

FUS-P525L Juvenile Amyotrophic Lateral Sclerosis and Intellectual Disability

Evidence for Association and Oligogenic Inheritance

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Abstract

Background and Objectives

Amyotrophic lateral sclerosis (ALS) is characterized by upper and lower motor neuron degeneration, with juvenile ALS (jALS) defined as disease with age at onset (AAO) before 25 years. We aimed to identify the genetic basis of 2 unrelated patients with jALS with very rapid deterioration and early age intellectual disability (ID) and to assess association of genetic findings with both phenotypes in a large cohort of patients with ALS and controls, and in the literature.

Methods

Exome sequencing was performed in 2 unrelated probands and their parents. Trio analyses included de novo, rare homozygosity, and compound heterozygosity analyses. A TaqMan genotyping assay was used to genotype ALS cohorts. A systematic literature review was conducted and additional information from authors obtained to assess prevalence of fused in sarcoma (*FUS*)-ALS associated with ID.

Results

A de novo mutation *FUS*-P525L was identified in both patients. Additional variations were identified in other genes related to intellectual disabilities. Among 8 additional unrelated juvenile patients, one carried the same *FUS* mutation and had a similar medical history of mild ID and fulminant ALS, whereas the others did not carry any *FUS* coding mutations and had no reported learning or intellectual disabilities ($p = 0.0083$). In addition, 486 patients with ALS with AAO ≥ 25 years were negative for this mutation. An extensive literature review showed that among all patients with *FUS*-related ALS with full phenotype reports, 10.3% exhibited additional learning/intellectual disabilities.

Discussion

FUS-P525L mutation was identified in 3 among 10 patients with jALS (30%) in our clinical cohort, all with a very aggressive disease course and ID. Together with literature reports, these results support a novel association between mutations in *FUS* and early life ID. Additional variations identified in genes related to ID and brain development in our patients (*GPT2*, *DNAH10*, and *SCUBE2*) may suggest a complex oligogenic inheritance for this phenotype. We propose that this mutation should be screened in patients with ALS with very early AAO, aggressive disease course, and sporadic occurrence, especially when ALS is accompanied by ID.

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Glossary

AAO = age at onset; **ACMG** = American College of Medical Genetics and Genomics; **ALS** = amyotrophic lateral sclerosis; **ASD** = autism spectrum disorder; **CADD** = Combined Annotation-Dependent Depletion; **CH** = compound heterozygosity; **fALS** = familial ALS; **FTD** = frontotemporal dementia; **FUS** = fused in sarcoma; **gnomAD** = Genome Aggregation Database; **jALS** = juvenile ALS; **LMN** = lower motor neuron; **LoF** = loss of function; **NLS** = nuclear localization signal; **PY** = proline-tyrosine; **sALS** = sporadic ALS; **UMN** = upper motor neuron; **WES** = whole-exome sequencing.

Amyotrophic lateral sclerosis (ALS) is characterized by upper and lower motor neuron (LMN) degeneration. The disease causes progressive paralysis, and most patients die within 3–5 years of symptom onset.¹ The prevalence of ALS is 4.1–8.4 cases per 100,000, with an annual incidence of 0.6–3.8 per 100,000 person-years.² Five to ten percent of patients with ALS develop comorbid symptoms of frontotemporal dementia (FTD), and up to 50% show mild cognitive impairments or behavioral changes that do not meet the FTD diagnostic criteria in parallel to their motor symptoms.³ However, congenital and early life developmental disturbances are not regarded as part of the phenotypic spectrum of the disease.

To date, over 40 genes associated with ALS were reported.⁴ One of the ALS-causative genes is fused in sarcoma (*FUS*). Since first identified as being associated with ALS,^{5,6} *FUS* mutations have been reported to be responsible for 4%–6% of familial ALS (fALS) and 0.7%–1.8% of sporadic ALS (sALS) cases.^{3,7} Over 50 autosomal dominant *FUS* mutations have been identified, mostly missense mutations, which are found in the Arg-Gly-Gly-rich region and the nuclear localization signal (NLS) as well as in the Gln-Gly-Ser-Tyr-rich and Gly-rich regions. These variants are mostly associated with early-onset and juvenile ALS (jALS).^{8,9}

jALS is defined as a disease with age at onset (AAO) before 25 years. These patients have usually a slower progression rate and prolonged survival. However, mutations in *FUS* are associated with jALS with a fast and aggressive disease progression.^{7,10}

Here, we describe 3 cases of jALS with a very fast and aggressive progression, all carrying the *FUS*-P525L mutation. Of interest, all 3 also have a history of early developmental intellectual disability (ID), whereas 7 additional jALS without developmental disorders did not carry this or any other coding mutations in *FUS*. We found additional variants in other genes in these patients, which are predicted to be functionally important; this might suggest oligogenic inheritance of the nonmotor phenotype. We further performed an extensive literature systematic review to assess the association of *FUS* mutations in general, and p.P525L specifically, with a broader neurologic phenotype, including learning disabilities and developmental ID.

Methods

Probands, Families, and Cohorts Studied

Two unrelated patients with jALS and their parents were studied by whole-exome sequencing (WES): one originated

from mixed Ashkenazi/North-Africa Jewish families (proband 1) and one from mixed Ashkenazi/Balkan families (proband 2). Libraries were prepared using the Illumina TruSeq Rapid Exome kit and sequenced on the Illumina NextSeq500 with 150 cycles (2 × 75; Illumina Inc. San Diego, CA).

The exomes of 4 additional patients with jALS were also sequenced (one with SureSelect v4.0, Agilent, sequenced on the Illumina HiSeq2500, including the parents, and 3 with Illumina Nextera Rapid Capture Exome, sequenced on the NextSeq500, 2 of them included also their parents).

A custom TaqMan genotyping assay for the *FUS*-P525L mutation (Applied Biosystems) was used to genotype 8 additional jALS. In addition, 486 patients with ALS with unknown mutations were genotyped for the *FUS*-P525L: 56 with early onset but not juveniles (AAO 25–40 years) and 430 with AAO > 40.

All patients with ALS were recruited between March 1, 2005, and March 1, 2018, and were followed at the ALS Clinic at Tel Aviv Sourasky Medical Center, Israel. All patients had a diagnosis of clinically definite or probable ALS according to the revised El Escorial criteria.¹¹ Demographic and clinical data were collected: ancestry, family history of ALS, dementia or other neurodegenerative diseases, AAO, and affected site at disease onset. Disease duration (defined from first recalled symptom to death or tracheostomy) was recorded for all patients.

Standard Protocol Approvals, Registrations, and Patient Consents

All participants provided written informed consent before DNA collection. DNA samples were coded and tested in an anonymous manner. The Institutional and National Supreme Helsinki Committees for Genetics Studies approved the study protocol and the informed consent form.

Bioinformatics Analysis

The NextSeq500 system generated .bcl files, followed by demultiplexing of indexed reads and generation of fastq files. Alignment to the hg19 reference genome was performed using BWA version 2.1 (BaseSpace onSite, version 2.13, Illumina Inc). We filtered out variants with less than 10 reads, less than 30 quality score, and with a Combined Annotation-Dependent Depletion (CADD) phred score of less than 20^{12,13} and included only coding and donor-acceptor sites variants. We continued with rare recessive, de novo, and compound heterozygosity (CH)-analyses using SNP &

Variation Suite v8.9 (Golden Helix, Inc., Bozeman, MT). Additional filtering was performed in each analysis (eFigure 1, links.lww.com/NXG/A535). In the de novo analysis, we excluded variants with an alternate allele reads/total reads ratio of less than 25%. In the rare recessive, we included variants that had an allele frequency of less than 1% in the Genome Aggregation Database (gnomAD v2.2.1),^{14,15} whereas in the CH analysis, we included those with less than 2%. All *FUS* exons that were sequenced by WES were evaluated for complete coverage and coding mutations' carrier status using GenomeBrowse (GoldenHelix v.2.0.3).

All de novo mutations were validated by Sanger sequencing, and the 15 coding exons of *FUS* were sequenced in 3 additional patients with jALS by Sanger Sequencing on Applied-Biosystems 3130xl Sequencer (eTable 1, links.lww.com/NXG/A535). Franklin tool by Genoox¹⁶ was used to retrieve the American College of Medical Genetics and Genomics (ACMG) scores for the final variants and for publications and association conditions literature search. In silico variant pathogenicity analysis was performed using AMINODE,¹⁷ Phyre2,¹⁸ I-MUTANT,¹⁹ and VarSite.²⁰

FMR1 and C9orf72

The 3 *FUS*-P525L mutation carriers were genotyped for the CGG repeat in the *FMR1* gene²¹ and the *C9orf72* hexanucleotide repeat expansion, as previously described.²²

Literature Investigation

An extensive literature search was performed in PubMed to identify all articles that describe patients with ALS with mutations in the *FUS* gene, published before May 10, 2020 (eFigure 2, links.lww.com/NXG/A535). The search terms "(ALS) AND (*FUS*)" AND "mutation" were used and then filtered with "humans." Articles were included based on language and clinical information (for a detailed description: eMethods, links.lww.com/NXG/A535). We then included only nonsynonymous or donor-acceptor sites variants. The remaining articles were included for further review and patients' selection.

In this article, we will use the term "ID" as an overall description of any of the symptoms observed in the patients reported in the literature according to the DSM-5 definition,²³ (for details: eMethods, links.lww.com/NXG/A535). Onset of intellectual and adaptive deficits should have been during early childhood. In case of missing data, we contacted the corresponding authors of the articles that did not report patients' cognitive status to obtain additional clinical information. If the corresponding author was unavailable, we reached out to a second author. We then analyzed only the patients for whom we obtained full phenotypic evaluations (motor and intellectual abilities): we collected data on sex, AAO, disease duration (until death or tracheostomy, the earlier), the specific mutation and its annotation, cognitive and mental developmental history, and whether there is a familial history or not.

Statistical Analysis

The Fisher exact test was used (2 tailed) to assess correlation between ID and null mutations and between ID and *FUS*-P525L mutation, and the level of significance was determined at $p < 0.05$. AAO and disease duration differences between groups were evaluated with the Mann-Whitney *U* test, using SPSS software V25 (SPSS Inc).

Data Availability

The research study protocol and the informed consent form that were approved by the Institutional and National Supreme Helsinki Committees do not allow us to share patient's information. However, when a specific request will be made by a qualified investigator, anonymized data will be shared after approval by our institutional review board (Helsinki committee) and after a MTA is signed between the institutions.

Results

Clinical Phenotype of Patients With jALS

Among our ALS clinic cohort, 10 patients developed motor symptoms before age 25 years (jALS). Proband 1 presented with action tremor at age 18 years (Table 1). In the following months, he developed rapidly progressive weakness in his limbs and recurrent falls. His neurologic examination revealed weakness in all limbs with upper motor neuron (UMN) and LMN signs. EMG showed denervation and reinnervation in all limbs. He underwent tracheostomy 12 months after disease onset. His medical history revealed a developmental delay in various domains that was first noted at age 10 months. At 6 years, he was diagnosed with mild autism spectrum disorder (ASD), and later, he was also diagnosed with Tourette syndrome and obsessive-compulsive disorder. He attended a special school until age 18. His parents and siblings do not have any relevant medical history.

Proband 2 presented at age 19 years (Table 1) with rapidly progressive weakness of both hands. His neurologic examination revealed proximal arm weakness, absent/weak deep tendon reflexes, and shoulder girdle atrophy, without pyramidal signs. EMG demonstrated severe denervation of upper extremities. His symptoms worsened rapidly, and he underwent tracheostomy 9 months after disease onset. In childhood, he had a delay in achieving psychomotor milestones, and he attended a support class at a regular school. At age 11, he was diagnosed with mild ASD. His parents and sister do not have any neurologic impairment.

Patient 3 was hospitalized at age 24 years because of rapidly progressive lower limb weakness and imbalance (Table 1). His neurologic examination revealed limb weakness with UMN and LMN signs, intention tremor of both hands, pes cavus, and some dysmorphic features (large ears). EMG showed diffuse denervation and reinnervation. He died 8 months after the initial presentation because of respiratory failure. He had been diagnosed with mild mental retardation

Table 1 Clinical and Demographic Data of the 10 Patients With Juvenile ALS in Our Cohort

Patient number	Origin	Sex	AAO (yrs)	Disease duration (mo)	Status ^a	fALS/sALS	Limb/Bulbar	Dementia	Early intellectual disability	p.P525L carrier (method)	Other nonsynonymous variants in <i>FUS</i> (method)
1 (proband 1)	Morocco/Tunis/Ashkenazi	M	18	12	D	sALS	L	No	Yes	Yes (WES, Sanger sequencing, TaqMan)	No (WES)
2 (proband 2)	Poland/Bulgaria/Germany	M	19	9	D	sALS	L	No	Yes	Yes (WES, Sanger sequencing, TaqMan)	No (WES)
3	Buchara	M	24	8	D	sALS	L	No	Yes	Yes (TaqMan, Sanger sequencing)	Not tested
4	Russia/Poland/Egypt/Yemen	F	20	201	D	fALS? ^c	L	No	No	No (TaqMan, PCR)	No (ES)
5	Syria/Buchara	F	21	383	D	sALS	L	No	No	No (TaqMan, PCR)	No (ES)
6	Germany/Romania	F	21	134 ^b	A	sALS	L	No	No	No (WES, TaqMan)	No (WES)
7	Egypt/Morocco	M	22	20	D	sALS	L	No	No	No (WES, TaqMan)	No (WES)
8	Poland/Morocco	M	22	115 ^b	A	sALS	L	No	No	No (WES, TaqMan)	No (WES)
9	Mixed	M	22	75	D	fALS? ^d	L	No	No	No (TaqMan, PCR)	No (ES)
10	Morocco	M	24	122 ^b	A	sALS	L	No	No	No (WES, TaqMan)	No (WES)

Abbreviations: AAO = age at disease onset; sALS = sporadic ALS; fALS = familial ALS; WES = whole-exome sequencing; ES = exon screening by Sanger sequencing.

^a A = alive, D = deceased or tracheostomy.

^b At the time of submission.

^c Her grandfather had ALS at age 81 years.

^d His father and 2 sisters were diagnosed with hereditary spastic paraparesis.

at age 6 years. He did not gain the ability to read or write. There was a family history of ID in a far cousin, without any motor dysfunction (Table 1).

Seven additional patients with jALS from our cohort who were analyzed in this study had normal cognitive development, no history of learning disabilities, graduated from high school, and were well integrated socially and at work before development of motor ALS symptoms (Table 1).

Exome Sequencing and Bioinformatics Analysis

Trio analyses for rare homozygotes, CH, and de novo variants identified several variations in each category for each trio. In proband 1, a de novo mutation in *FUS* (NP_004951.1: p.Pro525Leu) was identified. This mutation was previously established to be pathogenic in patients with jALS.^{5,24} In addition, CH in 4 genes was observed: *MROH2A* (p.M208V/c.4156-1G>C), *TRIM42* (p.G456S/p.V630M), *SCUBE2* (p.P235S/p.D371Y), and *DNAH10* (p.R3699C/p.W4348C). In proband 2, the same *FUS* mutation was identified, also de novo, together with an additional de novo loss-of-function

(LoF) variant in *CARD11* (NM_032415.4:c.1653+1G>A), and a rare variant in *GPT2* (NP_597700.1:p.D422Y) in a homozygous state.

FUS-P525L Is Associated With ID in jALS, With Full Motor Penetrance at an Early Age

We genotyped 8 additional patients with jALS for the *FUS*-P525L mutation (Table 1). Among them, 1 patient (patient 3, AAO = 24, disease duration = 8 months) carried this mutation, and he, as well, had ID and attended a special school for children with developmental learning disorders.

The association of *FUS*-P525L with learning and ID in our jALS group was significant ($p = 0.0083$). No other *FUS* mutations (nonsynonymous or LoF) were found in the coding sequence of these 10 patients with jALS.

We also analyzed the 4 jALS and 6 healthy parents with WES data and checked for the presence of deleterious variants (filter was set to CADD >13, coding variants, excluding synonymous and common variants) in 78 genes associated with ALS (eTable 2, links.lww.com/NXG/A535). We did not

observe de novo, recessive, or CH coding variants in the 3 trios that passed our filters, and the fourth patient with jALS did not carry a potentially deleterious variant that was not observed at least in one of the 6 healthy individuals.

We then screened the *FUS*-P525L mutation in 56 patients with ALS with early adult AAO (between 25 and 40 years) without known disease-causing mutations and 430 patients with AAO 41 years and older. None of these 486 patients carried the mutation, confirming the full penetrance at early age and association of the mutation with jALS ($p < 0.001$).

FMR1 and C9orf72 Repeats Expansion Genotyping

To exclude ALS due to additional *C9orf72* hexanucleotide repeat expansion mutation, the 3 *FUS*-P525L ALS carriers were genotyped for this expansion and were negative. To exclude developmental delay due to expansion in *FMR1*-CGG repeat expansion, the 3 patients were also genotyped for this mutation, but all carried an unexpanded allele (58, 30, and 40 repeats, respectively).

Pathogenicity Evaluation of Additional Variants

Among the additional 6 genes that passed the filter pipeline, variants in 4 genes (*SCUBE2*, *DNAH10*, *GPT2*, and *CARD11*) received an ACMG score of VOUS (variants of uncertain significance) or likely pathogenic, all with very low allele frequencies reported worldwide (Table 2).

Proband 1 carried 2 variants in *SCUBE2*, D371Y (allele frequency 4.4E-05), and P235S (a novel variant). The variations are located within different EGF-like domains, predicted to change the hydrophobicity of the protein, with the former having a high disease propensity score (1.44), and the latter having a large decrease of protein stability (delta-delta-Gibbs [DDG] of [-1.29]), and both have a high CADD score for predicted pathogenicity (32.0 and 28.0, respectively, Table 2). This proband also carried 2 rare variants in *DNAH10*, R3699C (allele frequency 6.2E-05), and W4348C (allele frequency 2.4E-05), both highly conserved, change a large amino acid to a tiny, have a high disease propensity score (1.71 and 2.21, respectively), with a large decrease effect of protein stability (DDG of [-0.99] and [-1.75], respectively). The hydrophobicity scale shows a large change for R3699C (from -1.01 to +1.54), and the CADD score of 32.0 for W4348C suggests a potential high deleterious effect (Table 2).

Proband 2 carried an additional de novo splice donor variant in *CARD11*, a gene predicted to be a LoF intolerant gene by gnomAD (observed/expected = 0.13, CI: 0.07–0.23). ACMG categorizes it as likely pathogenic. Of interest, proband 2 is also homozygote to a very rare missense variant in *GPT2* (D422Y, Table 2). *GPT2* is a mitochondrial enzyme that catalyzes the reversible transamination between alanine and 2-oxoglutarate to generate pyruvate and glutamate. The missense variant (rs140757319) is located in the aminotransferase domain (amino

acids 110–511 by pfam), has a high score of disease propensity (1.44), and is extremely rare worldwide (allele frequency of 0.0023 in Ashkenazi-Jews (AJ)-nonneuro and 0.000174 in all nonneuro cases). This might suggest that both parents of proband 2 are of AJ origin and may have a common ancestor (homozygosity block by exome data is 2.68 Mb; Chr16:46732095-49412531). The amino acid at position 422 is highly conserved and changes from nonaromatic to aromatic and from non-hydrophobic (-0.77) to hydrophobic (+0.96). Modeling the wild-type and mutant protein by Phyre2 predicts that the mutation may change the protein structure, omitting the prediction of transmembrane helix domains.

Literature Review

We identified 367 publications from PubMed, published before May 10, 2020 (eFigure 2, links.lww.com/NXG/A535). After selecting original articles with full text in English, we assessed 348 articles for relevance by titles, abstracts, methods sections, and tables, among which 91 met the eligibility criteria. We collected clinical and genetic data regarding patients whose early life cognitive status was reported in the article or was personally communicated to us (total of 56 scientific articles, eFigure 2, links.lww.com/NXG/A535). This yielded records of 175 patients (Figure 1). The cognitive status of 44 of them was obtained by personal communication.

Sixty patients were sALS and 115 fALS (from 64 unrelated families, 24 of them with more than one proband each). In total, 18 patients (10.3%, 8 females and 10 males) were reported to have had early life ID: 10 sALS (16.7%) and 8 fALS from 5 different families, with AAO 19.4 ± 6.5 years (median = 18, range = 13–34); their disease duration was 26.5 ± 52.7 months (median = 12, range = 6–216) (Figure 1A and Table 3). Fourteen had jALS, whereas the other 4 were young onset.

One hundred fifty-seven patients (89.7%) did not have early life ID (82 females, 73 males, and 2 unknown); 50 of them were sALS and 107 fALS from 60 different families; 27 (17.2%) were juvenile and 129 (82.2%) nonjuvenile (in one case, the AAO was not reported). Their AAO was older (39.6 ± 15.6 years, median = 37, range = 11–85), and their disease duration was 27.7 ± 23.1 months (median = 21, range = 6–138). When comparing the unrelated individuals from each group (one from each family), a significant earlier AAO and disease duration was observed in patients with ALS with ID (Mann-Whitney test, mean AAO = 17.5 ± 5.4 years compared with 37.1 ± 16.2 years, $U = 158.5$, $p = 5.09 \times 10^{-7}$, and disease duration = 30.0 ± 58.9 months compared with 29.9 ± 25.7 months, $U = 330.0$, $p = 0.048$, respectively). Although the mean of disease duration is similar, there is one outlier in the ID group with disease duration of 216 months, which shifts the mean higher. Among families with more than one described individual, in 21 families none of the affected had ID, in 2 families all affected members had additional ID and in 1 family the phenotype varied, with 1 proband with ID, whereas her mother had not.

Table 2 Pathogenicity Evaluation of Variants Identified in Trio Analyses

Gene	rs ID	Coding annotation	Protein annotation	Phred CADD score (hg37-v1.6)	gnomAD V2.1.1 allele frequency nonneuro		ACMG ^a	Conservation ^b	Cell compartment	Brain region with max expression (median TPM) ^c	Domain	Molecule type change	Hydrophobicity scale change	Disease propensity score ^b	Protein stability ^d
					AJ	Total									
SCUBE2	rs 768800446	NM_001330199.1: c.1111G>T	NP_001317128.1: p.Asp371Tyr	32.0	0	4.4E-05	VOUS	High (0.9)	EM	Cerebellum (6.397)	EGF-like	Negative small to aromatic large	(-0.77) to (+0.96)	1.44	-0.05, neutral stability
	Novel	NM_001330199.1: c.703C>T	NP_001317128.1: p.Pro235Ser	28.0	—	—	VOUS	High (0.5)			EGF-like	Rigid small to neutral tiny	(+0.72) to (-0.04)	0.59	-1.29, large decrease of stability
DNHA10	rs 77931549	NM_0207437.3: c.11095C>T	NP_997320.2: p.Arg3699Cys	24.6	0	6.2E-05	VOUS	High (0.9)	Cyt	Cerebellum (1.559)	—	Positive large to tiny	(-1.01) to (+1.54)	1.71	-0.99, large decrease of stability
	rs 1477800299	NM_0207437.3: c.13044G>C	NP_997320.2: p.Trp4348Cys	32.0	1.6E-4	2.4E-05	VOUS	High (0.9)			Dynein heavy chain C-terminal	Aromatic large to tiny	(+2.25) to (+1.54)	2.21	-1.75, large decrease of stability
GPT2	rs 140757319	NM_0133443.2: c.1264G>T	NP_597700.1: p.Asp422Tyr	25.7	2.3E-3	1.7E-04	VOUS	High (0.5)	Mit	Amygdala (54.86)	Aminotransferase	Negative small to aromatic large	(-0.77) to (+0.96)	1.44	+0.07, neutral stability
CARD11	Novel	NM_032415.4: c.1653+1G>A	—	33.0	—	—	Likely pathogenic	High (NA)	Cyt and Mem	Spinal cord (3.33)	NA	NA	NA	NA	NA

Abbreviations: ACMG = American College of Medical Genetics and Genomics; AJ = Ashkenazi Jews; CADD = Combined Annotation-Dependent Depletion; Cyt = cytoskeleton; EM = extracellular matrix; Mem = membrane; Mit = mitochondria; NA = not applicable; TPM = transcripts per million; VOUS = variant of uncertain significance.

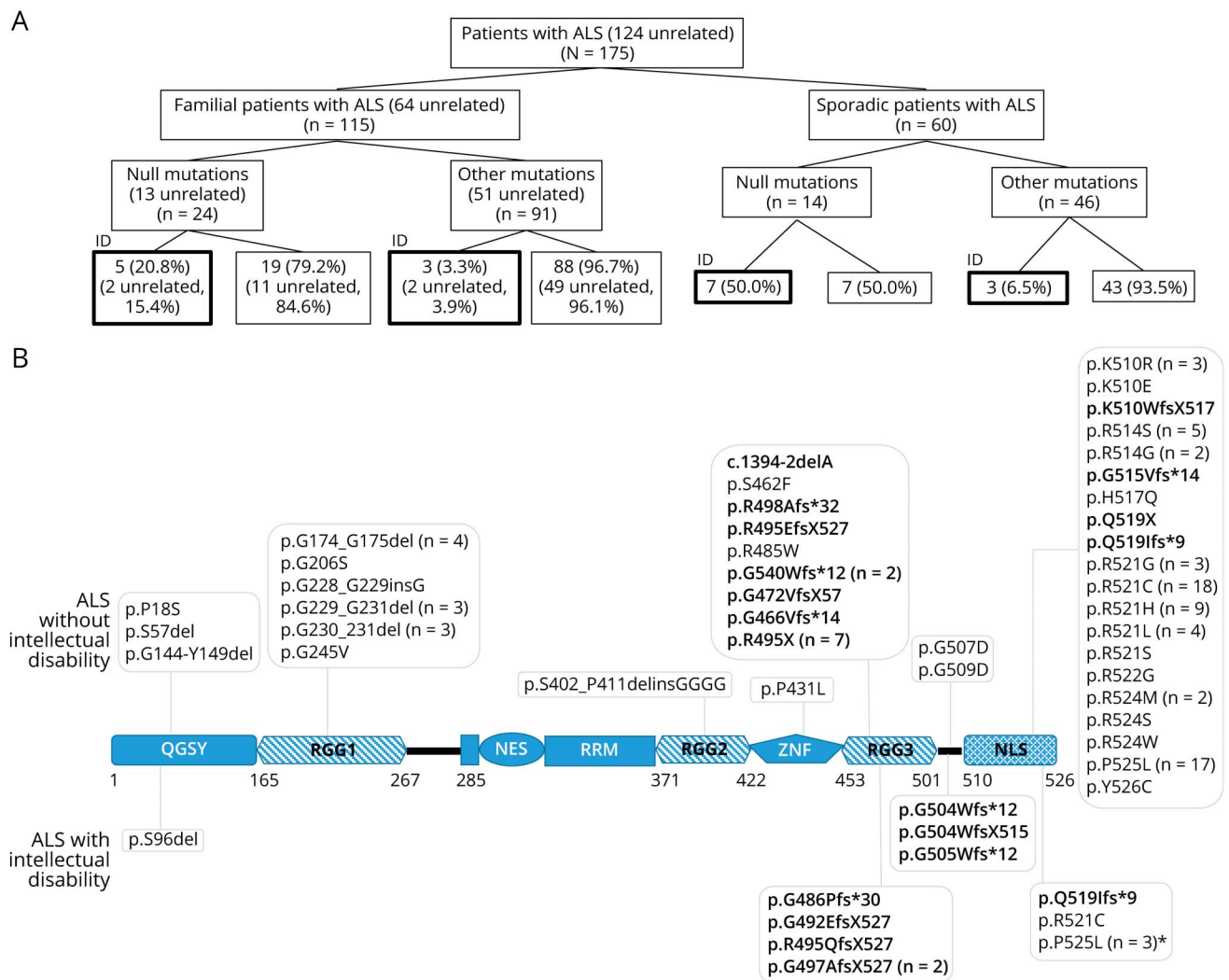
^a Via Franklin, Genoox.

^b Values from VarSite.

^c RNA expression levels from GTEX.

^d Delta-delta-Gibbs (DDG) predicted value (Kcal/mol) by I-Mutant.

Figure 1 Summary of 175 Patients With ALS With Full Phenotype From Literature Review



(A) Segregation to familial ALS/sporadic ALS and mutation type. In black thick squares are patients with intellectual disability (ID) phenotype. (B) *FUS* mutations in unrelated patients. Top panel: patients without intellectual disability; lower panel: patients with intellectual disability. In bold are null mutations. (*) = An additional patient with *FUS*-P525L-ALS was reported, who is a sibling of a patient with *FUS*-P525L-ALS without intellectual disability and, therefore, was omitted from this figure. *FUS* = fused in sarcoma; ALS = amyotrophic lateral sclerosis.

Patients with ID were more common in null *FUS* mutations carriers than in nonnull mutations carriers (missense or inframe indels): 33.3% (9/27) and 5.2% (5/97), respectively ($p = 0.0003$, Figure 1A). Specifically to *FUS*-P525L mutation, among the 21 carriers, 4 had ID (19%), with no significant difference in AAO or disease duration compared with the 17 carriers who did not have ID (Mann-Whitney U test, AAO = 14.8 ± 2.9 years, median = 13.5, range = 13–19, compared with 21.9 ± 8.9 years, median = 21, range = 11–45, respectively, $U = 14.0$, $p = 0.081$; Disease duration = 15.3 ± 6.4 months, median = 18, range = 8–20, compared with 15.9 ± 11.6 months, median = 12, range = 6–44, respectively, $U = 20.5$, $p = 0.616$).

Among all patients with ALS who carried missense mutations, there were significantly more patients with ID in the P525L carriers than in the other missense mutations carriers ($4/21 = 19\%$

compared with $1/62 = 1.6\%$, $p = 0.0135$). Within the NLS domain, this difference stays significant ($4/21$ vs $1/54$, $p = 0.0199$).

Discussion

We report a novel significant enrichment of *FUS*-P525L mutation in our cohort of jALS with early life ID, as well as a significant association with very early AAO of less than 25 years. Our literature search also suggests that among the patients with *FUS*-ALS, a relatively high percentage exhibit additional learning and ID (10.3%). Specifically to p.P525L mutation, 4 of 21 carriers had learning/early life intellectual disabilities (19.0%). This association is apparently unique to *FUS* gene mutations and was not described with mutations in any other ALS-causing gene or in ALS without known gene mutations. Of interest, among all patients with ALS who carried missense mutations, there were

Table 3 Eighteen Patients With ALS With *FUS* Mutation and Intellectual Disability Reported in Previous Publications

Patient number (family number)	Sex	Familial/sporadic ALS	Age at onset (y)	Disease duration (m)	Effect	Mutation—nucleotides	Mutation—protein	Intellectual/developmental disorder ^a	Ref
1 (F1)	F	fALS	27	~12	LoF	c.1485delA	p.G497AfsX527	Learning disorder	eRef 1
2 (F1)	M	fALS	18	11	LoF	c.1485delA	p.G497AfsX527	Learning disorder	
3 (F2)	M	fALS	29	12	LoF	c.1485delA	p.G497AfsX527	Learning disorder	eRef 2
4 (F2)	F	fALS	29	13	LoF	c.1485delA	p.G497AfsX527	Learning disorder	
5 (F2)	M	fALS	13	18	LoF	c.1485delA	p.G497AfsX527	Learning disorder	
6	M	fALS	~15 (PC)	216	Inframe deletion	c.287_291delCCTACinsAT	p.S96del	Intellectual disability	
7	F	fALS	34	7	Missense	c.1561C>T	p.R521C	Learning disorder	eRef 3
8	F	fALS	13	18	Missense	c.1574C>T	p.P525L	Mild intellectual disability	42
9	M	sALS	14	NA	Missense	NA	p.P525L	Mild intellectual disability (PC)	eRef 4
10	F	sALS	13	20	Missense	c.1574C>T	p.P525L	Speech and learning disorder	10
11	F	sALS	19	8	Missense	c.1574C>T	p.P525L	Learning disorder, ASD?	eRef 5
12	F	sALS	24	8	LoF	c.1507-1508delAG	p.G504WfsX515	Mild intellectual disability, additional cognitive decline after ALS-onset	eRef 6
13	F	sALS	18	25	LoF	c.1509_1510delAG	p.G504Wfs*12	Learning disorder	9, eRef 7
14	M	sALS	15	>120 (PC)	LoF	c.1456_1457GGdel	p.G486Pfs*30	Mild intellectual disability	eRef 8
15	M	sALS	17	NA	LoF	c.1475delG	p.G492EfsX527	Moderate intellectual disability	eRef 9
16	M	sALS	18	~12	LoF	c.1484delG	p.R495QfsX527	Learning disorder	eRef 10
17	M	sALS	18	6	LoF	c.1554_1557delACAG	p.Q519fs*9	Learning disorder	eRef 11
18	M	sALS	14	11	LoF	c.1510dupG	p.G505Wfs*12	Moderate intellectual disability	eRef 12

Abbreviations: ASD = autism spectrum disorder; PC = personal communication.

The level of disturbance (mild/moderate/severe) was not always possible to determine and might not be accurate. Learning disorder might in fact have been part of a broader syndrome of intellectual disability, in which the learning difficulties were emphasized in the clinical chart. Regarding patient 11, who was reported with ASD, we added a question mark because this diagnosis is probably overrepresented in clinical charts filled by nonspecialists.

^a For each patient, we attempted to establish the most accurate diagnosis based on the available (retrospectively gained) data.

significantly more patients with ID in the P525L carriers than in the carriers of other missense mutations ($p = 0.0135$), and this pattern remained significant when looking only within the NLS domain ($p = 0.0199$). The proline at position 525 is in the proline-tyrosine (PY) motif at the c-terminus of the gene, which is important for the binding of Kapbeta2,²⁵ a nuclear import protein. A significant reduction in binding affinity of *FUS* is reported with this mutation. The reduction is much higher compared with other missense mutations in the NLS that are not in the PY motif,²⁶ which might explain the differences in severe vs mild cytoplasmic accumulation of *FUS*. Very recently it has been shown that P525L mutation causes significant changes at the binding interface, and interrupts the molecular movement of Kapbeta2 affecting its nuclear import.²⁷ Altogether, these molecular observations may suggest that high effect on the nuclear transport may be necessary for the ID phenotype together with additional deleterious variants in ID-related genes.

The *FUS* gene encodes an RNA-binding protein consisting of 526 amino acids. The protein is primarily located in the nucleus and is involved in different functions and pathways such as transcription regulation at the RNA and microRNA level as well as DNA repair and cell proliferation.^{7,28,29} Dysfunction of *FUS* in the cytoplasm, especially in the neuron dendritic spines, can cause mRNA destabilization, cell maturation, effect on axonal transport, and morphological maintenance of neurons (review: 30).

Of interest, in a *FUS* mouse model,³¹ unbiased transcriptomic analysis identified changes in expression of a set of genes, among them, the *Sema5a*, a gene that is involved in axonal guidance during neural development and is known as an autism susceptibility gene.^{32,33} *FUS* binds directly to *Sema5a* mRNA and regulates *Sema5a* expression in a *FUS* dose manner. Data suggest that *FUS*-driven *Sema5a* deregulation may underlie the cognitive deficit in *FUS* transgenic mice.

Does *FUS* directly affect the cognitive and executive functions during development, in addition to the motor functions, and is it solely responsible for the ID phenotype presented here? We identified an additional recessive variant in *GPT2* in one proband and additional *DNAH10* variants (CH) in the second proband, which might be contributors to the ID phenotype.

The *GPT2* gene, also known as alanine aminotransferase 2 (*ALT2*), is a mitochondrial enzyme that reversibly catalyzes transamination between alanine and alpha-ketoglutarate to produce glutamate and pyruvate and is expressed in brain and muscle. Fourteen variants in *GPT2* were reported in association with neurodevelopmental disorder with spastic paraplegia and microcephaly, with severe or moderate ID (eTable 3, links.lww.com/NXG/A535, 10 homozygous mutations and 4 mutations in 2 cases of CH). Six missense mutations are within the aminotransferase domain, as our D422Y variant, with 4 showing profound reduction in enzyme activity in in vitro functional assays (S153R, C259R, N271T, and P272L) and 2 that were not evaluated for enzyme level activity. These cases support homozygosity/CH mode of inheritance for the neurodevelopmental disorder.

The *DNAH10* gene was previously associated with ASD. WES study in families with ASD identified a *DNAH10* de novo mutation in a proband,³⁴ whereas whole-genome-sequencing studies in families with ASD identified a *DNAH10* LoF variant in 2 affected siblings³⁵ and suggested it as a gene likely to affect ASD risk (enrichment in LoF mutations, false-discovery rate <0.30; odds ratio = 1.49).³⁶ Moreover, a recent study assessed genetic contribution to ASD by analyzing whole-genome sequences of 2,308 individuals from families with multiple affected children.³⁷ Results showed that *DNAH10* reached genome-wide significance under specific requirements, and variants in this gene (all LoF variants) exhibited overtransmission to affected children in the studied cohort. Because our proband carries 2 alleles with prediction of strong effect on protein stability and function, this case may also represent a LoF phenotype.

Because these variants have very low allele frequencies (estimated frequencies of 1:200,000 in AJs and 1:269 millions worldwide, for *GTP2*-p.D422Y homozygotes and *DNAH10*-R3699C/W4348C CH, respectively) and are predicted to be pathogenic, this may suggest that although *FUS*-P525L is a necessary and sufficient for the motor symptoms of ALS, additional genetic variants may be involved in the overall expression of ID phenotype. Unfortunately, we do not have any DNA samples from additional family members to establish additional genotype-phenotype correlations, nor do we have a large cohort of *FUS*-P525L carriers to statistically estimate the oligogenic model for ID. To show statistical support for oligogenic inheritance, a worldwide effort should be established to analyze the complete genome of all *FUS*-ALS carriers and compare the genomes of the ones with ID to the ones without ID, in the same manner as was done in the study by Pounraja and Girirajan.³⁸ This group developed a method to establish oligogenic inheritance in specific phenotypes among males on the autism spectrum and was able to identify mutated gene combinations significantly associated with ID phenotypes.

We identified a LoF de novo variant in the *CARD11* gene, changing a splice donor site, and predicted to be likely pathogenic by ACMG. Diseases associated with *CARD11* are mostly immunodeficiency diseases, and therefore, this LoF mutation may not be related to ALS/ID phenotype. However, assessment of postzygotic mosaic (PZM) mutations in ASD had found a significant enrichment in *CARD11* with recurrent non-synonymous PZMs in probands,³⁹ suggesting a possible link between *CARD11* and ASD. The effect of the specific *CARD11*-c.1653+1G>A variant should be further studied.

Finally, it is possible that 2 of the genes above also contribute to the motor symptoms observed in the jALS probands. *SCUBE2* has a nominal significant *p* value for burden of mutations in the Project MinE database (*p* value = 0.016, uncorrected for multiple testing),⁴⁰ suggesting, with caution, its involvement in ALS pathogenesis. *GPT2* affects motor neurologic symptoms associated with spastic paraplegia (eTable 3, links.lww.com/NXG/A535).

Our results emphasize the importance of full coding exon evaluation to accurately establish genotype-phenotype correlation in any neurodegenerative disease and specifically in ALS. Of note, most of the original articles that reported *FUS* mutations in patients with ALS had only sequenced the *FUS* gene or part of it. The attempt to conclude phenotype-genotype correlation based on partial mutation analysis results in a wide phenotypic heterogeneity, and careful assessment is needed when these correlations are made. It is known that about 5% of patients who undergo WES analysis and have a molecular diagnosis have 2 or more disease loci⁴¹; therefore, as more WES and whole-genome-sequencing are generated, close evaluation of the overall contribution of rare, deleterious variants to the specific subphenotypes is required.

In that respect, it is important to note that our trio analysis included the 3 standard modes of inheritance for healthy parents who passed the average AAO of the disease and have an affected young child (rare homozygosity, de novo, and CH), but additional dominant risk alleles might contribute to the overall phenotype of ALS/ID. As this mode of inheritance was not evaluated in our study, caution is needed when interpreting the complete genetic complexity of ALS/ID phenotype in jALS. Moreover, although we did not find *FUS*-P525L carriers in patients with ALS older than 25 in our cohort, a few later AAO carriers were reported in the literature,^{7,24,42,43} suggesting the possibility of additional genetic and/or environmental factors that can delay the onset by more than 10 years.

In the literature search conducted here, one of the main difficulties was to determine the prevalence of cognitive or developmental impairments among patients with ALS with *FUS* mutations because most publications lack information about early ID among patients with ALS because the cognitive evaluation of patients with ALS is based on clinical impression rather than on formal tests. A bias report might be present because evidence of mental disabilities is more likely to be noted than lack of it. On the other hand, early

life mental disabilities, especially if mild, might not be reported at all because this information is considered irrelevant to ALS. Therefore, published data might not reliably represent the rate of intellectual/mental disabilities among patients with ALS-FUS. The information that we received from authors regarding their patients helped to gain more reliable phenotype description, but this was not successful with all published cases, and therefore, we took into consideration only patients with full phenotypic description, which included also the intellectual and cognitive development. In the patients seen at our center, the clinical picture seems to be rather stereotype and compatible with the diagnosis of mild ID as defined by the DSM-5.

In conclusion, we observed a disproportionately high number of ID cases among our patients with juvenile-onset ALS, all with FUS-P525L mutation, and suggest that this phenotype should be added to the characteristic features of this genetic defect. The genetic basis of the ID phenotype may be oligogenic in nature. Functional studies on the additional variants identified in this study are warranted to evaluate their effect on proteins and enzymes functions, and further evidence from other populations is needed to elucidate the role of these genes to the global phenotype of ALS with ID. Further research on the association between FUS-specific mutations and early life cognitive impairment should be conducted. Finally, better characterization of this specific ALS phenotype may contribute to earlier diagnosis. This will have major clinical implications because options for tailored genetic treatment, such as the specific FUS antisense oligonucleotide Jacifusen (Ionis Pharmaceuticals, Inc.), are entering clinical trials.⁴⁴

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Vivian E Drory, MD	Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; Neuromuscular Diseases Unit, Department of Neurology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data

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Additional data, eReferences 1–12 available at: links.lww.com/NXG/AS35.

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