The role of genetics in amyotrophic lateral sclerosis: a large cohort study in Chinese mainland population

SUPPLEMENTARY MATERIALS

*These three supplementary tables were uploaded independently.

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Supplementary methods

Genetic analysis

DNA preparation, Repeat-primed polymerase chain reaction and whole exome sequencing

Genomic DNA was collected from peripheral blood leukocytes via standard phenol-chloroform procedures. The repeat-primed polymerase chain reaction (RP-PCR) was performed using triple primers listed in our previous study[1]. The intronic GGGGCC hexanucleotide repeat expansion (HREs) producing a characteristic "saw-tooth" pattern with a 6 base pair periodicity were identified, and more than 30 peaks were considered as potential large expansions. Genomic DNA of previous reported patients with HREs over 30 in *C9ORF72* was used as positive controls. For whole exome sequencing (WES), a total of 5ug DNA was fragmented to an average size of 350 bp using a Covaris LE220-plus Focused-Ultrasonicator, and the DNA library was constructed using a KAPA Library Amplification Kit according to the manufacture's instruction. WES was then performed using a NovaSeq 5000/6000 S2/S1 reagent kit (Illumina) as per standard protocols.

For WES, clean data were mapped to the reference genome (GRCh37/hg19) to obtain the bam file using the BWA Picard protocol[2]. Genotype calling was performed using Genome Analysis Toolkit's (GATK) Haplotype Caller software[3].

Sample quality control and variant quality control

The Samples with a high proportion of chimeric reads (>5%), high contamination (<5%), poor call rates (<90%), mean depth <10 X, or mean genotype-quality <65 were excluded from further analysis.

For variant quality control, we restricted the data to GENCODE coding regions, where Illumina exomes surpassed 10 X mean coverage. The "PASS" variants in GATK's variant quality score recalibration (VQSR) filter were included in further analysis. In addition, individual genotypes have to meet the following criteria: 1) genotype depth more than 10, 2) the allele balance (alternative allele cover/total allele cover) of heterozygous sites is between 0.2 and 0.8, and of homozygous sites > 0.8, 3) genotype quality (GQ) >20.

Allele frequency categorization

Allele frequencies were estimated using the public databases, the Exome Aggregation Consortium (ExAC), and the Genome Aggregation Database (GnomAD). We classified variants as rare variants using the following criteria: rare variants have a minor allele frequency (MAF) of <0.0001 in ExAC_EAS (East Asian) and GnomAD_ EAS.

Variants annotation

Variants of all the candidate genes (GI and GII), that met the above criteria were further analyzed. For rare variants, which were annotated as "missense", "inframe_deletion", "inframe_insertion", "frameshift", "stop-gain", or "splice region" were further classified as "deleterious" or "non-deleterious" based on the following criteria：1) all nonsense variants (stop-gain) and indels were regarded as deleterious; 2) splice variants were deleterious when they were predicted to affect splicing by at least one of two in silico tools, including Human Splicing Finder[4] and NetGene2[5]; 3) missense variants were only considered deleterious when they were predicted to be damaging by at least three of the 5 following in silico tools: Sorting Intolerant from Tolerant (SIFT)[6], Polymorphism Phenotyping v2 (PolyPhen-2)[7], Mutation Assessor[8], Functional Analysis through Hidden Markov Models (FATHMM)[9], Combined Annotation Dependent Depletion (CADD)[10]. For the known rare variants, pathogenicity as classified in the Clinvar database was considered to be important supporting evidence. Cosegregation analysis of candidate variants was performed on all available family members. Finally, in consideration of the varying degrees of genetic evidence for 41 involved genes in ALS, for the GI genes, rare variants were classified as pathogenic or likely pathogenic(P/LP), variant of uncertain significance (VUS), likely benign or benign according to the American College of Medical Genetics (ACMG) recommendations[11]; for the GII genes, rare variants were classified as deleterious and non-deleterious according to the five functional prediction software (for missense variants), criteria 1 (for nonsense variants) and criteria 2 (for splice variants).

Burden analysis

The sequencing kernel association test (SKAT) implemented in R packages AssotesteR were performed on the gene basis for genetic investigations into the collective risk of rare variants

in 40 genes for ALS (except C9orf72). Rare variants from 1866 in-house controls from WES were used as the control group (Figure 1). Rare variants were classified as damaging (P/LP variants in the GI genes and deleterious variants in the GII genes) and non-damaging (VUS, likely benign or benign in the GI genes and non-deleterious in the GII genes) variants in the ALS cohort and controls.

In order to explore which rare P/LP variants in the GI genes might contribute to the risk for ALS, the distribution of minor allele frequencies of rare P/LP variants in the GI genes between patients and controls were compared by standard fisher's exact test by two-stages analysis. In the initial stage, rare P/LP variants in each gene from 1587 cases and 1,866 in-house controls were analyzed, and the same analysis in the secondary stage were conducted including 1587 cases and controls from GnomAD-EAS (n=9977). Because a total of 95 rare P/LP variants in the GI genes were identified in our study, the p-value<5.3e-4 (0.05/95) was considered statistically significant after Bonferroni correction.

Haplotype analysis for variants identified in more than one patient

Due to all the patients involved in this study came from the unrelated families, to distinguish between the presence of a possible founder effect or a mutational hotspot for variants identified in more than one patient, we used polymorphisms-generated haplotypes to establish a common lineage. The SNP array platform used was Illumina Infinium Asian Screening Array-MD v1.0. Samples were processed using the Illumina manufacturer's recommended protocol. Genotyping and quality control were detailly were described in our previous study[12]. Haplotypes segregating with the disease were constructed to identify a possible ancestral haplotype.

Plasmid construction

The full-length cDNA of *TARDBP* was synthesized and separately cloned into pCMV-MCS vectors containing a 3*FLAG tag sequence. The mutations of G294V was generated from pCMV-MCS -3*Flag-TARDBP by the Q5® Site-Directed Mutagenesis Kit (NEB, USA, #E0554S).

Cell Culture and Transfection

HEK293T cells was obtained from the American Type Culture Collection (ATCC CRL-11268), which were cultured with Dulbecco's modified Eagle's medium (Sigma) supplemented with 10% fetal calf serum (Life Technologies). Cells were maintained in a humidified incubator at 37°C and 5% CO2. The plasmids were transfected with Lipofectamine 3000 (Invitrogen) for cell lines according to the manufacturer's protocol. After 48 h, transfected cells were collected for following experiments.

Immunofluorescence

Cells were fixed in 4% paraformaldehyde for 15 min and permeabilized with 0.3% Triton X-100 for 5 min. Cells were blocked with 5% bovine serum albumin for 30 min and incubated with primary antibodies overnight at 4℃. Mouse anti-FLAG (1:1000, Sigma cat# F1804) were used as primary antibodies. After incubation with the primary antibody, cells were washed three times with PBS, incubated with Alexa Fluor secondary antibodies (Invitrogen, 1:500) for 1 h at room temperature, and mounted with Vectashield hard-set mounting medium with DAPI (Vector Laboratories). Images were acquired using a laser scanning confocal microscope (Olympus).

Flow cytometric analysis

The Annexin V-FITC/PI (556547, BD, America) assay kit was used to detect the apoptosis. The experiments were conducted according to the user instructions. For apoptosis assay, after digesting by trypsin, the HEK-293T cells were collected and then washed twice with cold PBS. Subsequently, the cells were suspended by 400 μl Annexin V binding fluid, making the cell concentration 1×10^6 cells/ml. The cells suspension was then added into 5 μl Annexin V–FITC dyed liquid and incubated at 37℃ for 15mins. After that, the cells suspension was added into 10 μl PI dyed liquid and incubated at 37℃ for 5 min. Lastly, the samples were analyzed by cytomics FC 500 (Beckman, America).

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Table S1. Candidate ALS genes in this study

Genes	Protein	ALS	Inheritance	Locus	Refseq NM	OMIM*	PLI	First reported in ALS/FTD
Group 1 (GI, Well established causative genes)								
SOD1	Superoxide dismutase 1	ALS1	AD/AR	21q22.11	NM_000454.4	105400	0.18	Nature. 1993
FUS	Fused in sarcoma	ALS6	AD	16p11.2	NM_004960.3	608030	1.00	Science.2009
VAPB	Vesicle-associated membrane protein-associated protein B	ALS8	AD	20q13.32	NM_004738.5	608627	0.58	Am J Hum Genet. 2004
TARDBP	TAR DNA-Binding Protein, 43-Kd	ALS10	AD	1q36.22	NM_007375.3	612069	0.99	Science. 2008
OPTN	Optineurin	ALS12	AD	10p13	NM_021980.4	613435	0	Nature. 2010
VCP	Valosin Containing Protein	ALS14	AD	9p13.3	NM_007126.5	613954	1.00	Neuron. 2010
UBQLN2	Ubiquilin-2	ALS15	X-linked AD	Xp11.21	NM_013444.3	300857	0.85	Nature. 2011
C9orf72	Guanine nucleotide exchange C9orf72	ALS-FTD1	AD	9p21.2	NM_001256054.2	105550		Neuron.2011
PFN1	Profilin 1	ALS18	AD	17p13.2	NM_005022.4	614808	0.73	Nature. 2012
HNRNPA1	Heterogeneous nuclear ribonucleoprotein A1	ALS20	AD	12q13.13	NM_031157.4	615426	1	Nature. 2013
CHCHD10	Coiled-coil-helix-domain containing protein 10	ALS-FTD2	AD	22q11.23	NM_213720.2	615911	0	Brain. 2014
TBK1	Tank-binding kinase 1	ALS-FTD4	AD	12q14.2	NM_013254.3	616439	0.08	Science. 2015
NEK1	Never in mitosis gene A-related kinase 1	ALS24	AD	4q33	NM_012224.2	617892	0	Brain. 2016
ANXA11	Annexin 11	ALS23	AD	10q22.3	NM_001157.2	617839	0	Sci Trans Med. 2017
KIF5A	Kinesin family member 5A	ALS25	AD	12q13.3	NM_004984.3	617921	1	Neuron. 2018
DNAJC7[#]	DNAJ/HSP40 homolog, subfamily C, member 7	-	AD	17q21.2	NM_003315.3	-	0.99	Nat Neurosci. 2019
Group 2 (GII, new, needing to be confirmed genes, or risk factor)								
NEFH	Neurofilament heavy	-	AD, AR	22q12.2	NM_021076.3	105400	0	Nature. 1995
MAPT	microtubule associated protein tau	-	-	17q21.31	NM_016835.4	600274	0.01	Ann Neurol. 1997
ALS2	Amyotrophic lateral sclerosis 2, ALS2	ALS2	AR	2q33.1	NM_020919.4	205100	1.00	Nat Genet. 2001
SETX	Sentaxin	ALS4	AD	9q34.13	NM_015046.5	602433	0.95	Am J Hum Genet. 2004
DCTN1	Dynactin subunit 1	ALS	AD/AR	2q13	NM_004082.4	105400	0.08	Neurology. 2004

PRPH	Peripherin	-	AD, AR	12q13.12	NM_006262.3	105400	0	J Biol Chem. 2004
ANG	Angiogenin	ALS9	AD	14q11.2	NM_001145.4	611895	0.29	Nat Genet. 2006
GRN	granulin precursor	-	AD	17q21.31	NM_002087.3	607485	0.07	Nature 2006
CHMP2B	Charged multivesicular body protein 2b	ALS17	AD	3p11.2	NM_014043.4	614676	0	Neurology. 2006
PNPLA6	patatin like phospholipase domain containing 6	-	-	19p13.2	NM_006702.5	-	0	Am J Hum Genet. 2008
ELP3	elongator acetyltransferase complex subunit 3	-	-	8p21.1	NM_001284220.2	-	0	Hum Mol Genet. 2009
FIG4	Polyphosphoinositide 5- phosphatase	ALS11	AD	6q21	NM_014845.5	612577	0	Am J Hum Genet. 2009
DAO	D-amino-acid oxidase	ALS	AD	12q24.11	NM_001917.5	-	0	PNAS. 2010
SIGMAR1	Sigma non-opioid intracellular receptor	ALS16	AR	9p13.3	NM_005866.4	614373	0.17	Ann Neurol. 2010
SPG11	Spatacsin	ALS5	AR	15q21.1	NM_025137.4	602099	0.44	Brain. 2010
SQSTM1	Sequestosome-1	ALS-FTD3	AD	5q35.3	NM_003900.4	616437	0	Arch Neurol. 2011
EWSR1	protein associated factor 15	-	-	17q12	NM_139215.3	-	0.17	Hum Mol Genet. 2012
HNRNPA2B1	Heterogeneous nuclear ribonucleoprotein A2/B1	ALS-FTD	AD	7p15.2	NM_002137.4	-	1	Nature. 2013
ERBB4	Erb-b2 receptor tyrosine kinase 4	ALS19	AD	2q34	NM_005235.3	615515	1	Am J Hum Genet. 2013
MATR3	Martrin 3	ALS21	AD	5q31.2	NM_199189.2	606070	1	Nat Neurosci. 2014
TUBA4A	Tubulin, alpha-4A	ALS22	AD	2q35	NM_006000.2	616208	0.16	Neuron. 2014
CCNF	Cyclin F	-	AD	16p13.3	NM_001761.2	-	0.13	Nat Commun. 2016
TIA1	Cytotoxic granule-associated RNA binding protein	-	AD	2p13.3	NM_022173.3	-	0.27	Neuron. 2017
GLT8D1	Glycosyltransferase 8 domain-containing protein 1	-	AD	3p21.1	NM_152932.2	-	0	Cell Rep. 2019
CYLD	CYLD lysine-6 deubiquitinase	-	AD	16q12.1	NM_015247.2	-	1	Brain. 2020

*phenotype MIM number; #new gene having been identified by burden testing; pLI, probability of loss of function intolerance

Table S2. The geographical distribution of ALS patients.

Regions	N	Sex(M/F)
Sichuan	1293	760/533
Chongqing	81	38/43
Guizhou	46	30/16
Yunnan	45	22/23
Gansu	24	14/10
Xizang	19	14/5
Hubei	11	5/6
Henan	10	4/6
Xinjiang	8	4/4
Jiangxi	7	5/2
Shandong	6	3/3
Other provinces*	35	20/15
Unknown	3	3/0
Total	1587	922/665

*Other provinces including Shaanxi, Anhui, Hebei, Guangxi, Helongjiang, Jiangsu, Liaoning, Hunan, Qinghai, Shanxi, Zhejiang, Jilin, Fujian, Inner Mongolia.

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Table S3.The ethnical distribution of ALS patients.

Race	N	Sex(M/F)
Han	1530	889/641
Zang	30	19/11
Yi	6	3/3
Qiang	5	3/2
Hui	3	1/2
Buyi	3	1/2
Miao	2	1/1
Tu	2	1/1
Li	1	1/0
Man	1	1/0
Naxi	1	0/1
Yao	1	1/0
Dong	1	0/1
Zhuang	1	1/0
Total	1587	922/665

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Table S4. The information of 155 ALS patients carried P/LP variants of ALS causative genes

No.	Case ID	ALS	Genes	Chromosomal Position	Refseq ID	Exon	cDNA change	Amino Acid change	Variant Type	dbSNPs	In house controls	ExAC_EAS	GnomAD_EAS	Classification	Sex	AAO	FH	ACER	Status	Duration
1	2713	ALS1	*SOD1*	chr21:33032095	NM_000454.4	1	c.13G>A	p.Ala5Thr	missense	rs121912444	0	0	0	P	M	57.1	AD	94	Death	47.1
2	3001	ALS1	*SOD1*	chr21:33032096	NM_000454.4	1	c.14C>T	p.Ala5Val	missense	rs121912442	0	0	0	P	F	54.6	No	-	Death	14.2
3	3512	ALS1	*SOD1*	chr21:33032098	NM_000454.4	1	c.16G>T	p.Val6Leu	missense	-	0	-	-	LP	F	63.3	No	83	Alive	25.9
4	298	ALS1	*SOD1*	chr21:33036125	NM_000454.4	2	c.95T>C	p.Val32Ala	missense	-	0	-	-	LP	F	46.4	No	-	Death	168.4
5	728	ALS1	*SOD1*	chr21:33036145	NM_000454.4	2	c.115C>G	p.Leu39Val	missense	rs121912432	0	0	0	P	M	58.8	AD	-	Death	31.5
6	1164	ALS1	*SOD1*	chr21:33036145	NM_000454.4	2	c.115C>G	p.Leu39Val	missense	rs121912432	0	0	0	P	F	39.7	AD	70	Death	24.8
7	1967	ALS1	*SOD1*	chr21:33036145	NM_000454.4	2	c.115C>G	p.Leu39Val	missense	rs121912432	0	0	0	P	M	51.5	AD	72	Death	13.6
8	1285	ALS1	*SOD1*	chr21:33036146	NM_000454.4	2	c.116T>G	p.Leu39Arg	missense	-	0	-	-	P	M	32.4	AD	99	Death	24.3
9	1405	ALS1	*SOD1*	chr21:33036155	NM_000454.4	2	c.125G>A	p.Gly42Asp	missense	rs121912434	0	0	0	P	M	59.0	No	92	Death	18.2
10	912	ALS1	*SOD1*	chr21:33036161	NM_000454.4	2	c.131A>G	p.His44Arg	missense	rs121912435	0	0	0	P	F	47.5	AD	93	Death	9.2
11	3377	ALS1	*SOD1*	chr21:33036170	NM_000454.4	2	c.140A>G	p.His47Arg	missense	rs121912443	0	0	0	P	M	55.1	No	-	Alive	23.8
12	2852	ALS1	*SOD1*	chr21:33036170	NM_000454.4	2	c.140A>G	p.His47Arg	missense	rs121912443	0	0	0	P	F	43.3	AD	97	Alive	50.1
13	3618	ALS1	*SOD1*	chr21:33036170	NM_000454.4	2	c.140A>G	p.His47Arg	missense	rs121912443	0	0	0	P	M	34.1	AD	91	Alive	105.5
14	426	ALS1	*SOD1*	chr21:33036173	NM_000454.4	2	c.143T>C	p.Val48Ala	missense	-	0	-	-	LP	M	63.5	No	-	Death	5.1
15	467	ALS1	*SOD1*	chr21:33038765	NM_000454.4	3	c.173G>A	p.Cys58Tyr	missense	-	0	-	-	LP	M	73.1	No	-	Death	30.5
16	246	ALS1	*SOD1*	chr21:33038791	NM_000454.4	3	c.199C>A	p.Pro67Thr	missense	-	0	-	-	LP	M	37.6	No	-	Death	58.8
17	949	ALS1	*SOD1*	chr21:33038791	NM_000454.4	3	c.199C>T	p.Pro67Ser	missense	rs1356474292	0	-	-	P	F	47.3	No	54	Death	14.2
18	2373	ALS1	*SOD1*	chr21:33038791	NM_000454.4	3	c.199C>G	p.Pro67Ala	missense	-	0	-	-	P	F	64.6	No	-	Loss	16.0
19	3228	ALS1	*SOD1*	chr21:33038791	NM_000454.4	3	c.199C>G	p.Pro67Ala	missense	-	0	-	-	P	M	41.1	AD	81	Alive	46.5
20	3860	ALS1	*SOD1*	chr21:33038791	NM_000454.4	3	c.199C>T	p.Pro67Ser	missense	rs1356474292	0	-	-	P	F	39.8	No	-	Alive	16.0
21	3582	ALS1	*SOD1*	chr21:33038800	NM_000454.4	3	c.208A>G	p.Arg70Gly	missense	-	0	-	-	LP	F	57.1	No	78	Alive	56.8
22	3116	ALS1	*SOD1*	chr21:33038809	NM_000454.4	3	c.217G>A	p.Gly73Ser	missense	rs121912455	0	0	0	P	M	32.6	No	87	Death	14.5
23	1392	ALS1	*SOD1*	chr21:33038810	NM_000454.4	3	c.218G>A	p.Gly73Asp	missense	-	0	-	-	LP	M	44.1	No	86	Death	63.9
24	3670	ALS1	*SOD1*	chr21:33038810	NM_000454.4	3	c.218G>A	p.Gly73Asp	missense	-	0	-	-	LP	M	50.4	AD	78	Alive	8.0
25	2043	ALS1	*SOD1*	chr21:33039581	NM_000454.4	4	c.250G>C	p.Asp84His	missense	-	0	-	-	LP	M	57.9	No	93	Death	21.5
26	2893	ALS1	*SOD1*	chr21:33039581	NM_000454.4	4	c.250G>C	p.Asp84His	missense		0			LP	F	57.3	No	84	Death	13.3
27	3218	ALS1	*SOD1*	chr21:33039586	NM_000454.4	4	c.255G>C	p.Ala85Phe	missense	-	0	-	-	P	F	43.8	AD	75	Alive	26.3
28	692	ALS1	*SOD1*	chr21:33039591	NM_000454.4	4	c.260A>T	p.Asn87Ile	missense	-	0	-	-	LP	F	55.2	No	84	Death	41.6
29	2374	ALS1	*SOD1*	chr21:33039599	NM_000454.4	4	c.268G>A	p.Ala90Thr	missense	-	0	-	-	P	M	61.7	No	-	Death	43.7
30	2718	ALS1	*SOD1*	chr21:33039599	NM_000454.4	4	c.268G>A	p.Ala90Thr	missense	-	0	-	-	P	F	49.7	No	-	Loss	10.4
31	3831	ALS1	*SOD1*	chr21:33039633	NM_000454.4	4	c.302 A>G	p.Glu101Gly	missense	rs121912439	0	-	-	P	M	35.0	AD	-	Alive	32.4

32	2045	ALS1	*SOD1*	chr21:33039637	NM_000454.4	4	c.306T>G	p.Asp102Glu	missense	-	-	-	0	LP	F	55.6	No	93	Death	27.0
33	786	ALS1	*SOD1*	chr21:33039650	NM_000454.4	4	c.319C>T	p.Leu107Phe	missense	-	0	-	-	P	M	43.0	No	-	Death	27.4
34	425	ALS1	*SOD1*	chr21:33039666	NM_000454.4	4	c.335G>A	p.Cys112Tyr	missense	-	0	-	-	P	M	26.7	No	-	Death	16.6
35	1488	ALS1	*SOD1*	chr21:33039666	NM_000454.4	4	c.335G>A	p.Cys112Tyr	missense	-	0	-	-	P	M	38.8	No	-	Death	40.1
36	1756	ALS1	*SOD1*	chr21:33039672	NM_000454.4	4	c.341T>C	p.Ile114Thr	missense	rs121912441	0	0	0	P	F	54.4	No	-	Death	34.0
37	1697	ALS1	*SOD1*	chr21:33040784	NM_000454.4	5	c.358G>T	p.Val120Phe	missense	-	0	-	-	LP	M	32.1	No	80	Death	30.9
38	2431	ALS1	*SOD1*	chr21:33040789	NM_000454.4	5	c.363T>G	p.His121Gln	missense	-	0	-	-	P	M	49.8	No	67	Death	26.2
39	3534	ALS1	*SOD1*	chr21:33040809	NM_000454.4	5	c.383G>T	p.Gly128Val	missense	-	0	-	-	P	M	32.7	No	97	Death	10.4
40	3416	ALS1	*SOD1*	chr21:33040815	NM_000454.4	5	c.389_390del	p.Gly131Lysfs*2	frameshift	-	0	-	-	P	F	21.4	AD	94	Alive	23.8
41	3186	ALS1	*SOD1*	chr21:33040830	NM_000454.4	5	c.404G>A	p.Ser135Asn	missense	rs121912451	0	0	0	P	F	47.8	No	-	Death	5.0
42	505	ALS1	*SOD1*	chr21:33040869	NM_000454.4	5	c.443G>A	p.Gly148Asp	missense	-	0	-	-	LP	F	29.7	No	-	Alive	127.5
43	2691	ALS1	*SOD1*	chr21:33040881	NM_000454.4	5	c.455T>G	p.Ile152Ser	missense	-	0	-	-	LP	F	50.3	AD	-	Death	30.5
44	2980	ALS6	*FUS*	chr16:31193937	NM_004960.3	3	c.142T>C	p.Ser48Pro	missense	-	0	-	-	LP	M	55.0	No	53	Alive	11.4
45	2863	ALS6	*FUS*	chr16:31195209	NM_004960.3	4	c.221G>A	p.Gly74Glu	missense	-	0		-	LP	F	48.3	No	75	Death	35.8
46	1897	ALS6	*FUS*	chr16:31196472	NM_004960.3	6	c.736G>A	p.Gly246Ser	missense	rs1240931401	0	0	0	LP	M	60.5	No	-	Loss	3.3
47	995	ALS6	*FUS*	chr16:31202283	NM_004960.3	13	c.1394-1G>C	-	splicing	-	0	-	-	LP	F	49.4	No	92	Loss	3.3
48	1373	ALS6	*FUS*	chr16:31202373	NM_004960.3	14	c.1483C>T	p.Arg495*	stop-gain	rs387906627	0	0	0	P	F	25.3	No	-	Death	43.1
49	1994	ALS6	*FUS*	chr16:31202374	NM_004960.3	14	c.1483C>T	p.Arg495*	stop-gain	rs387906627	0	0	0	P	M	26.9	No	-	Alive	60.3
50	952	ALS6	*FUS*	chr16:31202375	NM_004960.3	14	c.1483C>T	p.Arg495*	stop-gain	rs387906627	0	0	0	P	M	26.6	No	83	Death	5.9
51	117	ALS6	*FUS*	chr16:31202739	NM_004960.3	15	c.1561C>T	p.Arg521Cys	missense	rs121909668	0	0	0	P	F	64.3	No	-	Death	30.5
52	2324	ALS6	*FUS*	chr16:31202739	NM_004960.3	15	c.1561C>T	p.Arg521Cys	missense	rs121909668	0	0	0	P	F	35.9	No	76	Loss	9.7
53	3263	ALS6	*FUS*	chr16:31202739	NM_004960.3	15	c.1561C>T	p.Arg521Cys	missense	rs121909668	0	0	0	P	F	27.6	No	98	Alive	26.1
54	2534	ALS6	*FUS*	chr16:31202739	NM_004960.3	15	c.1561C>T	p.Arg521Cys	missense	rs121909668	0	0	0	P	F	43.3	AD	74	Death	34.1
55	3822	ALS6	*FUS*	chr16:31202739	NM_004960.3	15	c.1561C>T	p.Arg521Cys	missense	rs121909668	0	0	0	P	M	31.9	AD	-	Alive	14.1
56	409	ALS6	*FUS*	chr16:31202740	NM_004960.3	15	c.1562G>A	p.Arg521His	missense	rs121909671	0.0003	0	0	P	F	43.1	No	-	Death	37.0
57	827	ALS6	*FUS*	chr16:31202740	NM_004960.3	15	c.1562G>A	p.Arg521His	missense	rs121909671	0.0003	0	0	P	M	42.8	No	-	Death	61.9
58	1893	ALS6	*FUS*	chr16:31202740	NM_004960.3	15	c.1562G>A	p.Arg521His	missense	rs121909671	0.0003	0	0	P	F	45.6	No	77	Death	22.8
59	2744	ALS6	*FUS*	chr16:31202740	NM_004960.3	15	c.1562G>A	p.Arg521His	missense	rs121909671	0.0003	0	0	P	F	45.0	No	86	Death	42.8
60	3329	ALS6	*FUS*	chr16:31202740	NM_004960.3	15	c.1562G>T	p.Arg521Leu	missense	-	0	0	0	P	M	22.5	AD	95	Alive	19.4
61	3217	ALS6	*FUS*	chr16:31202740	NM_004960.3	15	c.1562G>A	p.Arg521His	missense	rs121909671	0.0003	0	0	P	F	32.1	AD	-	Alive	14.4
62	661	ALS6	*FUS*	chr16:31202752	NM_004960.3	15	c.1574C>T	p.Pro525Leu	missense	rs886041390	0	0	0	P	M	24.6	No	-	Death	14.2
63	460	ALS8	*VAPB*	chr20:57019259	NM_004738.4	6	c.700G>A	p.Val234Ile	missense	rs149215094	0	0	0	LP	M	62.1	No	-	Death	5.6
64	3495	ALS10	*TARDBP*	chr1:11080633	NM_007375.3	5	c.691T>C	p.Phe231Leu	missense	-	0	0	0	LP	F	60.4	AD	-	Death	10.7
65	812	ALS10	*TARDBP*	chr1:11082347	NM_007375.3	6	c.881G>T	p.Gly294Val	missense	rs80356721	0	0	0	P	F	55.0	No	-	Death	42.7

66	913	ALS10	*TARDBP*	chr1:11082347	NM_007375.3	6	c.881G>T	p.Gly294Val	missense	rs80356721	0	0	0	P	M	72.2	No	81	Death	25.2
67	2411	ALS10	*TARDBP*	chr1:11082347	NM_007375.3	6	c.881G>T	p.Gly294Val	missense	rs80356721	0	0	0	P	M	46.3	No	-	Death	22.8
68	2414	ALS10	*TARDBP*	chr1:11082347	NM_007375.3	6	c.881G>T	p.Gly294Val	missense	rs80356721	0	0	0	P	M	57.3	No	96	Death	20.0
69	2755	ALS10	*TARDBP*	chr1:11082347	NM_007375.3	6	c.881G>T	p.Gly294Val	missense	rs80356721	0	0	0	P	F	62.4	No	-	Death	17.6
70	2858	ALS10	*TARDBP*	chr1:11082347	NM_007375.3	6	c.881G>T	p.Gly294Val	missense	rs80356721	0	0	0	P	F	51.7	No	-	Death	20.9
71	1468	ALS10	*TARDBP*	chr1:11082593	NM_007375.3	6	c.1127G>A	p.Gly376Asp	missense	-	0	0	0	LP	F	49.4	No	80	Death	20.2
72	2983	ALS10	*TARDBP*	chr1:11082598	NM_007375.3	6	c.1132A>G	p.Asn378Asp	missense	-	0	0	0	LP	M	52.6	No	78	Death	9.3
73	3026	ALS10	*TARDBP*	chr1:11082598	NM_007375.3	6	c.1132A>G	p.Asn378Asp	missense	-	0	0	0	LP	F	32.9	AD	-	Death	6.7
74	291	ALS10	*TARDBP*	chr1:11082599	NM_007375.3	6	c.1133A>G	p.Asn378Ser	missense	-	-	-	0	LP	M	39.8	No	-	Loss	79.7
75	2249	ALS10	*TARDBP*	chr1:11082599	NM_007375.3	6	c.1133A>G	p.Asn378Ser	missense	-	-	-	0	LP	M	63	No	-	Loss	10.1
76	1825	ALS10	*TARDBP*	chr1:11082613	NM_007375.3	6	c.1147A>G	p.Ile383Val	missense	rs80356740	0	0	0	LP	F	60.2	No	50	Death	51.8
77	3030	ALS10	*TARDBP*	chr1:11082613	NM_007375.3	6	c.1147A>G	p.Ile383Val	missense	rs80356740	0	0	0	LP	F	63.2	No	-	Death	9.63
78	3095	ALS10	*TARDBP*	chr1:11082613	NM_007375.3	6	c.1147A>G	p.Ile383Val	missense	rs80356740	0	0	0	LP	M	52.8	No	65	Death	14.4
79	757	ALS12	*OPTN*	chr10:13169854	NM_021980.4	11	c.1352T>C	p.Ile451Thr	missense	rs772864480	0	0	0	LP	M	65.3	No	-	Alive	99.3
80	1804	ALS12	*OPTN*	chr10:13169854	NM_021980.4	11	c.1352T>C	p.Ile451Thr	missense	rs772864480	0	0	0	LP	M	63.2	No	-	Alive	61.6
81	2148	ALS12	*OPTN*	chr10:13169899	NM_021980.4	11	c.1397delC	p.Ala466Valfs*34	frameshift	-	0	-	-	LP	F	41.5	AD	-	Death	20.5
82	2537	ALS12	*OPTN*	chr10:13174098	NM_021980.4	12	c.1433A>G	p.Glu478Gly	missense	rs267606929	0	-	-	LP	F	52.4	No	-	Death	16.2
83	2996	ALS12	*OPTN*	chr10:13175550	NM_021980.4	13	c.1583_1584del	p.Ser528*	Stopgain	-	0	-	0	LP	F	57.4	No	-	Loss	11.3
84	3362	ALS14	*VCP*	chr9:35061164	NM_007126.4	11	c.1207A>G	p.Thr403Ala	missense	-	0	-	-	LP	M	64.7	No	60	Death	9.7
85	3146	ALS14	*VCP*	chr9:35065361	NM_007126.4	5	c.463C>T	p.Arg155Cys	missense	rs121909330	0	-	-	P	F	49.8	AD	90	Alive	69.5
86	3339	ALS14	*VCP*	chr9:35066752	NM_007126.4	4	c.365C>T	p.Thr122Ile	missense	-	0	-	-	LP	M	46.4	AD	82	Alive	58.3
87	3368	ALS14	*VCP*	chr9:35067922	NM_007126.4	3	c.268A>G	p.Asn90Asp	missense	-	0	-	-	LP	M	38.6	No	90	Alive	49.2
88	1593	ALS15	*UBQLN2*	X:56591766	NM_013444.3	1	c.1460C>T	p.Thr487Ile	missense	-	0	-	0	LP	M	38.6	AD	78	Death	38.0
89	2064	ALS15	*UBQLN2*	X:56591766	NM_013444.3	1	c.1460C>T	p.Thr487Ile	missense	-	0	-	0	LP	F	44.4	No	-	Death	37.9
90	3500	ALS15	*UBQLN2*	X:56591796	NM_013444.3	1	c.1490C>T	p.Pro497Leu	missense	rs387906709	0	-	-	P	F	21.4	No	86	Alive	20.3
91	750	ALS15	*UBQLN2*	X:56591822	NM_013444.3	1	c.1516C>T	p.Pro506Ser	missense	-	0	-	0	P	F	32.9	No	-	Death	5.1
92	1922	ALS18	*PFN1*	chr17:4851616	NM_005022.3	1	c.74A>G	p.Tyr25Cys	missense	-	0	0	0	LP	M	81.8	No	71	Alive	66.0
93	333	A-F1	*C9orf72*	-	-	-	-	-	-	-	-	-	-	P	M	58.9	No	-	Death	4.3
94	488	A-F1	*C9orf72*	-	-	-	-	-	-	-	-	-	-	P	M	45.8	No	-	Death	28.2
95	503	A-F1	*C9orf72*	-	-	-	-	-	-	-	-	-	-	P	M	70.9	No	-	Death	24.9
96	663	A-F1	*C9orf72*	-	-	-	-	-	-	-	-	-	-	P	M	45.6	No	-	Loss	4.3
97	850	A-F1	*C9orf72*	-	-	-	-	-	-	-	-	-	-	P	M	47.6	No	75	Death	30.9
98	1056	A-F1	*C9orf72*	-	-	-	-	-	-	-	-	-	-	P	M	47.5	No	33	Death	24.3
99	1184	A-F1	*C9orf72*	-	-	-	-	-	-	-	-	-	-	P	F	46.8	No	60	Death	31.4

100	1398	A-F1	*C9orf72*	-	-	-	-	-	-	-	-	-	-	P	F	47.3	No	66	Death	17.2
101	1733	A-F1	*C9orf72*	-	-	-	-	-	-	-	-	-	-	P	M	55.4	No	-	Death	48.8
102	1833	A-F1	*C9orf72*	-	-	-	-	-	-	-	-	-	-	P	F	50.1	No	90	Death	37.4
103	2047	A-F1	*C9orf72*	-	-	-	-	-	-	-	-	-	-	P	M	54.0	No	81	Death	21.4
104	2408	A-F1	*C9orf72*	-	-	-	-	-	-	-	-	-	-	P	F	60.5	No	-	Death	42.6
105	2856	A-F1	*C9orf72*	-	-	-	-	-	-	-	-	-	-	P	M	69.5	No	-	Alive	33.9
106	2987	A-F1	*C9orf72*	-	-	-	-	-	-	-	-	-	-	P	F	66.1	No	94	Alive	29.9
107	3043	A-F1	*C9orf72*	-	-	-	-	-	-	-	-	-	-	P	M	59.9	No	72	Alive	32.7
108	3069	A-F1	*C9orf72*	-	-	-	-	-	-	-	-	-	-	P	F	61.8	No	-	Death	17.8
109	3200	A-F1	*C9orf72*	-	-	-	-	-	-	-	-	-	-	P	M	62.3	No	82	Alive	38.9
110	3446	A-F1	*C9orf72*	-	-	-	-	-	-	-	-	-	-	P	M	49.1	No	85	Alive	14.6
111	2817	A-F1	*C9orf72*	-	-	-	-	-	-	-	-	-	-	P	M	54.8	No	63	Death	27.0
112	2499	A-F1	*C9orf72*	-	-	-	-	-	-	-	-	-	-	P	M	51.8	No	-	Death	18.9
113	2625	ALS20	*HNRNPA1*	chr12:54677706	NM_031157.3	9	c.1018C>T	p.Pro340Ser	missense	-	-	-	-	LP	M	25.5	No	98	Alive	49.2
114	2336	A-F2	*CHCHD10*	chr22:24108215	NM_213720.2	4	c.410-2A>G	-	splicing	-	0	-	0	LP	M	67.9	No	-	Death	60.9
115	1243	A-F2	*CHCHD10*	chr22:24108418	NM_213720.2		c.306G>C	p.Gln102His	missense	-	-	-	0	LP	M	44.1	No	86	Death	63.5
116	1937	A-F4	*TBK1*	chr12:64858158	NM_013254.3	4	c.273T>A	p.Cys91*	Stopgain	-	0	-	-	LP	M	42.3	No	80	Death	26.9
117	3240	A-F4	*TBK1*	chr12:64860863	NM_013254.3	I5	c.540+1G>C	-	splicing	-	0	-	-	LP	M	62.0	No	60	Alive	14.3
118	2564	A-F4	*TBK1*	chr12:64868152	NM_013254.3	6	c.683G>A	p.Arg228His	missense	rs748622208	0	0	0	LP	M	40.9	No	90	Alive	11.9
119	1658	A-F4	*TBK1*	chr12:64873890	NM_013254.3	7	c.800G>A	p.Cys267Tyr	missense	-	0	-	-	LP	F	46.1	No	-	Death	18.8
120	2263	A-F4	*TBK1*	chr12:64875688	NM_013254.3	8	c.879G>A	p.Trp293*	Stopgain	-	0	-	-	LP	M	74.9	No	74	Death	10.1
121	1579	A-F4	*TBK1*	chr12:64875728	NM_013254.3	8	c.919C>T	p.His307Tyr	missense	-	0	-	-	LP	M	56.5	No	88	Death	19.2
122	1631	A-F4	*TBK1*	chr12:64875728	NM_013254.3	8	c.919C>T	p.His307Tyr	missense	-	0	-	-	LP	M	67.7	No	-	Death	21.3
123	2572	A-F4	*TBK1*	chr12:64879233	NM_013254.3	I9	c.1190-2A>T	-	splicing	-	0	-	-	LP	F	20.1	No	91	Alive	52.2
124	1821	A-F4	*TBK1*	chr12:64882308	NM_013254.3	12	c.1382_1385del	p.Thr462Lysfs*3	frameshift	-	0	-	-	P	M	45.0	No	-	Alive	86.4
125	2493	A-F4	*TBK1*	chr12:64889511	NM_013254.3	15	c.1676T>G	p.Met559Arg	missense	-	0	-	-	LP	F	34.1	No	95	Alive	45.1
126	2497	ALS24	*NEK1*	chr4:170315675	NM_012224.2	34	c.3764-1G>C	-	splicing	-	0	-	-	LP	M	49.8	No	93	Alive	43.0
127	3242	ALS24	*NEK1*	chr4:170321671	NM_012224.2	32	c.3630+1G>T	-	splicing	-	0	-	-	LP	F	68.0	No	-	Alive	33.9
128	1995	ALS24	*NEK1*	chr4:170322802	NM_012224.2	31	c.3499+1G>A	-	splicing	-	0	-	-	LP	M	61.8	No	-	Death	20.7
129	3367	ALS24	*NEK1*	chr4:170354729	NM_012224.2	27	c.2768G>A	p.Trp923*	Stopgain	-	0	-	-	LP	F	48.0	No	82	Death	17.7
130	465	ALS24	*NEK1*	chr4:170384477	NM_012224.2	25	c.2420C>T	p.Pro807Leu	missense	rs368699255	0	0	0	LP	F	53.0	No	-	Death	60.5
131	682	ALS24	*NEK1*	chr4:170384477	NM_012224.2	25	c.2420C>T	p.Pro807Leu	missense	rs368699255	0	0	0	LP	M	62.1	No	-	Death	40.6
132	1567	ALS24	*NEK1*	chr4:170384477	NM_012224.2	25	c.2420C>T	p.Pro807Leu	missense	rs368699255	0	0	0	LP	M	47.7	No	83	Loss	6.3
133	2107	ALS24	*NEK1*	chr4:170428268	NM_012224.2	21	c.1843C>T	p.Arg615Cys	missense	-	0	-	0	LP	M	67.8	No	91	Alive	69.5

134	2438	ALS24	*NEK1*	chr4:170429929	NM_001199398	19	c.1607_1608del	p.Lys536Argfs*18	frameshift	-	0	-	-	LP	F	78.5	No	-	Alive	61.4
135	2383	ALS24	*NEK1*	chr4:170482706	NM_012224.2	15	c.1192-1G>T	-	splicing	-	0	-	-	LP	F	48.1	No	67	Death	23.8
136	3202	ALS24	*NEK1*	chr4:170498223	NM_012224.2	11	c.876delA	p.Arg292Serfs*28	frameshift	-	0	-	-	LP	F	72.8	No	-	Alive	18.9
137	1525	ALS24	*NEK1*	chr4:170506597	NM_012224.2	9	c.710delA	p.Gln237Argfs*22	frameshift	-	0	-	-	LP	M	54.4	No	65	Loss	42.3
138	876	ALS24	*NEK1*	chr4:170506666	NM_012224.2	9	c.641A>G	p.Lys214Arg	missense	-	0.0003	5.8E-5	-	LP	M	41.9	No	90	Death	37.6
139	1415	ALS24	*NEK1*	chr4:170506666	NM_012224.2	9	c.641A>G	p.Lys214Arg	missense	-	0.0003	5.8E-5	-	LP	M	61.0	No	89	Death	68.0
140	1558	ALS24	*NEK1*	chr4:170506666	NM_012224.2	9	c.641A>G	p.Lys214Arg	missense	-	0.0003	5.8E-5	-	LP	F	46.1	No	84	Death	31.9
141	1864	ALS24	*NEK1*	chr4:170506666	NM_012224.2	9	c.641A>G	p.Lys214Arg	missense	-	0.003	5.8E-5	-	LP	F	48.9	No	-	Death	70.0
142	2425	ALS24	*NEK1*	chr4:170523180	NM_012224.2	3	c.193C>T	p.Gln65*	Stopgain	-	0	-	-	LP	M	67.1	No	-	Death	30.9
143	2599	ALS23	*ANXA11*	chr10:81923147	NM_001157.2	10	c.1044A>C	p.Glu348Asp	missense	rs756614787	0	0	0	LP	F	75.4	No	-	Death	40.7
144	3048	ALS23	*ANXA11*	chr10:81923147	NM_001157.2	10	c.1044A>C	p.Glu348Asp	missense	rs756614787	0	0	0	LP	M	49.5	No	86	Alive	31.5
145	3223	ALS23	*ANXA11*	chr10:81929004	NM_001157.2	4	c.282delC	p.Ser95Leufs*56	frameshift	rs777070421	0	0	0	LP	M	42.2	No	59	Alive	46.5
146	939	ALS23	*ANXA11*	chr10:81930590	NM_001157.2	3	c.137C>T	p.Ala46Val	missense	rs762084152	0	0	0	LP	F	61.1	No	-	Death	157.3
147	345	ALS23	*ANXA11*	chr10:81930608	NM_001157.2	3	c.119A>G	p.Asp40Gly	missense	-	0	-	0	LP	F	62.2	No	-	Death	90.9
148	811	ALS23	*ANXA11*	chr10:81930608	NM_001157.2	3	c.119A>G	p.Asp40Gly	missense	-	0	-	0	LP	M	66.4	No	-	Loss	10.0
149	2320	ALS23	*ANXA11*	chr10:81930608	NM_001157.2	3	c.119A>G	p.Asp40Gly	missense	-	0	-	0	LP	F	59.1	No	-	Death	51.6
150	2733	ALS25	*KIF5A*	chr12:57963461	NM_004984.3	11	c.1112G>T	p.Arg371Leu	missense	-	0	-	-	LP	M	55.6	No	-	Death	68.4
151	383	ALS25	*KIF5A*	chr12:57965871	NM_004984.3	14	c.1390G>C	p.Glu464Gln	missense	-	0	-	-	LP	M	53.7	No	-	Death	24.1
152	2722	ALS25	*KIF5A*	chr12:57976382	NM_004984.3	27	c.2993-3C>T	-	splicing	-	0	-	0	P#	M	52.5	AD	65	Death	22.2
153	2114	-	*DNAJC7*	chr17:40133978	NM_003315.3	12	c.1279A>T	p.Lys427*	Stopgain	-	0	-	0	LP	F	53.6	No	76	Death	32.9
154	1731	-	*DNAJC7*	chr17:40135592	NM_003315.3	10	c.1070_1073del	p.Thr357Argfs*10	frameshift	-	0	-	-	LP	F	50.8	No	-	Death	29.9
155	1107	-	*DNAJC7*	chr17:40169363	NM_003315.3	1	c.72delG	p.Lys25Argfs*31	frameshift	-	0	-	-	LP	M	64.6	AD	80	Death	22.3

AAO, Age at onset; ExAC_EAS, ExAC in east-Asian；GnomAD_EAS, GnomAD in East-Asian; AD, autosomal dominant inheritance; F, female; M, male; FH, family history; "-" in dbSNPs，ExAC_EAS or GnomAD_EASmeans this variant was not reported in these database.

Table S5. Top ten P/LP variants enriched in ALS by allelic Fisher's exact test

Allele	Initial OR	Initial p-value	Secondary OR[#]	Secondary p-value
C9orf72 G4C2 repeats	-(20,0)	1.7E-7*	-(20,0)	5.3E-18*
TARDBP p.Gly294Val	-(6,0)	9.4E-3	-(6,0)	7.0E-6*
FUS p.Arg521Cys	-(5,0)	0.02	-(5,0)	4.9E-5*
FUS p.Arg521His	5.9(5,1)	0.101	-(5,0)	4.9E-5*
FUS p.Arg495*	-(3,0)	0.097	-(3,0)	0.0026
SOD1 p.His47Arg	-(3,0)	0.097	-(3,0)	0.0026
SOD1 p.Leu39Val	-(3,0)	0.097	-(3,0)	0.0026
TARDBP p.Ile383Val	-(3,0)	0.097	-(3,0)	0.0026
ANXA11 p.Asp40Gly	-(3,0)	0.097	-(3,0)	0.0026
NEK1 p.Pro807Leu	-(3,0)	0.097	-(3,0)	0.0026

Initial stage, represented the comparation between ALS patients (n=1587) and in-house controls (n=1866); Secondary stage, represented the comparation between ALS patients (n=1587) and a lager controls group, coming from GnomAD_EAS (n=9977); OR, Odds ratios; Odds ratios and the counts in cases and controls are shown in brackets, *passed significance. -, infinite. The results displayed are from Fisher's exact test. Multiple testing was set at P <5.3E-4(0.05/95), 95 rare P/LP variants in the GI genes were identified in our study.

Table S6. phenotype and genotype analysis for ALS causative genes in Chinses ALS cohort

Genes	Frequency fALS/sALS	% relative contribution of mutations	Sex ratio(M/F)	Mean AAO	spinal onset (%)	Initial Spinal phenotype (upper vs lower)	Died/survival/ loss of follow up	Mean survival/disease duration*	Progression rate(fast%)	Cognitive function impairment[#]	Frontal behavior impairment[#]
SOD1	14/29	2.71	1.2 (23/20)	47.4	40(93%)	L>U (9/31)	29/12/2	31.3/45.2	0.93(44%)	5/24 (21%)	6/29(21%)
C9orf72	0/20	1.26	2.3(14/6)	55.5	15(79%)	U>L (8/7)	13/5/2	27.4/30.0	1.30(80%)	5/11(45%)	7/11(64%)
FUS	4/15	1.20	0.7(8/11)	39.5	14(74%)	U>L (8/6)	10/6/3	32.8/24.3	0.87(74%)	3/10(30%)	3/11(27%)
NEK1	0/17	1.07	1.1(9/8)	57.5	17(100%)	U>L (15/2)	10/5/2	40.2/45.3	0.60(41%)	2/9(22%)	1/7(14%)
TARDBP	2/13	0.95	0.7(6/9)	54.0	13(87%)	U>L (10/3)	14/0/1	20.9/-	1.72(86%)	2/6(33%)	3/6(50%)
TBK1	0/10	0.63	2.3(7/3)	49.0	8(80%)	U>L (5/3)	5/5/0	19.3/42.0	0.97(70%)	2/7(29%)	0/8(0.00%)
ANXA11	0/7	0.44	0.8(3/4)	59.4	5(71%)	U>L (3/2)	4/2/1	85.1/39.0	0.60(29%)	1/2(50%)	2/3(67%)
OPTN	1/4	0.32	0.7(2/3)	56.0	3(60%)	U>L (3/0)	2/2/1	18.4/80.4	1.16(100%)	-	-
VCP	2/2	0.25	3(3/1)	49.9	4(100%)	L>U (1/3)	1/3/0	9.7/59.0	0.78(25%)	1/4(25%)	2/4(50%)
UBQLN2	1/3	0.25	0.3(1/3)	34.3	3(75%)	U>L (2/1)	3/1/0	27.0/20.3	1.64(75%)	0/2(0%)	0/2(0%)
DNAJC7	1/2	0.19	0.5(1/2)	56.3	2(67%)	U>L (2/0)	3/0/0	28.4	1.11(33%）	0/2(0.00%)	1/2(50%)
KIF5A	1/2	0.19	M (3/0)	53.9	2(67%)	U>L (2/0)	3/0/0	38.2	0.60(33%)	1/1(100%)	1/1(100%)
CHCHD10	0/2	0.13	1.0(1/1)	56.0	2(100%)	U>L (2/0)	2/0/0	62.2	0.21(0%)	-	-
VAPB	0/1	0.06	M	62.1	1	L	1/0/0	5.6	3.21	-	-
PFN1	0/1	0.06	M	81.8	1	U	0/1/0	66.0	0.71	1/1(100%)	0/1(0.00%)
HNRNPA1	0/1	0.06	F	25.5	1	U	0/1/0	49.2	0.71	0/1(0.00%)	0/1(0.00%)
Total	26/129	9.77	1.2(83/72)	50.4	132 (85%)	U>L (73/59)	100/43/12	31.9/42.5	1.01(58%)	24/81 (30%)	28/88(32%)
None	37/1339	86.70	1.4(804/572)	54.2	1054(76.6%)	U>L (703/351)	842/420/114	36.4/50.3	0.84(61.8%)	196/607(32.3%)	239/712(33.65)

AAO, age at onset; *Mean survival means the mean survival time of dead patients, disease duration means the mean disease duration of survival patients when analysis; [#]means the ratio of cognitive function impairment or frontal behavior impairment in patients who were assessed using Addenbrooke's Cognitive Examination-revised (ACER), and Frontal Assessment Battery (FAB) tests; M, male; F, female; U, upper; L, lower.

Table S7. phenotype and genotype analysis for the GII genes in Chinses ALS cohort

Genes	Frequency fALS/sALS	% relative contribution of mutations	Sex ratio(M/F)	Mean AAO	spinal onset (%)	Initial Spinal phenotype (upper vs lower)	Died/survival/ loss of follow up	Mean survival/disease duration*	Progression rate(fast%)	Cognitive function impairment[#]	Frontal behavior impairment[#]
SETX	2/17	1.20	0.7 (8/11)	49.9	15(79%)	U>L (9/6)	10/9/0	41.2/58.9	0.77(758%)	2/7(29%)	3/10 (30%)
NEFH	0/10	0.63	2.3(7/3)	54.9	6(60%)	U>L (4/2)	9/1/0	31.7/42.0	1.23 (80%)	1/4 (25%)	2/4 (50%)
DAO	0/8	0.50	1.7 (5/3)	58.7	6(75%)	U>L (4/2)	3/4/1	47.4/54.0	0.28 (13%)	1/4 (25%)	2/5 (40%)
CCNF	0/8	0.50	0.6 (3/5)	57.7	5(63%)	U>L (3/2)	6/2/0	24.2/76.1	0.96 (63%)	1/4 (25%)	2/4 (50%)
PRPH	0/7	0.44	0.8 (3/4)	60.3	3(43%)	U>L (2/1)	6/0/1	30.6/-	0.86 (71%)	1/2 (50%)	0/3 (0%)
GRN	0/7	0.44	6 (6/1)	60.2	4(57%)	U>L (4/0)	6/1/0	33.9/127.7	0.94 (75%）	0/2 (0.00%)	1/3 (33%)
SQSTM1	0/5	0.32	0.7 (2/3)	55.7	5(100%)	U>L (3/2)	3/2/0	29.4/42.4	0.85 (80%)	2/4 (50%)	1/4 (25%)
FIG4	0/5	0.32	0.2 (1/5)	50.3	3(60%)	L>U (1/2)	2/3/0	47.9/29.2	0.63 (40%)	1/3 (33%)	1/3 (33%)
ERBB4	0/5	0.32	1.5 (3/2)	58.5	4(80%)	U>L (4/0)	2/3/0	41.6/20.0	0.68 (40%)	1/2 (50%)	2/3 (67%)
ELP3	0/4	0.25	0.3 (1/3)	46.0	3(75%)	U>L (2/1)	3/1/0	59.1/125.4	0.37 (50%)	1/2 (50%)	1/2 (50%)
EWSR1	0/3	0.19	0.5 (1/2)	60.4	3(100%)	U>L (3/0)	2/1/0	29.9/33.9	0.91 (67%)	1/2 (50%)	1/1(100%)
PNPLA6	0/3	0.19	0.5(1/2)	61.3	2(67%)	U=L (1/1)	2/1/0	31.2/34.2	0.83 (100%)	2/3 (67%)	0/3 (0%)
GTT8D1	0/3	0.19	- (3/0)	47.9	3(100%)	U>L (2/1)	2/1/0	22.9/14.6	0.72 (67%)	0/1(0%)	0/1(0%)

AAO, age at onset; *Mean survival means the mean survival time of dead patients, disease duration means the mean disease duration of survival patients when analysis; [#]means the ratio of cognitive function impairment or frontal behavior impairment in patients who were assessed using Addenbrooke's Cognitive Examination-revised (ACER), and Frontal Assessment Battery (FAB) tests; M, male; F, female; U, upper; L, lower.

TableS8. Haplotypes of genetic markers surrounding *SOD1* in patients harboring p.L39V and p.H47R variants

SNPs	Position(hg19)	Refer	Frequency of Refer in EAS	Patients with p.L39V				Patients with p.H47R			
				728	1164	1967	Share	3618	2852	3377	share
rs2070417	21:32638549	C	0.57	AC	CC	AA	-	CC	AC	AA	-
rs845967	21:32688912	T	0.97	TT	TT	TT	T	TT	TT	TT	-
rs2284510	21: 32780744	C	0.91	TC	TC	CC	C	CC	CC	CC	-
rs13052373	21:32844980	A	0.83	AA	AA	AG	A	AG	AA	AA	-
rs9981279	21: 32903888	A	0.86	AA	AA	AA	A	AA	AC	AA	-
rs999106	21:32968981	C	0.58	CC	CC	AC	C	AA	CC	AC	-
rs4465856	21: 32981750	C	0.60	CC	CC	TC	C	TT	CC	TC	-
rs4817415	21: 32991661	A	0.07	CC	CC	CC	C	CC	AC	CC	C
rs148022104	21:33007841	C	0.97	CC	CC	CC	C	TC	CC	CC	C
rs121912443	21:33036170(H47R)	A	-	AA	AA	AA	A	AG	AG	AG	G
rs567511139	21: 33040871	G	1.00	GG	GG	GG	G	GG	GG	GG	G
rs2833485	21: 33078925	G	0.53	AA	AG	AA	A	AG	AG	AG	G
rs7283466	21: 33193131	G	0.78	AA	AG	AA	A	GG	GG	GG	G
rs9305473	21: 33233766	A	0.23	GG	AG	GG	G	AG	GG	GG	G
rs75329558	21: 33254740	G	0.99	GG	GG	GG	G	GG	GG	GG	G
rs2833556	21: 33285300	G	0.73	AG	GG	AA	-	GG	GG	AG	G
rs3819152	21: 33331458	A	0.81	AG	AA	AG	-	AA	AA	AG	A
rs7280610	21: 33351149	A	0.69	AA	AA	GG	-	AA	AG	AA	A
rs8134939	21: 33409932	C	0.67	TC	CC	CC	-	TC	TC	TC	C
rs2833640	21: 33450081	G	0.34	AA	AG	AA	-	AA	GG	AA	-
The length of sharing haplotype				646.7kb				468.3kb			

The SNPs marked bold indicates this SNP located on *SOD1*; EAS, ExAC in east-Asian

Table S9. Haplotypes of genetic markers surrounding *FUS* in patients harboring p.R521C and p.R521H variants

SNPs	Position(hg19)	Refer	Frequency of Refer in EAS	Patients with p.R521C				Patients with p.R521H		
				3263	2534	2324	Share	2744	3217	share
rs12446288	16:30388709	A	0.08	-	-	-	-	GG	AA	-
rs142100728	16:30390417	T	1.00	-	-	-	-	TT	TT	T
rs1064524	16:30492823	C	1.00	-	-	-	-	CC	CC	C
rs61997235	16:30673889	C	1.00	-	-	-	-	AC	CC	C
rs104894518	16:30997933	G	1.00	GG	GG	GG	-	AG	GG	G
rs11150606	16:31099011	T	0.20	CC	CC	TT	-	TC	CC	C
rs7294	16:31102321	G	0.90	GG	GG	AG	G	GG	GG	G
rs2884737	16: 31105554	A	1.00	AA	AA	AA	A	AA	AA	A
rs9925964	16: 31129895	A	0.10	GG	GG	AG	G	GG	GG	G
rs377306867	16: 31160548	G	1.00	GG	GG	GG	G	GG	GG	G
rs267606831	16: 31202410	G	-	GG	GG	GG	G	GG	GG	G
***FUS*p.R521C/p.R521H**	16:31202739/31202740	C/G		CT	CT	CT	T	AG	AG	A
rs377300213	16: 31226253	T	1.00	TT	TT	TT	T	TT	TT	T
rs3815801	16: 31276937	G	0.30	AA	GG	AG	-	AA	AA	A
rs9937837	16: 31298939	T	0.99	TT	TT	TG	-	TT	TT	T
rs11860650	16: 31326706	C	0.99	CC	CC	TC	-	CC	CC	C
rs3925075	16: 31347748	T	0.33	CC	TT	TT	-	CC	CC	C
rs60339402	16: 31383522	G	0.78	GG	GG	GG	-	AG	AG	G
rs2070896	16: 31384554	T	0.33	TC	CC	CC	-	TT	CC	-
The length of sharing haplotype				177.9kb				995.7kb		

The SNPs marked bold indicates this SNP located on *FUS*; EAS, ExAC in east-Asia

Table S10. Haplotypes of genetic markers surrounding *TARDBP* in patients harboring p.G294V and p.N378D variants

SNPs	Position(hg19)	Refer	Frequency of Refer in EAS	Patients with p.G294V						Patients with p.N378D		
				2755	2858	2411	2414	913	share	2983	3026	share
rs12085319	1:11029478	G	0.54	TG	TG	TG	TT	TT	-	TT	GG	-
rs56102775	1:11029942	G	0.71	GG	GG	GG	AG	AG	-	AG	GG	G
rs11121667	1:11038476	C	0.70	TT	TC	TT	CC	TC	-	TC	TC	C
rs11121670	1:11042242	C	0.99	CC	CC	CC	CC	CC	C	CC	CC	C
rs7547006	1:11050459	A	0.58	AC	AA	AC	AA	AA	A	AC	CC	C
rs79373286	1:11060698	A	0.89	--	AA	AG	AA	AA	A	AA	AG	A
rs80356717	1:11078893	A	1.00	AA	AA	AA	AA	AA	A	AA	AA	A
rs80356719	1:11082325	G	1.00	GG	GG	GG	GG	GG	G	GG	GG	G
***TARDBP* p.G294V**	1:11082347	G	1.00	GT	GT	GT	GT	GT	T	GG	GG	G
rs2273346	1:11090897	A	0.78	--	AG	AA	AA	AG	A	AA	AG	A
rs2273342	1:11116052	C	0.96	TC	CC	CC	CC	CC	C	CC	CC	C
rs12140947	1:11179170	G	0.91	GG	GG	GG	GG	GG	G	GG	AG	G
rs17036414	1:11211395	G	0.91	GG	GG	GG	GG	GG	G	GG	AG	G
rs3765903	1:11260457	A	0.86	AA	AA	AA	AA	AA	A	AA	AG	A
rs7525957	1:11318236	C	0.12	TT	TT	TT	TT	TT	T	TT	TT	T
rs2788538	1:11359751	C	0.87	CC	CC	CC	CC	CC	C	CC	TC	C
rs2744827	1:11389233	C	0.98	CC	CC	CC	CC	CC	C	CC	CC	C
rs2982380	1:11395283	A	0.90	AC	AA	AA	AA	AA	A	CC	AA	-
rs2744854	1:11419868	C	0.22	TT	TC	TC	TT	TT	T	CC	TT	-
rs2335404	1:11435942	T	0.67	TT	TC	CC	TT	TT	-	CC	CC	-
The length of sharing haplotype				397.5kb						265.8kb		

The SNPs marked bold indicates this SNP located on *TARDBP*; EAS, ExAC in east-Asia

Table S11. Haplotypes of genetic markers surrounding *NEK1* in patients harboring p.K214R and p.P807L variants

SNPs	Position(hg19)	Refer	Frequency of	Patients with p.K214R				Patients with p.P807L			
			Refer in EAS	876	1558	1864	Share	1567	682	465	share
rs10021579	4:169680887	A	0.39	AA	AC	CC	-	-	-	-	-
rs142483450	4:169682157	T	0.98	TT	TC	TT	T	-	-	-	-
rs1318822	4:169764489	G	0.78	GG	AG	GG	G	GG	AG	AA	-
rs4396955	4:169845720	C	0.93	CC	TC	CC	C	CC	CC	CC	-
rs17658335	4:169998230	G	0.34	AG	AG	AG	G	AG	AG	AA	-
rs1471172	4:170017860	G	0.35	AG	AG	AA	A	AA	AA	AG	-
rs2706713	4:170089950	A	0.30	GG	GG	GG	G	GG	GG	AA	-
rs75744327	4:170140331	C	0.95	CC	CC	CC	C	CC	AC	CC	C
rs7671546	4:170262104	A	0.10	AG	GG	GG	G	GG	GG	GG	G
rs368699255	4:170384477	G	1.00	GG	GG	GG	G	AG	AG	AG	G
rs200161705	4:170506525	C	1.00	CC	CC	CC	C	CC	CC	CC	C
rs11132810	4:170534100	A	0.45	AC	AC	AC	A	AC	CC	AC	C
rs72696684	4:170660605	A	0.94	AA	AA	AA	A	AC	AA	AA	A
rs6810718	4:170778702	A	0.41	AC	AC	AC	A	AA	AC	AC	A
rs11132829	4:170814998	T	0.11	CC	CC	TC	C	TC	TC	CC	C
rs6842442	4:170933203	C	0.31	TC	TT	TC	T	TT	CC	TT	-
rs11734170	4:171271707	G	0.97	GG	GG	AG	G	GG	GG	GG	-
rs35270214	4:171620047	C	0.92	TC	TC	TC	C	-	-	-	-
rs967616	4:171888222	C	0.10	TC	TC	TC	C	-	-	-	-
rs2070371	4:172424391	A	0.30	CC	AG	GG	-	-	-	-	-
The length of sharing haplotype				2.74Mb				843.4kb			

The SNPs marked bold indicates this SNP located on *NEK1*; EAS, ExAC in east-Asia

TableS12. Haplotypes of genetic markers surrounding *ANXA11* in patients harboring p.D40G variants

SNPs	Position(hg19)	Refer	Frequency of Refer in EAS	Patients with p.D40G			
				345	811	2320	Share
rs7904954	10:81758995	A	0.35	AG	GG	AA	-
rs74725507	10:81781304	T	0.98	TT	TT	TT	T
rs10887392	10:81791482	A	0.61	AA	AG	AA	A
rs7083481	10:81884845	G	0.57	GG	AG	GG	G
rs745182	10:81901722	G	0.42	AG	AG	AG	G
rs184560325	10:81918018	G	0.99	GG	GG	GG	G
rs772725616	10:81926644	G	1.00	GG	GG	GG	G
rs188213562	10:81930990	G	0.99	GG	GG	GG	G
rs147286383	10:81964868	G	0.98	GG	GG	GG	G
rs12355463	10:82008561	G	0.51	AG	AG	AA	G
rs10887708	10:82027988	G	0.55	AG	AG	GG	G
rs4934027	10:82035560	C	0.55	TC	TC	CC	C
rs11202666	10:82118498	A	0.47	AG	AG	GG	A
rs10466235	10:82149655	T	0.14	CC	CC	CC	C
rs17105023	10:82182339	A	0.90	AA	AA	AA	A
rs116849573	10:82204368	T	0.96	TC	TT	TC	T
rs7080169	10:82232706	G	0.66	AG	AG	GG	G
rs12411484	10:82263954	A	0.71	AG	GG	AA	-
The length of sharing haplotype				504.9kb			

The SNPs marked bold indicates this SNP located on *ANXA11*; EAS, ExAC in east-Asia

Table S13. The number of patients with more than one rare variant of GI and GII genes

Genes	Pathogenicity	GI genes (number)			GII genes (number)		Total
		P/LP	VUS	Benign	Deleterious	Non-deleterious	
GI genes	P/LP	0	10	1	9	11	31
	VUS	-	1	0	2	3	6
	Benign	-	-	0	0	0	0
GII genes	Deleterious	-	-	-	7	3	10
	Non-deleterious	-	-	-	-	3	3

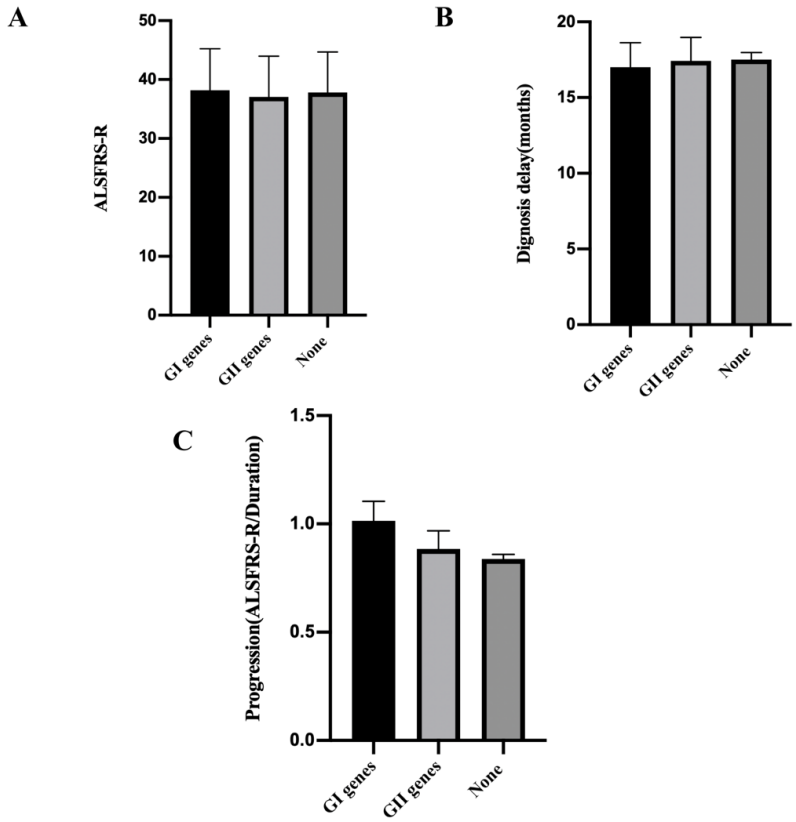

Figure S1. The comparison of ALSFRS-R, diagnosis delay and disease progression at baseline among patients with rare P/LP variants in the GI genes, rare deleterious variants in the GII genes and without rare damaging variants in the GI and GII genes. A) The Mean ± SD of ALSFRS-R is 38.2±7.0 in patients with rare P/LP variants of the GI genes (n=155), 37.1±6.9 in patients with rare deleterious variants of the GII genes (n=100) and 37.8±6.9 in patients without rare damaging variants in the GI and GII genes(n=1276). No significant differences in ALSFRS-R among patients with or without rare damaging variants of the GI and GII genes. **B**) The Mean ± SD of diagnosis delay is 17.0±20.0 months in patients with rare P/LP variants of the GI genes (n=155), 17.4±15.5 months in patients with rare deleterious variants of the GII genes (n=100) and 17.5±16.9 in patients without rare damaging variants in the GI and GII genes(n=1276). No significant differences in diagnosis delay between patients with or without rare damaging variants of the GI and GII genes. **C**) The Mean ± SD of progression rate is 1.0±1.1in patients with rare P/LP variants of the GI genes (n=155), 0.9±0.8in patients with rare deleterious variants of the GII genes (n=100), 0.8±0.7in patients without rare damaging variants in the GI and GII genes(n=1276). No significant differences in progression rate between patients with or without rare damaging variants of the GI and GII genes. SD, standard deviation.GI, ALS causative genes; GII, new, needing to be confirmed or risk genes

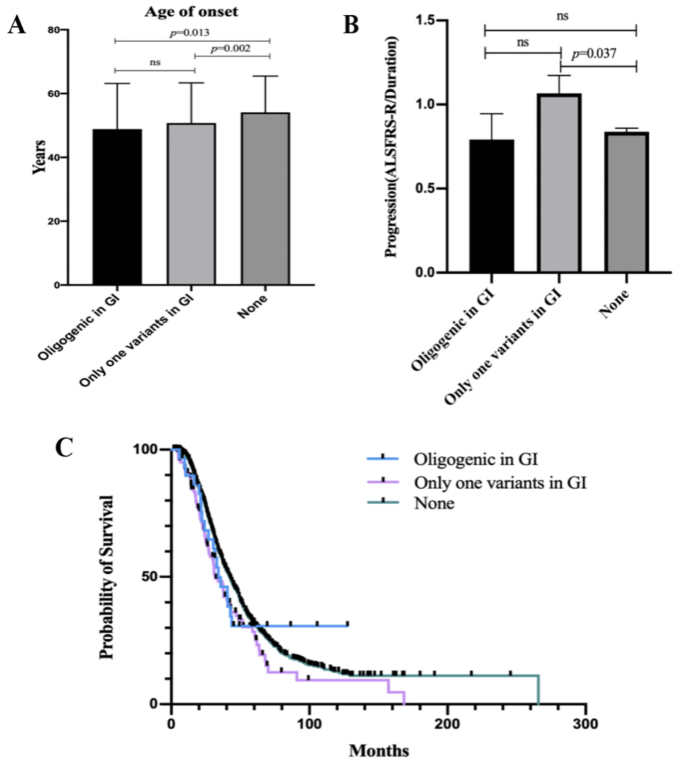

Figure S2. The comparison of age of onset, disease progression and median survival time among patients with more than one variant in the GI genes (at least one P/LP variants, n=31), only one rare P/LP variants in the GI genes (n=124), and without rare damaging variants in the GI and GII genes (n=1276). A) The Mean ± SD of age of onset is 48.9±14.3years in patients with more than one variant in the GI genes, 50.8±12.6 years in patients with only one rare P/LP variants in the GI genes and 54.1±11.4 years in patients without rare damaging variants in the GI and GII genes. No significant differences in age of onset between patients with more than one variant in the GI genes and with only one rare P/LP variants in the GI genes. **B**) The Mean ± SD of progression rate is 0.8±0.8 months in patients with more than one variant in the GI genes, 1.1±1.2 months in patients with only one rare P/LP variants in the GI genes and 0.8±0.7 in patients without rare damaging variants in the GI and GII genes. No significant differences in progression rate between patients with more than one variant in the GI genes and with only one rare P/LP variants in the GI genes. **C**) The median survival time is 34.1 (95%CI:23.1-45.2) months in patients with more than one variant in the GI genes, 31.9 (95%CI:25.7-38.1) months in patients with only one rare P/LP variants in the GI genes, 42.6 (95%CI:39.9-45.3) months in patients without rare damaging variants in the GI and GII genes. The significant difference was found between patients with only one rare P/LP variants in the GI genes and without rare damaging variants in the GI and GII genes (p=0.005), but no significant differences in median survival time between patients with more than one variant in the GI genes and with only one rare P/LP variants in the GI genes. SD, standard deviation. GI, ALS causative genes; GII, new, needing to be confirmed or risk gene

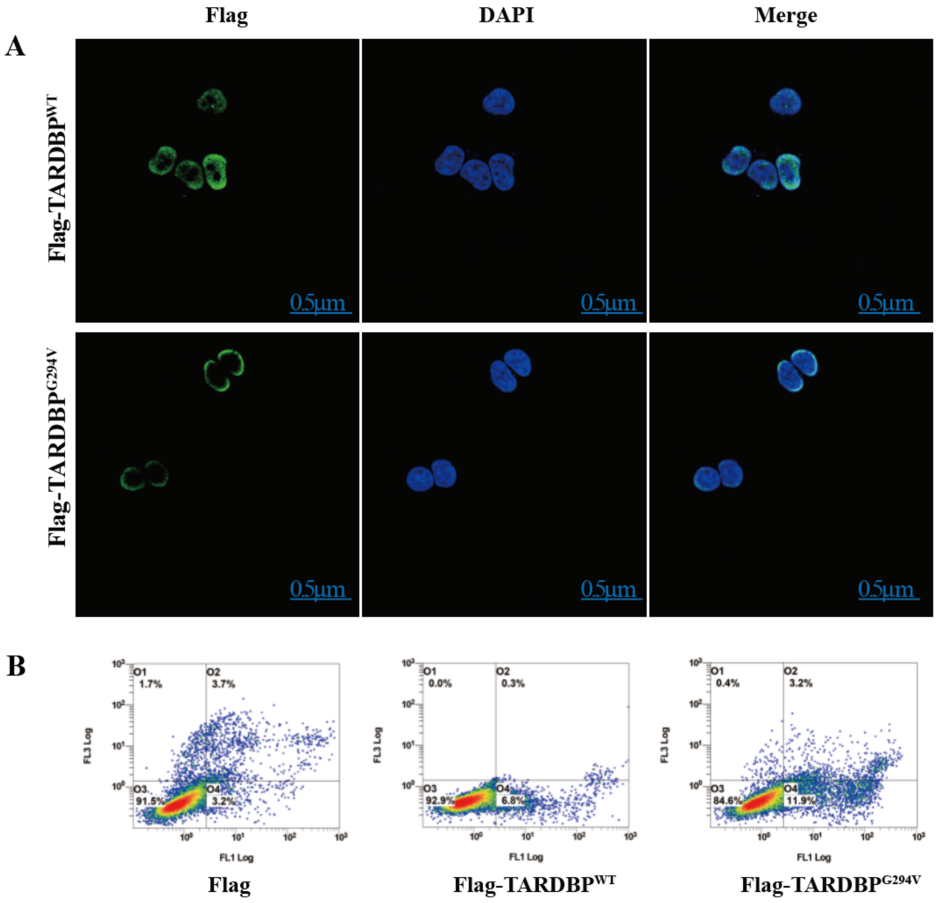

Figure S3. Functional analysis for p.Gly294Val of *TARDBP*. A) Immunocytochemistry analysis for the distribution of the mutant TARDBP (TARDBP^{G294V}) and wild-type TARDBP (TARDBP^{WT}). Mutant TARDBP^{G294V} mainly be around the nucleus or within the cytoplasm, but wild-type TARDBP diffusely distributed in the nucleus. HEK-293T Cells were transfected plasmids for 48 h, and stained with Flag-antibody (green) and DAPI (blue); **B**) Annexin V staining to measure apoptosis in HEK-293T cells when TARDBP mutates or not by flow cytometry. Cells transfected pCMV-MCS -3*Flag-TARDBP^{G294V} group showed much more ratio of the apoptosis cells than that in pCMV-MCS -3*Flag-TARDBP^{WT} and empty vector groups.