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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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. 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. 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. 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. One of the ALS-causative genes is fused in sarcoma (FUS). Since first identified as being associated with ALS, 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. 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).

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.

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. 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 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), 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 Genoonx16 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, Phyre2, I-MUTANT, and VarSite.

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

**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, (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
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. In addition, CH in 4 genes was observed: MROH2A (p.M208Y/c.4156-1G>C), TRIM42 (p.G456S/p.V630M), SCUBE2 (p.P235S/p.D371Y), and DNAH10 (p.P235S/p.D371Y). 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 Penetration 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

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**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 | fALS/ sALS | Limb/ Bulbar | Dementia | Early intellectual disability | p.P525L carrier (method) | Other nonsynonymous variants in FUS (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      | sALS      | 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      | sALS      | 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 = alive, D = deceased or tracheostomy.
+ At the time of submission.
^ Her grandfather had ALS at age 81 years.
§ His father and 2 sisters were diagnosed with hereditary spastic paraparesis.
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-P52SL 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-P52SL 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, having 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 × 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.
| Gene        | rs ID          | Coding annotation | Protein annotation | Phred CADD score (hg37-v1.6) | gnomAD V2.1.1 allele frequency nonneuro | Conservationb | Cell compartment | Brain region with max expression (median TPM)c | Molecule type change | Hydrophobicity scale change | Disease propensity scoreb | Protein stabilityd |
|-------------|----------------|-------------------|--------------------|------------------------------|----------------------------------------|--------------|------------------|-----------------------------------------------|----------------------|--------------------------|---------------------------|-------------------|
| 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              |
| DNHA10      | rs 77931549    | NM_207437.3:c.703C>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              |
|             | rs 1477800299  | NM_207437.3: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 | (−0.25) to (+1.54) | 2.21                       | −0.75, large decrease of stability |
| GPT2        | rs 140757319   | NM_133443.2: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              |
| CARD11      | Novel          | NM_032415.4:c.1653T>G>A | —                   | 33.0                         | — —                                 | Likely pathogenic | Cyt and Mem Spinal cord (3.33) | NA NA 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, Genoxx.
b Values from VarSite.
c RNA expression levels from GTEx.
d Delta-delta-Gibbs (DDG) predicted value (Kcal/mol) by I-Mutant.
Patients with ID were more common in 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 ALS 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...
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 catalyses transamination between alanine and alpha-ketoglutarate to produce glutamate and pyruvate and is expressed in the 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/AS35). 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 vitro and 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, whereas whole-genome-sequencing studies in families with ASD identified a DNAH10 LoF variant in 2 affected siblings and suggested it as a gene likely to affect ASD risk (enrichment in LoF mutations, false-discovery rate <0.30; odds ratio = 1.49). Moreover, a recent study assessed genetic contribution to ASD by analysing whole-genome sequences of 2,308 individuals from families with multiple affected children. 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. 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, 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), suggesting, with caution, its involvement in ALS pathogenesis. GPT2 affects motor neurologic symptoms associated with spastic paraplegia (eTable 3, links.lww.com/NXG/AS35).

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 loci; 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, 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 Jacobus (Ionis Pharmaceuticals, Inc.), are entering clinical trials.44

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