



# Gut microbiota links with cognitive impairment in amyotrophic lateral sclerosis: a multi-omics study

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**Supplementary Table 1 Inclusion and exclusion criteria**

Items	Criteria
Inclusion criteria	1. Clinically definite or probable ALS (revised El Escorial criteria) 2. 18 < age < 75 years 3. 18 < BMI < 28 kg/m <sup>2</sup>
Exclusion criteria	1. Any possible evidence for familial amyotrophic lateral sclerosis (fALS) 2. With other neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), etc. 3. With definite gastrointestinal diseases, heart failure, acute infection, tumor, immunodeficiency and autoimmune diseases 4. Any use of antibiotics within the last month 5. Regular drinking of commercial probiotics within the last year or any drinking of commercial probiotics within the last week 6. Obvious symptoms of dysphagia or dyspnea

**Supplementary Table 2 Relative abundance at phylum level between ALS and HC**

Phylum	ALS	HC	P-value
Actinobacteria	5.63 (1.66, 13.73)	2.87 (0.87, 5.85)	0.053
Bacteroidetes	2.62 (0.42, 9.65)	3.99 (1.37, 15.57)	0.125
Firmicutes	78.78 (62.53, 86.54)	66.55 (45.64, 81.42)	0.075
Proteobacteria	1.56 (0.87, 10.33)	7.78 (1.22, 33.45)	0.026
Verrucomicrobia	0 (0, 0.01)	0 (0, 0)	0.689
F/B	29.07 (6.47, 138.63)	18.32 (3.72, 64.07)	0.092

Data are presented as median (25% quantile, 75% quantile). P-values were calculated by Mann-Whitney U-test. ALS: amyotrophic lateral sclerosis; HC: healthy control; F/B: Firmicutes/Bacteroidetes.

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Received: 07 September 2022; Revised: 05 November 2022; Accepted: 22 November 2022; Published online: 28 December 2022

CLC number: R744.8, Document code: A

The authors reported no conflict of interests.

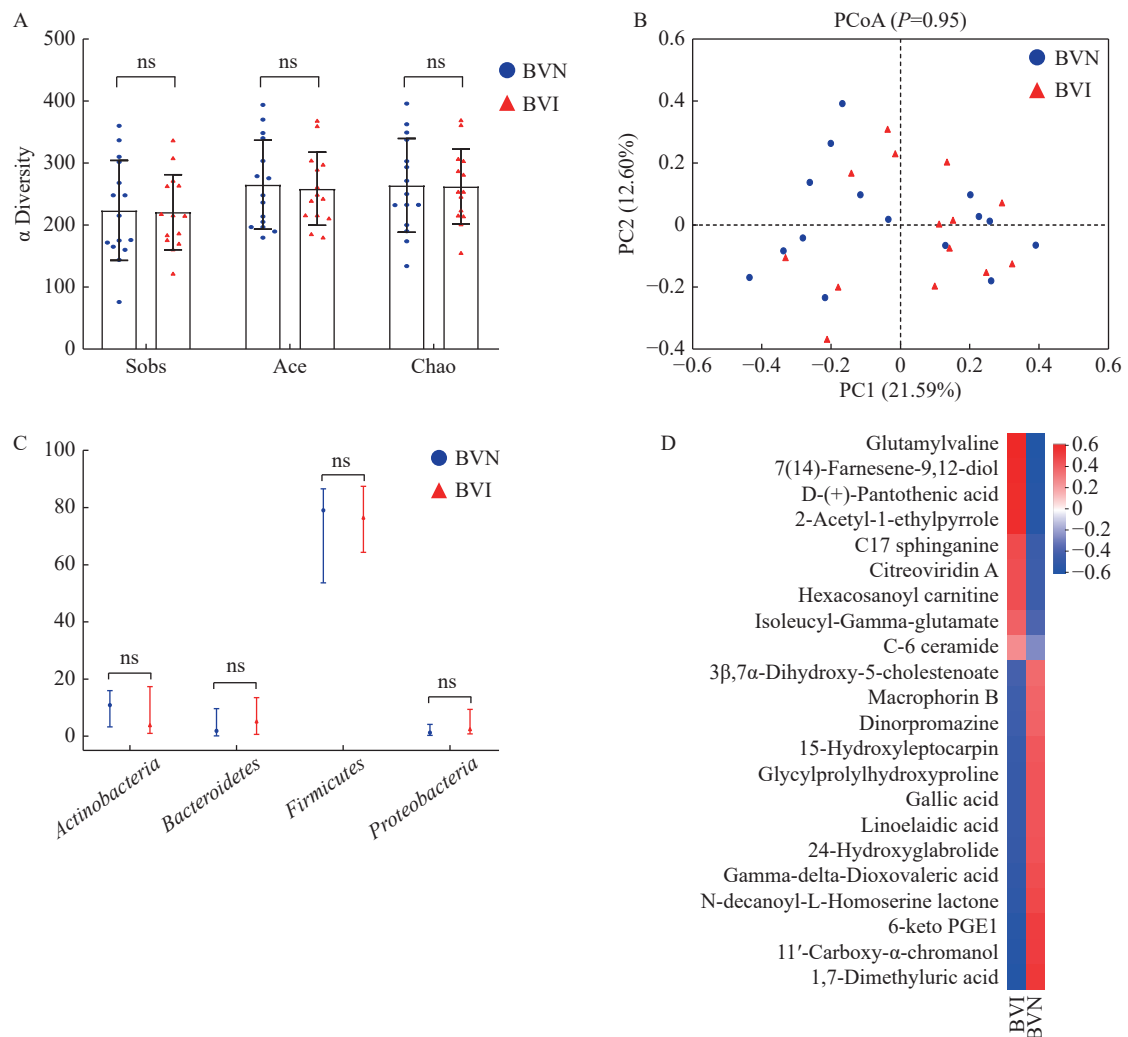
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Phylum	CN	CI	P-value
Actinobacteria	10.88 (5.63, 26.45)	1.9 (1.27, 9.20)	0.066
Bacteroidetes	1.79 (0.21, 8.49)	8.95 (2.35, 16.14)	0.039
Firmicutes	80.69 (62.54, 86.85)	74.68 (52.59, 84.97)	0.383
Proteobacteria	1.29 (0.32, 5.67)	1.51 (1.14, 14.35)	0.215
Verrucomicrobia	0 (0, 0)	0 (0, 43.20)	0.033
F/B	45.55 (8.59, 368.50)	9.10 (3.47, 32.30)	0.022

Data are presented as median (25% quantile, 75% quantile). P-values were calculated by Mann-Whitney U-test. CN: normal cognition; CI: cognitive impairments; F/B, Firmicutes/Bacteroidetes.

Phylum	BVN	BVI	P-value
Actinobacteria	10.92 (3.30, 15.97)	3.90 (1.03, 17.38)	0.106
Bacteroidetes	1.91 (0.15, 9.68)	5.18 (0.61, 13.49)	0.348
Firmicutes	79.04 (53.69, 86.55)	76.47 (64.32, 87.47)	0.485
Proteobacteria	1.24 (0.32, 4.14)	2.49 (0.82, 9.40)	0.256
Verrucomicrobia	0 (0, 0.09)	0 (0, 0)	0.114
F/B	45.40 (6.58, 368.69)	18.79 (5.61, 174.30)	0.458

Data are presented as median (25% quantile, 75% quantile). P-values were calculated by Mann-Whitney U-test. BVN: behavior normal; BVI: behavior impaired; F/B, Firmicutes/Bacteroidetes.



**Supplementary Fig. 1** Difference in microbial community and fecal metabolites between patients with BVN or BVI. ns: not significant; BVN: behavior normal; BVI: behavior impaired.

**Supplementary Table 5 Comparison of 46 fecal bile acids between CN and CI**

Bile acid	CN (ng/g)	CI (ng/g)	Effect size	P-value
Deoxycholic acid	19 303.91 (11 950.51)	16 824.23 (8 588.38)	0.580	0.567
Hyodeoxycholic acid	4 257.27 (2 358.58)	2 947.85 (2 192.8)	1.454	0.157
Alpha-Muricholic acid	36.88 (19.05)	17.45 (14.45)	2.818	0.009
Lithocholic acid	20 577.58 (16 757.16)	22 749.48 (13 842.67)	-0.351	0.728
Isoodeoxycholic acid	0.58 (0.26)	0.65 (0.27)	-0.691	0.495
Glycocholic acid	440.68 (163.06, 1 255.82)	99.83 (39.4, 577.28)	-2.294	0.022
Glycochenodeoxycholic acid	746.89 (398.69, 1 124.03)	218.68 (100.81, 428.34)	-2.845	0.004
Taurocholic acid	179.14 (58.02, 800.7)	59.28 (11.74, 232.33)	-1.606	0.108
Taurochenodeoxycholic acid	220.9 (120.53, 407.36)	77.85 (32.10, 223.88)	-2.294	0.022
Glycoursodeoxycholic acid	268.64 (106.39, 602.2)	28.84 (23.06, 267.43)	-2.661	0.008
Allocholic Acid	1 591.32 (212.42, 2 767.59)	152.17 (74.74, 1 402.8)	-2.065	0.039
Ursodeoxycholic acid	6 091.26 (1 900.69, 9 848.19)	2 003.6 (708.65, 7027)	-1.652	0.099
Tauroursodeoxycholic acid	24.58 (7.75, 95.88)	2.04 (1.15, 32.88)	-2.340	0.019
apocholic acid	10.08 (0, 26.02)	8.65 (0, 31.35)	-0.048	0.962
Glycodeoxycholic acid	402.58 (37.63, 790.16)	96.53 (63.79, 486.55)	-1.101	0.271
Glycolithocholic acid	32.33 (2.50, 96.5)	18.96 (3.71, 70.08)	-0.367	0.714
Beta-Muricholic acid	988.52 (398.5, 2 676.31)	265.4 (226.12, 1 015.36)	-1.973	0.048
7-ketoLithocholic acid	3 267.56 (1 118.73, 7 681.85)	992.11 (398.15, 6 928.47)	-1.285	0.199
Tauro- $\alpha$ -muricholic acid	3.1 (1.16, 33.16)	3.33 (1.15, 26.78)	0.000	1.000
Tauro- $\beta$ -muricholic acid	1.97 (1.27, 13.89)	1.79 (0.73, 5.50)	-0.918	0.359
Omega-Muricholic acid	519.93 (68.46, 1 856.2)	64.27 (30.5, 545.63)	-1.468	0.142
murideoxycholic acid	168.62 (81.52, 382.15)	153.25 (44.74, 267.45)	-0.826	0.409
Taurohyodeoxycholic acid	25.91 (10.84, 120.13)	2.44 (0.94, 33.75)	-2.478	0.013
Taurohyocholic acid	1.04 (0.48, 3.39)	1.04 (0.28, 2.30)	-0.596	0.551
taurolithocholic acid	16.58 (4.34, 23.81)	8.56 (1.47, 12.04)	-1.422	0.155
Taurodeoxycholate acid	38.45 (26.23, 175.27)	18.72 (1.4, 47.88)	-1.606	0.108
Cholic acid	17 476.93 (3 392.46, 31 117.48)	2 298.26 (402.65, 15 086.72)	-2.111	0.035
Chenodeoxycholic acid	13 405.17 (4 045.43, 23 799.13)	2 222.42 (541.38, 13 613.2)	-2.386	0.017
hyocholic acid	1 466.61 (361.96, 2 853.8)	624.34 (157, 1 510.36)	-1.331	0.183
norcholic acid	131.32 (84.62, 299.36)	55.98 (35.71, 157.43)	-2.019	0.044
Glycohyocholic acid	2.4 (1.36, 5.34)	0.39 (0.34, 1.03)	-3.349	0.001
23-Nordeoxycholic acid	9.41 (0.55, 26.8)	15.46 (0.80, 30.39)	-0.322	0.747
isolithocholic acid	14 864.25 (231.85, 26 994.75)	22 587.64 (9 516.01, 37 160.78)	-0.918	0.359
12-ketolithocholic acid	54.94 (38.6, 131.63)	70.61 (40.55, 126.74)	-0.184	0.854
dehydrolithocholic acid	956.96 (131.08, 4 816.54)	3 270.94 (2 033.57, 6 014.95)	-0.780	0.435
Lithocholic acid 3-sulfate	2 337.19 (941.51, 8 830.24)	461.87 (122.87, 3 939.11)	-2.111	0.035
Chenodeoxycholic acid-3- $\beta$ -D-glucuronide	99.43 (65.40, 343)	86.85 (50.45, 137.36)	-1.055	0.291
3 $\beta$ -Ursodeoxycholic acid	2 322.27 (546.43, 4 058.25)	1 046.1 (165.36, 4 834.84)	-0.780	0.435
3-Dehydrocholic acid	472.57 (114.00, 4 138.09)	102.71 (12.98, 792.39)	-1.927	0.054
Chenodeoxycholic Acid 24-Acyl- $\beta$ -D-glucuronide	3.14 (1.22, 9.08)	0.39 (0.20, 4.19)	-2.065	0.039
12-ketochenodeoxycholic acid	1 768.2 (369.24, 16 349.26)	406.47 (109.68, 1 929.66)	-1.698	0.090
7,12-Diketolithocholic acid	80.62 (25.98, 221.79)	26.79 (8.34, 62.82)	-1.927	0.054
Dehydrocholic acid	2.27 (1.38, 3.62)	1.85 (1.04, 4.53)	-0.849	0.396
Ursocholic acid	10 507.16 (1 377.77, 23 380.96)	1 094.41 (202.85, 9 942.72)	-1.514	0.130
7-ketodeoxycholic acid	2 642.46 (1 795.27, 17 525.9)	1 216.89 (338.84, 9 486.95)	-1.331	0.183
3 $\beta$ -Cholic acid	4 237.48 (769.19, 9 479.69)	1 037.88 (137.85, 5 457.19)	-1.331	0.183

Data are presented as median (25% quantile, 75% quantile). P-values were calculated by Mann-Whitney U-test. CN: normal cognition; CI: cognitive impairments.

Bile acid	BVN	BVI	Effect size	P-value
Ursodeoxycholic acid	6 199.46 (4 564.13)	4 524.07 (3 635.19)	1.088	0.286
Deoxycholic acid	17 531.73 (11 921.98)	19 431.47 (9 840.22)	-0.466	0.645
Hyodeoxycholic acid	4 253.34 (2 547.17)	3 326.18 (2 104.63)	1.064	0.297
Alpha-Muricholic acid	29.53 (19.37)	30.89 (20.79)	-0.183	0.856
Dehydrocholic acid	1.9 (1.02)	3.8 (3.03)	-2.233	0.040
Glycocholic acid	424.7 (146.61, 897.75)	297.86 (84.73, 747.25)	-0.611	0.541
Glycochenodeoxycholic acid	496.08 (391.04, 1 124.03)	383.57 (179.72, 1 089.59)	-0.786	0.432
Taurocholic acid	325.08 (24.96, 787.05)	80 (37.63, 351.23)	-0.655	0.513
Taurochenodeoxycholic acid	220.9 (103.44, 585.21)	101.48 (70.35, 281.97)	-1.047	0.295
Glycoursodeoxycholic acid	268.09 (45.77, 383.54)	151.62 (28.09, 561.74)	-0.349	0.727
Allocholic Acid	1 215.52 (119.89, 2 767.59)	639.79 (104.84, 1 875.25)	-0.742	0.458
Tauroursodeoxycholic acid	24.58 (3.08, 95.88)	16.4 (2.12, 29.85)	-0.480	0.631
apocholic acid	10.08 (0, 26.02)	8.65 (0, 31.16)	0.000	1.000
Glycodeoxycholic acid	331.64 (37.63, 734.99)	346.07 (73.91, 783.53)	-0.262	0.793
Glycolithocholic acid	9.44 (1.99, 96.5)	35.95 (5.96, 70.84)	-0.742	0.458
Beta-Muricholic acid	733.54 (254.82, 2 676.31)	680.26 (263.99, 1 071.89)	-0.262	0.793
7-ketoLithocholic acid	5 575.11 (616.49, 7 681.85)	2 602.89 (830, 4 803.18)	-0.524	0.600
Tauro- $\alpha$ -muricholic acid	3.1 (1.16, 46.42)	3.78 (1.35, 15.59)	-0.175	0.861
Tauro- $\beta$ -muricholic acid	1.97 (1.18, 13.89)	2.11 (0.97, 5.17)	-0.349	0.727
Omega-Muricholic acid	426.57 (30.57, 2 071.08)	250.69 (45.24, 672.18)	-0.436	0.663
murideoxycholic acid	183.13 (56.95, 382.15)	152.46 (71.67, 332.42)	-0.393	0.694
Taurohyodeoxycholic acid	29.2 (4.40, 120.13)	15.94 (2.81, 28.57)	-0.698	0.485
Taurohyocholic acid	1.05 (0.57, 1.8)	0.85 (0.29, 3.07)	-0.436	0.663
tauroolithocholic acid	8.25 (1.69, 16.77)	12.12 (5.69, 33.38)	-1.528	0.127
Taurodeoxycholate acid	29.66 (8.54, 62.64)	44.53 (14.14, 182.28)	-0.742	0.458
Lithocholic acid	14 529.43 (1 612.12, 38 816.5)	27 715.41 (18 640, 30 730.43)	-0.786	0.432
Cholic acid	25 852.42 (903.55, 31 117.48)	7 784.96 (977.27, 18 338.82)	-1.004	0.315
Chenodeoxycholic acid	18 067.76 (2 562.88, 23 246.58)	4 818.78 (1 966.83, 15 016.37)	-0.960	0.337
hyocholic acid	446.78 (253.43, 2 558.18)	1 157.54 (569.66, 3 218.12)	-0.960	0.337
norcholic acid	151.76 (57.89, 224.69)	89.85 (47.67, 178.17)	-0.873	0.383
Glycohyocholic acid	1.49 (0.68, 3.03)	1.29 (0.40, 4.61)	-0.044	0.965
23-Nordeoxycholic acid	15.51 (0.55, 27.53)	9.64 (3.65, 26.11)	-0.175	0.861
isolithocholic acid	4 499.56 (231.85, 37 278.71)	20 873.81 (10 639.70, 28 829.47)	-0.742	0.458
12-ketolithocholic acid	53.38 (38.60, 112.34)	73.75 (40.63, 138.57)	-0.567	0.570
dehydrolithocholic acid	460.18 (34.53, 4 816.54)	2 913.2 (842.86, 5 523.13)	-0.786	0.432
Lithocholic acid 3-sulfate	1 982.18 (642.94, 7 149.78)	1 547.74 (365.88, 6 979.24)	-0.349	0.727
Chenodeoxycholic acid-3- $\beta$ -D-glucuronide	97.7 (55.50, 239.3)	96.95 (50.45, 376.51)	-0.175	0.861
3 $\beta$ -Ursodeoxycholic acid	1 875.87 (439.07, 4 315.57)	1 733.41 (451.54, 4 076.74)	-0.131	0.896
3-Dehydrocholic acid	712.52 (37.96, 4 138.09)	199.85 (58.99, 850.56)	-0.524	0.600
Chenodeoxycholic Acid 24-Acyl- $\beta$ -D-glucuronide	3.74 (0.43, 31.85)	0.89 (0.38, 3.51)	-1.528	0.127
12-ketochenodeoxycholic acid	1 671.12 (107.12, 16 349.26)	893.87 (322.93, 3 059.98)	-0.044	0.965
7,12-Diketolithocholic acid	37.05 (16.77, 126.75)	57.79 (22.29, 221.36)	-0.524	0.600
Ursocholic acid	7 879.37 (242.21, 30 668.33)	6 522.81 (1 344.95, 12 188.63)	-0.175	0.861
7-ketodeoxycholic acid	6 695.79 (265.09, 17 525.9)	2 459.53 (667.74, 6 547.47)	-0.480	0.631
Isodeoxycholic acid	0.54 (0.40, 0.85)	0.56 (0.46, 0.71)	-0.524	0.600
3 $\beta$ -Cholic acid	1 811.27 (143.74, 9 635.12)	2 260.49 (316.05, 5 541.74)	-0.044	0.965

Data are presented as median (25% quantile, 75% quantile). P-values were calculated by Mann-Whitney U-test. BVN: behavior normal; BVI: behavior impaired.