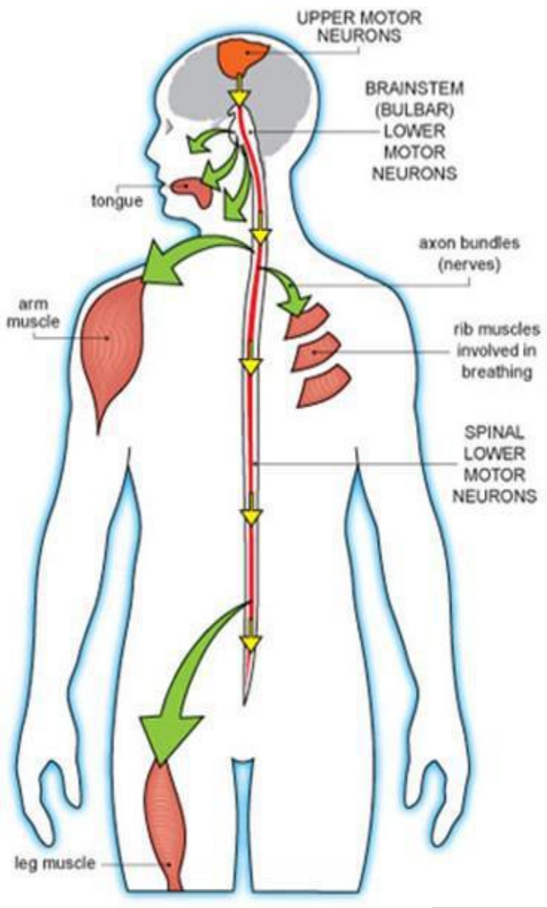
S. De Benedetti[1], G. Lucchini[2], A. Marocchi[3], S. Penco[3], C. Lunetta[4], S. Iametti[1], E. Gianazza[5], F. Bonomi[1]

[1]DeFENS, [2]DiSAA, [5]DiSFeB - University of Milan, [3]Medical Genetics Unit, Department of Laboratory Medicine, [4]NEuroMuscular Omnicentre (NEMO), Fondazione Serena Onlus - Niguarda Ca' Granda Hospital.

stefano.debenedetti@unimi.it

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 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.



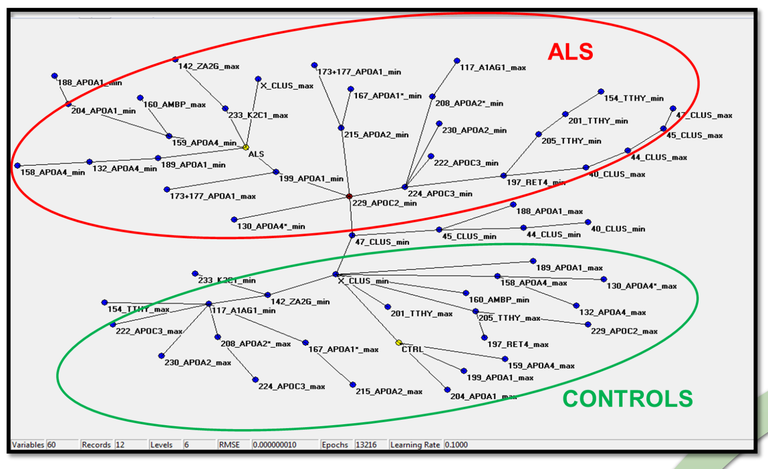
Materials and Methods:

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 the Auto CM algorithm[14]. For proteomic analyses, immobilized pH gradient strips for the 1st d were prepared. Both reducing conditions (1% 2-mercaptoethanol), and non reducing conditions were evaluated. The 2nd dimension was run on a gradient polyacrylamide gel. Selected spots were identified by Mass Spectrometry (MS). Comet assay was performed on 5µL of whole blood.

Results:

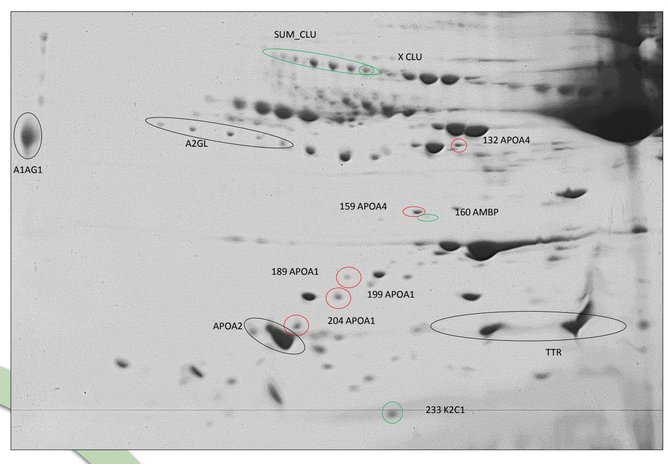
Proteomics

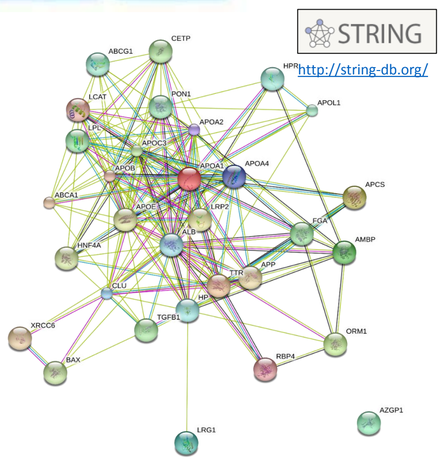
DECREASED ABUNDANCE IN PATIENTS	INCREASED ABUNDANCE IN PATIENTS
APOA1	ANT3
APOA2	AMBP
APOA4	K2C1
RET4	CLU
TTR	
INCREASED DURING DISEASE COURSE	
A1AG1	A2GL



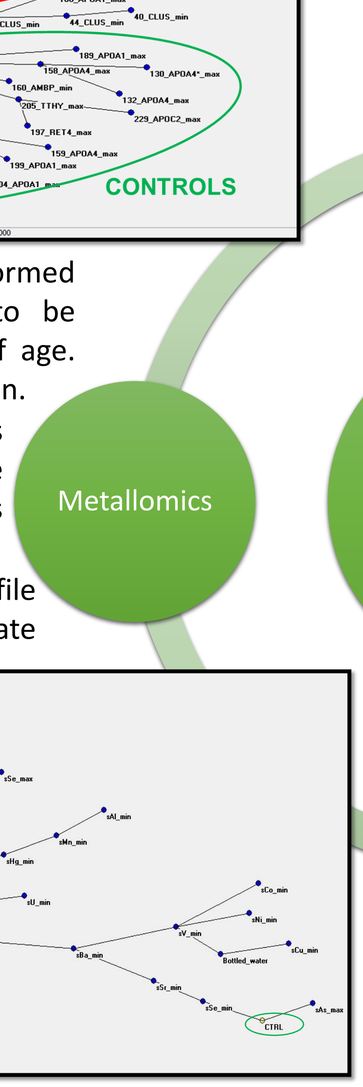
The Table reports the combined results of the 2-DEs performed in the two described conditions. RET4 was found to be decreased only in patients with onset after 60 years of age. A1AG1 and A2GL positively correlated with disease duration. TTR and APOA2 were significantly decreased in runs performed in reducing conditions and showed a negative correlation with disease course in the experiments performed in non reducing conditions.

Auto-CM analyses helped to define the peptidomic profile characteristic for ALS patients; it was also able to associate fragments of the same proteins.





The 2DE performed in non-reducing conditions shows the localization of proteins/peptides found with differential abundance. STRING software analysis shows the close functional interconnections between them.



Metallomics

ICP-MS analyses showed high concentrations of Al, Ni, Cr, Ba and V in serum and Mn, Zn, Co and Cr in whole blood both in patients and controls, in comparison with reference values for Italian population[15] serum concentration.

Only the serum concentration of As was significantly lower in patients.

Auto-CM associated higher levels of metals analyzed with the ALS group, except for arsenic. Tap water consumption was associated with ALS group.

APOE Genotyping

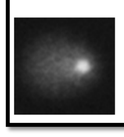
Population	Allele			Genotype					
	ε2	ε3	ε4	ε2/ε2	ε2/ε3	ε3/ε3	ε2/ε4	ε3/ε4	ε4/ε4
Caucasian	0.08	0.78	0.14	0.01	0.12	0.61	0.02	0.22	0.02
Study Controls	0.10	0.80	0.10	0.00	0.20	0.60	0.00	0.20	0.00
Study Patients	0.00	0.71	0.29	0.00	0.00	0.43	0.00	0.57	0.00

APOE genotype was evaluated, since the protein is involved in lipid homeostasis and has been associated to neurological disorders. Despite the low number of subjects, frequencies in controls are comparable to those reported for the Caucasian population.

The risk allele ε4 is more frequent in the ALS cohort than in control subjects.

Comet Assay

Patients % DNA in tails = 8,30 ± 2,52 — p = 0,943 — Controls % DNA in tails = 8,18 ± 3,08



No significant differences between patients and controls
No correlations with metal levels

Conclusions:

Altered metals' concentrations could be possibly related to environmental exposure, due to the presence in the area the subjects where from 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 specific (yet unknown) body districts/tissues, where they exert toxic effects. Besides, metals can compete for binding sites in some metalloproteins, such as those containing iron-sulfur clusters[17].

Regarding proteomics data, proteins found to be altered are involved in the Acute Phase Response. 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. Higher APOE4 allelic frequency in ALS patients gives an interesting link between lipids homeostasis and neurodegeneration, at least in this cohort of subjects.

The analyses performed with Artificial Neural Networks gave very promising results in evaluating different variables at the same time, providing an insight in proteomic and metallomic profile in ALS, that must be more deeply evaluated.

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

Bibliography:

1. Crichton et al. (2006). *England: John Wiley&Sons.*
2. Hadzhieva et al. (2014) *Neuropathol Appl Neurobiol.*
3. Carrí et al. (2003) *Brain Res .*
4. Garzillo et al. (2014) *J Occup Environ Med.*
5. Roos et al. (2013) *Biol Trace Elem Res.*
6. Bocca et al. (2015) *J Neurol Sci.*
7. Peters et al. (2016) *Neurotoxicology.*
8. Kruger et al. (2013) *Proteomics - Clinical Applications*
9. Diana Caballero-Hernandez et al (2016) *Trends in Molecular Medicine*
10. Nardo et al. (2011) *PLoS One.*
11. Brettschneider et al. (2011) *Neuroscience Letters*
12. Liu et al (2013) *PLoS ONE*
13. Mendonça et al. (2012) *Neurological research*
14. Buscema et al. (2012) *Neurol Res Int.*
15. ISTISAN et al. (2010) Report 10/22: Accessed May 2016.
16. Marescotti P et al. (2010) *Environmental Earth Sciences*.
17. De Benedetti et al. (2016) *Peptidomics*
18. Fergani et al. (2007) *Journal of lipid research*