

Supplementary results

Supplementary Table 1 – diagnosis of mimic conditions

Supplementary Table 2 – ANOVA of chitinase protein levels controlling for age.

Supplementary Table 3 – Cox proportional hazards modelling of individual chitinase proteins and pNFH

Supplementary Figure 1 – correlation of chitinase proteins with biochemical parameters.

Supplementary Figure 2 – survival curves of patients with ALS and PLS stratified by chitinase protein level at initial visit.

Supplementary Table 1. Diagnosis of patients with ALS-mimicking conditions.

	<i>n</i>
Conduction block neuropathy	3
Multifocal acquired demyelinating sensory and motor neuropathy	3
Kennedy's disease	1
Cervical spondylotic myeloradiculopathy	1
Benign fasciculation syndrome	1
Progressive multiple sclerosis (motor symptoms only)	1
Brachial plexopathy	1
Progressive lower limb weakness with gait initiation failure	1

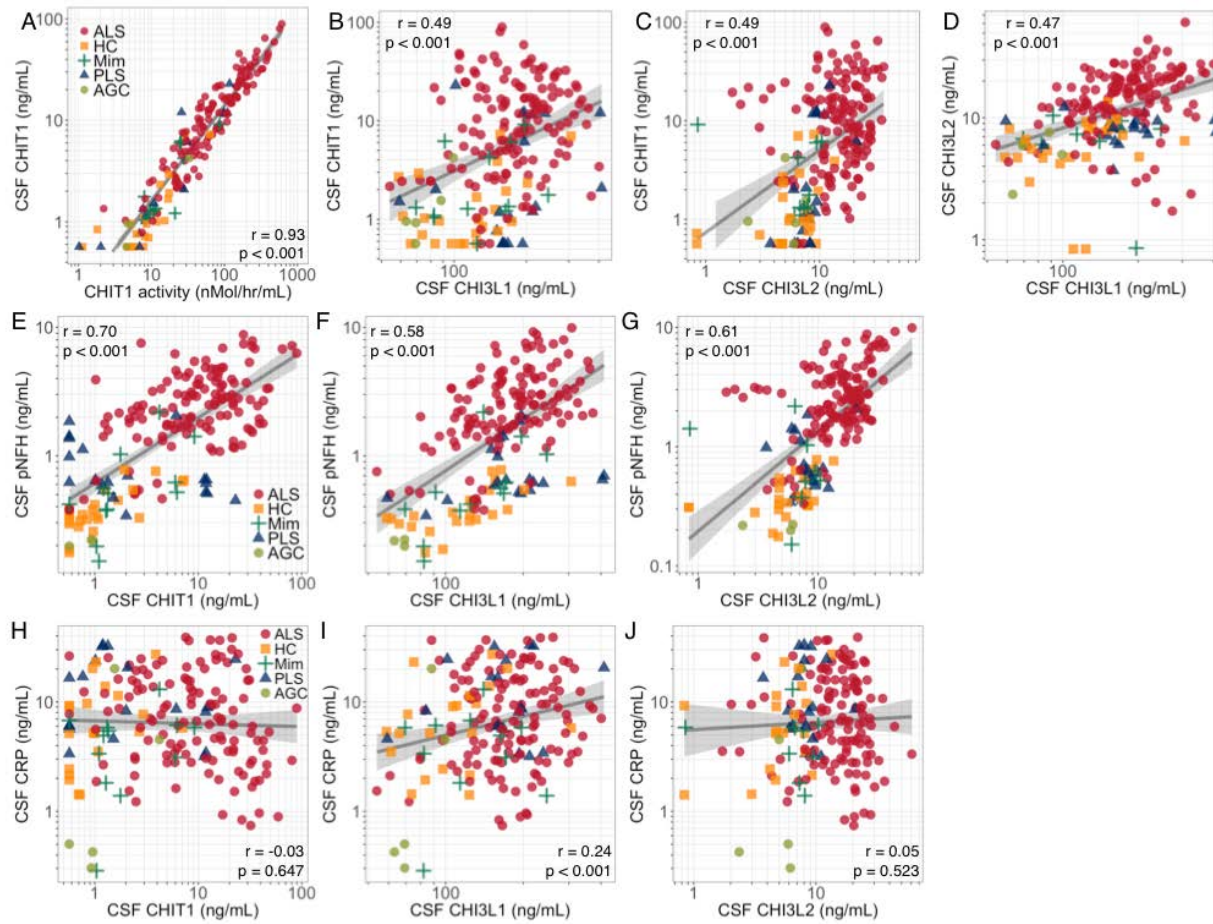
Supplementary Table 2. Cross-sectional analysis of chitinase protein levels using Mann-Whitney *U* test and ANCOVA of log-transformed protein concentration with Benjamini-Hochberg *p*-value adjustment for multiple comparisons. ALS – amyotrophic lateral sclerosis; PLS – primary lateral sclerosis; HC – healthy control; AGC – asymptomatic gene carrier; CHIT1 – chitotriosidase 1; CHI3L1 – chitinase 3-like protein 1; CHI3L2 – chitinase 3-like protein 2; MWU – Mann-Whitney U test *p*-value; ANCOVA – analysis of covariance *p*-value

	CHIT1		CHI3L1		CHI3L2	
	MWU	ANCOVA	MWU	ANCOVA	MWU	ANCOVA
ALS vs Mimic	<0.001	<0.001	0.017	0.002	<0.001	0.002
ALS vs HC	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ALS vs PLS	0.021	0.004	0.417	0.207	<0.001	0.012
ALS vs AGC	0.003	<0.001	<0.001	<0.001	0.002	0.011
HC vs AGC	0.844	0.524	0.657	0.027	0.578	0.587

Supplementary Table 3. Cox proportional hazards modelling of factors known to be associated with survival and individual chitinase proteins and pNFH separately. CSF – cerebrospinal fluid; HR – hazard ratio; CI – confidence interval; CHIT1 – Chitotriosidase-1; CHI3L1 – Chitinase 3-like protein 1; CHI3L2 – Chitinase 3-like protein 2; pNFH – phosphorylated Neurofilament heavy chain.

	HR	95% CI	<i>p</i>	HR	95% CI	<i>p</i>	HR	95% CI	<i>p</i>	HR	95% CI	<i>p</i>
Gender (male)	0.567	0.216-1.490	0.250	0.696	0.273-1.772	0.447	0.667	0.261-1.703	0.397	0.431	0.152-1.227	0.115
Onset site (spinal)	0.955	0.295-3.095	0.939	0.970	0.291-3.236	0.960	0.875	0.256-2.984	0.831	0.796	0.242-2.623	0.708
Age at onset (years)	1.065	0.998-1.135	0.056	1.047	0.987-1.111	0.126	1.041	0.997-1.107	0.290	1.027	0.966-1.091	0.401
CHIT1	4.844	1.675-14.008	0.004	-	-	-	-	-	-	-	-	-
CHI3L1	-	-	-	1.014	0.058-17.662	0.993	-	-	-	-	-	-
CHI3L2	-	-	-	-	-	-	2.192	0.235-20.411	0.491	-	-	-
pNFH	-	-	-	-	-	-	-	-	-	1.386	1.110-1.732	0.004

Supplementary Figure 1. Correlation of chitinase proteins with biochemical parameters. A Correlation of CHIT1 level and CHIT1 activity. B-D correlation of different chitinase proteins. E-G correlation of chitinase proteins with pNFH. H-J correlation of chitinase proteins with CRP. ALS – amyotrophic lateral sclerosis; PLS – primary lateral sclerosis; Mim – mimic conditions; AGC – asymptomatic gene carriers; HC – healthy control; CHIT1 – chitotriosidase 1; CHI3L1 – chitinase 3-like protein 1; CHI3L2 – chitinase 3-like protein 2; pNFH – phosphorylated neurofilament heavy chain; CRP – C-reactive protein



Supplementary Figure 2. Survival curves of patients with ALS and PLS stratified by chitinase protein level above or below (or equal to) median level from time of sampling. P-values indicate log-rank test. ALS – amyotrophic lateral sclerosis; PLS – primary lateral sclerosis; CHIT1 – chitotriosidase 1; CHI3L1 – chitinase 3-like protein 1; CHI3L2 – chitinase 3-like protein 2.

