DISSECTING SPORADIC ALS IN A GEOGRAPHICAL CLUSTER OF PATIENTS: A MULTIDISCIPLINARY STUDY

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Introduction

Neurodegenerative disorders such as Amyotrophic Lateral Sclerosis (ALS) have been linked to iron and metals through the years[1]. Several studies analyzed different groups of patients with non similar environmental exposure by investigating metals in different tissues, but producing contrasting results[2-4]. At present, few studies on gel-based proteomics in ALS are reported, performed on different tissues[5-7], but none on serum. This poster reports the results of a multidisciplinary 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.

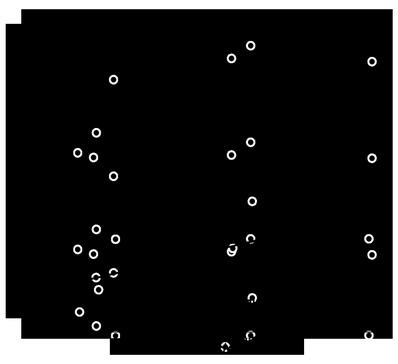
Materials and Methods

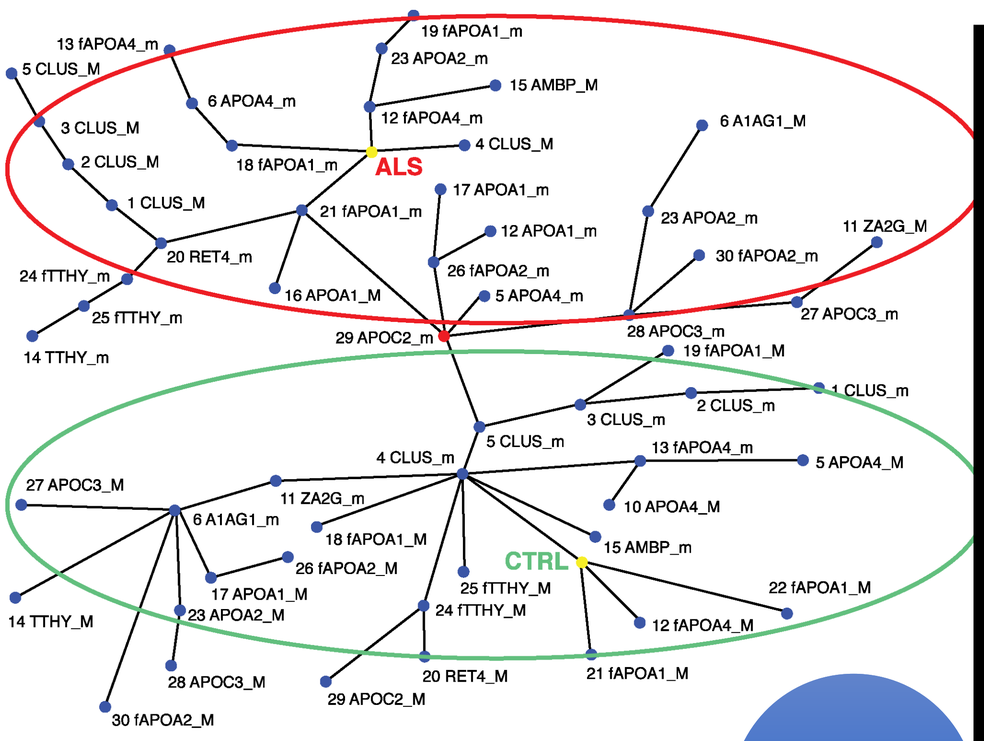
Blood was collected from all subjects. ALS diagnosis was in accordance with El Escorial criteria for clinically defined sporadic cases. All patients were genotyped for the main ALS genes (SOD1, FUS, TARDBP, C9ORF72). Samples of serum were analyzed by ICP-MS for metal quantification and results have been evaluated both through classical statistical methods and with the ANN-based Auto CM algorithm[8]. For proteomic analyses, immobilized pH gradient strips for the 1std were prepared. Both reducing conditions (1% 2-mercaptoethanol), and non reducing conditions were evaluated. The 2nd dimension was run on a porosity gradient polyacrylamide gel. Selected spots were identified by Mass Spectrometry (MS). Comet assay was performed on 5µL of frozen whole blood.

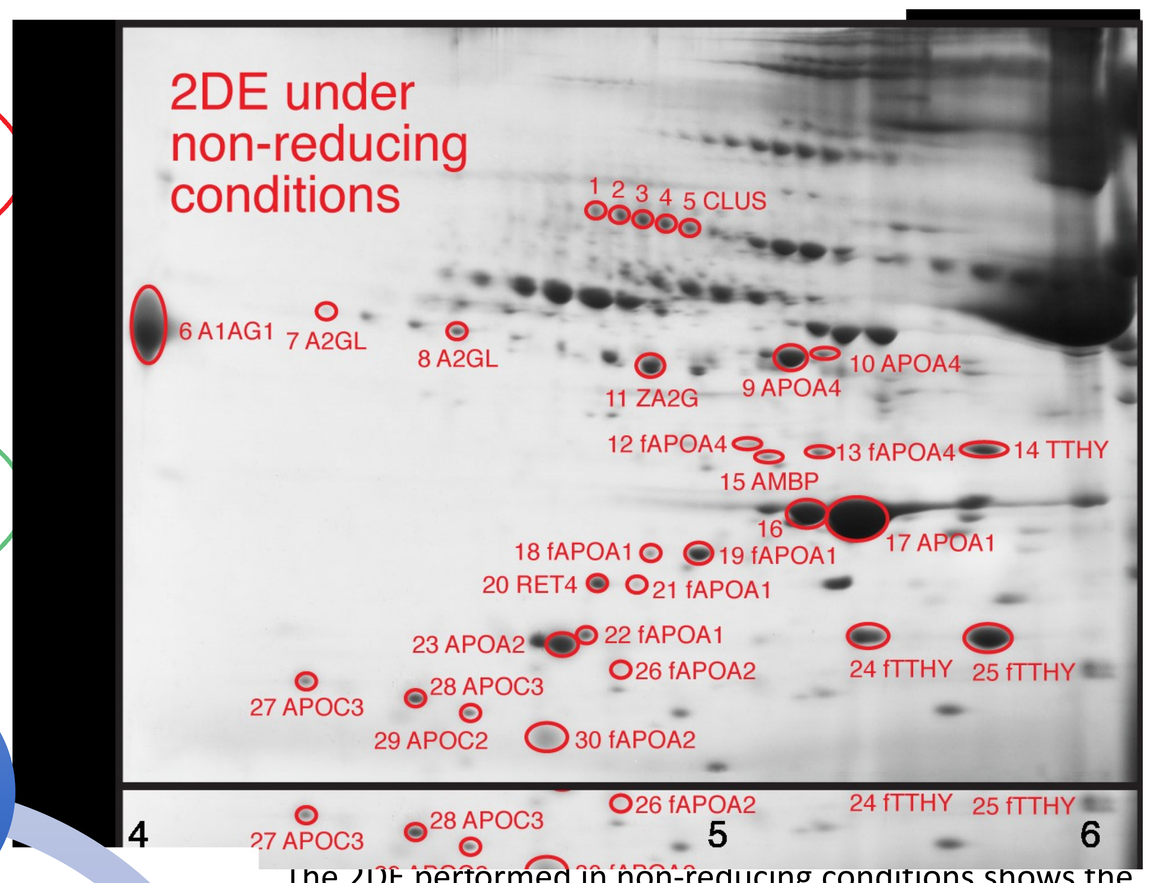
Results

Proteomics

DECREASED ABUNDANCE IN PATIENTS	INCREASED ABUNDANCE IN PATIENTS
APOA1	ANT3
APOA2	AMBP
APOA4	CLU
TTR	



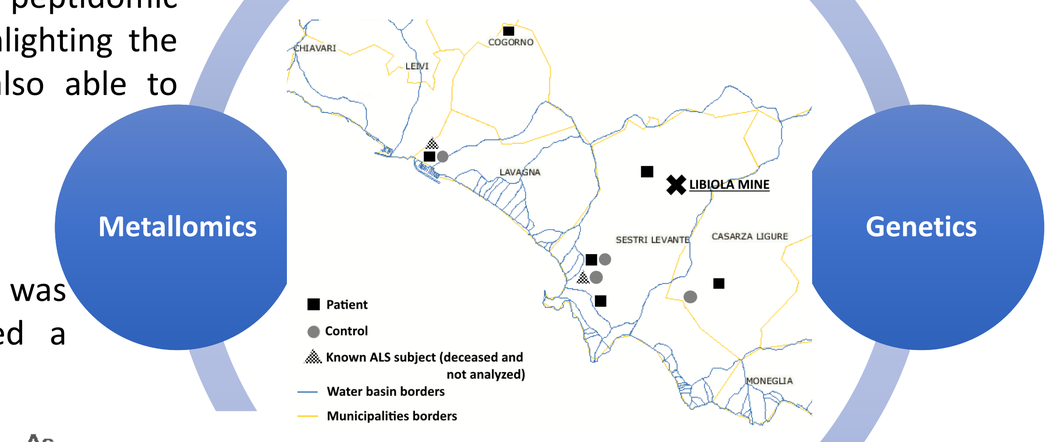




The 2DE performed in non-reducing conditions shows the localization of proteins/peptides found with differential abundance.

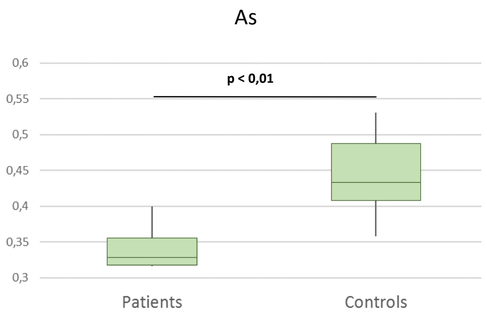
The Table reports the combined results of the 2-DEs performed in the two described conditions. A1AG1 and A2GL abundance positively correlated with disease duration.

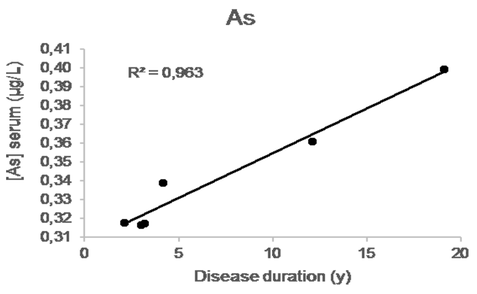
The map reported here, created with an Artificial Neural Network based software, helps in defining the peptidomic profile characteristic for ALS patients by highlighting the association between the variables; it was also able to associate fragments of the same proteins.



Metallomics

Only the serum concentration of As was significantly lower in patients, and showed a positive correlation with disease duration.





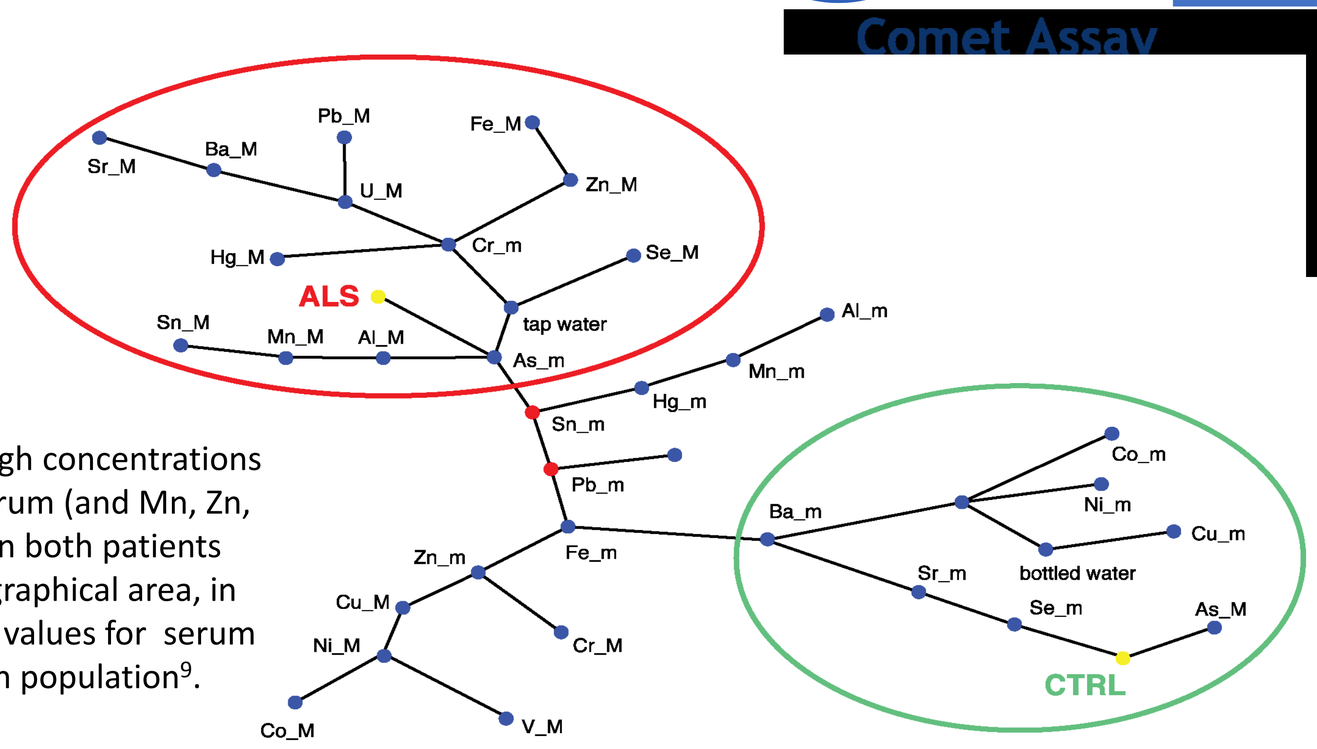
APOE Genotyping

The APOE genotype was evaluated in detail, since this protein is involved in lipid homeostasis and it 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.

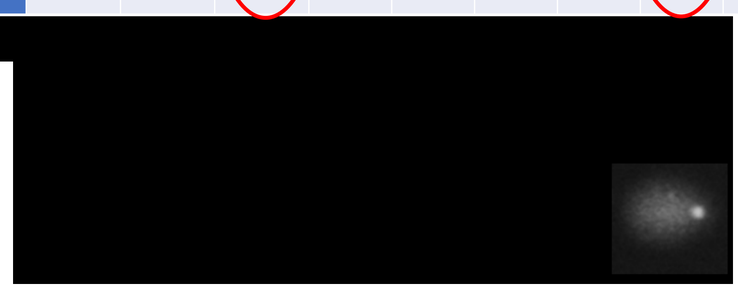
Population	Allele			Genotype					
	ε2	ε3	ε4	ε2/ε2	ε2/ε3	ε3/ε3	ε2/ε4	ε3/ε4	ε4/ε4
Caucasian	0.08	0.78	0.14	0.01	0.12	0.61	0.02	0.22	0.02
Study Controls	0.10	0.80	0.10	0.00	0.20	0.60	0.00	0.20	0.00
Study Patients	0.00	0.71	0.29	0.00	0.00	0.43	0.00	0.57	0.00

Analyses based on Artificial Neural Network software associated high levels of metals with the ALS group, except for arsenic. Also, tap water consumption turns out to be associated with the ALS group.

ICP-MS analyses showed high concentrations of Al, Ni, Cr, Ba and V in serum (and Mn, Zn, Co and Cr in whole blood) in both patients and controls form this geographical area, in comparison with reference values for serum concentrations in the Italian population[9].



Comet Assay



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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 polluted by metals, due to **Acid Mine Drainage**[10]. Cellular metabolism of **Arsenic**, found in lower levels in patients, elicits the generation of oxidative stress. Metals found in lower concentration in patients' sera could reflect their accumulation in specific (but yet unknown) body districts/tissues, where they exert toxic effects[11]. Besides, metals can compete for binding sites in some metalloproteins, such as those containing iron-sulfur clusters[12].
- 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[13]. All the proteins found with differential abundance 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[14].
- 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.

Analyzing well defined cohorts of human patients, despite being small in number, could be a reliable model for studying sporadic ALS.