Independent recapitulation of sphingomyelin (d18:2/24:1) as an amyotrophic lateral sclerosis marker in a third cohort

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Abstract

Sphingomyelin (d18:2/24:1) has been reported by a single group as elevated in amyotrophic lateral sclerosis (ALS) and as associated with functional decline, with Mendelian randomization supporting a causal contribution. Independent confirmation has been lacking. We tested the species in ST004318, a Metabolon serum cohort of 34 ALS patients stratified by progression rate, used by neither prior report. SM(d18:2/24:1) separates fast from slow progressors at area under the curve 0.883 (Cohen's d = +1.24, P = 0.0003), surviving adjustment for sex (P = 0.0031) and repeated cross-validation (0.872 ± 0.148). The direction matches both prior reports. We additionally recompute effect sizes from the originating group's published case-control distributions (Cohen's d = +0.585 and +0.530 across their two cohorts), and report a co-analyzed candidate, 2-hydroxypalmitate, that fails to replicate under the same procedure. SM(d18:2/24:1) shows concordant direction across three independent cohorts and two analytical platforms.

Background

Serum lipid markers for ALS have been proposed repeatedly and confirmed rarely. Among the more promising is sphingomyelin (d18:2/24:1), hereafter SM 42:3, reported by the University of Michigan group in two studies: a case-control analysis across two cohorts (1) and a longitudinal analysis of 317 patients that additionally applied Mendelian randomization (2).

Both reports originate from a single group and a single analytical platform. No confirmation in a cohort outside that group has been published. This report supplies one.

We arrived at SM 42:3 independently, through reanalysis of the public case-control cohort MTBLS13295. That derivation is excluded from the evidence presented here: the cohort acquired all 36 patients in acquisition positions 1–36 and all 32 controls in positions 37–68, so acquisition order alone predicts diagnosis at area under the curve 1.000 and no case-control contrast from it is identifiable.

Methods

Cohort. ST004318 (Metabolomics Workbench), 34 serum samples from ALS patients, 12 fast-progressing (≥1.5 ALSFRS-R points/month) and 22 slow-progressing (≤0.5), profiled by Metabolon ultra-high-performance liquid chromatography–tandem mass spectrometry. Neither prior report uses this cohort. Abundances were log2-transformed and standardized. Group separation was assessed by Mann-Whitney U, by area under the receiver operating characteristic curve, and by logistic regression with 5-fold cross-validation repeated 20 times. Sex was imbalanced across groups (8/12 fast female vs 6/22 slow, Fisher P = 0.036) and was therefore included as a covariate in a linear model on log abundance.

Recomputed case-control effect sizes. The originating case-control report publishes per-metabolite distributions rather than per-metabolite tests. Cohen's d was computed from the published group means and pooled standard deviations. These are our computation, not the authors' statistics, and are uncorrected for multiple comparisons.

No new samples were collected or measured. All primary data are public.

Results

SM 42:3 separates progression groups in an independent cohort. In ST004318, SM 42:3 is higher in fast progressors: area under the curve 0.883, Cohen's d = +1.24, Mann-Whitney P = 0.0003. The association survives adjustment for sex (P = 0.0031) and repeated cross-validation (0.872 ± 0.148).

Direction is concordant across all three cohorts and both platforms. Table 1 assembles the evidence. The species is elevated in patients relative to healthy controls in both originating cohorts, associated with worse functional status and shorter time to decline in the longitudinal cohort, and elevated in faster progressors here.

A co-analyzed candidate fails the same test. 2-hydroxypalmitate was the single strongest analyte in ST004318 (area under the curve 0.894, d = +1.32, sex-adjusted P = 0.0003), marginally exceeding SM 42:3. It does not replicate. In the 317-patient longitudinal cohort it is null for functional status (P = 0.65) and for both time-to-decline

endpoints, with hazard ratios of 0.92 and 0.89, point estimates opposite in direction to the discovery result. Across the two case-control cohorts it gives d = +0.094 (P ≈ 0.53) and d = +0.413, inconsistent between cohorts of the same study.

This calibrates the SM 42:3 result. ST004318 uses an extreme-phenotype design, which inflates effect sizes; d = +1.32 in 34 samples proved uninformative about the underlying association. That SM 42:3 replicates under the same procedure while 2-hydroxypalmitate does not demonstrates that the test discriminates between them.

Table 1. SM(d18:2/24:1) across three cohorts

Cohort	n	Contrast	Result	Source
Case-control 1	125 / 71	ALS vs healthy	d = **+0.585**	recomputed from (1)
Case-control 2	225 / 104	ALS vs healthy	d = **+0.530**	recomputed from (1)
Longitudinal	317 (548 samples)	functional status	padj = **1.9×10^{-9}**	as published (2)
Longitudinal	317	time to 20% decline	HR 1.19 (1.04–1.37)	as published (2)
Longitudinal	317	time to 50% decline	HR 1.22 (1.07–1.40)	as published (2)
Mendelian randomization	32 instruments	genetic liability to ALS	OR **1.079** (1.016–1.146)	as published (2)
ST004318	**34**	**fast vs slow progression**	**AUC 0.883, d = +1.24, P = 0.0003**	**this report**

Direction is consistent throughout: higher SM 42:3 accompanies disease and worse trajectory.

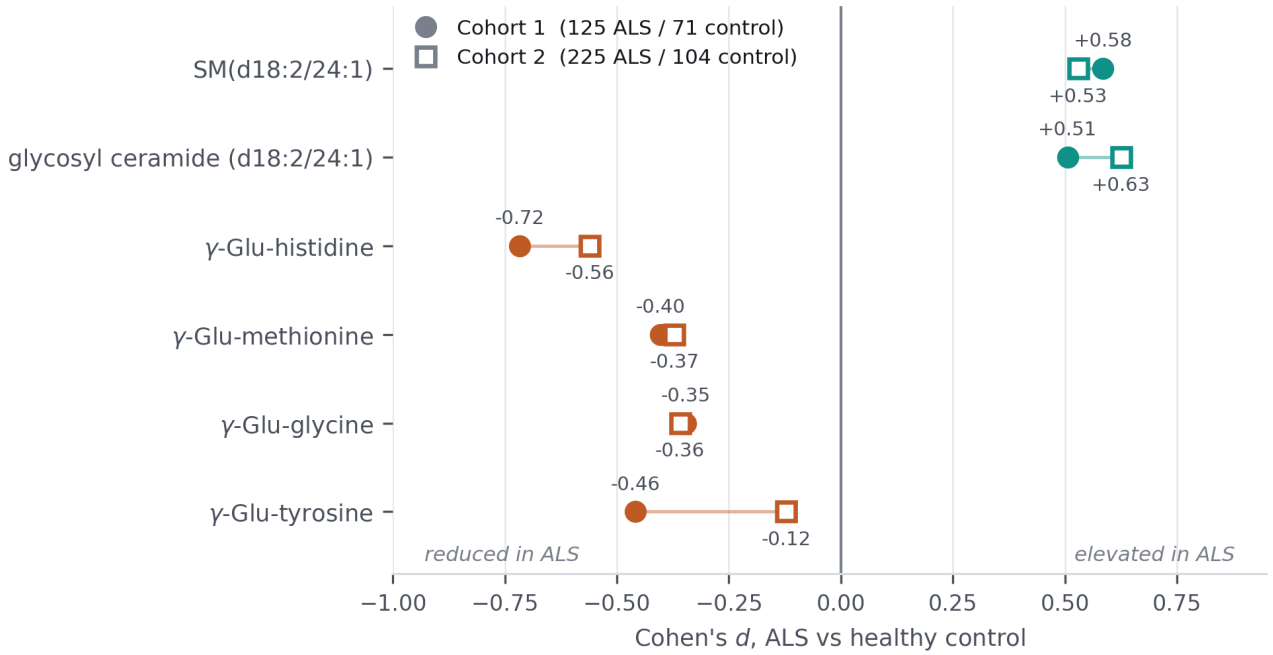

Figure 1

Figure 1. Effect sizes (Cohen's d) in ALS relative to healthy control, recomputed from the published distributions of (1). Filled circles, cohort 1 (125 ALS/71 control); open squares, cohort 2 (225 ALS/104 control). SM(d18:2/24:1) and its backbone-sharing partner glycosyl ceramide (d18:2/24:1) are elevated; gamma-glutamyl amino acids, shown for context, move in the opposite direction.

Discussion

SM 42:3 was first reported by the group cited in (1) and (2). The confirmation presented here is independent on three counts: a cohort neither report used, a different contrast (progression rate rather than disease status), and an analysis performed without access to their per-sample data.

Concordance across three cohorts is stronger evidence than any single result, and the Mendelian randomization in (2) argues the association is not merely a consequence of illness. Together these make SM 42:3 the best-supported serum lipid marker in ALS.

Four limitations bound the result. The recomputed case-control effect sizes derive from published summary statistics rather than per-sample data and should be verified against the authors' own tests. ST004318 is small (n = 34) and uses an extreme-phenotype design that inflates effect sizes. Its contrast is progression rate within diagnosed patients, not diagnosis, so this report does not independently confirm the diagnostic claim, only its direction. And no cohort examined here includes neurological disease controls, so specificity for ALS as against neurodegeneration generally remains untested.

That last point is the natural next step, and it requires a cohort holding both.

References

1. Goutman SA, Guo K, Savelieff MG, Patterson A, Sakowski SA, Habra H, Karnovsky A, Hur J, Feldman EL. Metabolomics identifies shared lipid pathways in independent amyotrophic lateral sclerosis cohorts. Brain 2022;145(12):4425–4439. doi:10.1093/brain/awac025
2. Guo K, Savelieff MG, Jang DG, Teener SJ, Zhao L, Hur J, Goutman SA, Feldman EL. Longitudinal metabolomics in amyotrophic lateral sclerosis implicates impaired lipid metabolism. Ann Neurol 2025;98:19–34. doi:10.1002/ana.27208

Data availability

ST004318, Metabolomics Workbench. MTBLS13295, MetaboLights. Effect sizes in Table 1 rows 1–2 were computed from the supplementary material of reference (1). Analysis code available on request.

Competing interests

Baja Bio, Inc. has filed a provisional patent application covering a two-axis index in which SM(d18:2/24:1) is one component. No claim is made to SM(d18:2/24:1) alone, which is prior art per references (1) and (2).