

ribosomal DNA, RNA metabolism, neurite outgrowth and axonal pathfinding. Cell survival assays show that wild type ANG is capable of rescuing cells containing ANG mutations from death when challenged with toxic agents, suggesting ANG is a potent neuroprotective factor.<sup>3</sup> Functional studies have demonstrated that most mutations result in a loss of function.<sup>3</sup>

A small study demonstrated elevated serum ANG levels in patients with ALS,<sup>4</sup> which could however not be replicated in a later study.<sup>5</sup> Here, we compared serum ANG levels in a large cohort of patients with ALS and PD with controls.

## METHODS

Two hundred and sixty-five serum samples were available from patients with sporadic ALS, all of which were referred to the University Medical Center Utrecht (UMCU) and diagnosed according to the revised El Escorial criteria. One hundred and sixty-three serum samples were available from patients with PD, all of which were referred to the Radboud University Nijmegen Medical Center (RUNMC) and diagnosed according to the UK Brain bank criteria. Samples from 462 controls were available through a nationwide, population-based study on ALS, in which family practitioners are asked to recruit matching controls for each patient with ALS in their practice.

Details are provided in table 1. Samples were drawn at the initial visit to the outpatient clinic. All cases were negative for ANG mutations. All participants gave written informed consent and the study was approved by the relevant ethical committees.

Concentrations of ANG were measured using commercially available ELISA kits (Quantikine) from R&D Systems (Abingdon, UK) according to manufacturer's guidelines. Venous blood samples were drawn and subsequently cells were

removed by centrifugation. Samples were stored at -80°C until the assays were performed. Assays were performed in triplicate. The intra-assay and interassay coefficients of variation were <2.0%.

The normality of data distribution was tested using the Shapiro-Wilk test. Initial comparisons between ALS, PD and controls were done by using independent-samples t test or Mann-Whitney test as appropriate. Because age, sex and body mass index (BMI) influence serum ANG levels, the data were further explored by analysis of covariance (ANCOVA) with these variables as covariates.<sup>4</sup> The data for the ALS group were also analysed stratified according to the site of disease onset, because a previous report only found elevated ANG levels in spinal-onset cases.<sup>4</sup> Association between ANG levels and survival in ALS was tested using Cox regression. Sensitivity and specificity were analysed by receiver operator curves (ROC) analyses (see online supplementary figure S2).

## RESULTS

We observed elevated ANG levels in patients with ALS compared with controls. No difference between patients with PD and controls was seen. Multivariate modelling accounting for the covariates (age, sex and BMI) demonstrated significantly elevated levels for patients with ALS ( $p=1.74\times10^{-3}$ ), patients with bulbar onset ALS ( $p=0.02$ ), patients with spinal onset ALS ( $p=2.90\times10^{-3}$ ), but not in patients with PD ( $p=0.72$ ). Gender was the only covariate found to influence levels ( $p=1.90\times10^{-3}$ ) (table 1). Serum ANG levels were not significantly higher in patients with spinal onset ALS compared with bulbar onset cases ( $p=0.13$ ). Serum ANG levels were also significantly higher in patients with ALS compared with patients with PD ( $p=1.29\times10^{-4}$ ). There was no influence of serum ANG levels on survival in ALS with  $p=0.67$ .

## Serum angiogenin levels are elevated in ALS, but not Parkinson's disease

### INTRODUCTION

Mice lacking the hypoxia responsive element in the promoter of vascular endothelial growth factor (VEGF) develop a phenotype with weakness and pathological reflexes that resemble amyotrophic lateral sclerosis (ALS). A subsequent study demonstrated an association between polymorphisms in the promoter of VEGF and ALS in humans. Therefore other angiogenic genes were investigated in ALS, which showed mutations in *angiogenin* (ANG) in patients with familial and sporadic ALS.<sup>1</sup> A recent study confirmed this association and also demonstrated that ANG mutations predispose to Parkinson's disease (PD).<sup>2</sup> The association with PD has independently been replicated.

It is not known how mutations in ANG lead to neurodegeneration. The ANG protein is involved in the transcription of

**Table 1** Baseline characteristics and results

	No.	Male/female (No.)	Age (year) (range)	BMI (range)	Mean ANG serum level (ng/mL)	SD	p Value
ALS	265	148/117	63 (23–84)	24.7 (17.3–37.6)	425.3	111.3	$1.74\times10^{-3}$
Bulbar ALS	87	40/47	66 (41–84)	24.4 (17.3–35.1)	410.9	115.5	0.02
Spinal ALS	178	112/66	61 (23–83)	24.7 (17.5–37.6)	433.2	109.5	$2.90\times10^{-3}$
PD	163	112/51	64 (34–88)	25.0 (15.9–41.1)	399.7	76.4	0.72
Controls	462	254/208	68 (33–95)	26.9 (16.6–37.7)	401.6	95.9	–

Age is at sample collection, BMI, body mass index, p values were calculated using ANCOVA with age, gender, BMI as covariates. Also see online supplementary figure S1 and supplementary table S1.

ALS, amyotrophic lateral sclerosis; ANCOVA, analysis of covariance; ANG, angiogenin; PD, Parkinson's disease.

## DISCUSSION

In this study we measured serum levels of a protein derived from a gene in which mutations are strongly associated with a higher risk for disease (the OR of ANG mutations for ALS is 9.2 and 6.7 for PD).<sup>2</sup> Many functional studies have demonstrated that ANG mutations result in (complete) loss of protein function and have also shown that the wild type protein is a very potent neuroprotective factor.<sup>3</sup> In this study, we show that serum ANG levels are higher in patients with ALS without ANG mutations compared with healthy controls. We postulate that the elevated serum levels of a strong neuroprotective factor in ALS are due to a compensatory mechanism of the motor system in response to degeneration.

The interesting question then becomes, why are ANG serum levels elevated in ALS but not in PD (considering the gene is involved in both disorders)? Especially, considering that ANG has recently been demonstrated to be protective in experimental models of PD. The exact mechanism through which ANG is neuroprotective is unclear. It has been suggested that ANG has paracrine or autocrine effects. Supporting this view, is data from a recent study in ALS mice (SOD1-G93A) showing upregulation of neuroprotective genes (including ANG) in the motor neurons of mice with a slowly progressive phenotype.

If indeed ANG acts through an autocrine or a paracrine mechanism, it would seem reasonable to assume that any compensatory upregulation of ANG in PD would take place in the basal ganglia and perhaps therefore cannot be demonstrated in serum. It would therefore be interesting to measure ANG levels in cerebrospinal fluid (CSF) of patients with PD. The elevated serum levels in ALS may thus be a reflection of more widespread disease (also including the peripheral nervous system). This might explain the higher levels

observed in patients with spinal ALS compared with patients with bulbar ALS (observed in our study and previously),<sup>4</sup> as loss of motor neurons is usually more generalised in spinal ALS.

Although, the neuroprotective mechanism of ANG is not completely understood, we interpret the consistent finding of elevated serum levels this potent neuroprotective factor reflective of a compensatory mechanism of the motor system in response to neurodegeneration. Therefore ANG could be an interesting therapeutic target.

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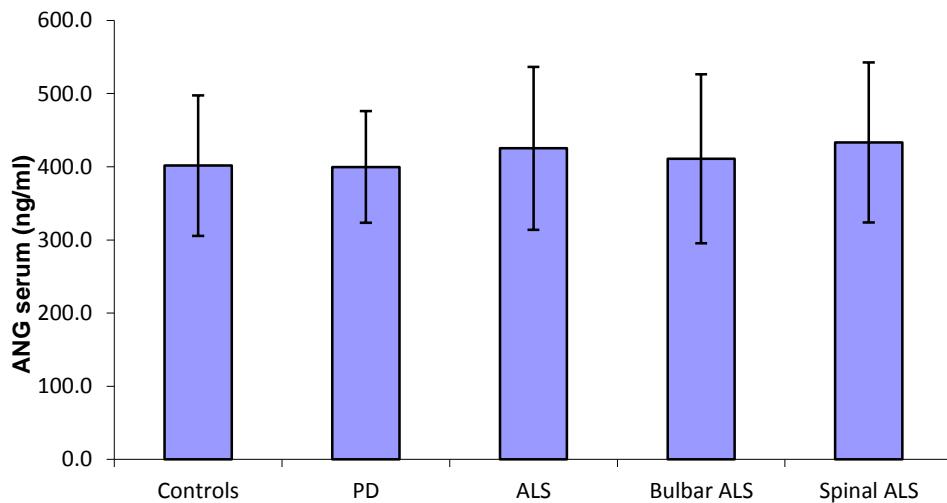
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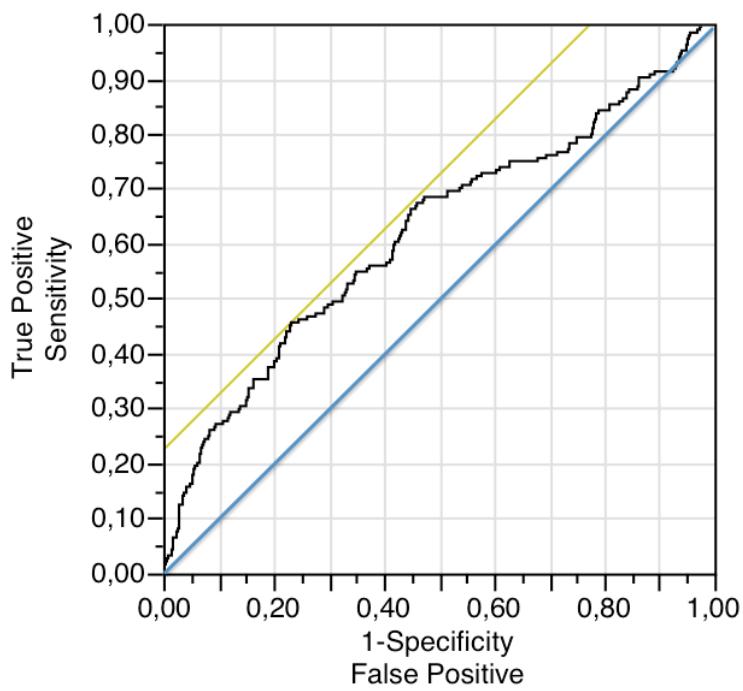
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**Supplementary data to Serum angiogenin levels are elevated in ALS, but not Parkinson's disease**



**Supplementary Figure 1.** Distribution of ANG levels



**Supplementary figure 2.** Receiver operating characteristics (ROC) curve analysis for ALS patients compared to healthy controls and PD patients demonstrating sensitivity and specificity of angiogenin serum levels with respect to a predicting case or control status. The area under the curve is 0.61.

**Supplementary table 1. Raw data**

Status	Gender	Age	BMI	Site of onset	Serum angiogenin level
1	0	75	16,6	0	270,6
1	0	86,9	17,3	0	685,5
1	0	92,7	18,7	0	405,7
1	0	65,5	20,3	0	656,7
1	0	93,2	20,4	0	319,7
1	0	69,5	21,3	0	330,9
1	1	84,6	21,7	0	399,3
1	0	87,2	21,8	0	388,2
1	1	84,1	22	0	494,5
1	0	60,3	22,2	0	397
1	0	80,5	22,2	0	288,6
1	0	52,1	22,6	0	257,5
1	1	75,5	22,6	0	358
1	0	48,1	22,7	0	462,1
1	0	44,9	22,7	0	360,6
1	0	60,8	22,7	0	473
1	0	70,2	22,8	0	318,7
1	0	79,6	22,8	0	402,5
1	0	52,7	23,3	0	338,4
1	0	77,1	23,3	0	491
1	0	59,7	23,4	0	420,8
1	1	57,4	23,6	0	332,9
1	1	85,4	23,7	0	383,4
1	0	34	23,7	0	368,1
1	0	65,2	23,7	0	328,1
1	1	67,6	23,8	0	327
1	0	71,2	23,8	0	467
1	0	59,4	23,9	0	321,3
1	1	36,9	23,9	0	442,3
1	0	77,1	24,2	0	287,3
1	0	47,6	24,2	0	366,8
1	0	85,1	24,2	0	404,5
1	1	76,5	24,2	0	347,8
1	1	39,3	24,3	0	394,5
1	0	65	24,4	0	228,8
1	0	42,6	24,4	0	296,5
1	0	76,5	24,4	0	317,6
1	0	56,3	24,4	0	256,6
1	0	62,7	24,5	0	257,3
1	0	65	24,5	0	421,8
1	1	77,6	24,7	0	296,2
1	1	79,6	24,7	0	282,7

1	0	70,3	24,8	0	552,6
1	1	62,8	24,8	0	292,7
1	1	70,5	24,9	0	441,2
1	0	89,2	24,9	0	419
1	1	82,9	25	0	411,5
1	1	50,2	25	0	488,5
1	0	64,3	25,1	0	244,7
1	0	89,6	25,1	0	441,4
1	1	59,5	25,1	0	302,8
1	1	53,5	25,2	0	339,3
1	0	42,2	25,2	0	441,3
1	1	78,6	25,3	0	399,9
1	1	74,4	25,3	0	391,8
1	1	58,5	25,6	0	344,9
1	1	54	25,7	0	525,7
1	1	81,1	25,7	0	600,6
1	1	74,1	25,9	0	472
1	0	66,5	26	0	391,8
1	0	66,4	26,1	0	203,9
1	1	64,1	26,3	0	559,5
1	1	83	26,3	0	802,8
1	0	58,7	26,3	0	399,7
1	0	43,4	26,4	0	343,8
1	1	67,8	26,5	0	517,2
1	0	56,5	26,6	0	557,3
1	1	33,7	26,6	0	422,8
1	0	60,6	26,8	0	466,3
1	0	52	27,1	0	267,5
1	0	39,8	27,3	0	393,6
1	0	65,3	27,4	0	358,3
1	1	78,9	28,3	0	563,7
1	0	48,4	28,3	0	325,8
1	1	67,9	28,4	0	459,5
1	0	61,4	28,7	0	472,3
1	1	33,3	29,2	0	513,4
1	0	54	29,7	0	459,5
1	0	77,5	30,4	0	206,8
1	1	35,7	30,6	0	440,2
1	0	55,8	31	0	494,2
1	0	61,8	31,2	0	545,3
1	1	58,2	31,3	0	230,2
1	0	60,3	31,9	0	381,6
1	0	73,1	32,9	0	228,5
1	1	61,1	33,7	0	368,1
1	0	71,1	34,7	0	364,2

1	1	70,1	37,2	0	443,3
1	0	65,3	-9	0	423,5
1	1	59,6	-9	0	354,6
1	0	66,1	-9	0	269,6
1	1	62,4	-9	0	429,7
1	1	75,3	-9	0	379,3
1	1	60,6	-9	0	412,8
1	0	44,8	-9	0	352,8
1	1	68	-9	0	512,7
1	0	55,9	-9	0	317,6
1	1	63,9	-9	0	477,3
1	1	62	-9	0	253,1
1	1	64,8	-9	0	344,4
1	0	39,6	-9	0	337,5
1	0	73,8	-9	0	341,8
1	1	60,2	-9	0	354,8
1	0	58,6	-9	0	504,6
1	0	60,2	-9	0	476,4
1	0	62,7	-9	0	465,2
1	0	68,5	-9	0	316,4
1	1	79,1	-9	0	403,3
1	0	74,5	-9	0	322,2
1	0	50,4	-9	0	281,1
1	1	54,7	-9	0	497,4
1	0	60,3	-9	0	400,2
1	1	84,4	-9	0	543
1	0	77,2	-9	0	380,8
1	1	71	-9	0	416,1
1	0	80,3	-9	0	281,6
1	1	55	-9	0	369,2
1	0	82	-9	0	460,3
1	1	62,7	-9	0	326,8
1	0	84,1	-9	0	393,4
1	1	57,2	-9	0	324,6
1	0	56	-9	0	477,2
1	0	59,3	-9	0	259,4
1	0	60	-9	0	394,7
1	0	69,8	-9	0	369,2
1	1	71,3	-9	0	561,3
1	1	86,5	-9	0	450,2
1	1	80,2	-9	0	400,4
1	0	78,3	-9	0	287,7
1	0	75,3	-9	0	327,8
1	0	76,2	-9	0	368,3
1	0	73,6	-9	0	498,8

1	1	53,9	-9	0	528,1
1	1	71,7	-9	0	325,1
1	1	70,6	-9	0	527,5
1	1	68,8	-9	0	271
1	1	73,3	-9	0	372,5
1	1	69	-9	0	398
1	1	69,9	-9	0	404,8
1	0	67,2	-9	0	563,6
1	1	68,6	-9	0	399,2
1	1	65,7	-9	0	296,5
1	1	62,1	-9	0	424,7
1	1	67	-9	0	338,6
1	1	65,2	-9	0	365,9
1	1	65,1	-9	0	354,8
1	0	64,5	-9	0	516,4
1	1	61,3	-9	0	446,6
1	0	62,1	-9	0	325,2
1	1	58,9	-9	0	431,3
1	1	58,9	-9	0	604,8
1	1	62,1	-9	0	435,8
1	1	58,8	-9	0	317,8
1	1	58,1	-9	0	477,1
1	1	55,8	-9	0	469,6
1	1	56,7	-9	0	325
1	0	56,6	-9	0	268,2
1	0	56,4	-9	0	357,9
1	1	52,9	-9	0	699,1
1	1	51,7	-9	0	478,4
1	1	52	-9	0	491,2
1	0	48,3	-9	0	477,4
1	1	49,2	-9	0	487
1	0	58,2	-9	0	387
1	0	46,6	-9	0	354,5
1	1	40	-9	0	386,8
1	0	45,9	-9	0	390,2
1	1	39,3	-9	0	374
1	1	39,3	-9	0	502,6
1	1	75,9	-9	0	509,2
1	1	78,6	-9	0	337,1
1	1	79,1	-9	0	475,2
1	0	55,8	-9	0	278,1
1	1	72,3	-9	0	382,3
1	1	71,2	-9	0	523,3
1	0	68,1	-9	0	515,1
1	0	33,3	-9	0	448,2

1	0	61,3	-9	0	413,3
1	0	60,7	-9	0	483,5
1	0	63,5	-9	0	292,7
1	1	60,7	-9	0	401,4
1	1	60,7	-9	0	535,9
1	0	57,4	-9	0	510,2
1	0	55,9	-9	0	371,8
1	0	48,4	-9	0	501,2
1	0	52,5	-9	0	541,4
1	1	51,3	-9	0	420,4
1	0	52,5	-9	0	327,2
1	0	49,6	-9	0	311,6
1	1	46,2	-9	0	329
1	1	35,4	-9	0	393,1
1	0	74,4	-9	0	462,9
1	0	62,7	-9	0	424,5
1	1	72,2	-9	0	471,8
1	1	45,8	-9	0	325,5
1	1	71	-9	0	299,2
1	1	52,4	-9	0	433,5
1	0	50,8	-9	0	438,5
1	1	49,6	-9	0	339,6
1	1	45	-9	0	268,2
1	0	43,9	-9	0	355,5
1	1	33,5	-9	0	371,5
1	1	51,8	-9	0	421,5
1	1	41,4	-9	0	403,5
1	0	52	-9	0	465,9
1	0	54,8	-9	0	419,3
1	0	63,1	-9	0	804,7
1	1	47	-9	0	552
1	1	62	-9	0	462,1
1	1	50,7	-9	0	449,7
1	0	66,2	-9	0	352,1
1	1	58,6	-9	0	502,7
1	0	72,4	-9	0	441,4
1	1	43,8	-9	0	363,1
1	0	49,9	-9	0	352,7
1	1	67,1	-9	0	402,5
1	0	66,8	-9	0	259,9
1	0	62,2	-9	0	271
1	1	73,7	20,3	0	484,8
1	0	50,9	20,6	0	490,5
1	0	40,9	20,8	0	419,5
1	1	61,8	20,8	0	286,3

1	0	65,5	22,1	0	404,1
1	1	58,3	22,2	0	425,4
1	1	71,6	22,7	0	310,5
1	0	81,1	23	0	357,9
1	0	56,7	23,5	0	225,1
1	0	65,5	23,9	0	603
1	1	81,1	24,3	0	270,4
1	0	58,9	24,7	0	374,5
1	0	55,7	24,7	0	423,7
1	0	70,1	24,8	0	289,2
1	1	72,8	25	0	392,7
1	1	67	25,1	0	520,5
1	1	87,4	25,2	0	368,1
1	1	89,2	25,8	0	438,7
1	0	45,2	26	0	321,6
1	0	79,2	26,4	0	370,4
1	0	54,3	26,7	0	450,9
1	1	62,9	27,3	0	386,5
1	1	85,5	27,4	0	527,3
1	0	83	27,4	0	369
1	0	64,1	27,9	0	455,9
1	1	65,7	28,1	0	449,7
1	1	70,7	28,7	0	362,1
1	0	34	28,7	0	407,9
1	0	60,2	29,6	0	745,2
1	1	36,1	30	0	288
1	0	65,9	30,1	0	271,9
1	1	51,8	30,7	0	388,9
1	0	70,9	-9	0	347,6
1	1	56,6	-9	0	463
1	0	69,5	-9	0	423,2
1	0	62,1	-9	0	255,9
1	0	65,4	-9	0	392,5
1	0	76,6	-9	0	395,3
1	0	64,8	-9	0	339
1	1	84,3	-9	0	356,7
1	1	62,3	-9	0	568,9
1	0	77,5	-9	0	527,4
1	0	47,8	-9	0	337
1	1	56,9	-9	0	588,5
1	0	90,8	-9	0	398
1	0	93	-9	0	462,9
1	0	58,3	-9	0	312,7
1	0	69,8	-9	0	431,2
1	0	74,5	-9	0	536,7

1	0	80,3	-9	0	393,1
1	0	75,4	-9	0	305,1
1	0	69	-9	0	361,5
1	0	68,8	-9	0	454,2
1	1	65	-9	0	738,6
1	0	63,4	-9	0	477,6
1	1	60,1	-9	0	690,3
1	0	56,7	-9	0	364,8
1	0	47,3	-9	0	423,4
1	1	43,4	-9	0	458,5
1	0	70,9	-9	0	295,9
1	1	73,1	-9	0	308,1
1	0	58,1	-9	0	303,1
1	0	66,7	-9	0	341,6
1	0	63,5	-9	0	349,8
1	1	58,2	-9	0	299,3
1	0	50,4	-9	0	524,5
1	0	40,3	-9	0	385,1
1	1	77,1	-9	0	370,6
1	1	83,2	-9	0	421,5
1	0	37	-9	0	299,8
1	1	55,9	-9	0	382,6
1	1	36,9	-9	0	411
1	1	80,8	-9	0	453,7
1	1	50,7	-9	0	421,8
1	1	66,7	-9	0	422,4
1	1	79,3	24	0	334,3
1	1	71,6	27	0	461,8
1	1	65,2	28,4	0	426
1	1	82,8	22,9	0	271,2
1	0	82,7	23,6	0	368,8
1	1	73,9	26	0	333,1
2	1	75,3	29,4	1	839,6
2	1	62,4	22,8	1	395,7
2	0	44,4	-9	1	382,3
2	0	83,7	26,4	1	295,7
2	1	66,8	25,7	1	533,4
2	1	47,6	-9	1	611,9
2	0	64,9	27	1	443,2
2	1	70,1	23,5	1	616,1
2	0	74,7	24,3	1	569,6
2	1	65,7	23,1	1	611,2
2	0	73,3	23,9	1	460,5
2	1	56,3	-9	1	275,2
2	0	58,3	18,9	1	274,8

2	0	77,8	18,7	1	294,8
2	0	57,3	26,7	1	421,8
2	0	63	-9	1	379,5
2	0	72,4	-9	1	384,5
2	0	70,9	32,4	1	350,4
2	1	55,5	19,4	1	413,8
2	1	61,1	26,1	1	535,9
2	1	67,3	20,3	1	434,9
2	1	70,1	25,4	1	364,1
2	0	75,3	-9	1	337,4
2	1	56,8	19,6	1	369,6
2	0	56,5	20,1	1	516,4
2	0	83,5	-9	1	252,4
2	0	83	-9	1	310,2
2	0	82,9	27	1	443,5
2	0	80,9	34	1	397,5
2	1	80,7	24,5	1	348,9
2	1	78	26,4	1	340
2	1	72,5	24,2	1	435
2	0	72	30,4	1	605,5
2	0	65,8	-9	1	472,8
2	0	65,7	22	1	395,3
2	1	64,6	29,7	1	364,1
2	0	63,1	-9	1	284,5
2	1	63,1	-9	1	370,4
2	1	61,4	-9	1	408,3
2	0	58,4	23,3	1	484,2
2	0	67,4	-9	1	355,6
2	1	53,5	23,8	1	449,2
2	1	52,1	-9	1	320,9
2	1	61,6	27,6	1	352,4
2	0	74,8	-9	1	393,6
2	1	60,7	26	1	410,8
2	1	73,8	22,1	1	392,6
2	0	61,6	24,8	1	252,5
2	0	69,5	-9	1	392
2	1	72,7	25,4	1	320,8
2	1	69,9	25,4	1	418,6
2	0	57,2	21	1	296,2
2	1	41	-9	1	346,2
2	1	60,6	27,5	1	410,1
2	0	80,3	22,7	1	529,5
2	0	77,3	22	1	348,6
2	0	57	-9	1	316,7
2	0	55,4	17,3	1	366,9

2	0	53,8	-9	1	399,2
2	0	69,4	-9	1	302,4
2	0	51,4	18,2	1	652,1
2	1	65,4	21,7	1	616,9
2	0	51,1	22,6	1	298,1
2	0	59,2	-9	1	253,3
2	0	61,2	-9	1	387,6
2	0	69,3	35,1	1	355,8
2	0	72	22	1	472,9
2	0	82,8	24,1	1	708,7
2	1	58,9	-9	1	329,2
2	1	52,2	-9	1	324,9
2	0	62,7	20,9	1	332,4
2	0	78,3	24,4	1	332,1
2	0	77,5	-9	1	331,2
2	0	76,9	-9	1	377,3
2	0	74,9	17,3	1	432
2	1	58	26,6	1	669,1
2	1	56,7	-9	1	406,7
2	1	53,8	-9	1	384,3
2	0	74,9	24,8	1	641,2
2	0	60,8	34,7	1	288,9
2	1	62,1	25,2	1	480
2	1	64,7	26,1	1	408
2	0	54,9	18,9	1	351,9
2	0	51,5	22,5	1	280
2	0	62,3	23,8	-9	298,4
2	1	66,8	23,5	-9	477,1
2	1	72,9	25,9	-9	314,9
2	1	62	29,6	-9	330,1
2	1	77,4	25,7	2	479,5
2	1	44,5	21,7	2	591
2	0	70,2	27,3	2	338,1
2	0	62,7	-9	2	476,9
2	1	56,6	25,4	2	576,3
2	1	61,1	22,1	2	630
2	1	33,4	29,7	2	519
2	1	63	27,5	2	489,3
2	0	58	25,8	2	332,3
2	0	53,4	22,5	2	545,3
2	1	53,4	21,4	2	351,9
2	1	44,9	22	2	514,2
2	1	77,6	26,8	2	460,3
2	0	69	26,3	2	354
2	0	70,8	21,5	2	651,3

2	1	75,8	23	2	633,6
2	1	74,7	-9	2	474,7
2	0	59,8	22,8	2	624,9
2	0	66,6	24,8	2	267,5
2	1	45	25,3	2	314
2	1	35,8	-9	2	333,4
2	1	55,4	-9	2	428,7
2	0	43,2	-9	2	293,8
2	1	82,5	-9	2	562,6
2	1	75,2	31,4	2	531,4
2	1	71	24,1	2	271,4
2	1	72,5	22,6	2	331,1
2	0	59,9	23,8	2	548,9
2	1	68,6	20	2	327,4
2	0	61,5	23,7	2	437,3
2	0	68,2	25,4	2	539,1
2	1	72	20,6	2	391,6
2	0	67	-9	2	279,4
2	1	66,2	31,5	2	347,4
2	0	52,2	22,6	2	403,2
2	1	59,9	-9	2	418,2
2	1	53,6	19	2	340,6
2	1	56,7	-9	2	504
2	0	52,4	23,8	2	354,4
2	0	70,5	26,4	2	405,3
2	0	33,9	21,7	2	527,9
2	0	78,5	20	2	454,9
2	0	56,3	22	2	512,3
2	0	63,4	20,2	2	443,3
2	1	32,6	25,5	2	512,7
2	1	59,3	26,7	2	534,5
2	0	41,8	37,6	2	442,3
2	0	54,3	22,9	2	305,2
2	1	57,7	22,4	2	542,6
2	0	66,5	26,7	2	359,4
2	0	67,4	19,2	2	525,8
2	1	68,3	-9	2	502,4
2	1	72,4	24,5	2	283,1
2	1	66,5	25,4	2	401,4
2	1	62,7	-9	2	345,2
2	0	55,9	-9	2	459,7
2	0	56,2	-9	2	389,7
2	1	56,7	-9	2	320,3
2	0	55,2	21,8	2	440,1
2	1	47,6	-9	2	409,9

2	0	48,1	-9	2	309,9
2	1	58,8	24,1	2	372,9
2	0	80,1	-9	2	336,3
2	1	77,7	-9	2	472,9
2	1	67,7	25	2	275,5
2	1	76,4	-9	2	328,2
2	0	58,9	28,4	2	448,5
2	0	74,9	23,1	2	424,5
2	1	74,9	23,6	2	367
2	1	59,9	24	2	479,7
2	1	42,1	22	2	313,8
2	1	74,2	22,2	2	476,3
2	0	72,8	26,4	2	304
2	1	72,3	-9	2	600,6
2	1	62	20,8	2	262,4
2	1	70,5	-9	2	647
2	1	70,1	32,3	2	530,3
2	1	69,6	20,9	2	587,3
2	0	70,5	24,2	2	355,6
2	1	65,1	25,7	2	449
2	0	69,5	-9	2	465,2
2	0	68,8	20	2	309,4
2	1	68	25,6	2	580,4
2	0	67	25,1	2	420,4
2	1	69,8	21,8	2	461,8
2	0	66,6	-9	2	331,2
2	1	63,2	-9	2	465,8
2	1	65,7	-9	2	504
2	1	62,9	27,5	2	595
2	1	60	26	2	406,3
2	0	56	23,2	2	246,1
2	1	55,6	-9	2	357
2	1	55,5	-9	2	388,3
2	0	54,9	31,1	2	652,6
2	0	54,3	-9	2	365,9
2	1	46,7	24,6	2	642,3
2	1	38,8	-9	2	553,2
2	1	35,6	24,9	2	490
2	1	73,3	-9	2	434,3
2	0	73,7	-9	2	292,2
2	0	70	17,5	2	273,8
2	1	67,5	25,6	2	327
2	1	74,2	23,7	2	443
2	1	62,2	22,1	2	263,3
2	1	46,8	20,8	2	479,7

2	1	51,9	21,3	2	427,1
2	1	41,6	18,4	2	365,3
2	1	58,4	28,4	2	317,3
2	1	66,6	30,3	2	460,5
2	1	53,7	31	2	288,9
2	0	23,4	21,5	2	419,5
2	1	40,4	-9	2	393,8
2	1	52,7	-9	2	311,9
2	1	57,3	22,4	2	318,3
2	1	75	24,3	2	487,6
2	1	72,7	-9	2	361,7
2	0	50,4	-9	2	456,7
2	0	64,7	25,1	2	420,2
2	1	66,2	-9	2	488,5
2	1	74,2	25,1	2	325
2	0	77,9	32,1	2	359,9
2	1	62,4	26,6	2	706,1
2	1	46	23,5	2	543,4
2	1	35,3	27,8	2	410,4
2	0	76,8	-9	2	276,7
2	1	53,9	22	2	328,2
2	1	43,3	-9	2	515,5
2	1	66,3	30	2	503,1
2	0	55,7	25,3	2	465,6
2	1	61,1	27,5	2	679,7
2	1	64,3	-9	2	533
2	1	55,3	-9	2	567,5
2	1	59,6	-9	2	402,6
2	0	66,2	21,5	2	393
2	1	74,3	27,8	2	400,5
2	0	59,2	23,9	2	318,9
2	0	44,1	26,8	2	346
2	1	64,1	-9	2	400,8
2	0	33	-9	2	309,1
2	1	75,2	25,5	2	406,7
2	1	38,5	30,9	2	611,2
2	0	65,4	27,7	2	410
2	1	52,7	-9	2	429,1
2	1	70,8	-9	2	313,4
2	1	59,7	23,8	2	321,9
2	1	67,4	24,9	2	521,8
2	0	60,3	31,1	2	817,1
2	0	76	-9	2	403,2
2	0	79,4	23,8	2	380,2
2	1	66,8	-9	2	555,7

2	1	45,6	-9	2	360,8
2	1	83,2	22,3	2	440,5
2	0	75,5	27	2	389,7
2	1	74,7	30,4	2	567,5
2	1	71,7	27,4	2	542,6
2	1	69,9	-9	2	561,4
2	0	69,8	-9	2	311,3
2	0	68,1	-9	2	390,5
2	0	80,5	21,6	2	439,8
2	1	43,2	26,8	2	618,7
2	0	60,2	30,8	2	331,6
2	1	71	25,9	2	308,7
2	0	67,2	23,9	2	407,6
2	0	54,4	21,3	2	460,7
2	1	67,7	-9	2	247,9
2	1	78,8	22,8	2	434,4
2	1	57,5	21,5	2	473,1
2	1	71,1	24,6	2	292,2
2	0	60,5	23,5	2	252
2	1	40,2	24,5	2	484,3
2	1	64,8	23,5	2	665,6
2	1	50,7	25,2	2	433,7
2	1	54,3	23,8	2	513,9
2	0	53,9	-9	2	438,2
2	1	74,7	23,5	2	491,5
2	0	61,9	28,5	-9	456,5
2	0	68,4	30,1	-9	506,2
3	1	62	23,57	0	386,2
3	1	46	26,57	0	494,0
3	1	66	24,06	0	331,0
3	1	48	18,99	0	572,3
3	1	68	23,53	0	448,0
3	0	78	25,91	0	462,0
3	1	67	24,45	0	386,2
3	0	73	27,43	0	466,6
3	0	63	25,22	0	481,7
3	1	71	28,40	0	518,8
3	0	49	20,05	0	320,7
3	1	74	26,42	0	569,4
3	1	56	22,63	0	378,6
3	1	54	22,60	0	353,4
3	1	66	23,51	0	495,2
3	0	67	26,89	0	355,3
3	1	65	20,75	0	441,8
3	1	53	26,47	0	418,6

3	1	70	23,12	0	304,4
3	1	65	20,06	0	360,9
3	1	59	23,67	0	343,3
3	0	60	24,80	0	359,9
3	0	50	22,49	0	369,2
3	1	52	24,73	0	381,4
3	1	64	21,97	0	350,6
3	0	59	41,12	0	225,0
3	0	51	22,68	0	245,8
3	1	42	22,82	0	417,5
3	1	73	23,23	0	484,9
3	0	59	21,09	0	231,3
3	0	60	25,03	0	445,6
3	1	59	26,81	0	388,8
3	1	66	29,76	0	384,7
3	1	52	23,24	0	397,0
3	1	67	20,57	0	354,4
3	1	68	23,88	0	391,5
3	1	61	29,22	0	427,2
3	1	53	23,04	0	345,0
3	1	67	28,72	0	385,1
3	1	68	23,77	0	328,4
3	0	70	22,94	0	358,6
3	1	62	28,41	0	444,7
3	1	68	25,71	0	356,5
3	1	69	25,06	0	404,4
3	1	59	21,50	0	402,2
3	0	52	23,31	0	470,1
3	0	75	23,73	0	351,8
3	1	57	28,98	0	434,2
3	1	76	19,71	0	429,1
3	0	61	28,96	0	363,4
3	0	61	25,82	0	325,8
3	1	75	27,04	0	357,6
3	0	64	22,66	0	416,0
3	1	50	30,19	0	476,2
3	1	34	20,44	0	444,3
3	1	56	20,99	0	323,9
3	0	68	25,21	0	441,3
3	1	62	27,76	0	371,3
3	1	60	23,12	0	426,5
3	0	77	29,38	0	320,9
3	0	59	27,10	0	427,5
3	0	53	26,42	0	470,8
3	1	69	24,62	0	581,7

3	1	67	27,17	0	419,2
3	1	74	20,96	0	464,4
3	1	48	23,12	0	398,5
3	1	61	26,56	0	405,7
3	0	68	25,86	0	459,1
3	1	75	22,58	0	458,0
3	1	66	28,07	0	383,1
3	0	49	19,38	0	369,7
3	1	40	22,21	0	316,3
3	1	68	24,34	0	250,8
3	1	61	27,44	0	460,7
3	0	69	25,24	0	411,8
3	1	54	20,48	0	396,1
3	0	69	20,83	0	503,1
3	1	67	25,25	0	419,3
3	0	69	25,22	0	392,5
3	1	70	24,81	0	402,6
3	0	59	18,37	0	299,8
3	1	57	20,22	0	529,5
3	0	68	24,06	0	318,2
3	1	46	24,31	0	555,3
3	1	55	20,48	0	430,8
3	1	54	25,96	0	366,5
3	0	76	26,49	0	269,3
3	0	77	23,94	0	434,0
3	1	49	29,32	0	364,5
3	1	48	27,70	0	297,5
3	1	47	25,74	0	473,4
3	1	57	23,42	0	473,4
3	0	68	22,31	0	397,4
3	1	76	23,18	0	368,5
3	1	69	25,71	0	373,6
3	1	64	25,06	0	424,7
3	1	49	27,17	0	315,1
3	0	61	26,37	0	320,1
3	1	73	25,25	0	428,9
3	1	69	24,98	0	483,7
3	0	72	23,31	0	459,9
3	1	66	25,56	0	406,8
3	1	55	27,47	0	438,5
3	1	55	24,76	0	401,6
3	1	65	26,67	0	401,6
3	1	66	23,85	0	477,2
3	0	82	28,98	0	422,5
3	1	71	29,65	0	584,5

3	1	88	22,49	0	355,1
3	1	56	25,13	0	387,5
3	0	61	18,59	0	560,9
3	1	46	25,46	0	475,6
3	1	57	21,62	0	293,1
3	0	68	24,98	0	332,9
3	1	66	21,26	0	277,3
3	0	73	20,70	0	399,5
3	0	81	24,88	0	412,5
3	1	45	25,18	0	304,4
3	0	78	15,92	0	478,1
3	1	56	27,73	0	315,6
3	0	59	24,80	0	394,5
3	1	76	22,28	0	514,0
3	1	71	25,35	0	381,1
3	1	67	30,93	0	438,5
3	0	76	27,10	0	359,7
3	0	74	26,99	0	414,3
3	1	73	24,39	0	431,1
3	1	61	26,42	0	423,8
3	0	70	23,89	0	521,7
3	1	65	26,56	0	473,8
3	1	52	24,92	0	341,5
3	1	71	25,69	0	601,0
3	1	69	25,83	0	292,6
3	1	81	26,47	0	255,2
3	1	56	26,09	0	445,8
3	1	64	23,57	0	274,3
3	0	43	21,97	0	249,3
3	0	67	23,53	0	338,4
3	1	51	20,68	0	321,6
3	0	75	39,52	0	369,2
3	1	67	23,51	0	377,8
3	1	73	19,69	0	393,0
3	1	66	25,71	0	399,9
3	1	60	27,38	0	404,3
3	1	78	26,47	0	360,3
3	1	56	33,14	0	284,7
3	0	67	21,48	0	340,8
3	1	77	22,84	0	365,8
3	1	58	29,28	0	405,5
3	0	63	23,80	0	429,0
3	1	72	28,08	0	457,3
3	0	84	32,14	0	314,1
3	0	69	27,64	0	253,9

3	1	55	30,19	0	416,6
3	1	75	27,99	0	652,5
3	1	59	25,98	0	373,5
3	1	72	22,34	0	446,3
3	1	62	24,81	0	324,4
3	1	70	26,99	0	390,4
3	1	69	24,80	0	414,6
3	1	75	26,89	0	437,1
3	1	61	25,50	0	399,5
3	0	70	26,67	0	452,5
1	1	62	23,66	0	474,1
1	1	44	27,46	0	452,7
1	1	66	24,69	0	403,2
1	1	48	25,06	0	405,9
1	1	68	23,74	0	301,3
1	0	78	23,05	0	282,9
1	1	67	24,49	0	523,6
1	0	73	28,44	0	414,1
1	0	49	24,91	0	197,5
1	1	71	30,86	0	366,6
1	0	49	20,57	0	231,4
1	1	74	26,58	0	383,5
1	1	56	23,89	0	434,0
1	1	54	23,04	0	316,1
1	1	66	25,01	0	401,3
1	0	69	26,17	0	330,5
1	1	65	22,09	0	508,3
1	1	53	26,02	0	430,2
1	1	70	23,04	0	409,9
1	1	65	22,79	0	509,3
1	1	59	26,83	0	543,9
1	0	60	23,12	0	403,2
1	0	50	22,09	0	283,2
1	1	53	27,17	0	375,8
1	1	64	21,91	0	298,1
1	0	59	31,10	0	254,9
1	0	51	25,83	0	417,5
1	1	42	22,94	0	325,1
1	1	73	23,37	0	384,7
1	0	59	21,80	0	311,5
1	0	60	26,79	0	501,0
1	1	59	27,78	0	372,6
1	1	66	30,47	0	357,6
1	1	53	27,47	0	529,3
1	1	67	22,34	0	213,6

1	1	68	24,28	0	386,2
1	1	61	29,04	0	423,9
1	1	55	27,08	0	312,0
1	1	67	26,87	0	527,5
1	1	68	25,71	0	348,1
1	0	70	22,59	0	430,4
1	1	62	29,38	0	403,3
1	1	68	25,93	0	372,3
1	1	69	23,81	0	422,8
1	1	60	21,97	0	461,2
1	0	52	24,61	0	379,8
1	0	75	23,44	0	310,7
1	1	57	26,09	0	283,6
1	1	76	21,20	0	395,0
1	0	61	27,24	0	439,2
1	0	61	25,65	0	380,1
1	1	75	26,09	0	431,1
1	0	64	23,11	0	475,2
1	1	50	31,56	0	405,9
1	1	36	22,59	0	279,9
1	1	58	24,57	0	357,6
1	0	68	24,30	0	390,0
1	1	62	26,78	0	616,1
1	1	60	23,12	0	459,1
1	0	77	29,07	0	700,4
1	0	59	26,93	0	383,1
1	0	53	24,80	0	290,6
1	1	69	23,77	0	412,0
1	1	67	25,01	0	371,9
1	1	74	21,15	0	444,3
1	1	48	29,32	0	469,7
1	1	61	26,23	0	426,5
1	0	69	25,77	0	319,9
1	1	75	19,03	0	392,4
1	1	66	27,73	0	462,3
1	0	50	19,83	0	456,9
1	1	42	21,33	0	357,9
1	1	68	26,30	0	423,0
1	1	61	27,46	0	445,6
1	0	69	24,62	0	250,8
1	1	55	27,73	0	515,8
1	0	68	18,04	0	309,3
1	1	67	23,30	0	318,9
1	0	69	26,04	0	436,1
1	1	70	24,69	0	385,1

1	0	59	18,44	0	289,5
1	1	57	24,11	0	494,4
1	0	68	23,71	0	391,2
1	1	46	22,99	0	535,4
1	1	57	25,16	0	444,6
1	1	54	25,36	0	376,7
1	0	75	26,81	0	332,1
1	0	75	24,80	0	312,2
1	1	50	24,38	0	373,7
1	1	48	32,47	0	341,2
1	1	47	25,68	0	500,4
1	1	57	24,22	0	320,2
1	0	68	22,32	0	326,1
1	1	77	24,82	0	662,5
1	1	69	27,77	0	349,1
1	1	64	24,86	0	394,3
1	1	50	24,21	0	492,5
1	0	61	25,51	0	357,2
1	1	73	23,53	0	389,1
1	1	69	23,24	0	488,1
1	0	72	26,22	0	312,2
1	1	66	25,46	0	435,3
1	1	55	27,34	0	350,1
1	1	55	24,76	0	405,7
1	1	66	26,79	0	493,5
1	1	66	26,04	0	383,9
1	0	82	31,92	0	310,0
1	1	71	25,48	0	324,3
1	1	88	25,06	0	341,6
1	1	56	25,77	0	446,9
1	0	61	19,96	0	422,5
1	1	45	20,20	0	377,6
1	1	57	24,86	0	333,9
1	0	68	23,59	0	352,2
1	1	66	20,81	0	331,9
1	0	73	23,63	0	370,8
1	0	80	23,50	0	359,0
1	1	47	22,60	0	253,5
1	0	76	19,27	0	462,0
1	1	56	27,17	0	367,8
1	0	59	23,89	0	396,6
1	1	79	22,59	0	425,9
1	1	71	24,72	0	375,0
1	1	67	34,69	0	433,2
1	0	77	26,84	0	471,6

1	0	74	24,80	0	431,1
1	1	73	20,99	0	421,7
1	1	61	25,22	0	376,0
1	0	70	22,10	0	350,5
1	1	66	27,17	0	408,0
1	1	58	25,93	0	339,4
1	1	71	22,20	0	395,1
1	1	69	28,65	0	320,5
1	1	82	26,79	0	325,8
1	1	56	26,58	0	462,6
1	1	64	23,52	0	565,7
1	0	44	20,52	0	312,2
1	0	67	21,09	0	584,5
1	1	58	25,44	0	784,6
1	0	73	32,72	0	283,4
1	1	67	23,25	0	328,9
1	1	74	21,71	0	263,2
1	1	66	26,26	0	357,0
1	1	60	27,73	0	467,6
1	1	78	26,18	0	679,5
1	1	56	29,98	0	386,6
1	0	68	22,15	0	317,3
1	1	77	22,79	0	409,9
1	1	58	29,35	0	324,8
1	0	63	23,74	0	298,3
1	1	72	27,06	0	471,0
1	0	83	33,53	0	402,1
1	0	71	27,08	0	357,0
1	1	55	32,37	0	381,1
1	1	74	27,78	0	363,5
1	1	60	26,09	0	387,4
1	1	72	22,72	0	395,5
1	1	62	25,10	0	275,8
1	1	70	27,13	0	421,7
1	1	70	25,20	0	344,8
1	1	75	24,82	0	472,2
1	1	61	25,25	0	479,4
1	0	70	23,05	0	340,0

Legend to supplementary table 1. Status: 1 = healthy controls, 2 = ALS, 3 = PD; Gender: 0 = female, 1 = male; Age: age at onset for ALS and PD or age at sampling for healthy controls; BMI: -9 = missing; Site of onset: 0 = not applicable for PD and controls, 1 = bulbar ALS, 2= spinal ALS.