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Abstract: Amyotrophic Lateral Sclerosis (ALS) is a multifactorial disease characterized by the interplay of genetic and environmental factors. In the majority of cases, ALS is sporadic (SALS), whereas familial forms (FALS) occur in less than 10% of patients. Herein, we present the results of molecular analyses performed in a large cohort of Italian ALS patients, focusing on novel and already described variations in ALS-linked genes. Our analysis revealed that more than 10% of tested patients carried a mutation in one of the major ALS genes, with C9orf72 hexanucleotide expansion being the most common mutation. In addition, our study confirmed a significant association between ALS patients carrying the ATNX-1 intermediate repeat and the pathological C9orf72 expansion, supporting the involvement of this risk factor in neuronal degeneration. Overall, our study broadens the known mutational spectrum in ALS and provides new insights for a more accurate view of the genetic pattern of the disease.

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Rende, July 21st, 2020

Dr Peter R. Rapp,

Editor-in-Chief, Neurobiology of Aging

Baltimore, Maryland, United States

Re: Ms. No.: NBA 20-397R1

Thank you for your email dated June 20, 2020 regarding the manuscript entitled "Molecular screening in ALS patients of South Italy: a two-decade analysis", submitted by Ungaro and colleagues as a *Regular Article* to *Neurobiology of Aging*.

Enclosed please find the updated and revised manuscript. We are very grateful to the reviewers for comments and suggestions.

I hope we have fully addressed all the concerns kindly raised by the reviewers.

We wish to thank you very much for your attention.

All my best Regards,

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Genetic investigation of ALS patients in South Italy: a two-decade analysis

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Disclosure

The authors declare no competing interests.

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Genetic investigation of ALS patients in South Italy: a two-decade analysis

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Abstract

Amyotrophic Lateral Sclerosis (ALS) is a multifactorial disease characterized by the interplay of genetic and environmental factors. In the majority of cases, ALS is sporadic (SALS), whereas familial forms (FALS) occur in less than 10% of patients. Herein, we present the results of molecular analyses performed in a large cohort of Italian ALS patients, focusing on novel and already described variations in ALS-linked genes. Our analysis revealed that more than 10% of tested patients carried a mutation in one of the major ALS genes, with *C9orf72* hexanucleotide expansion being the most common mutation. In addition, our study confirmed a significant association between ALS patients carrying the *ATNX*-1 intermediate repeat and the pathological *C9orf72* expansion, supporting the involvement of this risk factor in neuronal degeneration. Overall, our study broadens the known mutational spectrum in ALS and provides new insights for a more accurate view of the genetic pattern of the disease.

Keywords: Amyotrophic Lateral Sclerosis; molecular analysis; Sanger sequencing

1. Introduction

ALS is an adult, fatal neurodegenerative disease affecting primarily both upper and lower motor neurons and leading to muscular denervation, atrophy and, ultimately, paralysis of skeletal muscles. The incidence of ALS is reported to be around five cases per 100,000 population/year in most countries (Chiò et al., 2013; McCauley et al., 2019). Approximately 5-10% of patients newly diagnosed with ALS report a positive family history and are classified as FALS, often with an autosomal dominant pattern of inheritance, while the remaining 90-95% of cases are considered as sporadic (SALS) (Rowland et al., 2001). Although useful, there is a consensus that this classification is unreliable because of incomplete penetrance in family histories, unclear relatedness, early death of close relatives, and since every established FALS gene has also been implicated in SALS (Turner et al., 2017; Gibson et al., 2017). Indeed, a SALS patient with a FALS mutation is very often a FALS patient with a non-recognized family history.

Although more than 100 genes have been associated with ALS (https://alsod.ac.uk/), only a few of them are linked to a significant percentage of ALS cases. Together, *SOD1, C9orf72, TARDBP* and *FUS* genes account for about 50% of FALS and 6% of SALS in the world (*major genes*), while frequencies of single gene mutations in other genes are very rare (≤1% of patients) (Zou et al., 2017; Müller et al., 2018; Lamp et al., 2018). Nevertheless, many genetic variants not directly causing ALS could enhance susceptibility to the disease, modifying the clinical phenotype (Chiò et al., 2012a; Millecamps et al., 2012). Among these, the independent contribution of *ATXN1* and *ATXN2* as ALS risk factors has been proposed (Elden et al., 2010; Conforti et al., 2012), and the action of these genes supports the theory by which several variants strictly drive the interaction between genes, in a way that promotes disease onset and progression (Renton et al., 2014).

Achieving a detailed and accurate molecular investigation of the various genetic aberrations associated with ALS may help broaden our vision on the role of genetics in ALS pathogenesis. With this aim, here we present the results of our own experience in the molecular genetic testing of

ALS-related genes performed in a large cohort of ALS patients referred to our Institute during the past two decades.

2. Patients and methods

2.1 Patients

Informed consent was obtained from each study subject or from a close relative if the subject was too severely disabled to give written consent. Nine hundred and ninety-seven patients of Italian descent, except for two single SALS cases, one of French origin and the second of Arabian origin, were prospectively and randomly recruited at the Institute of Neurological Sciences-CNR, Mangone (CS), and DNA samples were collected from January 1999 to December 2018. All patients underwent a full neurological evaluation to establish the clinical diagnosis of ALS according to the El Escorial criteria (Brooks et al., 2000) and the recently proposed guidelines for FALS classification (Byrne et al., 2012). A population characterized by 296 age- and sex- matched Italian individuals without neurodegenerative disease was used as control sample. The characteristics of ALS patients and controls are reported in Table 1.

2.2 Genetic analysis

Mutational analysis of *C9orf72* (MIM: 614260), *SOD1* (MIM: 147450), *TARDBP* (MIM: 605078), *FUS* (MIM: 137070), *ANG* (MIM: 105850), *VAPB* (MIM: 605704), *VCP* (MIM: 601023), and *ATXN1* (MIM: 164400) was performed according to standard procedures. Purified amplicons were directly sequenced on an ABI Prism 3130XL genetic analyzer (Applied Biosystems, Foster City, CA), using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA). Analysis of repeat expansion in *C9orf72* and *ATXN1* was performed using fluorescent-labeled primer PCR with capillary electrophoresis on an ABI Prism 3130XL genetic analyzer and analyzed with GeneMapper Software, version 4.0 (Applied Biosystems, Foster City, CA).

2.3 Statistical analysis

Statistical differences between mutated cases and ALS patients without genetic mutations were evaluated with a 2-tailed t-test for continuous variables (such as age at symptom onset) and χ^2test for discrete variables (such as gender distribution, family history, and site of onset).

We also assessed associations of polyQ repeats in *ATXN1* gene and *C9orf72* hexanucleotide repeat expansions in different groups of ALS patients using a chi-square test. All p-values were computed using the R software (R Foundation for Statistical Computing) and adjusted using Welch's correction in a 2-tailed t-tests and Yates' continuity correction in a chi-square tests. A $p<0.05$ was considered statistically significant.

3. Results

Molecular analyses revealed that more than 10% of tested patients carried a mutation of one of the major ALS genes, with C*9orf72* hexanucleotide expansion being the most common mutation (Figure 1). Table 2 summarizes demographic, clinical and genetic data of ALS patients carrying pathogenic mutations in ALS-related genes. Thirty-seven out of the 66 patients (56.1%) with FALS carried a mutation in one of the tested ALS genes. In contrast, only 66 (7.1%) out of the 931 apparently SALS cases had a genetic mutation. Mean age at symptom onset was similar among patients carrying *SOD1* mutations, *TARDBP* mutations and *C9orf72* hexanucleotide repeat expansion. In contrast, patients with *FUS* mutations manifested symptoms at a much younger age and this difference was found to be statistically significant (Unpaired t test with Welch's correction, p=0.0022). There was a significant difference in gender, family history and site of onset between C*9orf72* positive and negative groups (Table 3), while no statistically significant difference was observed between patients with and without mutations in other ALS-linked genes. The distribution of the C*9orf72* repeat in our cohort of ALS patients is shown in Figure 2. All mutations detected, except for two in *SOD1* and *FUS* genes, were previously reported (Table 4). The new *SOD1* missense substitution D83V (according to HGVS nomenclature: c.251A>T, p. Asp84Val) was neither found in the Genome Aggregation Database-gnomAD (http:// gnomad.broadinstitute.org/)

nor in the 1000G database (http://www.1000genomes.org/) and it was absent in our control subjects. Yet, the mutation was predicted to be pathogenic by 3 different programs, Sift (https://sift.bii.a-star.edu.sg/), Polyphen2 (http://genetics.bwh.harvard.edu/pph2) and MutationTaster (http://www.mutationtaster.org/). The patient carrying this mutation was a 44-year-old man without a relevant family history, who initially presented with a slowly progressive muscle weakness of the lower extremities with upper motor neuron signs. He showed mild dyspnea but no dysphagia, nor dysphonia. He suffered from mild dysarthria, worsened until he had difficulty with walking, showing impairment of lower motor neurons, and he died two years after disease onset.

The novel *FUS* mutation Gly246DUPL (c.738_740dupl AGG), identified in exon 6 of the gene, was neither found in the above mentioned databases and *in silico* analysis predicted the mutation as "disease-causing". The patient carrying this mutation is a 35-year-old obese man, who noticed fasciculation in both shoulders and arms, together with weakness and atrophy in the left proximal arm muscles. Disease duration is to date 48 months, with a relatively slow progression. Bulbar functions are normal and he does not show dysphagia nor dysarthria.

TARDBP analysis identified the previously described G376D mutation (Conforti et al. 2011), in a large family in which all affected individuals showed a rapid progressive disease. Interestingly, the reconstruction of the genealogic tree led us to a large collection of DNAs from family members, either affected or not, and the segregation analysis revealed a dominant pattern of transmission, even though the penetrance appeared incomplete (Figure 3).

The screening of VAMP/synaptobrevin-associated membrane protein B (*VAPB*), Angiogenin (*ANG*) and Valosin Containing Protein (*VCP*) genes revealed no novel mutations but many single nucleotide polymorphisms (SNPs) and a previously described *ANG* gene mutation in one sporadic patient (Conforti et al., 2008). A list of SNPs related to all the genes analyzed in ALS patients is reported in Table 5.

Finally, a cohort of 703 ALS patients (49 FALS and 654 SALS) underwent *ATXN*-1 repeat analysis to evaluate the frequency of *ATXN-1* expansion in *C9orf72* carriers. We considered 33 as

the cut-off to discriminate between normal and intermediate repeats. Results showed that 10/51 *C9orf72* positive cases (19.6%) had at least 1 allele with a polyQ repeat length ≥33, revealing a statistically significant association between *ATXN1* and *C9orf72* repeat expansions in ALS patients (fixed-effect model odds ratio = 2.28, 95% confidence interval = 1.12-4.7, p = 0.0446). In particular, 27.8% (5/18) of FALS patients and 15.15% (5/33) of SALS patients with *C9orf72* repeat expansions showed ≥33 polyQ repeats in the *ATXN1*, with no significant differences between the 2 groups (FALS p= 0.1123, SALS p= 0.3624).

4. Discussion

This study summarizes the results of genetic analyses of ALS patients performed at the Institute of Neurological Sciences-CNR, Mangone (CS) during the past two decades. A large cohort of patients was investigated using Sanger sequencing analysis of well-established ALS-related genes: *SOD1, C9orf72, TARDBP, FUS, ANG*, *VAPB*, *VCP* and *ATXN1*.

The highest frequency of positive cases was obtained in *C9orf72 (6%),* followed by *SOD1(2%), TARDBP (1.5%)* and *FUS (1%)*. In particular, *C9orf72* repeat expansion analysis revealed the presence of the pathogenic intronic $(GGGGCC)_n$ repeat expansion in 22 out of 65 FALS patients (33.8%) and 37 out of 913 SALS patients (4%), confirming this mutation as the most frequent alteration in ALS Italian patients (Chiò et al., 2012a). Pathogenic *C9orf72* repeat expansion frequencies vary greatly by ethnicity/geographic origin. The highest frequencies are reported in northern European countries (FALS 40% and SALS 8%), with low frequencies reported in Asian countries (FALS 2.3% and SALS 0.3%) (Cruts et al., 2015). In our cohort, patients carrying the expansion were more likely to be female, with a family history of disease and a bulbar-onset, which is consistent with previous findings (Majounie et al., 2012; Umoh et al., 2016). Moreover, 11 *C9orf72*-carrier patients showed clinical FTD to primary diagnosis, confirming that expansions are commonly observed in patients with FTD/ALS (van Blitterswijk et al., 2013).

SOD1 molecular investigation revealed a mutational frequency of 10% for FALS cases and 1.4% for SALS cases. These the results are similar to the frequencies observed in population-based studies of ALS in Italy but are slighter lower than those reported in other countries, supporting a different geographic distribution for these mutations (Chiò et al., 2012a; Battistini et al., 2012; Conte et al., 2012; Lattante et al., 2012). We identified the novel heterozygous D83V missense mutation in a sporadic ALS patient. Segregation analysis in the patient's family revealed that it was present in the healthy father, suggesting the non-pathogenicity or the incomplete penetrance of this variation. Unfortunately, because the patient was unavailable for further study, it was not possible to confirm the predicted effects of the c.251A>T on the SOD1 protein by functional analysis.

Mutational frequencies observed in *TARDBP (*10.3% FALS and 0.9% SALS) and *FUS* (5% FALS and 0.78% SALS) were consistent with previous findings (Lattante et al., 2013; Chiò et al., 2012b; Sproviero et al., 2012; Polymenidou and Cleveland, 2017). We confirmed that *FUS* mutations are associated with an earlier onset of the disease in comparison with the general mean age of approximately 60 years reported for ALS (Chiò et al., 2012a). None of these patients showed signs of cognitive impairment. Approximately 5% of patients with ALS also develop frontotemporal dementia (Groen et al., 2010) and, to date, only a small number of ALS/dementia patients with FUS mutations have been described, although cognitive dysfunction has been reported to be absent or rare in *FUS*-mediated ALS (Blair et al., 2010; Lagier-Tourenne and Cleveland, 2009; Yan et al., 2010).

In addition, regarding the new variation c.738_740 dupl AGG (p. Gly246Dupl) identified in a sporadic male ALS patient, we were unable to demonstrate its segregation with the disease, and we did not perform functional assays but prediction methods suggested a pathogenic role of this variation. However, this new variant should be interpreted cautiously considering that it is located in a region of the *FUS* gene (exon 6) where many of the mutations detected represent susceptibility factors or variants with incomplete penetrance in FALS (Deng et al., 2014), in contrast with most of

the mutations located in exon 12-15 (C-term) of the gene that were shown to be pathogenic in FALS and SALS cases.

Considering that ALS is a complex and multigenic disease, it is plausible that multiple variants cooperate in influencing disease onset, severity or duration. To this regard, the investigation of *ATXN1* as a potential disease modifier in *C9orf72* expansion carriers revealed a statistically significant association between ALS patients bearing the expanded poliQ *ATNX1* and those with the pathological expansion in *C9orf72*. A similar result was reported in a recent independent study (Lattante et al., 2018). These data suggest that mutant *ATNX1* may predispose carriers of *C9orf72* expansions to ALS development, therefore influencing their phenotype.

In summary, this report gives a picture of a two-decade traditional genetic investigations of ALS patients in the south of Italy, confirming not only *C9orf72* as the most frequent genetic alteration in this population, but also supporting the role of *ATNX1* intermediate expansions in predisposing to development of ALS in *C9orf72* -related patients. However, due to the complex genetic architecture of ALS, a more accurate genomic characterization of patients needs to be ensured for the development of new-targeted strategies in clinical practice and personalized medicine.

Disclosure

The authors declare no competing interests.

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Author contributions

C.C. Investigation and Writing - Original Draft; T.S. Investigation and Writing - Original Draft; G.M. Formal analysis, Investigation and Data curation; B.P. Investigation; A.G.S. Formal analysis; I.L.S. Resources; F.T. Resources; R.S. Data Curation;V.L.B. Resources and Review & Editing; S.A. Supervision; S.C. Review & Editing; F.L.C. Conceptualization, Editing and Supervision.
All authors have read and approved the final version of this manuscript and agreed to be accountable for all aspects of the work.

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Figure legends

Figure 1. Percentage of mutations in ALS; (a) distribution of mutated gene in whole ALS cohort; (b) prevalence of mutations in the 103 ALS cases.

Figure 2. Histogram of *C9orf72* repeat sizes in ALS patients (n= 978). Total number of patients is shown as regards the repeat number on the X-axis.

Figure 3. Pedigree of the *TARDBP*-G376D family with a history of ALS showing an autosomal dominant pattern of inheritance. Square indicates male; circle female; slash deceased; black symbols patients affected by ALS.

Table 1. Main clinical characteristics of ALS patients and controls

Overall subjects (n)	**Gender (female) n (%)**	**Median age at onset[§]/inclusion[#] y (range)**	**Form (FALS) n (%)**	**Site of onset (bulbar) n (%)**	**FTD n (%)**
Patients (997)	421 (42.2%)	59.3 (17-89)	66 (6.6%)	182 (22%)	16 (1.9%)
Controls (296)	125 (42.2%)	60.5 (31-80)	–	–	–

[#] Age at onset of ALS and age at inclusion for controls.

[§] Data not available for site of onset for 174 patients.

Table 2. Demographic, clinical and genetic data of ALS patients carrying pathogenic mutations.

Patients with mutations							
Gene	**No. (%) of cases**	**Form (SALS), n (%)[≠]**	**Gender (female), n (%)**	**Median age at onset, y (range)**	**Site of onset (spinal), n (%)**	**FTD, n (%)**	**Overall patients, n**
SOD1[¥]	19 (2%)	13 (68.4%)	9 (47%)	54.8 (33–85)	18 (95%)	no	997
TARDBP	14 (1.5%)	8 (57%)	8 (57%)	59.7 (36-79)	10 (71%)	no	928
FUS‡	10 (1.0%)	7 (70%)	5 (50%)	36.5 (21-75)	8(80%)	no	953
C9orf72[§]	59 (6.0%)	37 (62.7%)	34 (57.6%)	57.7 (36-80)	31 (52.5%)	11(18.6%)	978

[§]Data not available for site of onset for 10 patients. [¥] Data not available for age of onset for 1 patient. ‡Data not available for site of onset for 1 patient. [≠] Calculated on overall patients. The case with ANG mutations is not indicated in the table.

Table 3.

Descriptive statistics of amyotrophic lateral sclerosis patients with pathogenic *C9orf72* expansion (*C9orf72* positive) and without expansion (*C9orf72* negative).

	***C9orf72* positive (n=59)**	***C9orf72* negative (n=919)**	**P value**
Gender, n (%)			
Female	34 (57.63%)	382 (41.57%)	**0.0224**
Male	25 (42.37%)	537 (58.43%)	
Family history, n (%)			
FALS	22 (37.29%)	43 (4.68%)	**<0.0001**
SALS	37 (62.71%)	876 (95.32%)	
Site of onset, n (%)			
Bulbar	18 (36.73%)	163 (21.28%)	**0.0190**
Spinal	31 (63.27%)	603 (78.72%)	
Mean age at onset (years)	57.75	59.48	0.2247

Values in bold show statistically significant differences

Table 4

Table 4. Mutations in ALS patients and clinical phenotypes

Patient	Aminoacid change	Variant	ExAC/ GnomAD	*Clin Var (Last reviewed)	FALS/SALS	Gender M/F	Site of onset (S/B)	Age of onset (yrs)	Disease duration (yrs)	Reference
SOD1 NM_000454.4										
1	N19S	c.59A>G	0.00008/0.0084	Uncertain significance[A] (Jun 15, 2018)	SALS	M	B	45	1.1	Anderson (2003)
2	N19S	c.59A>G	0.00008/0.00008	Uncertain significance[A] (Jun 15, 2018)	SALS	M	S	85	1.2	Anderson (2003)
3	Q22L	c.68A>T	-/-	Likely pathogenic[B] (Mar 31, 2020)	SALS	F	S	48	2	Anderson (2003)
4	G61R	c.184G>C	-/-	Not reported	SALS	F	S	56	n/a	Conforti (2011)
5	D83V	c.251A>T	-/-	Not reported	SALS	M	S	44	2	This report
6	D90A	c.272A>C	0.00112/ 0.00143	Likely pathogenic[B] (Dec 12, 2016)	SALS	F	S	55	Years	Anderson (1995)
7	D90A	c.272A>C	0.00112/ 0.00143	Likely pathogenic[B] (Dec 12, 2016)	FALS	F	S	46	n/a	Anderson (1995)
8	D90A	c.272A>C	0.00112/ 0.00143	Likely pathogenic[B] (Dec 12, 2016)	FALS	M	S	49	n/a	Anderson (1995)
9	D90A	c.272A>C	0.00112/ 0.00143	Likely pathogenic[B] (Dec 12, 2016)	FALS	F	S	55	Years	Anderson (1995)
10	D90A	c.272A>C	0.00112/ 0.00143	Likely pathogenic[B] (Dec 12, 2016)	FALS	M	S	63	n/a	Anderson (1995)
11	D90A	c.272A>C	0.00112/ 0.00143	Likely pathogenic[B] (Dec 12, 2016)	SALS	M	S	33	24	Anderson (1995)
12	D90A/hete	c.272A>C	0.00112/ 0.00143	Likely pathogenic[B] (Dec 12, 2016)	SALS	F	S	54	2	Anderson (1995)
13	D90A/hete	c.272A>C	0.00112/ 0.00143	Likely pathogenic[B] (Dec 12, 2016)	SALS	M	S	52	2.4	Anderson (1995)
14	G93D	c.281G>C	-/-	Not reported	FALS	F	S	63	1.8	Esteban (1994)
15	G93D	c.281G>C	-/-	Not reported	SALS	F	S	36	n/a	Esteban (1994)
16	L106P	c.320T>C	-/-	Not reported	SALS	M	S	77	3	Valentino (2005)
17	R115C	c.346C>T	-/-	Not reported	SALS	M	S	73	n/a	Tortelli (2013)
18	L144F	c.435G>T	-/0.00001	Pathogenic[B] (Mar 31, 2020)	SALS	F	S	n/a	n/a	Weber (2012)

Table 4. Mutations in ALS patients and clinical phenotypes

19	I149T	c.449T>C	-/0.00008	Not reported	FALS	M	S	41	2	Pramatarova (1995)
TARDBP NM_007375.3										
20	G294V	c.881G>T	-/-	Pathogenic[C] (Apr 23, 2009)	SALS	F	B	63	1.3	Corrado (2009)
21	G294V	c.881G>T	-/-	Pathogenic[C] (Apr 23, 2009)	SALS	M	B	68	1.2	Corrado (2009)
22	G294V	c.881G>T	-/-	Pathogenic[C] (Apr 23, 2009)	SALS	M	S	48	1.6	Corrado (2009)
23	G294V	c.881G>T	-/-	Pathogenic[C] (Apr 23, 2009)	FALS	M	S	61	n/a	Corrado (2009)
24	G294V	c.881G>T	-/-	Pathogenic[C] (Apr 23, 2009)	FALS	F	S	58	0.3	Corrado (2009)
25	G294V	c.881G>T	-/-	Pathogenic[C] (Apr 23, 2009)	FALS	M	B	59	3.3	Corrado (2009)
26	G294V	c.881G>T	-/-	Pathogenic[C] (Apr 23, 2009)	FALS	F	S	63	1	Corrado (2009)
27	G294V/homo	c.881G>T	-/-	Pathogenic[C] (Apr 23, 2009)	SALS	F	S	78	1.2	Corrado (2009)
28	G295R	c.883G>A	-/-	Pathogenic[C] (Mar 12, 2015)	SALS	F	S	58	19	Corrado (2009)
29	G295R	c.883G>A	-/-	Pathogenic[C] (Mar 12, 2015)	SALS	F	B	51	n/a	Corrado (2009)
30	G376D	c.1127G>A	-/-	Not reported	FALS	F	S	58	n/a	Conforti (2011)
31	S379A	c.1135T>G	-/ 0.00003	Not reported	SALS	F	S	79	0.7	Sprovieri (2019)
32	A382T	c.1144G/A	-/0.00003	Likely pathogenic[B] (Mar 31, 2020)	SALS	M	S	52	6	Kabashi (2008)
33	A382T	c.1144G/A	-/0.00003	Likely pathogenic[B] (Mar 31, 2020)	FALS	M	S	36	3	Kabashi (2008)
FUS NM_004960.3										
34	Gly174_Gly175del	c.518_523del	-/-	Not reported	SALS	M	S	65	n/a	Kwiatkowski (2009)
35	R216C	c.646C>T	0.00002/ 0.00012	Pathogenic[C] (Aug 10, 2012)	SALS	M	n/a	60	n/a	Kwiatkowski (2009)
36	Gly246DUPL	c.738_740duplAGG	-/-	Not reported	SALS	M	S	75	2.2	This report
37	R521G	c.1561C>G	-/0.00001	Pathogenic[A] (Mar 5, 2018)	FALS	M	S	31	n/a	Kwiatkowski (2009)

Table 4. Mutations in ALS patients and clinical phenotypes

38	R521C	c.1561C>T	-/ 0.000012	Pathogenic[C] (Aug 31, 2010)	SALS	F	S	35	n/a	Kwiatkowski (2009)
39	R521C	c.1561C>T	-/ 0.000012	Pathogenic[C] (Aug 31, 2010)	SALS	F	S	53	5	Kwiatkowski (2009)
40	R521C	c.1561C>T	-/ 0.000012	Pathogenic[C] (Aug 31, 2010)	FALS	F	S	26	5	Kwiatkowski (2009)
41	P525L	c.1574 C>T	-/ 0.000004	Pathogenic[B] (Mar 31, 2020)	SALS	F	S	45	3	Kwiatkowski (2009)
42	P525L	c.1574 C>T	-/ 0.000004	Pathogenic[B] (Mar 31, 2020)	SALS	M	S	26	1	Kwiatkowski (2009)
43	P525L	c.1574 C>T	-/ 0.000004	Pathogenic[B] (Mar 31, 2020)	FALS	F	B	21	1.6	Kwiatkowski (2009)
ANG NM_001145.4										
44	M-1I	c.3G>A	0.000199/0.000216	Uncertain significance[B] (Oct 1, 2018)	SALS	M	S	63	n/a	Conforti (2008)

FALS: familial amyotrophic lateral sclerosis; SALS: sporadic amyotrophic lateral sclerosis; FTD: frontotemporal dementia; Site of onset S/B: Spinal /Bulbar; n/a data not available. ExAC, Exome Aggregation Consortium; GnomAD, genomes aggregation database *Clin Var: Variant interpretation and assertion criteria according to A- Nykamp K et al. (Genet Med 2017); B: ACMG Guidelines by Richards et al., 2015; C: no assertion criteria provided.

Table 5

Table 5. SNPs detected in ALS patients and controls.

Overall patients n	dbSNP	cDNA alteration	Amminoacid change	Func.refGene	ALS patients (%)	Control patients (%)	allele frequency ExAC	allele frequency GnomAD	*ClinVar
	SOD1 NM_000454.4								
997	rs1804447	c.*2C>T	-	3'UTR	0.1	-	-	0.25	Benign
	rs112510394	-	-	5'UTR	0.1	-	-	-	-
	rs143100660	c.423T>A	A141A	exonic	0.1	-	0.00046	0.00022	Benign
	rs373888553	c.180T>C	S60S	exonic	0.1	-	0.00001	0.00006	Benign
	rs2234694	c.239+34A>C	-	intronic	5.3	-	0.03857	0.04007	Benign
	-	c.357+42del [TACA]	-	intronic	0.1	-	-	-	-
	TARDBP NM_007375.3								
928	rs61730366	c.198T>A	A66A	exonic	0.1	-	0.00640	0.00178	Benign
	FUS NM_004960.3								
953	rs80301724	c.*41G>A	-	3' UTR	1.0	-	0.00691	0.00405	Benign
	rs757651881	c.G230_G231del	-	Inframe Deletion	0.1	-	-	-	Uncertain significance
	rs13331793	c.1393+34G>T	-	Intronic	0.1	-	-	1.2	-
	-	c.669_671del GGcGGc	Gly226_Gly227del	Inframe Deletion	-	-	-	-	Benign
	-	c.335-15del [TTTT]	-	Intronic	0.1	-	-	-	-
	rs138901914	c.1566G>A	R522R	exonic	0.2	-	0.00123	0.00081	Benign
	ANG NM_001145.4								
390	rs11701	c.330T>A	G110G	exonic	28	22	-	0.12600	Benign
	rs2228653	c.363A>G	T121T	exonic	0.25	0.3	0.00488	0.01278	Benign
	rs121909541	c.208A>G	I70V	exonic	0.5	0	0.000609	0.00080	Uncertain significance
	rs17560	c.250A>G	K84E	exonic	0.25	0.3	0.0015	0.0038	Benign
	VCP NM:007126.5								
300	rs10972300	c.129+47G>A	-	intronic	18.3	13	0.1458	0.1634	Benign
	rs757728490	c.576+10C>G	-	intronic	0.3	0	-	-	Likely benign
	-	c.1194+38T>C	-	intronic	4.6	0	-	-	-
	-	c.1082-21INS[TTGTGTACTGT]	-	intronic	16.6	5	-	-	-
	rs142577424	c.1704A>G	Q568Q	exonic	1.3	0	0.0026	0.0024	Benign
	rs563516701	c.1722A>G	L574L	exonic	0.3	0	8e-06	-	-
	VAPB NM_004738.4								
154	rs2234487	c.315+35C>T	-	intronic	54	43	0.41674	0.45475	Benign
	rs2234488	c.315+138A>G	-	intronic	43	27	-	0.36118	-

Table 5. SNPs detected in ALS patients and controls.

	rs374376908	c.547C>T	L183L	exonic	0.6	0	8e-06	-	-
	rs146459055	c.390T>G	D130E	exonic	2	2.4	0.00135	0.00051	Benign

Abbreviations: SNPs, single nucleotide polimorfisms; dbSNP, database of single Nucleotide; cDNA, complementary deoxyribonucleic acid; ExAC, Exome Aggregation Consortium; GnomAD, genomes aggregation database; n, number; -, not present or zero.

*According to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/)

Figure 1
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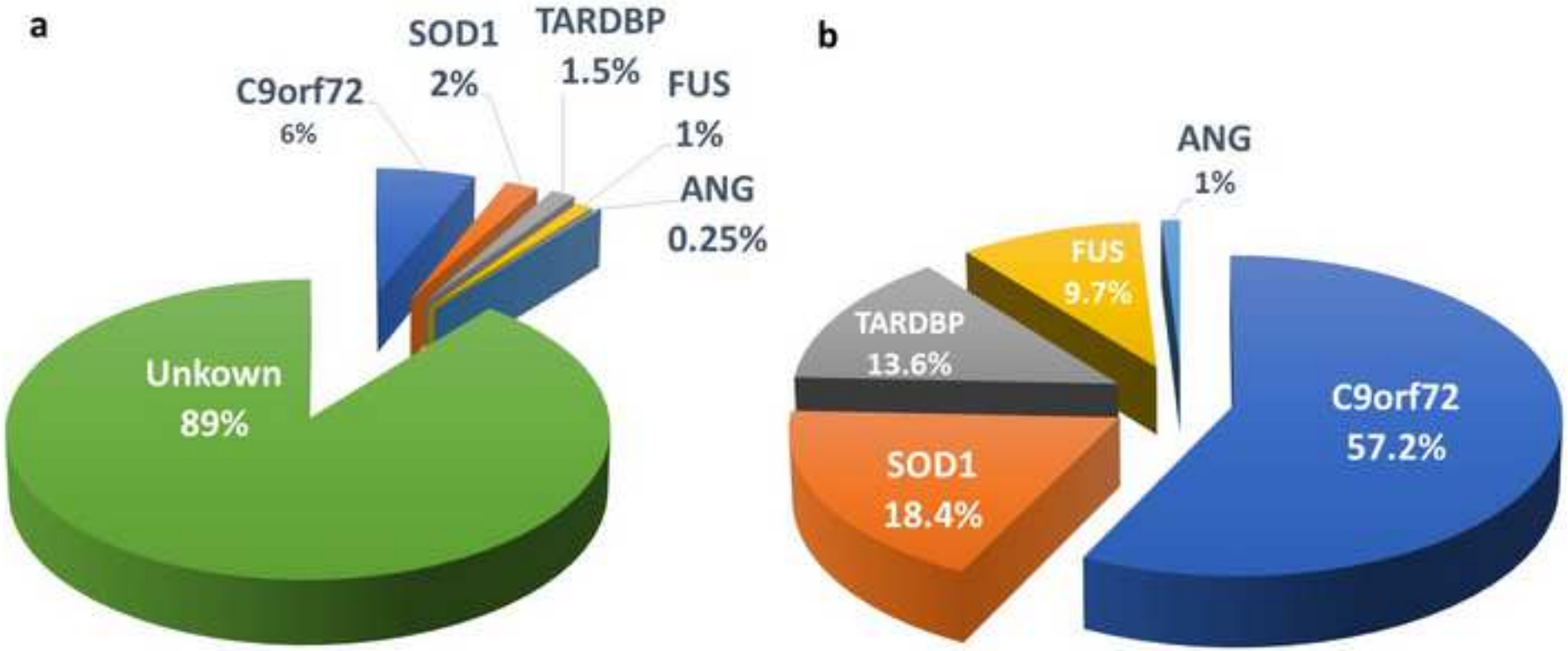

Figure 2

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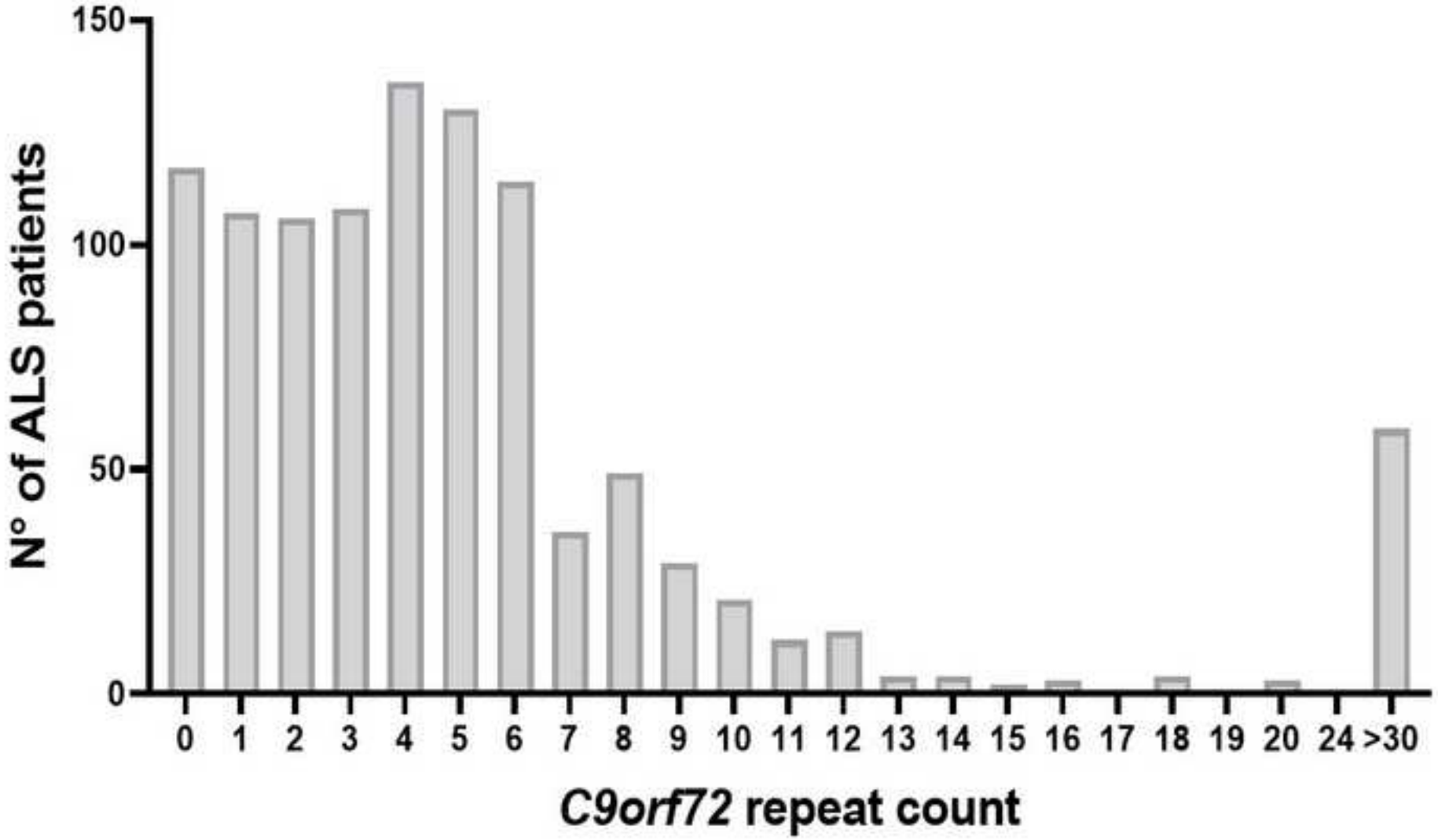

Figure 3

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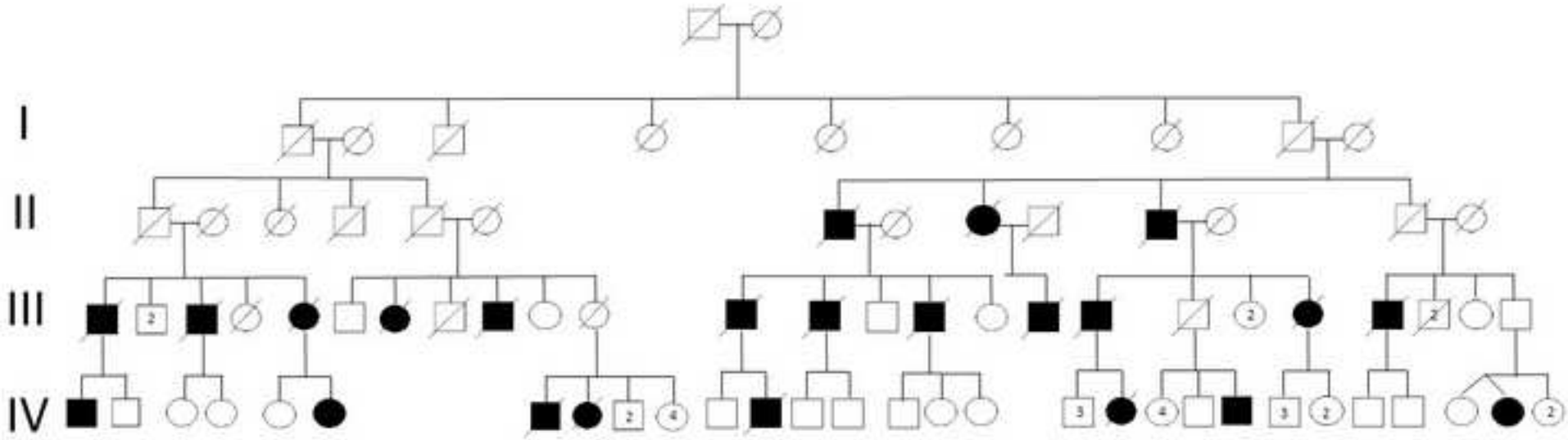

Highlights

- Genetics of ALS in southern Italy similar to most other populations;
- Novel variants of unknown pathogenicity discovered;
- Confirmation of a significant *C9orf72/ATXN1* association;
- Involvement of *C9orf72* expansion as risk factor in neuronal degeneration.

*Credit Author Statement

Genetic investigation of ALS patients in South Italy: a two-decade analysis

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