

Micronutrients In A Neurodegenerative Disorder Such As Amyotrophic Lateral Sclerosis

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This PhD thesis research project is aimed at investigating the role of micronutrients in Amyotrophic Lateral Sclerosis. Their levels will be correlated with the disease status to evaluate how micronutrients and their regulatory pathways could influence the manifestation and the difference of clinical signs of disease in pre-symptomatic subjects thus explaining the wide phenotypic spectrum of disease. This will be performed through different approaches: clinical, biochemical and genetic in order to obtain a nutritional outcome. This could help to elucidate the role of micronutrients and its metabolism in the pathogenesis and/or in the progression of ALS.

Il Ruolo Dei Micronutrienti In Una Malattia Neurodegenerativa Come La Sclerosi Laterale Amiotrofica

Questo progetto di tesi di dottorato si propone di indagare il ruolo dei micronutrienti nella Sclerosi Laterale Amiotrofica. Durante questo studio si cercherà di correlarne i livelli corporei con lo stato di malattia in termini di età di esordio e prognosi e di valutare come essi potrebbero influenzare la manifestazione e l'eterogeneità di segni clinici di malattia. Questo verrà effettuato con diversi approcci: clinici, biochimici e genetici al fine di valutarne l'apporto nutrizionale. Ciò potrebbe contribuire a chiarire il ruolo dei micronutrienti e dei loro meccanismi di regolazione nella patogenesi e/o nella progressione della SLA.

1. State-of-the-Art

Amyotrophic Lateral Sclerosis (ALS) is a rare neurodegenerative disorder with an incidence of about 1/100.000 case per year. This progressive disease is characterized by a selective degeneration of both upper and lower motor neurons in the brain, brainstem and spinal cord resulting in paralysis due to muscle weakness and atrophy, leading to death in 3-5 years since the first manifestations of symptoms (Mitchell et al., 2007). In a small percentage of the cases, dementia is observed.

Genetic and environmental factors are involved in the pathogenesis of this complex disease. In familial forms, mutations in several genes have been found segregating with the pathology in dominant, recessive and X-Linked pattern, giving to ALS the characteristic of an oligogenic disease.

Neurodegenerative disorders such as ALS have been linked to iron and metals metabolism in different studies through the years (Crichton et al., 2006; Hadzhieva et al., 2013). Transition metal induced toxicity has been proposed to be involved in ALS (Carrì et al., 2003) and higher concentrations of metals and proteins that regulate metal homeostasis have been described in ALS patients (Roos et al., 2012). However, it is difficult to establish the real role of metals in ALS aetiology and progression since, when the pathology is diagnosed, all the motoneurons are already dead. In this context when we analyze micronutrients we don't know if we are measuring something that is partial cause of disease or if we are observing the endpoint of an unknown process started years before. Moreover the iron content of formulas usually employed in ALS Home Enteral Nutrition is significantly higher than the recommended daily intake for healthy individuals. Thus this PhD project will aim to disclose the role of micronutrients in ALS in a cohort of patients bearing all the same mutation in TARDBP gene. This mutation leads to heterogeneous phenotypes (typical ALS, FTD, ALS and FTD, no symptoms) even in subjects with comparable ages.

Table 1 *Main ALS causative genes*

Gene	Locus	Inheritance	Prevalence in fALS (%)	Clinical features
C9ORF72	9q21.2-p13.3	AD	35	ALS with FTD
SOD1	21q22.1	AD/AR	20	Typical ALS
FUS/TLS	16q12	AD/AR	4	Typical ALS
TARDBP	1p36	AD	5	ALS with FTD
VCP	9p13.3	AD	1-2	Adult onset, with or without FTD
UBQLN2	Xp11.21	X-linked	Rare	UMN signs preceding LMN signs

AD: Autosomal Dominant; AR: Autosomal Recessive; fALS: Familial Amyotrophic Lateral Sclerosis;
FTD: Fronto-Temporal Dementia; UMN: Upper Motor Neuron; LMN: Lower Motor Neuron

These subjects have a common geographical origin in a geographical restricted area (Acese - Catania), they share common diet, water and environment exposure.

The analysis of micronutrients' concentrations, the evaluation of proteins that are involved in their homeostasis and the genetic analysis of the genes coding for these proteins, in different subjects with an heterogeneous phenotype, could help to clarify the effective role of micronutrients in ALS. Recent studies indicate that the properties of Epigallocatechin Gallate may account for its neuroprotective capacity (Mandel et al., 2004). A previous study performed on transgenic mouse model of ALS showed that an oral treatment with EGCG should improve clinical symptoms (Zhihao et al., 2006). Dietary implementation of these natural iron chelators should be taken into account as a strategy to prevent iron overload in relatives of patients with ALS, bearing the same mutation, without clinical symptoms.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 2:

- A1) **Sample collection** of blood, urine and liquor when available, of patients belonging to the cohort in analysis comprising mutated symptomatic patients, mutated asymptomatic patients and non mutated patients. All the mutated patients share the same mutation in TARDBP gene. Samples will be collected every 6 months.
- A2) **Biochemical analysis** to evaluate micronutrients (Fe, Cu, Zn, Al, Mn) concentrations by ICP-MS in available biological fluids (A2.1) and plasmatic concentrations of Ferritin, Transferrin, Ceruloplasmin and Lactoferrin by 2D-Electrophoresis and Mass Spectrometry (A2.2). Measurements will be performed after each sample collection. Statistical analysis to assess any correlation of data obtained with the presence of the mutation in TARDBP and other vital parameters (A2.3).
- A3) **Genetic analysis** of Hemocromatosis (HFE) will be performed in order to exclude that high levels of iron are attributable to variants in this gene by FRET technology (A3.1). DNA extraction, PCR amplification and sequencing of genes coding for the previously mentioned proteins will be performed with specific primers flanking the coding exons and the regulatory regions (A3.2).
- A4) **Cellular model** will be created by transfecting transiently with either the wild type and the TARDBP mutated gene a human neuronal cellular model (SH-SY5Y neuroblastoma) (A4.1). Evaluation of expression and regulation of different intracellular proteins will be performed on this model (A4.2).
- A6) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 2 Gantt diagram for this PhD thesis project.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
A1) Samples collection		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
A2) Biochemical analysis		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
1) Micronutrients' concentrations		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
2) Plasmatic proteins concentrations		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
3) Statistical analysis		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
A3) Genetic analysis		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
1) HFE Genotyping		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
2) DNA extraction, PCR, Sequencing		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
A4) Cellular model		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
1) Transfection and culture		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
2) Intracellular proteins evaluation		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
A5) Statistical analysis		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
A6) Thesis and Paper Preparation		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

3. Selected References

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