Supplement

eMethods

Literature investigation

An extensive literature search was performed in PubMed, to identify all articles that describe ALS patients with mutations in the *FUS* gene, published before May 10th, 2020 (eFigure 2). The search terms "(ALS) AND (*FUS* or "fused in sarcoma") AND "mutation" were used, and then filtered with "humans". First, we omitted articles if they were (i) written in a language different than English, (ii) reviews, (iii) basic science studies, unless a detailed clinical description of individual ALS-*FUS* patients was included, (iv) cases that have previously been reported in another article, (v) missing the required data and noted that it could not be retrieved, (vi) using data from public databases or tissue banks that did not report the cognitive status. We then included only nonsynonymous or donor-acceptor sites variants and excluded synonymous, 5' and 3' untranslated regions (UTR) variants. The remaining articles were included for further review and patients' selection. As cognitive, intellectual, learning, developmental and behavioral history are not specified under these categories in most publications, we looked after descriptions of deficits in intellectual functions, such as reasoning, problem solving, planning, abstract thinking, judgment, academic learning, learning from experience, deficits in adaptive functioning with lack of personal independence and social responsibility that may limit functioning in one or more activities of daily life, such as communication, social participation, and independent living, as these features are characteristic for early intellectual disability according to DSM-5.[22] In this paper we will use the term "intellectual disability" (ID) as an overall description of any of the symptoms above observed in the patients reported in the literature according to DSM-5. 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, in order 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 which 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.

eTable 1. Primers used to sequence *FUS* coding exons and to validate the *de novo* mutations *FUS*-P525L, and *CARD11*-c.1653+1G>A.

Gene	**Forward primer**	**Reverses primer**	**Product size (bp)**	**Sequencing primer**
FUS-Exon 1	CTGCCTGCTCGGACCCTCTAC	CGACACCAGCCTCCTCCAGTTAC	392	Forward and Reverse
FUS-Exon 2	TGGTCCTTTTTATTCATCAGTGCTTG	AGCTGAGACAGCACCACTGCAC	495	Forward and Reverse
FUS-Exon 3				Forward and Reverse
FUS-Exon 4	CTTCTGAGAGGCTGGCTTTATGAGT	CCTCAGCAACAGAGACAGAGCAAG	742	Forward
FUS-Exon 5				Forward
FUS-Exon 6	TGGCACTTGTCAAACCTTTTCAAAC	AAAAGAAAGTCACTGCCCCCAAAT	479	Reverse
FUS-Exon 7	GGAAGAACGACCAAGGAAAATGG	GCTCCAGGTTAGCACACACCAG	498	Forward and Reverse
FUS-Exon 8	TTGGAGTGAGGCTGTGAGCACTTA	GCTGGCAACAACCACTAAGACTTG	1176	TCCACATTTTGAAAGAACCACTGC
FUS-Exon 9				Reverse
FUS-Exon 10	AAGTAACTGGGAAGAGGGGAGCTG	TTGGCTAAATCTGACCCCAACAGT	986	Forward
FUS-Exon 11				Forward and Reverse
FUS-Exon 12				Reverse
FUS-Exon 13	CTAGGTCTTGCCTATTCCCCATCG	GGTCACTTTTAATGGGAACCAGAGG	907	Forward
FUS-Exon 14				Forward
FUS-Exon 15				Reverse
FUS-P525L mutation in probands 1 and 2	GCAGTTGAACAGAGGCCATAGGAT	GAAAGTGAAAAGGGGGAAGAGGAA	493	Reverse
CARD11- LoF mutation in proband 2	GTTAGAAGGAGGTTGGGGATGAT	ATACATTGCGAGGCTGTGGAA	654	Forward and Reverse

eTable 2. Seventy-eight genes screened in non-*FUS* jALS patients.

ABCD1, ABHD12, ALS2, ANG, ARHGEF28, C9ORF72, CHCHD10, CHMP2B, CRYM, DAO, DCTN1, ERBB4, FIG4, FUS, GRN, HNRNPA1, HNRNPA2B1, LUM, MAPT, MATR3, NEFH, NEK1, OPTN, PFN1, PRPH, PSEN1, SETX, SIGMAR1, SOD1, SPART, SPG11, SQSTM1, TAF15, TARDBP, TBK1, TREM2, TRPM7, TUBA4A, UBQLN2, UNC13A, VAPB, VCP, VEGFA, KIF5A, TFG, ANXA11, HEXA, ABCA7, APOE, APP, ARSA, ATL1, ATP7B, ATXN2, BSCL2, CCNF, CP, CSF1R, CYLD, CYP27A1, EWSR1, FTL, GLE1, HSPD1, ITM2B, NOTCH3, NPC1, PANK2, PRNP, PSEN2, REEP1, SLC52A3, SNCA, SORL1, SPAST, TYROBP, UBE3A, WASHC5.

eTable 3. *GPT2* mutations reported in literature

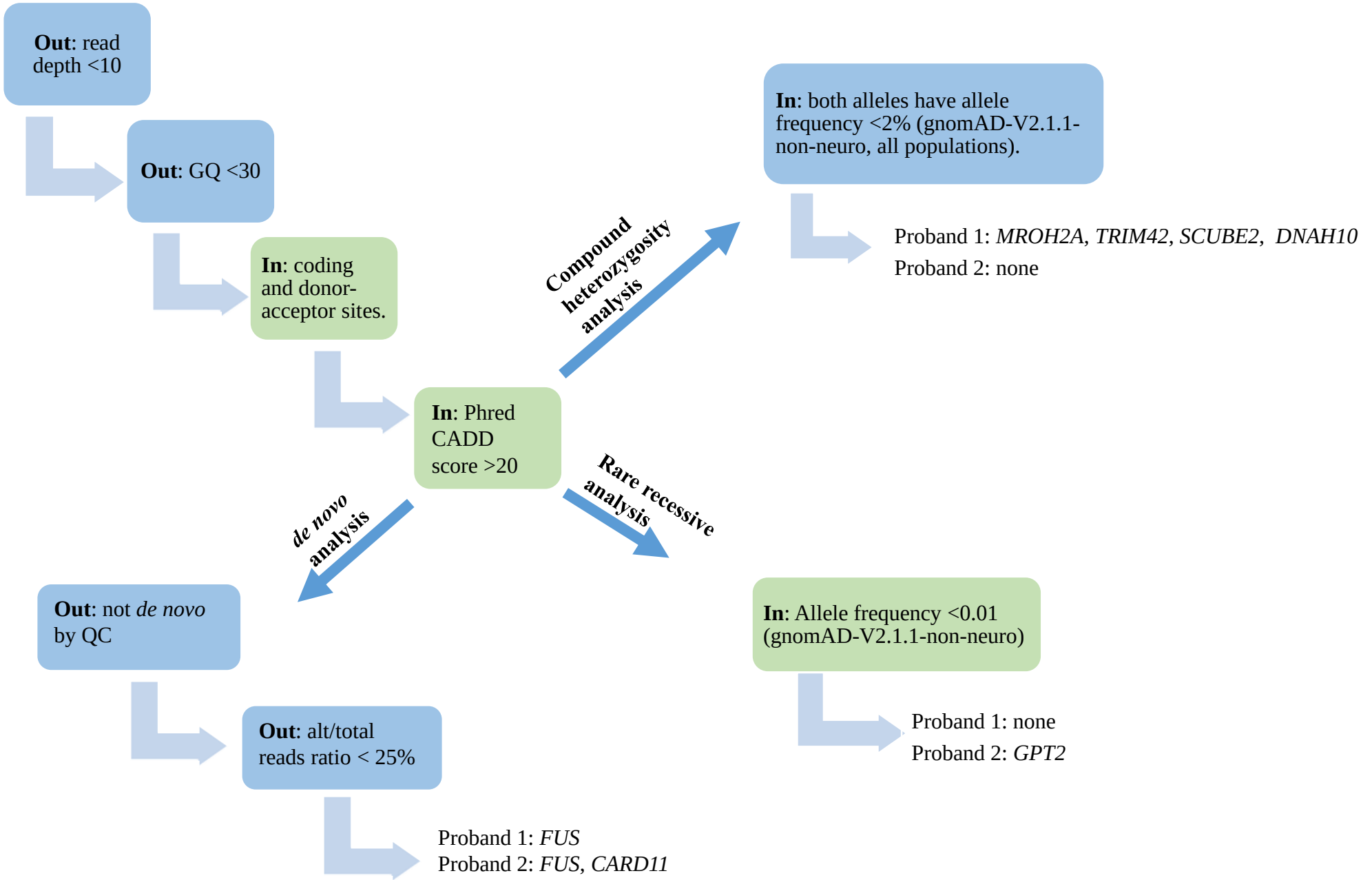
Protein change	Reference (PMID number)	Mode of inheritance	Family, segregation, and phenotype	Functional experiments	CADD	rs ID, hg38 position, ClinVar
S153R	Celis et al., 2015 (25758935)	Homozygote	3 siblings, born to consanguineous Mizrahi Jewish parents, with neurodevelopmental disorder with spastic paraplegia and microcephaly. Mutation segregated in the family.	Functional in vitro analyses in bacteria and 293HEK cells demonstrated a severe loss of enzymatic function	25.1	rs786203999, Chr16: 46906858- C-G, ClinVar=pathogenic
R404X	Ouyang et al., 2016 (27601654)	Homozygote	Large consanguineous family, 5 affecteds, segregated with the disorder in the family. ID, microcephaly, and variable progressive spasticity.	HeLa cells transfected with the mutations showed almost undetectable enzyme activity, consistent with a loss of function	36	rs115352435, Chr16: 46922414-C-T, ClinVar=pathogenic
P272L		Homozygote	Large consanguineous family, 9 affecteds, segregated with the disorder in the family. ID, microcephaly, and variable progressive spasticity.	HeLa cells transfected with the mutations showed almost undetectable enzyme activity, consistent with a loss of function	25.8	rs886038199, Chr16: 46909922-C-T clinVar=VOUS
G96R	Lobo-Prada et al., 2016 (28130718)	Homozygote	4 siblings out of 12, full segregation, with non-syndromic severe ID, spastic deplegia, pyramidial dysfunction.	Low allele frequency in ExAC, Crystal structure model suggest effect on catalitic activity. Evolutionary highly conserved. No functional assay.	29.8	rs778445616, Chr16: 46897690-G-A, no report in ClinVar
R134C and V479M	Kaymakcalan et al., 2018 (29226631)	Compound Heterozygosity	6 patients from a large, partially consanguineous Turkish family with NEDSPM, segregated with the disorder in the family.	Neither variant was found in the dbSNP or ExAC databases. Both mutations occurred at highly conserved residues in the aspartate aminotransferase family domain and were predicted to be pathogenic, but functional studies of the variants and studies of patient cells were not performed.	23.6/28.7	R134C: rs1420397443, Chr16: 46900748-C-T, ClinVar=pathogenic; V479M: rs1372862248, Chr16: 46926991-G-A, no ClinVar

Q24X	Hengel et al., 2018 (29882329)	Homozygote	In 5 patients from 2 consanguineous Palestinian families with NEDSPM, segregated with the disorder in the families, homozygosity block.	Extremely rare allele. Functional studies of the variant and studies of patient cells were not performed, but the variant was predicted to result in a loss of function.	34	rs1437184398, Chr16: 46884785-C-T, ClinVar=pathogenic
R404X	Ouyang et al., 2019 (31471722)	Homozygote	3 sibs, born to consanguineous Pakistani parents, with NEDSPM. The patients had global developmental delay with severely to profoundly impair intellectual development, speech delay, oromotor dysfunction, poor growth with microcephaly, hyperreflexia of the lower limbs, and motor incoordination.	Was reported in Harripaul et al, 2018 (PMID: 28397838).	36	rs115352435, Chr16: 46922414-C-T, ClinVar=pathogenic
N271T and c.1432_1433delGT (Val478ArgfsTer73)		Compound Heterozygosity	2 sibs, born to unrelated Caucasian parents, with neurodevelopmental disorder with NEDSPM, segregated with the disorder in the family. The patients had global developmental delay, poor overall growth with microcephaly, dysarthria, spastic diplegia, and childhood-onset seizures.Moderate ID.	Expression of the mutations in HeLa cells showed that both resulted in reduced protein expression, decreased localization of GPT2 to mitochondria, and significantly decreased enzymatic activity compared to controls, comparable to cells transfected with an empty vector. These findings were consistent with a loss of function.	26.2/NA	N271T: no rs. Chr16: 46909919-A-C; V478Rfs*73: Chr16: 46926988-989
C259R		Homozygote	In a 17-year-old boy, born to unrelated parents (family 2), with neurodevelopmental disorder with NEDSPM. Segregated with the disorder in the family. The patient had developmental delay with microcephaly, speech delay, and abnormal gait, but he did not have overt spastic paraplegia. Moderate ID.	Expression of the mutation in HeLa cells showed that it resulted in reduced protein expression, decreased localization of GPT2 to mitochondria, and significantly decreased enzymatic activity compared to controls, comparable to cells transfected with an empty vector. These findings were consistent with a loss of function.	26.6	rs368711025, Chr16: 46909882-T-C, ClinVar=pathogenic

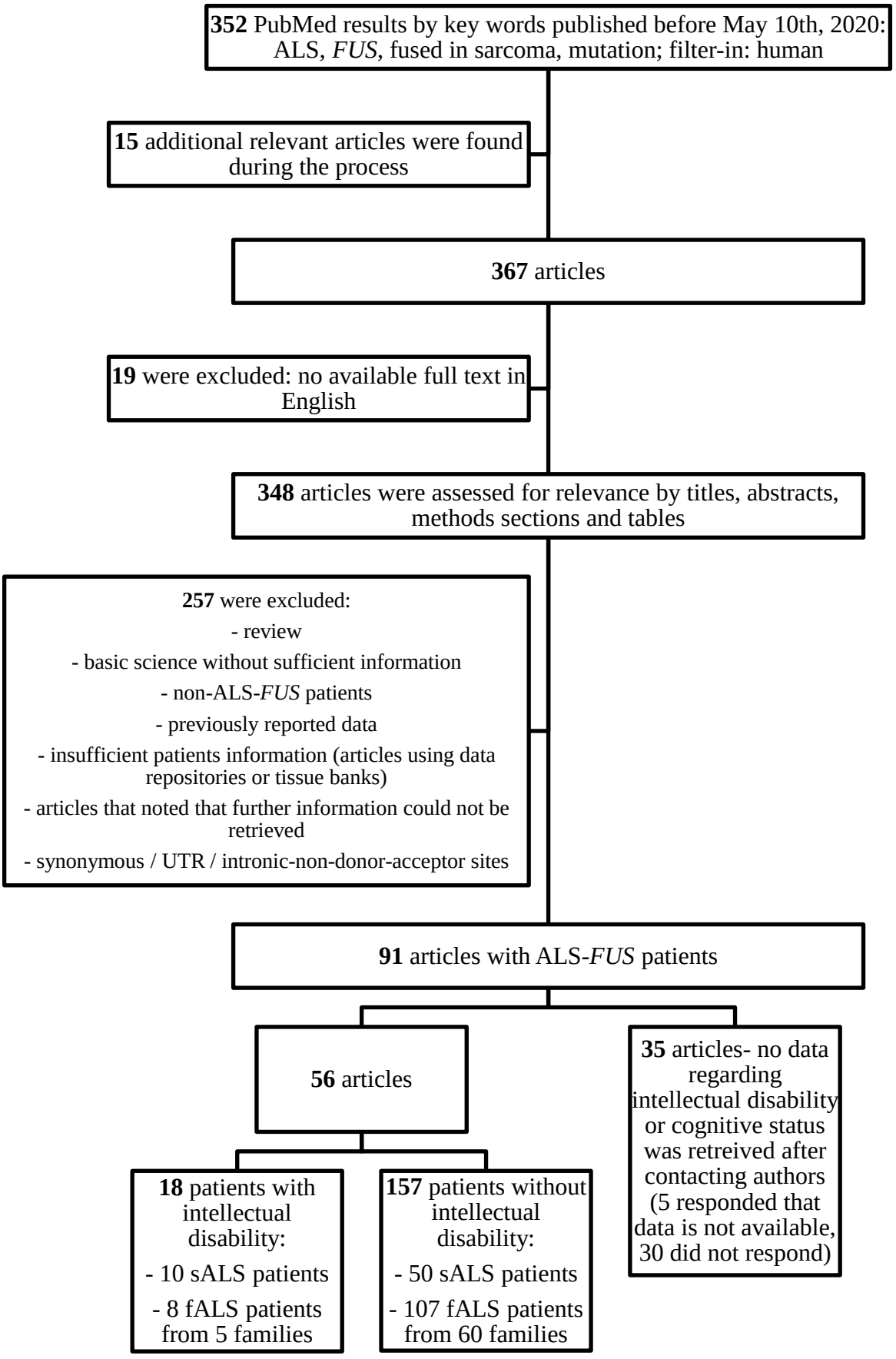
Q80E		Homozygote	Intellectual and developmental disabilities.	All expreiments showed no effect on protein level, localization, or enzyme activity. In Zebrafish there is E in this position.	20.7	No rs. Chr16: 46884953-C-G
p.G345=		Homozygote	4 siblings (out of 6) were homozygotes, with ID in the mild to moderate range. Mutation segregates in the family.	Trapping assay show that it does introduce a new splicing site, that cause 4 bp deletion in the RNA, and the new site does change the splicing.	26.2	rs778652804. Chr16: 46918755-C-T. No report in ClinVar.
E89G	Binaafar et al., 2020 (31978613)	Homozygote	4 sibs, full segregation in Iranian family, with non-progressive mild to severe ID.	No functional assay.	31	no rs. Chr16: 46897670-A-G
Asp189* (c.564dup)	Debs et al., 2021 (33501685)	Homozygote	1 child to a non-consanguineous parents. Syndromic peripheral neuropathy with severe developmental delay/ID. Progressive demyelinating motor neuropathy.	No functional assay.	NA	no rs. Chr16: 46906936-Tdup

ID= intellectual disability; NEDSPM=neurodevelopmental disorder with spastic paraplegia and microcephaly.

eFigure 1. Filter pipeline for variants inclusions, for *de novo*-, compound heterozygosity-, and rare recessive- analyses.



eFigure 2. Flow-chart of literature review



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