Original Paper

Neurodegener Dis 2017;17:171–180
DOI: 10.1159/000460253

Received: October 28, 2016
Accepted after revision: February 6, 2017
Published online: May 6, 2017

Elevated Levels of Selenium Species in Cerebrospinal Fluid of Amyotrophic Lateral Sclerosis Patients with Disease-Associated Gene Mutations

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Keywords
Amyotrophic lateral sclerosis · Gene mutations · TUBA4A mutation · Selenium · Selenium species · Cerebrospinal fluid · Environment · Genetics

Abstract
Background: Although an increasing role of genetic susceptibility has been recognized, the role of environmental risk factors in amyotrophic lateral sclerosis (ALS) etiology is largely uncertain; among neurotoxic chemicals, epidemiological and biological plausibility has been provided for pesticides, the heavy metal lead, the metalloid selenium, and other persistent organic pollutants. Selenium involvement in ALS has been suggested on the basis of epidemiological studies, in vitro investigations, and veterinary studies in which selenium induced a selective toxicity against motor neurons. ***Objective:*** Hypothesizing a multistep pathogenic mechanism (genetic susceptibility and environmental exposure), we aimed to study selenium species in ALS patients carrying disease-associated gene mutations as compared to a series of hospital controls. ***Methods:*** Using advanced analytical techniques, we determined selenium species in cerebrospinal fluid sampled at diagnosis in 9 ALS patients carrying different gene mutations (C9ORF72, SOD1, FUS, TARDBP, ATXN2, and TUBA4A) compared to 42 controls. ***Results:*** In a patient with the tubulin-related TUBA4A mutation, we found highly elevated levels (in μg/L) of glutathione-peroxidase-bound selenium (32.8 vs. 1.0) as well as increased levels of selenoprotein-P-bound selenium (2.4 vs. 0.8), selenite (1.8 vs. 0.1), and selenate (0.9 vs. 0.1). In the remaining ALS patients, we detected elevated selenomethionine-bound selenium levels (0.38 vs. 0.06). ***Conclusions:*** Selenium compounds can impair tubulin synthesis and the cytoskeleton structure, as do tubulin-related gene mutations. The elevated selenium species levels in the TUBA4A patient may have a genetic etiology and/or represent a pathogenic pathway through which this mutation favors disease onset, though unmeasured confounding cannot be excluded. The elevated selenomethionine levels in the other patients are also of in-

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terest due to the toxicity of this nonphysiological selenium species. Our study is the first to assess selenium exposure in genetic ALS, suggesting an interaction between this environmental factor and genetics in triggering disease onset.

Introduction

In recent years, a large number of gene mutations have been described and have remarkably added knowledge to the pathogenic mechanisms underlying amyotrophic lateral sclerosis (ALS) [1, 2]. Mutations linked to protein misfolding (SOD1, FUS, TARDBP, VCP, and C9ORF72), impaired autophagy and degradation systems (TBK1, SQSTM1, VCP, and VAPB), oxidative processes (SOD1 and CHCHD10), transcription factor impairment (FUS and TDP43), intracellular trafficking (OPTN), and alterations affecting the cytoskeleton architecture and dynamics (DCTN1, PRPH, NEFH, PFN1, TUBA4A, and MATR3) have been suggested to be involved in ALS [1–4]. However, not all carriers of these mutations develop ALS, due to the incomplete penetrance of these mutations, even for that (C9ORF72) which has demonstrated the strongest association with disease risk [5].

Actually, although a role of genetic susceptibility in ALS etiology has been increasingly recognized, a role of environmental risk factors is also considered likely though still largely undefined with reference to specific determinants, such as exposure to neurotoxic chemicals [6–8]. In particular, epidemiological and biological plausibility has been provided for pesticides, the heavy metal lead, the metalloid selenium [9] and other persistent organic pollutants [10]. Selenium is a trace element widely recognized for the complex and heterogeneous nutritional and toxicological activities of its various chemical species and whose involvement in ALS etiology has been suggested on the basis of epidemiological studies [11–13] and in vitro investigations [14]. In veterinary medicine studies, some selenium species have been shown to be selectively toxic against motor neurons, an apparently unique feature among neurotoxic chemicals, and a few laboratory investigations have supported a specific ability of selenium to impair locomotor activity [14].

Recently, in patients with sporadic ALS we described elevated levels of inorganic tetravalent selenium (selenite) in the fluid nearest to the central nervous system, i.e., cerebrospinal fluid (CSF), thus apparently confirming the hypothesis of a causal relation between selenium exposure and the disease due to the excess disease risk observed in subjects exposed to environmental selenium [15]. However, we are not aware of studies on selenium and selenium species levels in ALS patients carrying ALS-linked gene mutations. Hypothesizing a multistep pathogenic mechanism (genetic susceptibility and environmental exposure), we used advanced analytical speciation methods to determine CSF selenium species levels in ALS patients carrying disease-associated gene mutations as compared to a series of hospital controls.

Methods

Study Subjects

ALS patients were recruited from 3 major Italian ALS referral centers (Milan, Modena, and Rome) from among all patients who were diagnosed with definite or probable ALS, according to revised El Escorial criteria [16], since 2002 and who underwent lumbar puncture (LP) at diagnosis. Among 164 CSF samples of consecutive ALS patients, we selected those carrying an ALS-related gene mutation and having at least 1 mL of CSF still stored and available for the present study.

Eligible controls were Italian residents who underwent LP because of suspected but later unconfirmed neurological disease and whose sample (≥1 mL of CSF) was still available. Among these individuals, we randomly selected 42 subjects matched to ALS patients for age (±10 years). Signs or symptoms that led to neurological examination and LP were: headache (n = 20), paresthesia/vertigo (n = 18), and isolated cranial nerve palsy (n = 4). The use of CSF specimens for this study was approved by the Modena Ethics Committee.

Sample Collection

We collected CSF via LP performed according to an established procedure in the 3 ALS centers. The neurologist located the L3-L4 interspace together with the interspaces above and below it, and wore nonsterile gloves and a mask, cap, and coat as did the assisting nurse. The widest space was marked with a thumbnail, and then sterile gloves were put on and sterile polypropylene tubes set up. The procedure then included skin swabs and antiseptic solution to clean the skin in a circular fashion and an adhesive sterile drape to create a sterile field on the patient. After application of a local anesthetic, a 20-gauge needle was used to withdraw CSF, orienting the bevel parallel to the longitudinal dural fibers to increase the chances that the needle would separate the fibers rather than cut them. Withdrawal of the stylet yielded 6–8 mL of CSF (depending on diagnostic/clinical requirements) which was collected in sterile polypropylene tubes. After CSF collection, the stylet was replaced and the needle removed and eliminated.

Sample Storage

The 6–8 mL of CSF collected from each patient were immediately stored at –80°C in sterile polypropylene tubes. To maintain blindness, all collected samples from ALS and healthy subjects were subjected to a rigorous labelling system that removed any clinical-demographic data and an alphanumeric code was assigned to each sample. A 1-mL aliquot of the anonymized samples was

Table 1. Characteristics of the ALS patients included in this study

Gene mutation	Disease type	Gender	Age at ALS onset, years	Type of onset	Disease phenotype	Cognitive impairment	Survival, months
C9ORF72	Sporadic	M	52	Bulbar	Pyramidal	No	24
C9ORF72	Familial	M	45	Spinal	Classic	No	42
C9ORF72	Sporadic	F	56	Spinal	Classic	No	66
SOD1	Sporadic	M	55	Spinal	Flail leg	No	82
SOD1	Sporadic	M	58	Spinal	Flail leg	No	24
FUS	Sporadic	F	12	Spinal	Classic	No	14
TARDBP	Sporadic	F	50	Spinal	Classic	No	24
ATXN2	Familial	F	64	Spinal	Classic	No	9
TUBA4A	Sporadic	M	57	Spinal	Flail leg	No	168

ALS, amyotrophic lateral sclerosis; M, male; F, female.

transported by air courier, deep frozen in dry ice, to the element speciation laboratory at the Helmholtz Zentrum München and kept continuously frozen until use.

Selenium Speciation Analyses

We determined the total selenium and the selenium species selenite (Se-IV), selenate (Se-VI), selenomethionine-bound selenium (Se-Met), selenocysteine-bound selenium (Se-Cys), thioredoxin reductase-bound selenium (Se-TrxR), glutathione-peroxidase-bound selenium (Se-GPx), selenoprotein-P-bound selenium (Se-PP), and albumin-bound selenium (Se-HSA) in the CSF samples using ion exchange chromatography coupled with inductively coupled plasma sector field mass spectrometry (ICP-sf-MS) in high-resolution mode in analogy to methodologies previously established for CSF [17, 18].

A Beckman 127 NM gradient HPLC system was connected to an AS-11 ion exchange column (250 × 4 mm i.d.) from Thermo Dionex (Idstein, Germany) for species separation. The sample volume was 50 µL. The mobile phases were: eluent A: 10 mM Tris-HAc and 5% MeOH, pH 8.0, and eluent B: 50 mM Na_2CO_3, 20 mM NH_4Ac, 5% MeOH, pH 8.0. The gradient elution expressed as a percent was: eluent A: 0–3 min, 100–80%; 3–23 min, 80–45%; 23–29 min, 45–0%; 29–40 min, 0%; and 40–46 min, 100%. The flow rate was 0.80 mL · min^{-1}.

The experimental settings for ICP-sf-MS (ELEMENT II; Thermo Scientific) were: radio frequency power, 1,260 W; plasma gas flow, 16 L Ar/min; auxiliary gas flow, 0.85 L Ar/min; nebulizer gas flow, 1.085 L Ar/min; daily optimized dwell time, 300 ms, and ions monitored, ^{77}Se and ^{78}Se (high-resolution mode).

The total selenium was measured with ICP dynamic reaction cell (DRC)-MS. The experimental settings for ICP-DRC-MS (Perkin Elmer NexIon) after optimization were: radio frequency power, 1,250 W; plasma gas flow, 15 L Ar/min; auxiliary gas flow, 1.05 L Ar/min; nebulizer gas flow, 0.98 L Ar/min; daily optimized dwell time, 300 ms; ions monitored, ^{77}Se, ^{80}Se, and ^{103}Rh (internal standard); DRC reaction gas, CH_4 reaction at 0.58 mL/min; and DRC rejection parameter q, 0.6. Five-point calibration curves from 0–5,000 ng/L were linear with r^2 (^{77}Se) = 0.999943 or r^2 (^{80}Se) 0.999942.

Data files from selenium chromatograms were exported from the ELEMENT software and processed with PeakFit™ software for peak area integration.

Data Analysis

We reported the mean and 25th, 50th, and 75th percentiles of analytical results after inputting for values below the limit of detection half that limit. We compared the results for all of the familial cases except for the TUBA4A case and the control subjects using a 2-tailed *t* test for independent samples, and we computed the odds ratio (as an estimate of the relative risk [RR]) of ALS using an unconditional logistic regression model adjusting for age and sex.

Results

The 9 ALS patients included 5 men and 4 women, with a mean age at disease onset of 50 years (range 12–64), who underwent LP during the diagnostic process. The 42 age-matched controls had a mean age of 46 years (range 15–68). The clinical features of ALS patients are shown in Table 1.

Overall, the total concentration of selenium and organic and inorganic selenium and those of the single selenium species were considerably higher in patients compared to controls, although this was entirely due to the exceptionally high levels of some selenium forms in 1 patient who was carrying the TUBA4A mutation (Fig. 1, 2). Therefore, after removing this patient from the case series, the subsequent comparison between the remaining patients and the controls revealed little difference in selenium species levels, with only one exception, i.e., the organic form Se-Met, the concentration of which was considerably higher among patients (Fig. 2; Table 2). In such

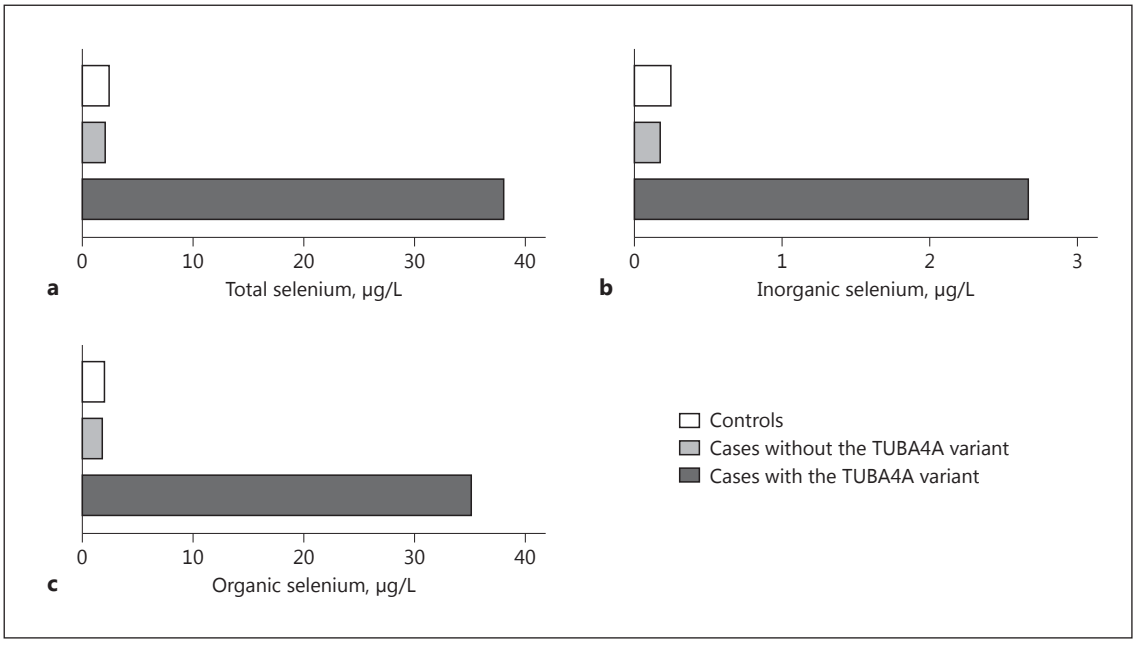

Fig. 1. Distribution of mean levels of total, inorganic, and organic selenium in the cerebrospinal fluid of the 9 patients with amyotrophic lateral sclerosis-related gene mutations (with and without the TUBA4A variant case) and the 42 controls.

a population, there was little evidence of any increased or decreased RR of disease according to levels of total selenium, inorganic and organic selenium, and single species of the element, with the exception of a considerably increased though statistically imprecise risk associated with Se-Met (Table 3).

In the ALS patient carrying a TUBA4 mutation, the overall selenium levels as well as the concentrations of most single selenium species and of the 2 subcategories of organic and inorganic selenium showed an extremely strong increase in CSF concentrations (Table 4; Fig. 1). Conversely, in this patient the levels of the 2 organic forms Se-Met and Se-TrxR did not increase, but they were even lower in this patient compared to the control group as well as compared to the remaining ALS case population (Fig. 2).

Discussion

The 2 main findings of our study are the increased Se-Met content in the CSF of most of the ALS patients, compared to a series of hospital-referred individuals as the reference group, and the dramatic and entirely unexpected increase in most selenium species in the ALS patient carrying the TUBA4A mutation. These observations appear to suggest an involvement of some selenium species in ALS etiology in subjects carrying disease-associated gene mutations, with a distinctive pattern for 1 extremely rare mutation, i.e., the TUBA4A mutation. Differently from what was observed in a population of ALS patients affected by the sporadic form of the disease, we were unable to find an excess content of inorganic selenium, and selenite in particular, in patients, with the exception of the TUBA4A case [15]. In that previous study, however, the Se-Met content was not determined.

Se-Met has been shown to be neurotoxic in veterinary medicine studies, where it was able to induce paralytic signs and lesions of symmetrical poliomyelomalacia [14], and in some though not all laboratory studies is has been shown to be one of the most toxic selenium species [19–21]. The exact mechanisms through which Se-Met exerts its powerful toxicity are still not well understood, but they may include nonspecific incorporation into proteins by entering the methionine pool and thus inducing translational changes [22]. Furthermore, Se-Met may exert its toxicity by increasing the oxidative stress [23–25], and oxidative stress has long been recognized as a major toxic effect of selenium species, in intriguing coexistence with the antioxidant activity of selenium-containing en-

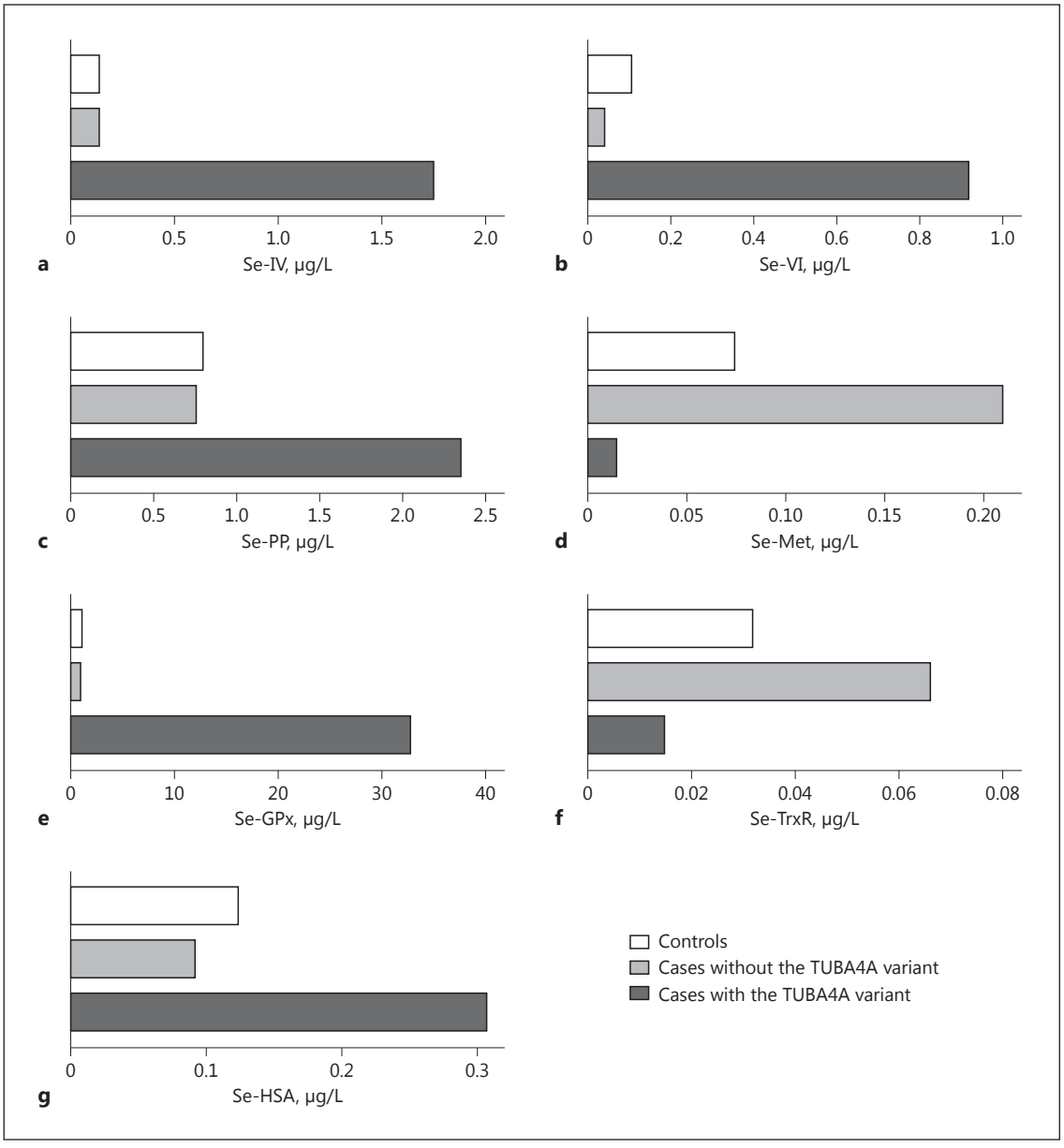

Fig. 2. Distribution of mean selenium species levels in the cerebrospinal fluid of the 9 patients with amyotrophic lateral sclerosis-related gene mutations (with an without the TUBA4A variant) and the 42 controls. Se-IV, selenite; Se-VI, selenate; Se-PP, selenoprotein P-bound selenium; Se-Met, selenomethionine-bound selenium; Se-GPx, glutathione-peroxidase-bound selenium; Se-TrxR, thioredoxin-reductase-bound selenium; Se-HSA, albumin-bound selenium.

zymes, inducible by selenium itself and several other stressors [26]. Therefore, it is possible that our finding of an excess Se-Met content in the CSF of our ALS patients represents a relevant factor in disease etiology.

We found extremely high levels of a few selenium forms, particularly Se-GPx, Se-PP, Se-IV and Se-VI, in the TUBA4A patient. The TUBA4A gene mutation is a recently described very rare mutation that has been linked to ALS and has a prevalence of around 1% in familial ALS [27]. The TUBA4A gene encodes for a member of the α-tubulin family (the tubulin α 4A protein), which is ubiquitously expressed in human tissues, with its highest expression in the brain. Cells transfected with mutant TUBA4A (and not cells transfected with wild-type

TUBA4A) display ubiquitin-positive aggregates and ALS-specific features but not TUBA4A protein aggregation [28]. TUBA4A mutations are also associated with an abnormal microtubule network and dynamics [28]. It has also been shown that, while most tubulin subunits are expressed particularly during brain development and decrease with age, TUBA4A levels increase dramatically (>50-fold) with age. However, the exact phenotype and clinical features of tubulin-related diseases are still not well defined [29].

Paradoxically mirroring the effects of tubulin-related genes mutations [30], selenium compounds themselves can induce derangements of tubulin synthesis and cytoskeleton structure. In particular, selenium species adversely affect tubulin [31, 32]. Se-IV has been shown to inhibit tubulin polymerization and microtubule synthesis by oxidizing sulfhydryl groups of tubulin and inducing the arrangement of disulfide bridges, with the final formation of "selenotubulin" [31]. Recent studies have also shown the ability of Se-IV to induce sequestration of insoluble tubulin and microtubule depolymerization in cancer cell lines [33, 34], indicating that microtubules are among the targets of the toxicity of this compound.

An effect of selenium on tubulin and therefore cytoskeleton synthesis and structure, however, is only one of the possible mechanisms linking its extremely high CSF

Table 2. Distribution of selenium species in the CSF of the 8 ALS patients carrying the C9ORF72, SOD1, FUS, TDP-43, and ATXN mutations (excluding the TUBA4A case) and the 42 controls

	n	Mean	SD	25th percentile	50th percentile	75th percentile	Mean difference (SE)	*p* value
Total selenium							0.203 (0.459)	0.661
Cases	8	2.108	1.102	1.585	1.765	2.070		
Controls	42	2.310	1.204	1.600	2.105	2.830		
Inorganic selenium							0.044 (0.102)	0.906
Cases	8	0.172	0.150	0.073	0.109	0.252		
Controls	42	0.246	0.279	0.03	0.159	0.276		
Organic selenium							0.074 (0.370)	0.473
Cases	8	1.879	0.768	1.406	1.749	1.918		
Controls	42	1.923	0.989	1.407	1.731	2.597		
Se-IV							0.007 (0.069)	0.915
Cases	8	0.133	0.144	0.058	0.084	0.151		
Controls	42	0.141	0.184	0.015	0.071	0.200		
Se-VI							0.066 (0.055)	0.231
Cases	8	0.039	0.040	0.015	0.015	0.060		
Controls	42	0.105	0.153	0.015	0.043	0.114		
Se-Met							0.136 (0.055)	0.018
Cases	8	0.210	0.266	0.015	0.103	0.377		
Controls	42	0.074	0.110	0.015	0.015	0.062		
Se-PP							0.048 (0.240)	0.843
Cases	8	0.754	0.708	0.217	0.599	1.003		
Controls	42	0.801	0.607	0.405	0.566	1.240		
Se-HSA							0.032 (0.076)	0.679
Cases	8	0.116	0.218	0.015	0.015	0.015		
Controls	42	0.124	0.193	0.015	0.015	0.180		
Se-GPx							0.167 (0.022)	0.557
Cases	8	0.849	0.424	0.748	0.852	1.003		
Controls	42	1.015	0.771	0.450	0.843	1.306		
Se-TrxR							0.034 (0.022)	0.122
Cases	8	0.066	0.119	0.015	0.023	0.040		
Controls	42	0.032	0.037	0.015	0.015	0.033		

Values are expressed as micrograms per liter unless otherwise stated. CSF, cerebrospinal fluid; ALS, amyotrophic lateral sclerosis; Se-IV, selenite; Se-VI, selenate; Se-Met, methionine-bound Se; Se-PP, selenoprotein P-bound Se; Se-HSA, albumin-bound Se; Se-GPx, glutathione-peroxidase-bound Se; Se-TrxR, thioredoxin-reductase-bound Se.

content in our TUBA4A patient with disease etiology, possibly adding to the effects induced by this gene mutation on the encoded protein. Additional mechanisms might be prooxidant and mitochondrial damage, particularly for species such as Se-IV, Se-VI, and Se-Met [26, 34–36], copper/zinc superoxide-dismutase translocation into mitochondria, and increased inducible nitric oxide synthase [37], DNA damage [38], and P38-P53 activation [39]; all mechanisms are potentially involved in ALS etiopathogenesis [3, 40], and more generally functional alterations due to nonspecific incorporation of selenium species into proteins and to adverse effects on lipid metabolism and protein synthesis [41].

Table 3. Multivariate OR of ALS, adjusted for sex and age, according to a 1-μg/L increase in CSF selenium species

	OR	95% CI	*p* value
Total selenium	0.8	0.4–1.7	0.607
Inorganic selenium	0.1	0.0–7.3	0.329
Organic selenium	1.0	0.4–2.2	0.913
Se-IV	0.6	0.0–59.3	0.807
Se-VI	0.0	0–394.1	0.214
Se-Met	175.0	1.5–19,858.1	0.032
Se-PP	0.9	0.2–3.2	0.842
Se-HSA	0.3	0.0–36.9	0.645
Se-GPx	0.7	0.2–2.3	0.556
Se-TrxR	1,653.2	0.0–∞	0.202

The analysis was limited to the 8 patients carrying ALS-linked mutations (excluding the TUBA4A case) and the 42 controls. ALS, amyotrophic lateral sclerosis; CSF, cerebrospinal fluid; Se-IV, selenite; Se-VI, selenate; Se-Met, methionine-bound Se; Se-PP, selenoprotein P-bound Se; Se-HSA, albumin-bound Se; Se-GPx, glutathione-peroxidase-bound Se; Se-TrxR, thioredoxin-reductase-bound Se.

Our data do not allow hypothesizing regarding the reasons underlying the excess selenium levels observed in the ALS patients, including that carrying the TUBA4A mutation. Such high levels may derive from a common genetic background linking these mutations to alterations of genes involved in selenium metabolism, or they may represent a pathogenetic pathway through which the mutation related to ALS favors disease onset. Alternatively, they might represent an "innocent bystander" in our patients, including the TUBA4A one, or they may really reflect an excess exposure to some selenium species in these subjects carrying ALS-associated gene mutations. From such a perspective, ALS development would be favored in these patients by the concurrent occurrence of both a high selenium exposure and genetic susceptibility. However, it appears unlikely that environmental exposure to selenium species induced differences as large as those that we detected between ALS patients and controls in CSF. In fact, the only biomarker of human exposure to single selenium species for which evidence is available, i.e., the serum levels of these compounds, appears to be either slightly or not influenced by factors such as age, body mass index, the use of dietary supplements, and smoking [42]. In addition, in the Italian population the consumption of such supplements appears to be rare [42] and the dietary selenium intake tends to be rather homogeneous [43]. We also geocoded in a geographical information system the addresses (current and historical) of the patients and controls included in the present study; this allowed us to rule out a previous exposure to drinking water with an unusually high (inorganic) selenium content distributed in the city of Reggio Emilia, near Modena [13, 44], while the selenium content in the tapwater distributed in Italy is always very low [45]. In addition, we found no mention of occupational exposure to high-selenium environments or consumption of selenium supple-

Table 4. Distribution of selenium species in the CSF of the patient carrying the TUBA4A gene mutation and the 42 controls

	Total selenium	Inorganic selenium	Organic selenium	Se-PP	Se-Met	Se-GPx	Se-TrxR	Se-IV	Se-VI	Se-HSA
TUBA4A case	38.100	2.667	35.156	2.356	0.015	32.770	0.015	1.749	0.917	0.307
Controls (n = 42)										
Mean	2.310	0.246	1.923	0.801	0.074	1.015	0.032	0.141	0.105	0.124
SD	1.204	0.279	0.989	0.607	0.110	0.771	0.037	0.184	0.153	0.193

Values are presented as micrograms per liter. CSF, cerebrospinal fluid; Se-IV, selenite; Se-VI, selenate; Se-Met, methionine-bound Se; Se-PP, selenoprotein P-bound Se; Se-HSA, albumin-bound Se; Se-GPx, glutathione-peroxidase-bound Se; Se-TrxR, thioredoxin-reductase-bound Se.

ments in the clinical records of our study subjects. Overall, there was therefore little evidence of excess exposure to this metalloid for the study subjects, and neither the environmental nor the lifestyle factors that usually affect the selenium status would have likely been able to determined differences in selenium species levels as large as those that we detected between patients and controls. Therefore, it seems plausible that the abnormally high levels of selenium compounds we found in ALS patients were in some way related to their specific genetic background and/or to the disease.

We could reasonably rule out the occurrence of selenium contamination of the CSF sample for the TUBA4A patient and more generally in all of our samples due to the quality controlled standard operative procedures we followed, but also for a few specific reasons. First, the increases we observed occurred for most but not all of the selenium species in our cases. In addition, although the plastic vials used for CSF sampling and storage had not been acid-washed during the manufacturing process as the most optimal methodology would have required [46], we found negligible (undetectable) levels of Se in blank samples of ultrapure water using the same analytical procedures adopted for the samples [47]. Moreover, the levels of other elements we analyzed in the study samples (i.e., the metals Fe, Mn, and Cu; data not shown) were fully within the normal range, without any abnormally high value compared to the reference data [18, 48]. Finally, factory-available quality control data about extractables from the vials we used (kindly provided by the manufacturer Biosigma srl, Cona, Verona, Italy) allowed us rule out any significant releases of the trace elements tested (Ti, Cr, Mn, Ni, Cu, As, Mo, Ag, Cd, Pb, and Sn), with values always below the limit of quantification.

We also consider it unlikely that such strongly increased selenium species levels were due to a compensatory response to a condition of oxidative stress, a mechanism which is well known to occur following environmental stressors including selenium itself [26] and which has been suggested to explain the excess selenoprotein P content in Alzheimer's disease brains [49]. In fact, in such a case we would have expected an increase limited to anti-oxidant selenium compounds, such as Se-GPx, Se-PP, and Se-TrxR, and not the other forms (such as Se-HSA and the inorganic ones, i.e., Se-IV and Se-VI) not belonging to the usual metabolic pathways for antioxidant selenoproteins synthesis in humans. In addition, we could not detect any increase in Se-TrxR in this patient despite the antioxidant properties of such enzymes [50].

Further investigation of the selenium species status of ALS patients or unaffected individuals carrying the TUBA4A mutation is clearly required to confirm our findings, although this may be very difficult due to the extremely rare occurrence of the mutation (around 2 cases per 10 million people/year [27, 28], with no additional case currently available within the 2 Italian networks on genetic ALS, i.e., "SLAGEN" and "ITALSGEN"), in addition to the rare availability of CSF in ALS patients. In fact, the assessment of selenium status in ALS patients should focus on the selenium content in the CSF or in other central nervous system compartments [15, 51], since they are the target tissues for the degenerative process and the CSF content of some selenium species does not correlate with their peripheral levels, including blood concentrations [15, 18].

In conclusion, in the present study, the first to investigate biomarkers of selenium status in genetic ALS, we found abnormally high levels of selenomethionine in the CSF of patients carrying various disease-associated gene mutations. We also found very high levels of organic and inorganic selenium compounds in a patient carrying the extremely rare TUBA4A mutation. Such increases in potentially neurotoxic selenium compounds might represent an innocent bystander due to a common genetic background or unmeasured confounding, or alternatively they might play an independent and relevant role in the etiopathogenesis of the disease.

Acknowledgements

The authors are grateful to the 2 Italian networks for genetic ALS, i.e., SLAGEN and ITALSGEN, and to their coordinators Prof. Vincenzo Silani and Prof. Adriano Chiò for granting data on patients' genetics.

Financial support for this study was provided by the National, Modena, and Reggio Emilia sections of the Italian Amyotrophic Lateral Sclerosis Association (AISLA), the Local Health Unit of Reggio Emilia, and the Vignola Foundation (to Dr. Vinceti), the DAAD-SPbSU Dmitrij Mendeleev-Programme (2015, grant No. 91591663), and the Russian Foundation of Basic Research (grant 16-33-60004 mol_a_dk (to Dr. Solovyev).

Disclosure Statement

The authors declare no conflicts of interest.

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