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TUBA4A gene analysis in sporadic amyotrophic lateral sclerosis: identification of novel mutations

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Conflicts of interest The authors have no competing interest.

Ethical standard On behalf of all the authors, the corresponding author states that we acted in accordance with the ethical standards of the 1964 Declaration of Helsinki.

Informed consent Each patient gave informed consent prior to inclusion in the study.

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Dear Sirs,

Amyotrophic lateral sclerosis (ALS) is a late-onset disease caused by motor neuron degeneration with no effective therapies [1]. Approximately, 5–10 % of cases are familial (FALS), whereas the majority of patients are sporadic (SALS). ALS exhibits an extreme genetic heterogeneity and at least 25 genes are associated to familial forms, with *C9orf72* and *SOD1* representing the most common mutated genes [2].

Recently, by performing an exome-wide, case-control burden analysis of rare variant in FALS index cases, we identified an excess of patient variants (7/635) in *TUBA4A* gene, encoding for a member of the alpha-tubulin family [3]. Functional studies showed that *TUBA4A* mutations exert deleterious effects on microtubule network and dynamics in primary motor neurons. By extending *TUBA4A* genetic analysis to 1355 sporadic cases of different origin, we identified one additional variant (p.Gly43Val) with a mild effect on microtubule cytoskeleton in an Italian patient [3].

These data led us to further assess the involvement of *TUBA4A* gene in sporadic cases by analyzing a large cohort of 1106 SALS of Italian origin, including 43 patients with concomitant fronto-temporal dementia (ALS-FTD).

Our mutational screening revealed the presence of four novel heterozygous variants in four patients (Table 1). Three were missense mutations (p.Val7Ile; p.Thr349Ser and p.Asp438Asn) which determined amino-acid substitutions at evolutionarily conserved residues ("Online Resource"), while the fourth variant (c.226+4A>G) was a donor splice site mutation in in-tron 2. These variants were absent in 3960 in-house Italian controls as well as in 7595 individuals from public databases (1000 Genome project and NHLBI GO Exome Sequencing Project). Other synonymous variants and previously reported polymorphisms were also detected ("Online Resource").

In silico analysis predicted a possibly damaging effect of three novel missense mutations on TUBA4A protein and of the c.226+4A>G variant on exon 2 splicing (Table 1). We proved that this variant abolished the original donor splice site resulting in exon 2 skipping using a minigene splicing assay (Fig. 1).

All patients carrying *TUBA4A* mutations had a classical ALS phenotype, with upper and lower motor neuron signs (full clinical information is listed in “Online Resource”). Notably, one case also showed mild cognitive impairment, adding further evidence that *TUBA4A* mutations in ALS may be associated to the ALS-FTD continuum, as previously observed [3].

In conclusions, here we report the identification of novel *TUBA4A* variants with predicted deleterious effects on protein function in a series of SALS Italian patients. Although functional studies are needed to determine their pathological effect on microtubule network, our results further support the role of *TUBA4A* gene in ALS. Together with *PFN1* gene [4], mutations in *TUBA4A* indicate that defects in neuronal cytoskeleton architecture represent one of the pathogenic mechanisms triggering neurodegeneration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Wijesekera LC, Leigh PN (2009) Amyotrophic lateral sclerosis. Orphanet J Rare Dis 4:3 [PubMed: 19192301]
2. Marangi G, Traynor BJ (2014) Genetic causes of amyotrophic lateral sclerosis: new genetic analysis methodologies entailing new opportunities and challenges. Brain Res S0006–8993:01361–01364
3. Smith BN, Ticozzi N, Fallini C et al. (2014) Exome-wide rare variant analysis identifies TUBA4A mutations associated with familial ALS. Neuron 84:324–331 [PubMed: 25374358]
4. Wu CH, Fallini C, Ticozzi N et al. (2012) Mutations in the profilin 1 gene cause familial amyotrophic lateral sclerosis. Nature 488:499–503 [PubMed: 22801503]

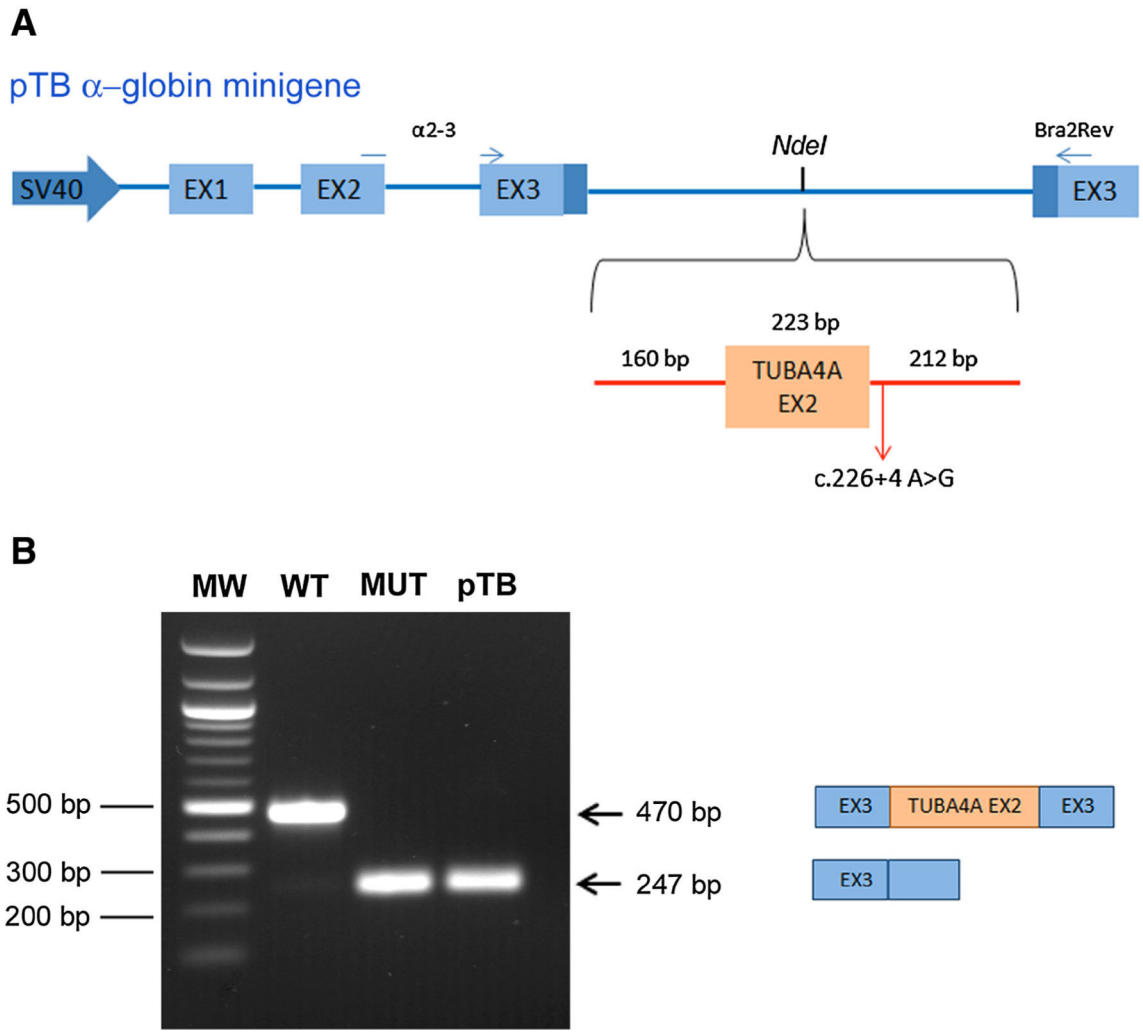

Fig. 1.

Minigene splicing assay for *TUBA4A* intronic variant c.226+4A>G. **a** Schematic representation of the pTB minigene system, in which *light blue boxes* represent a-globin exons and *thick lines* are introns. *TUBA4A* exon 2 *(orange box)* along with part of flanking introns was subcloned into the *NdeI* restriction site of the pTB vector. The primers a 2–3 and Bra2rev, used for RT-PCR analysis, are indicated as *thin blue arrows* in the pTB map. **b** HEK293 cells were transfected with wild-type (WT) or mutant (MUT) *TUBA4A* hybrid minigene, or with the empty vector (pTB), as indicated. The size of the transcripts, with *TUBA4A* exon 2 inclusion or exclusion, is indicated on the right side of the gel. The molecular weight marker (100pb DNA ladder, Life Technologies) is reported

Table 1

TUBA4A variants identified in SALS Italian patients

Sample ID	Sex	Age at onset	Disease duration	Site of onset	Cognitive impairment	Variant	SIFT prediction	Mutation taster	PolyPhen2 prediction	BDGP Wild type/mutant	ASSP wild type/mutant
B1103	M	57	14 years	Spinal	No	IVS2+4A>G c.226+4A>G	n/a	n/a	n/a	0.76/-	10.264/7.612
P937	F	n/a	n/a	n/a	n/a	p.Val7Ile c.19C>A	Tolerated	Disease causing	Benign	n/a	n/a
N5214	F	62	12 months	Bulbar	No	p.Thr349Ser c.l045A>T	Damaging	Disease causing	Possibly damaging	n/a	n/a
N6287	M	59	26 months	Bulbar	Yes	p.Asp438Asn c.1312G>A	Damaging	Disease causing	Benign	n/a	n/a

BDGP Berkeley Drosophila Genome Project: Splice Site Prediction by Neural Network, *ASSP* Alternative Splice Site Predictor, n/a not available, - the constitutive splice site is not recognized in mutant sequence