A novel p.Leu106fs*15 *SOD1* mutation leading to the formation of a premature stop codon in an apparently sporadic ALS patient: insights into the underlying pathomechanisms

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Introduction

Some *SOD1* mutations leading to a premature stop codon have been reported. We report a novel *SOD1* p.Leu106fs*15 mutation, predicted to cause a premature protein truncation.

Methods

SOD1 enzymatic activity
The dismutation activity was analysed in erythrocytes by a direct spectrophotometric method.

PBMC isolation
Blood sample diluted (1:1) in phosphate-buffered saline (PBS) was slowly layered over Lymphoprep™ and centrifuged at 600*g* without brake at room temperature for 20 min. PBMC layer was collected, pelleted and washed twice with PBS.

Extraction of detergent-insoluble proteins
An aliquot of PBMC was resuspended in an ice-cold buffer containing 0.05 M HEPES pH 7.3, 0.15 M NaCl, 0.1 M EDTA, 0.1 mM DTT, 1% protease inhibitor cocktail, 1% Triton X-100 and centrifuged at 15000g for 15 min at 4°C. The obtained supernatant was designated the soluble fraction; the pellet was washed twice with PBS and designated the insoluble fraction. Both fractions were treated with a urea buffer (8 M deionized urea, 0.05 M Tris-HCl pH 6.8, 5% w/v sodium dodecyl sulfate, 2% v/v 2-mercaptoethanol).

Treatment of PBMC with MG132
An aliquot of PBMC was resuspended in RPMI-1640 medium, supplemented with 10% fetal bovine serum and 1X penicillin/streptomycin cocktail and incubated in a 24-wells plate (5x105 cells/well) at 37°C and 5% CO_2. Cells were exposed to 0.01 M of the proteasome inhibitor for 3 and 12 hours, washed twice with PBS and treated as described below.

WI analysis
PBMC were lysed in the above mentioned urea buffer. 50 µg of protein extract was loaded on each lane of polyacrylamide 4-15% precast gel. Membranes were blocked with 2% bovine serum albumin (BSA) in Tris-buffered saline added with 0.02% (v/v) Tween 20 (TBST buffer). Membranes were exposed overnight at 4°C to the rabbit polyclonal SOD1 antibody FL-154 raised against the full-length human SOD1 protein, to the goat polyclonal SOD1 antibody N-19 targeting only the N-terminal region of human SOD1, and to the rabbit polyclonal SOD1 antibody A303-811 raised specifically against an epitope between amino acids 54 and 104. Probed blots were then incubated at 4°C with HRP-conjugated secondary antibodies, washed in TBST buffer and incubated with enhanced chemiluminescent reagent. The immunostained bands were visualized using C-Digit® Blot Scanner. Membranes were reprobed with an antibody against β-actin for equal protein loading.

RT-PCR analysis of SOD1 mRNA and cDNA analysis
RNA was extracted from lymphocytes of the patient and two controls (a healthy person and an ALS patient with a p.D109Y *SOD1* mutation). Reverse transcription was performed. cDNA was amplified by end point PCR. We sequenced the amplified products.

Case report

The proband showed bulbar onset at 64. Family history was negative. We found a heterozygous p.Leu106fs*15 *SOD1* mutation causing a frameshift and a premature stop codon. The patient is alive, ~7 years from onset.

WI analysis
All the antibodies detected the full-length SOD1. The intensity of SOD1 band in the patient was lower than in controls (Fig.1a), ~50% of the mean intensity detected in 3 healthy controls, 3 sALS cases and 3 *SOD1*-mutated patients (Fig.1b). None of the antibodies revealed any band specific for the mutant protein encoded by the p.Leu106fs*15 allele (Fig.1a). The wtSOD1 was detected only in the soluble fraction. The treatment with MG132 did not reveal any additional band in the sample from the p.Leu106fs*15 patient.

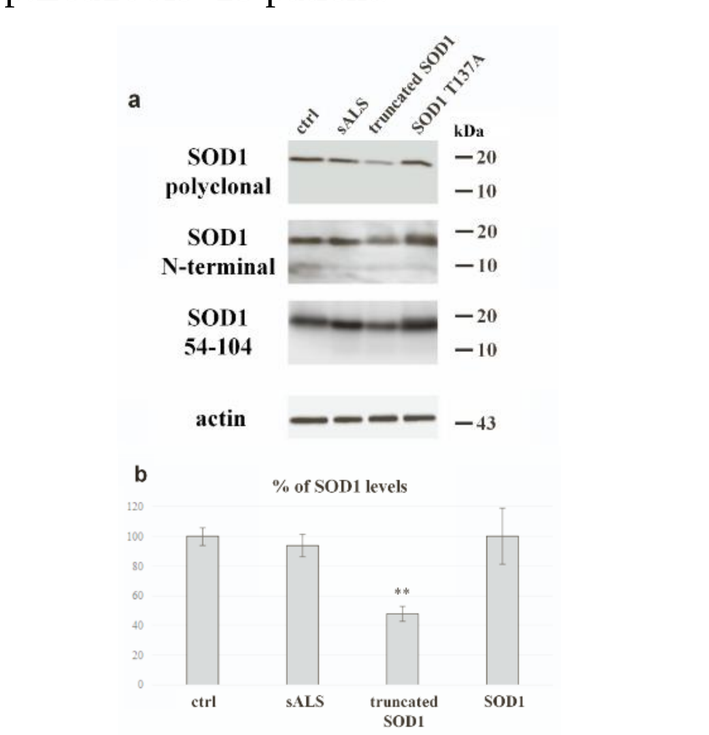

Figure 1. (a) WI of SOD1 protein in PBMC; (b) SOD1 levels, detected with the antibody against the N-terminal region of the protein.

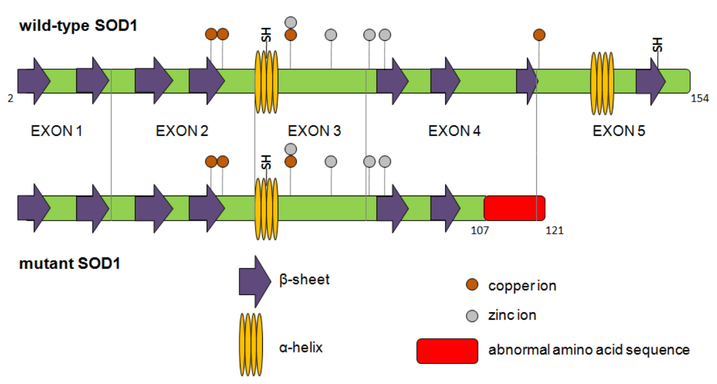

Figure 2. Comparison between wildtype SOD1 and the protein encoded by the allele carrying the frameshift mutation.

RT-PCR analysis of SOD1 mRNA
The product of end point PCR obtained from patient RNA was not qualitatively different from products obtained from a healthy person and an ALS patient with a p.D109Y *SOD1* mutation. We conclude that the allele carrying the p.Leu106fs*15 mutation is expressed at levels similar to the wild type allele.

SOD1 enzymatic activity
The dismutation activity was halved (26.46 U/mg Hb - normal 55,36±6,22).

Discussion

Halved dismutation activity in erythrocytes has been reported also in four members of a family heterozygous for the p.Gly127insTGGG mutation, that introduces a premature stop codon in position 133. Minute (<0.5% of SOD1 content in controls) amount of the mutant protein could be detected in spinal cord of an autopsied patient.[1]
The p.Leu106fs*15 mutant can result in a neopeptide sequence that is heavily positive-charged compared to wtSOD1 that will reduce the overall net negative charge of SOD1 to almost zero. The mutant protein should accordingly have less repulsive forces resulting in a greatly increased propensity to aggregate compared to wtSOD1. The p.Leu106fs*15 mutation will result in the loss of essential residues for binding the catalytic copper and the stabilizing zinc, for stabilizing the monomer and for the formation of a stable dimer interface. The predicted p.Leu106fs*15 mutant might be a highly unstable aggregation-prone protein with no superoxide dismutation capability and unable to form a heterodimer with wtSOD1. Some studies suggest that minute amounts of mutant SOD1 misfold forming cytoplasmic species with prion-like behavior.[2,3] Our data may help in elucidating which sequence of the SOD1 molecule is essential for prion-like propagation.
The mutant protein might have a conformation very different from the wtSOD1 used to immunize the animals to obtain antibodies, thus explaining its apparent absence in WI.

References
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