# SOD1-related cerebellar ataxia and motor neuron disease: A Cp modifier?

**Luca Marsili** ( luca.marsili@uc.edu )
University of Cincinnati
**Jennie L. Davis**
University of Washington-Valley Medical Center
**Alberto J. Espay**
University of Cincinnati
**Jonathan Gilthorpe**
Umeå University
**Chloe Williams**
Umeå University
**Marcelo A. Kauffman**
Centro Universitario de Neurología José María Ramos Mejía
**Aleksey Porollo**
Cincinnati Children's Hospital Medical Center

**Short Report**

**Keywords:** cerebellar ataxia, amyotrophic lateral sclerosis, SOD1, ceruloplasmin, neurodegeneration

**Posted Date:** November 22nd, 2022

**DOI:** https://doi.org/10.21203/rs.3.rs-2278154/v1



**Additional Declarations:** No competing interests reported.

**Version of Record:** A version of this preprint was published at The Cerebellum on February 9th, 2023. See the published version at https://doi.org/10.1007/s12311-023-01527-3.

# Abstract

We describe a novel superoxide dismutase (*SOD1*) mutation-associated clinical phenotype of cerebellar ataxia and motor neuron disease with a variant in the ceruloplasmin (*Cp*) gene which may have contributed to the phenotype. Our conclusions are supported by the genetic and protein structure analyses of the case.


# Introduction

We describe a novel superoxide dismutase (*SOD1*) mutation-associated clinical phenotype characterized by combined cerebellar ataxia and motor neuron disease, which was unrecognized until shortly before death, masked under a picture dominated by sensory neuropathy. We also highlight the potential contribution to the observed clinical phenotype, of a concomitant variant of uncertain significance in the ceruloplasmin (*Cp*) gene.

# Case Report

**Clinical presentation.** A 61-year-old Caucasian man presented to us with a 19-year history of progressive neurological decline. Onset of disease occurred at the age of 42 years with painful paresthesia of the left lower extremity. The disease progressed slowly over the first 14 years, only to accelerate over the subsequent 5 years with the development of appendicular and gait ataxia with lower limb atrophy, followed by cerebellar tremor, head titubation, dysphagia, aprosody, and a slow, scanning speech. Incoordination, impaired dexterity, and tremor impeding fine motor skills, such as using a knife and playing the oboe, were also present. Ataxia and increasing weakness forced the patient to transition from using a cane to a wheelchair at 61. During the last 12 months of the patient's life there was unintentional weight loss of fifty-five pounds and bowel and bladder incontinence. Family history was positive for thyroid (mother and brother) and cognitive (father, in his eighties) disorders. His mother had recurrent miscarriages. He also had history of levothyroxine-treated hypothyroidism and essential hypertension.

Our first neurological examination took place 19 years after symptom onset, at age 61. It revealed an ataxic syndrome with scanning speech, head titubation, right arm action and postural tremor, and bilateral leg weakness. Ocular movements were normal. Reflexes were diminished in the upper extremities, absent in the ankles but with brisk patellar responses and positive Babinski sign bilaterally. Vibration and proprioception were absent in the ankles (**Supplementary Video**).

Laboratory investigations revealed decreased motor amplitude over the left extensor digitorum brevis on electromyography (EMG)/nerve conduction studies. One year later, left thigh atrophy and pain prompted another EMG which revealed chronic denervation of the left quadriceps and iliopsoas. Extensive workup revealed elevated anti-gliadin antibody levels. Duodenal biopsy was consistent with a gluten-sensitive enteropathy. A metabolic panel showed high triglycerides (298 mg/dL), low HDL (30 mg/dL), high VLDL (60 mg/dL), and high cholesterol/HDL ratio (5.1). Serum copper (58 ug/dL; normal, 72–166 ug/dL) and

ceruloplasmin 12.5 mg/dL (normal, 16–31 mg/dL) were low. Ferritin was elevated at 923 ng/mL (normal, 30–400 ng/mL). Plasmatic iron and zinc levels were normal. Cerebrospinal fluid (CSF) analysis (age 61) showed increased proteins (52 mg/dL), normal glucose (67 mg/dL), and two nucleated cells. Cytology and IgG index were normal. The autoimmune movement disorder panel on serum was negative. Levels of ANA, CRP, and ESR on serum were also normal. HIV and syphilis tests were negative. A brain MRI at age 61 showed cerebellar atrophy and prominent T2 hyperintensities in the middle cerebellar peduncles (MCP) (Fig. 1).

There was no response to gluten-free diet or to oral and intravenous copper. A one-month trial of deferasirox partially and transiently improved his speech and hand tremors. He developed diffuse venous thrombosis and died at age 62 from distributive (vasodilatory) shock secondary to obstructive pancreatitis, after a hospitalization for deep venous thrombosis (he was bedridden). Autopsy was not pursued.

**Genetic evaluation.** Post-mortem whole-exome sequencing (WES) found a likely pathogenic heterozygous variant in the *SOD1* gene (NM_000454:exon4:c.347G > A:p.R116H) and a heterozygous variant of uncertain significance (VUS) in the *Cp* gene (NM_000096.3(CP):c.2684G > C p.Gly895Ala) according to ACMG criteria. The amino acid change in SOD1 is conservative with the arginine residue changed to histidine. The variant is not present in the databases of healthy controls, including the gnomAD database, dbSNP or Exome Variant Server or the ALSoD database of genetic variatnts connected to ALS [1]. Arginine at codon 11 is highly conserved in across kingdoms. The bioinformatics tools SIFT and PolyPhen-2 predict the p.R116H substitution to be “deleterious” and “probably damaging”, respectively.

**Protein structure analysis.** Experimentally resolved 3D structures of human *Cp* and *SOD1* proteins were retrieved from the Protein Databank – PDB IDs: 2J5W and 2C9V, respectively. Figure 2A-B demonstrates the location of the *Cp* G895 variant, which is inside the globular fold and distant from functional sites of the protein, such as metal binding sites. *Cp* G895A mutant was generated using AlphaFold2 [2]. Structure alignment of the mutant with the reference structure yielded only 0.031Å RMSD. Collectively, the structural analysis suggests that the introduction of methyl group at position G895 did not cause conformational changes.

The likely pathogenicity of the *SOD1* R116H variant was supported by virtue of its location in a known mutational hotspot with at least two alternative pathogenic variants described in the same codon [3]. Figure 2C shows that the R116 position is located on the surface of *SOD1*. Analysis of the resolved structure with SPPIDER [4] indicates that R116 is part of the protein-protein interaction interface of the *SOD1* homodimer. Subsequent in silico assessment of the change in free binding energy of the homodimer upon R116H mutation using SSIPe server [5] suggested a strongly unfavorable mutation ($\Delta\Delta G_{bind} = 4.566$). Therefore, we hypothesize that the R116H variant most likely destabilizes the protein, impeding its normal function in the dimeric form.

# Discussion

Pathogenic variants in *SOD1* are causative for familial and sporadic amyotrophic lateral sclerosis (ALS) under a dominant model of inheritance [6]. We describe a patient with symptoms of atypical ALS carrying a novel heterozygous SOD1 R116H mutation. Previous studies have identified three different missense mutations in SOD1 at position 116 (R116G, R116C, and R116S) [3] resulting ALS with a rapid disease progression. *SOD1*-ALS has been reported to include cerebellar deficits [7] and shared functional pathways are compromised in ALS and hereditary ataxias [8]. Furthermore, evidence of structural abnormalities in cerebellar structures and cerebellar peduncles (as presented by this patient) have been reported in ALS patients [9].

The effect of R116 substitution has previously been investigated. Changes were reported in the ability of R116 to form hydrogen bonds with residues E50 and C112 changing their relative positions within monomeric SOD1 and potentially leading to SOD1 misfolding and aggregation. Our structural analysis suggests that R116 plays a critical role in the stabilization of the SOD2 homodimer, and we predict that the R116H mutation hinders the formation of the SOD1 enzyme as a dimer.

Conversely, although the pathogenicity of the *Cp* variant is questionable, the *Cp*-G895A exists in the gnomAD database, dbSNP, and Exome Variant Server, and could have contributed to the phenotype. Cerebellar ataxia and hypo/aceruloplasminemia have been reported in patients with heterozygous and homozygous *Cp* variants (in the latter case, also associated with diabetes mellitus, retinal degeneration, involuntary movements, and cognitive impairment), respectively [10]. However, we are the first to report middle cerebellar peduncle (MCP) hyperintensities in hypoceruloplasminemia, associated with marked cerebellar atrophy in the absence of iron deposition in the basal ganglia, ferritin abnormalities, or muscle wasting [11].

To be enzymatically active SOD1 must be in its dimeric form, in a complex with copper and zinc ions. It is unknown whether or not Cp insufficiency might affect availability of copper for the normal folding and activity of SOD1. Previous reports in Down syndrome [12] and Wilson's disease [13] suggest that SOD1 activity is not affected by Cp levels. However, astrocyte proteomics in SOD1-ALS models have demonstrated Cp upregulation as a likely compensatory/stress response [14]. In the present case, this response could have been compromised. Our patient's laboratory abnormalities of low copper, low ceruloplasmin, and high ferritin (albeit with normal iron), along with a transient response to iron chelation, suggest that a functional hypoceruloplasminemia could have contributed to this patient's phenotype.

From a clinical standpoint, the finding of a cerebellar syndrome associated with high-intensity signal in the MCP on imaging brought two other diagnostic possibilities before a potentially pathogenic *SOD1* variant was uncovered: neuronal intranuclear inclusion disease due to *NOTCH2NLC* variants and autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS, usually with pontine hypointensity) [15]. If ataxia and neuropathy had not been associated with MCP hyperintensity, *CMT2A* (mitofusin 2 mutation, which includes an overlapping phenotype of progressive distal limb muscle weakness and atrophy, stepping gait, distal sensory loss, and mobility impairment) or gluten ataxia would have been considerations [15].

In conclusion, we describe a novel pathogenic *SOD1* variant causing an unusual late-onset cerebellar ataxia with unilateral ALS phenotype and suggest a possible modifying role for genetic hypoceruplasminemia from a variant of uncertain significance isolated in the *Cp* gene whose effect is suggested from laboratory abnormalities. Further studies in cellular and animal models of ALS should explore the enzymatic function of *SOD1*-R116H and its propensity for misfolding and aggregation. Additional studies are required to examine *in vivo* the relationship between *Cp* and *SOD1* to clarify their possible concurrent activity.

# Declarations


### Acknowledgements

We thank the patient and his family for the participation in the study.

### Funding

This study was funded by The Parkinson's Patient Diagnostic Genetic Assistance Fund at the University of Cincinnati located in Cincinnati, OH, USA.


### Conflict of Interest

The authors declare that they have no conflict of interest related to the research covered in this article.

Dr. Luca Marsili has received honoraria from the International Association of Parkinsonism and Related Disorders (IAPRD) Society for social media and web support.

Dr. Alberto J. Espay has received grant support from the NIH and the Michael J Fox Foundation; personal compensation as a consult-ant/scientific advisory board member for Abbvie, Neuroderm, Neurocrine, Amneal, Adamas, Acadia, Acorda, Kyowa Kirin, Sunovion, Lundbeck, and USWorldMeds; publishing royalties from Lippincott Williams & Wilkins, Cambridge University Press, and Springer; and honoraria from USWorldMeds, Acadia, and Sunovion. Dr. Espay is cofounder of REGAIN Therapeutics, owner of a provisional patent on compositions and methods for treatment and/or prophylaxis of proteinopathies.

Dr. Marcelo A. Kauffman is an employee of the CONICET and has received grant support from the Ministry of Science and Technology of Argentina and the Ministry of Health of Buenos Aires.

Drs. Davies, Gilthorpe, Williams, and Porollo have nothing to report.

### Ethics approval

All study procedures were performed in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments. Informed patient consent was obtained for this work. The authors confirm that they have read the Journal’s position on issues involved in ethical publication and affirm that this work is consistent with those guidelines.

### Consent to Participate

Written informed consent was obtained from the participant included in the study.

### Consent to Publish

The authors affirm that the participant provided informed consent for publication of the images and videos.

### Data and/or Code Availability

The dataset used and analyzed for this study is available from the corresponding author upon request.

### Authors' Contributions

All authors contributed to the study conception and design.

(1) Research Project: A. Conception, B. Organization, C. Execution; (2) Manuscript Preparation: A. Writing of the First Draft, B. Review and Critique.

LM, JD 1 A,B,C; 3 A,B

AJE 1 A,B; 3 A,B

JG, CW, KDS 1 B; 3B

MAK 1 A, B,C; 3B

AP 1C; 3A,B

## References


1. Al-Chalabi NL A, Radunovic A, Gowrie I, Abel O, Opie-Martin S. ALSOD. 2021.
2. Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, et al. Highly accurate protein structure prediction with AlphaFold. Nature. 2021;596:583–9.
3. Wen X, Zhu W, Xia NL, Li Q, Di L, Zhang S, et al. Missense Mutations of Codon 116 in the SOD1 Gene Cause Rapid Progressive Familial ALS and Predict Short Viability With PMA Phenotype. Front Genet. 2021;12:776831.
4. Porollo A, Meller J. Prediction-based fingerprints of protein-protein interactions. Proteins 2007;66:630–45.
5. Huang X, Zheng W, Pearce R, Zhang Y. SSIPe: accurately estimating protein-protein binding affinity change upon mutations using evolutionary profiles in combination with an optimized physical energy function. Bioinformatics. 2020;36:2429–37.

6. Mathis S, Goizet C, Soulages A, Vallat JM, Masson GL. Genetics of amyotrophic lateral sclerosis: A review. J Neurol Sci. 2019;399:217–26.
7. Yasser S, Fecto F, Siddique T, Sheikh KA, Athar P. An unusual case of familial ALS and cerebellar ataxia. Amyotroph Lateral Scler. 2010;11:568–70.
8. Scoles DR, Dansithong W, Pflieger LT, Paul S, Gandelman M, Figueroa KP, et al. ALS-associated genes in SCA2 mouse spinal cord transcriptomes. Hum Mol Genet. 2020;29:1658–72.
9. Bharti K, Khan M, Beaulieu C, Graham SJ, Briemberg H, Frayne R, et al. Involvement of the dentate nucleus in the pathophysiology of amyotrophic lateral sclerosis: A multi-center and multi-modal neuroimaging study. Neuroimage Clin. 2020;28:102385.
10. Miyajima H, Kono S, Takahashi Y, Sugimoto M, Sakamoto M, Sakai N. Cerebellar ataxia associated with heteroallelic ceruloplasmin gene mutation. Neurology. 2001;57:2205–10.
11. Mehta SH, Parekh SM, Prakash R, Morgan JC, Miyajima H, Sethi KD. Predominant ataxia, low ceruloplasmin, and absent K-F rings: hypoceruloplasminemia or Wilson's disease. Mov Disord. 2010;25:2260–1.
12. Tórsdóttir G, Kristinsson J, Hreidarsson S, Snaedal J, Jóhannesson T. Copper, ceruloplasmin and superoxide dismutase (SOD1) in patients with Down's syndrome. Pharmacol Toxicol. 2001;89:320–5.
13. Tórsdóttir G, Gudmundsson G, Kristinsson J, Snaedal J, Jóhannesson T. Ceruloplasmin and superoxide dismutase (SOD1) in heterozygotes for Wilson disease: A case control study. Neuropsychiatr Dis Treat. 2009;5:55–9.
14. ALS Cell Atlas. https://alscellatlas.org. Last accessed Nov 15 2022.
15. Manto M, Gandini J, Feil K, Strupp M. Cerebellar ataxias: an update. Curr Opin Neurol. 2020;33:150–60.

## Figures

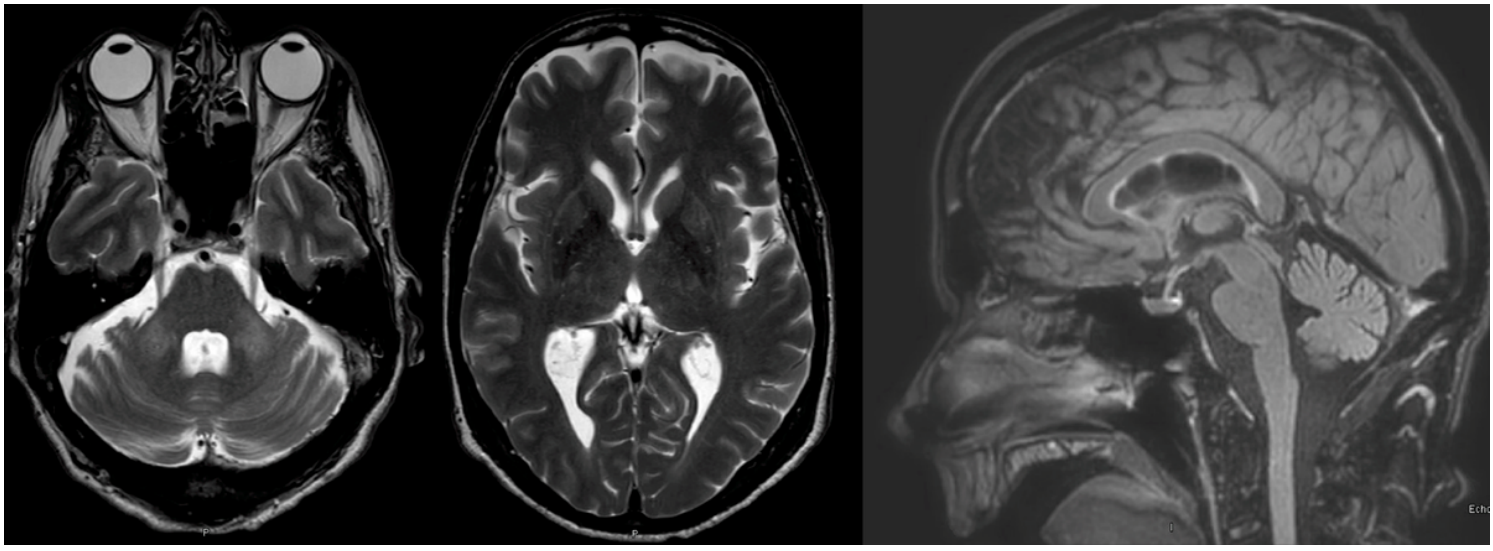

**Figure 1**

**Brain MRI nineteen years after symptom onset.** Axial T2-weighted images show bilateral hyperintensity in the middle cerebellar peduncles with hemispheric cerebellar atrophy (left) but without brain atrophy or basal ganglia iron deposition (center); midsagittal T1-weighted MRI shows normal cerebellar vermis (right).

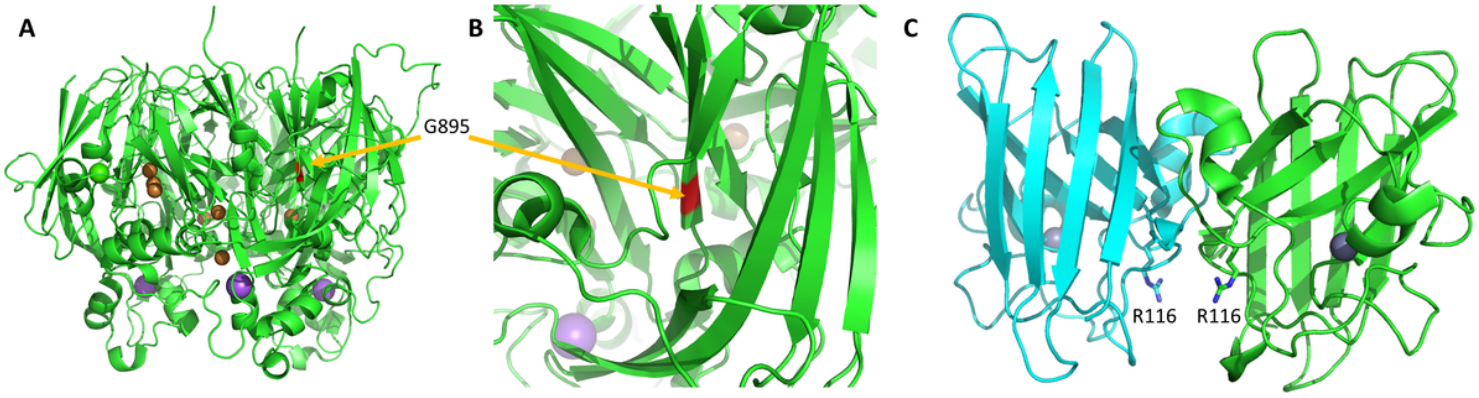


**Figure 2**

**Experimentally resolved structures of human Cp and SOD1 proteins.** The monomer structure of *Cp* (A) (Protein Databank ID: 2J5W) showing G895 without side chain in close up (B). The homodimer complex of *SOD1* (C) (Protein Databank ID: 2C9V) is shown with positions of interest rendered as sticks. Spheres in all panels represent metal ions.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- MWW62yoM1.mp4