Running title: Analysis of known amyotrophic lateral sclerosis and frontotemporal dementia genes reveals a substantial genetic burden in patients manifesting both diseases not carrying the *C9orf72* expansion mutation

Supplementary table 1. List of known ALS and/or FTD genes analyzed in this study.

Gene	Location	Inheritance	Phenotype
TARDBP	1p36.22	AD	ALS, FTD, ALS/FTD
TBK1	12q14.2	AD	ALS, FTD, ALS/FTD
FUS	16p11.2	AD/AR	ALS, FTD, ALS/FTD
SQSTM1	5q35.3	AD/Risk	ALS, FTD, ALS/FTD
VCP	9p13.3	AD	ALS, FTD, ALS/FTD
OPTN	10p13	AD/AR	ALS, FTD, ALS/FTD
UBQLN2	Xp11.21	XL	ALS, FTD, ALS/FTD
DCTN1	2p13.1	AD	ALS, FTD, ALS/FTD
CHCHD10	22q11.2	AD	ALS, FTD, ALS/FTD
CHMP2B	3p11.2	AD	FTD, ALS/FTD
MAPT	17q21.31	AD	FTD, ALS/FTD
MATR3	5q31.2	AD	ALS, ALS/FTD
ANG	14q11.2	AD	ALS, ALS/FTD
TUBA4A	2q35	AD	ALS, ALS/FTD
CCNF	16p13.3	AD	ALS, ALS/FTD
SOD1	21q22.11	AD/AR	ALS, ALS/FTD
PFN1	17p13.2	AD	ALS
ALS2	2q33.1	AR	ALS
SETX	9q34.13	AD	ALS
SPG11	15q21.1	AR	ALS
VAPB	20q13.33	AD	ALS
EWSR1	22q12.2	AD	ALS
TAF15	17q12	AD	ALS
FIG4	6q21	AD	ALS
SIGMAR1	9p13.3	AR	ALS
ANXA11	10q22.3	AD	ALS
DAO	12q24.1	AD	ALS
HNRNPA1	12q13.1	AD	ALS
HNRNPA2B1	7p15.2	AD	ALS
ERBB4	2q34	AD	ALS
NEK1	4q33	AD	ALS
GRN	21q22.1	AD	FTD
TREM2	6p21.1	AD/AR	FTD

AD: autosomic dominant inheritance; AR: autosomic recessive inheritance; XL: X-linked inheritance; ALS: amyotrophic lateral sclerosis: FTD: frontotemporal dementia.

Supplementary table 2. List of rare variants of unknown significance found in this study.

Gene	cDNA position	Aminoacid change	Case number	GnomAD NFE_MAF	SP_MAF	ALS/FTD_MAF
ANXA11	c.832A>G	p.I278V	14	0.005	0.008	0.009
DAO	c.992G>A	p.G331E	15	0.0001	0	0.009
DCTN1	c.526A>G	p.I196V	16	0.006	0.009	0.009
ERBB4	c.1122T>G	p.H374Q	17	0.002	0	0.009
FUS	c.404G>A	p.S135N	4, 18, 19	0.0002	0.015	0.028
GRN	c.329G>A	p.R110Q	14	$6x10^{-5}$	0	0.009
GRN	c.1297C>T	p.R433W	20	0.004	0.004	0.009
MAPT	c.637G>A	p.G213R	10	0.001	0.01	0.009
MAPT	c.953C>T	p.S318L	1, 10	0.001	0.009	0.019
MAPT	c.1280C>T	p.S427F	21	0.002	0.008	0.009
NEK1	c.1021G>A	p.A341T	3, 22	0.003	0.001	0.019
NEK1	c.2235T>G	p.N745K	9	0.006	0.001	0.009

Case number corresponds to the same that in table 1. GnomAD NFE_MAF: Minor allele frequency in the Non-Finnish European population from the Genome Aggregation Database; SP_MAF: aggregated minor allele frequency from Dopazo et al. .and the 1000 Genomes Project Consortium Spanish individuals; ALS/FTD_MAF: minor allele frequency in the group of ALS/FTD patients from this study.

Supplementary Methods

Genetic analyses

Whole-exome sequencing raw data was processed following GATK 3.4-46 best practices[1]. Reads were mapped to the human reference GRCh37.p13 build using the Burrows-Wheeler Aligner 0.7.10[2]. Duplicate reads were flagged using Picard Tools 1.119 (http://picard.sourceforge.net) and realignment of insertions and deletions (Indels) as well as base quality score recalibration was performed with GATK. Variant calling was executed with the Haplotype Caller tool of GATK. High quality single nucleotide variants and Indels (defined in our final data set as having a VQSLOD truth sensitivity of 99%) were kept in the final vcf file which was annotated using Annovar[3] and dbNSFP v3.0[4].

Neuropathological assessment

Neuropathological examination was performed according to standardized protocols at the Neurological Tissue Bank of the Biobanc-Hospital Clinic-IDIBAPS[5] and the Neuropathology Unit of the Hospital Universitario Fundación Alcorcón. Neuropathological examination of this latter case was completed at the CIEN Tissue Bank. At least 25 representative brain areas were embedded in paraffin and included frontal, temporal, parietal, and primary motor and visual regions, anterior and posterior basal ganglia, anterior, middle and posterior thalamic nuclei, amygdala, hippocampus, midbrain, pons, medulla oblongata, spinal cord (available for 14 cases), and cerebellum. For histologic evaluation, 5-µm-thick sections were stained with hematoxylin & eosin and luxol fast blue in selected areas including spinal cord. Immunohistochemistry was performed in selected areas using various primary antibodies: anti-bA4-amyloid (DAKO, Glostrup, Denmark, mc, clone 6F/3D), anti-phosphorylated tau (Thermo Scientific, Rockford, IL, USA; mc, clone AT8), anti-ubiquitin (DAKO, pc), anti-alpha-synuclein (Novocastra, Newcastle, UK; mc, clone KM51), anti-TDP-43 (Abnova, Taipei, Taiwan; mc, clone 2E2-D3), anti-FUS (Sigma Aldrich HPA008784, St. Louis, MO, USA, and Lifespan Biosciences, Seattle, WA, USA) anti-neurofilaments (Novocastra, clone RT97), anti-RD3 (Millipore, Temecula, CA, USA; mc, clone 8E6/C11), anti-RD4 (Millipore, mc, clone 1E1/A6), anti-alpha-internexin (Invitrogen, CA, USA; mc, clone 2E3), anti-p62 (BD Transduction Laboratories TM, NJ, USA; mc, clone 3/p62 lck ligand), anti-Transportin 1 (Abcam, clone D45) and anti-TAF15 (TAFII68, Bethyl laboratories Inc, Montgomery, TX, USA).

Supplementary references

1. McKenna A, Hanna M, Banks E, *et al.* The genome analysis toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res* 2010;20:1297-303.

2. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics* 2010;26:589-95.

3. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res* 2010;38:e164.

4. Liu X, Wu C, Li C, Boerwinkle E. dbNSFP v3.0: A One-Stop Database of Functional Predictions and Annotations for Human Nonsynonymous and Splice-Site SNVs. *Hum Mutat* 2016;37:235-41.

5. Gelpi E, Lladó A, Clarimón J, *et al.* Phenotypic variability within the inclusion body spectrum of basophilic inclusion body disease and neuronal intermediate filament inclusion disease in frontotemporal lobar degenerations with FUS-positive inclusions. *J Neuropathol Exp Neurol* 2012;71:795-805.