CLINICAL/SCIENTIFIC NOTES OPEN ACCESS

Differential subcellular expression of ^{P525L}FUS as a putative biomarker for ALS phenoconversion

Maria Caputo, MD, Vincenzo La Bella, MD, PhD, and Antonietta Notaro, PhD

Neurol Genet 2020;6:e410. doi:10.1212/NXG.0000000000000410

Correspondence
Dr. La Bella
vincenzo.labella@unipa.it

^{P525L}Fused-in-Sarcoma (*FUS*) mutation is associated with a specific amyotrophic lateral sclerosis (ALS) phenotype characterized by a juvenile-onset and a severe course.[1] This harmful point mutation is located in the nuclear localization signal (NLS) domain at the protein C-terminal.[2] Although wild-type FUS protein is expressed almost exclusively in the nucleus, the ^{P525L}*FUS* mutation leads to a protein mislocalization into the cytoplasm[3,4] because of its loss of capacity to bind its transporter karyopherin-2 and to be transferred back to the nucleus.[3]

Here, we compare FUS expression and localization in skin fibroblasts of 2 sisters, both carriers of a ^{P525L}*FUS* mutation, belonging to a Sicilian family with this mutation.[4,5]

The first sister (DC) was seen at age 21 years when she was asymptomatic. After almost 2-year follow-up, she developed a bulbar form of ALS and died 13 months after disease onset. In both conditions, i.e., asymptomatic (DC-A, at the time of the first visit) and symptomatic (DC-S, soon after disease onset), skin fibroblasts were purified and cultured. The other sister (DL) was seen when she was aged 25 years; on that occasion, fibroblasts were also purified. She is at present asymptomatic.

We studied the expression and subcellular localization of FUS protein in fibroblasts from the 2 ^{P525L}*FUS* carriers. Concerning DC, we analyzed FUS expression in the fibroblasts when she was asymptomatic and after the disease onset. A patient with sporadic clinically definite ALS with no known ALS-related gene mutations (sporadic ALS [S-ALS]) and a healthy control (HC) were used as controls.

All individuals and patients involved in this study signed informed consent for the genetic testing and the skin biopsy. The experimental protocol was approved by the Ethics Committee Palermo 1 (July 2017).

Fibroblasts were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% calf serum and antibiotic/antimycotic solution. Cells were plated on glass coverslips to perform immunofluorescence by using a polyclonal FUS antibody (11570-1-AP, Proteintech Group, Chicago, IL). The analysis of subcellular FUS expression was made through a Zeiss LSM5 confocal microscope. Cell counting for subcellular FUS expression was performed as described by Lo Bello et al.[4]

As expected, FUS mislocalized to the cytoplasm in almost all fibroblasts carrying the ^{P525L}FUS mutation.[4] Conversely, control fibroblasts (S-ALS and HC) expressed FUS only in the nucleus (figure, A).

After a more careful inspection, we observed important differences in the nucleus-cytoplasm distribution of FUS protein between the 2 FUS mutants (DC and DL). By visual

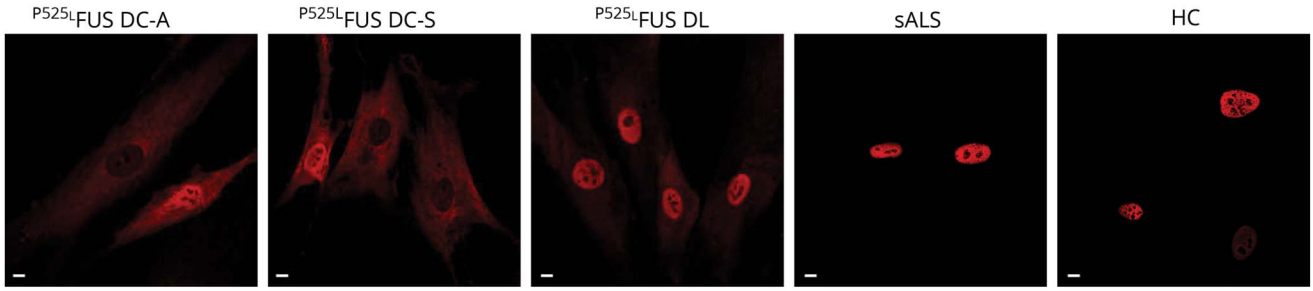
From the ALS Clinical Research Center and Laboratory of Neurochemistry, Department of Biomedicine Neuroscience and Advanced Diagnostics (Bi.N.D.), University of Palermo, Italy.

Go to Neurology.org/NG for full disclosures. Funding information is provided at the end of the article.

The Article Processing Charge was funded by the authors.

Figure Expression and differential subcellular distribution of P525LFUS in fibroblasts of asymptomatic and symptomatic mutation carriers

A



B

FUS staining	Percent of total number of cells				
	DC-A	DC-S	DL	S-ALS	HC
Nucleus only	0	0	0	94 ± 0.56	92 ± 1.41
Nucleus + cytoplasm	64 ± 0.8	30 ± 1.7	99 ± 0.35	6 ± 0.96	8 ± 1.61
Cytoplasm only	36 ± 1.6[a]	70 ± 0.9[a]	1 ± 0.52[a]	0	0

(A) Representative confocal images of immunofluorescence experiments performed with a polyclonal anti-FUS antibody on fibroblasts from the P525LFUS mutation carrier DC, either in the asymptomatic (DC-A) or symptomatic (DC-S) stage, the asymptomatic P525LFUS mutation carrier sister (DL), a patient with sporadic ALS (S-ALS), and HC. Note that while in the P525LFUS mutation carriers, the protein is mislocalized to the cytoplasm; in sALS and HC, FUS remains almost exclusively nuclear. Bar, 10 μm. (B) Percent of FUS expression in the nucleus, in the cytoplasm, or in both the nucleus and the cytoplasm of fibroblasts from the P525LFUS mutation carriers, sALS and HC. Data are expressed as percentage of the total counted cells (mean ± SD of 2 separate experiments performed in duplicate wells). [a]$p < 0.05$, percent of cells with FUS expression exclusively in the cytoplasm vs cells with FUS expression in both the nucleus and the cytoplasm from DC-A, DC-S, and DC-L. One-way analysis-of-variance with a post hoc Holm-Sidak analysis. FUS = fused-in-sarcoma; HC = healthy control; sALS = sporadic amyotrophic lateral sclerosis.

counting, most DL fibroblasts showed a combined nuclear and cytoplasmic FUS localization. Exclusive cytoplasmic staining was instead seen in over 35% of fibroblasts from DC in her asymptomatic stage (DC-A), which increased to 70% after the disease onset (DC-S, figure, B). Thus, a higher number of cells with an exclusively FUS cytoplasmic localization seems to be related to the phenotype conversion.

Discussion

This study confirms that in P525LFUS fibroblasts, taken from asymptomatic mutation carriers, the protein is mislocalized to the cytoplasm.[3,4] However, it also shows that after phenoconversion, in the large majority of mutant cells the protein disappear from the nucleus. This occurred to DC fibroblasts, whose number of cells expressing FUS solely in the cytoplasm doubled from some 35% in the asymptomatic phase (DC-A) to over 70% after ALS onset (DC-S). The 2 sisters were biopsied at the same time when they were in their asymptomatic phase. Two years after the first biopsy, when first symptoms of ALS appeared to DC, DL was still asymptomatic with almost all cells showing a nucleocytoplasmic FUS localization.

The presence of an appreciable number of cells from DC-A showing only cytoplasmic FUS expression is intriguing, as it might suggest a change from a stable asymptomatic phase to incoming disease onset. Therefore, we hypothesize that although asymptomatic, DC could have been in a no-return point already 2 years before clinical onset.

It would be interesting to verify whether other FUS mutations, especially those in the NLS at the C-terminal, show a similar subcellular redistribution after disease onset.

A question arises about the meaning of the reduced nuclear FUS expression in mutant P525L*FUS* cells near to and after the disease onset. This abnormal subcellular FUS redistribution might express a loss of its nuclear physiologic function; this might in turn contribute to motoneuron dysfunction and thus to the disease onset.[6]

We suggest that the lack of FUS expression in the nucleus of fibroblasts of asymptomatic P525L*FUS* mutation carriers might signal an incipient disease onset, being, therefore, a specific biomarker of phenoconversion. Our report also highlights the importance of the skin changes as representative of concurrent neuronal/glial biological modifications occurring in the disease.[7]

Author contributions

M. Caputo was involved in study concept, data analysis, and writing of the manuscript. V. La Bella was involved in study concept, data analysis, critical revision of the manuscript, and

study supervision. A. Notaro was involved in writing of the manuscript and study supervision.

Study funding

This study was in part supported by a liberal donation from the Italian Association of Amyotrophic Lateral Sclerosis (AISLA), Palermo Unit.

Disclosure

Disclosures available: Neurology.org/NG.

Publication history

Received by *Neurology: Genetics* November 7, 2019. Accepted in final form January 31, 2020.

References

1. Conte A, Lattante S, Zollino M, et al. P525L FUS mutation is consistently associated with a severe form of juvenile amyotrophic lateral sclerosis. Neuromuscul Disord 2012; 22:73–75.
2. Kwiatkowski TJ Jr, Bosco DA, Leclerc AL, et al. Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. Science 2009;323: 1205–1208.
3. Dormann D, Rodde R, Eddbauer D, et al. ALS-associated fused in sarcoma (FUS) mutations disrupt transportin-mediated nuclear import. EMBO J 2010;29:2841–2857.
4. Lo Bello M, Di Fini F, Notaro A, et al. ALS-related mutant FUS protein is mislocalized to cytoplasm and is recruited into stress granules of fibroblasts from asymptomatic FUS P525L mutation carriers. Neurodegener Dis 2017;17:292–303.
5. Chiò A, Restagno G, Brunetti M, et al. Two Italian kindreds with familial amyotrophic lateral sclerosis due to FUS mutation. Neurobiol Aging 2009;30:1272–1275.
6. Ishigaki S, Sobue G. Importance of functional loss of FUS in FTLD/ALS. Front Mol Biosci 2018;5:44.
7. Paré B, Gros-Louis F. Potential skin involvement in ALS: revisiting Charcot's observation—a review of skin abnormalities in ALS. Rev Neurosci 2017;28: 551–572.

Differential subcellular expression of ^{P525L}FUS as a putative biomarker for ALS phenoconversion

Maria Caputo, Vincenzo La Bella and Antonietta Notaro

Neurol Genet 2020;6;
DOI 10.1212/NXG.0000000000000410