

Hippocampal proton magnetic resonance spectroscopy and symptom severity in medication-naive children with attention-deficit/hyperactivity disorder: an exploratory retrospective cross-sectional study

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Abstract

Background

Hippocampal circuits contribute to contextual processing, memory and attentional control, but within-cohort associations between hippocampal proton magnetic resonance spectroscopy (^1H -MRS) measures and symptom severity in childhood attention-deficit/hyperactivity disorder (ADHD) remain uncertain.

Methods

This retrospective cross-sectional study included 72 medication-naïve children aged 6–13 years with ADHD who underwent clinically acquired bilateral hippocampal single-voxel ^1H -MRS on a 3.0-T GE Discovery MR750 system in 2021. NAA/Cr, NAA/Cho and NAA/(Cho + Cr) ratios were examined across the hippocampal head, body and tail. Pearson correlations with Benjamini–Hochberg false-discovery-rate (FDR) correction were applied to the SNAP-IV correlation matrix (72 tests), followed by age- and sex-adjusted regression for FDR-significant associations. Sensitivity analyses included Spearman correlations, post hoc exclusion of observations with unusually high ratios (> 3.0), and Cook's distance influence diagnostics. Quantitative spectral-quality metrics were unavailable from the retrospective dataset.

Results

The cohort comprised 65 boys and 7 girls (mean age 8.93 ± 1.55 years); 55 children had both SNAP-IV data and a usable left hippocampal head spectrum. In the initial Pearson analysis, lower left hippocampal head NAA/Cr was associated with higher inattention ($r = -0.521$, $q = 0.003$) and SNAP-IV mean scores ($r = -0.455$, $q = 0.012$), while lower NAA/(Cho + Cr) was associated with higher inattention ($r = -0.467$, $q = 0.012$) and mean scores ($r = -0.437$, $q = 0.016$). These associations remained significant after adjustment for age and sex. None remained statistically significant in Spearman analyses or after post hoc exclusion of observations with ratios > 3.0 , and one to three observations per regression model exceeded the Cook's distance screening threshold of $4/n$. No association involving QCD or CPRS-48 scores survived FDR correction.

Conclusions

Initial parametric analyses suggested associations between lower NAA-based ratios in the left hippocampal head and greater inattention and overall ADHD symptom severity, but these associations were sensitive to influential observations and were not reproduced in rank-based or post hoc exclusion analyses. In the absence of quantitative spectral-quality metrics and a typically developing comparison

group, the findings remain inconclusive and should be considered hypothesis-generating. Prospective studies incorporating quantitative spectral-quality assessment, motion monitoring and typically developing comparison groups are needed to determine the clinical relevance of hippocampal metabolite ratios in ADHD.

Background

Attention-deficit/hyperactivity disorder (ADHD) is a common neurodevelopmental disorder of childhood, with pooled prevalence estimates of approximately 5% and substantial variation by diagnostic method and setting [1]. ADHD is defined by developmentally inappropriate inattention and/or hyperactivity-impulsivity and is often accompanied by academic, emotional and social impairment [2]. Current evidence supports ADHD as a heterogeneous neurodevelopmental disorder involving distributed neural systems rather than a single focal brain abnormality [2, 3].

Structural and functional neuroimaging studies have implicated fronto-striatal, fronto-cerebellar, parietal and limbic networks in ADHD [4]. Large-scale work from the ENIGMA ADHD consortium identified small subcortical volume differences, particularly in children, but neuroimaging findings remain heterogeneous and are not sufficiently specific for clinical diagnosis [5]. Examining associations between imaging measures and symptom dimensions may therefore provide complementary information to conventional case-control comparisons.

The hippocampus has established roles in memory and contextual processing and interacts with prefrontal and limbic systems involved in attention and emotional regulation. A multimodal MRI study in medication-naïve children with ADHD suggested that hippocampal morphology may be relevant to affective symptoms in ADHD [6]. Hippocampal neurochemistry, however, has been examined less frequently than frontal or striatal regions in pediatric ADHD.

Proton magnetic resonance spectroscopy (^1H -MRS) provides in vivo estimates of brain metabolites. N-acetylaspartate (NAA) is commonly interpreted in relation to neuronal integrity and mitochondrial metabolism, choline-containing compounds reflect aspects of membrane turnover, and creatine (Cr) is frequently used as an internal reference. MRS studies in ADHD have reported heterogeneous findings involving NAA, choline, creatine and glutamate/glutamine across brain regions, age groups, medication exposures, acquisition protocols and quantification methods [7–14]. Against this background, the present study examined NAA-based metabolite ratios in the bilateral hippocampal head, body and tail of medication-naïve children with ADHD and evaluated their associations with SNAP-IV symptom severity using multiple-comparison correction and complementary sensitivity analyses. Because no typically developing control group was available, the study focused on within-cohort symptom-metabolite associations rather than ADHD-control differences.

Methods

Study design and setting

This single-center retrospective cross-sectional study used pre-existing clinical rating-scale and neuroimaging data from children evaluated for ADHD at Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology between January and December 2021. For each child, one index clinical record linked to the MRI/MRS examination was used for the cross-sectional analysis. Reporting was guided by the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [15].

Participants

The source dataset comprised 72 unique children aged 6–13 years with a clinical diagnosis of ADHD documented as meeting DSM-5 criteria and an index clinical record linked to MRI/MRS. All children were medication-naïve before the MRI/MRS examination. Eligibility criteria were: (1) a clinical diagnosis of ADHD according to DSM-5 criteria; (2) age 6–13 years at the index assessment; and (3) available hippocampal MRS and clinical rating-scale data for at least one analysis. Children were excluded if the clinical record documented a neurological disorder affecting brain structure or function, intellectual disability, or a clinically significant structural abnormality on conventional MRI.

Spectra with an unstable baseline or visually unacceptable spectral quality were excluded from the corresponding subregional analysis rather than resulting in exclusion of the child from the entire cohort. Likewise, participants with missing rating-scale data were excluded only from the analysis requiring that measure. Formal IQ testing at the index visit was available for only two children; therefore, intellectual disability was based on existing clinical documentation and could not be independently reassessed using a uniform IQ threshold. Because the study used retrospectively acquired clinical data, the number of available observations differed across hippocampal subregions and clinical measures.

No a priori sample-size calculation was performed. The sample comprised all eligible children with available index MRI/MRS-linked records during the prespecified January–December 2021 study period.

MRI and ¹H-MRS acquisition

Imaging was performed on a 3.0-T GE Discovery MR750 system (GE Healthcare, Milwaukee, WI, USA) using the manufacturer's clinical head coil; the specific coil model and channel count could not be recovered from the archived records. Conventional MRI included axial, sagittal and coronal T1-weighted and T2-weighted imaging and axial fluid-attenuated inversion recovery imaging. A sagittal three-dimensional T1-weighted brain-volume acquisition was obtained for anatomical evaluation and MRS voxel localization (TR/TE = 8.16/3.18 ms; inversion time = 450 ms; flip angle = 12°; field of view = 256 × 256 mm; matrix = 256 × 256; slice thickness = 1 mm; no interslice gap; 165 sagittal slices).

Single-voxel ^1H -MRS was acquired using the vendor SVS-SE-30 sequence (TR/TE = 1500/30 ms; flip angle = 90° ; 256 signal averages; acquisition bandwidth = 1000 Hz). Separate voxels measuring $1.5 \times 1.0 \times 0.75$ cm (approximately 1.1 mL) were positioned in the head, body and tail of the left and right hippocampi when technically feasible. Automatic prescanning was used for gain adjustment, shimming and water suppression. Metabolite peaks and peak areas were obtained using the spectroscopy analysis software integrated into the GE workstation; the software version could not be recovered from the archived dataset. Nominal chemical-shift positions were N-acetylaspartate (NAA) at 2.0 ppm, creatine (Cr) at 3.0 ppm, choline-containing compounds (Cho) at 3.2 ppm and myo-inositol (ml) at 3.5 ppm. MRS acquisition and analysis are reported in accordance with MRSinMRS and clinical brain MRS recommendations to the extent permitted by the retrospective archived data [16, 17]. A representative hippocampal spectrum and representative voxel placement are shown in Figs. 4 and 5, respectively.

Quantitative spectral-quality metrics, including signal-to-noise ratio (SNR), full width at half maximum (FWHM) and Cramér–Rao lower bounds (CRLB), could not be recovered from the vendor-exported retrospective dataset. Spectra were assessed visually for baseline stability and lineshape, and spectra with unstable baselines or visually unacceptable quality were excluded from the corresponding subregional analysis. Because hippocampal spectroscopy is susceptible to magnetic-field inhomogeneity and the voxel volume was small, the absence of quantitative spectral-quality metrics limits confidence in individual metabolite estimates. Additional sources of uncertainty included variable spectral availability across subregions, reliance on visual rather than quantitative spectral-quality screening and unavailable motion metrics. These limitations were considered in the interpretation of the findings and were partially explored through available-case reporting and sensitivity and influence analyses, but they could not be directly quantified or eliminated.

Metabolite ratios

Three NAA-based metabolite ratios were analyzed for each available hippocampal subregion: NAA/Cr, NAA/Cho and NAA/(Cho + Cr). Although the acquisition included the myo-inositol (ml) resonance, the exported analytic dataset contained only the NAA-based ratios used in the present analysis. Absolute metabolite concentrations were not available. Accordingly, these ratio-based measures depend on the relative stability of the denominator metabolites and cannot distinguish changes in NAA from changes in Cr, Cho, or both.

Clinical assessments

ADHD symptom severity was assessed using the Chinese parent form of the Swanson, Nolan and Pelham-IV Rating Scale (SNAP-IV), which includes inattention, hyperactivity/impulsivity and oppositional domains [18]. In routine clinical practice, a trained assessor recorded the parent's responses. The Questionnaire-Children with Difficulties (QCD) total score was used to assess daily functional difficulties in the available subset [19]. Behavioral and emotional symptoms were assessed using the 48-item

Conners Parent Rating Scale (CPRS-48), including conduct problems, psychosomatic symptoms, anxiety, learning problems, impulsive-hyperactive symptoms and the hyperactivity index [20]. Rating-scale data from the index MRI/MRS-linked visit were preferentially used; when same-visit data were unavailable, the assessment closest in time to the MRI/MRS examination was selected. Later follow-up measurements were not included in the cross-sectional analyses.

Statistical analysis

Continuous variables were summarized using mean $\pm$ standard deviation and median with interquartile range, and categorical variables as counts and percentages. Pearson correlation coefficients were initially calculated between hippocampal MRS ratios and SNAP-IV scores. The Benjamini–Hochberg false-discovery-rate (FDR) procedure was applied across the complete SNAP-IV correlation matrix (6 subregions $\times$ 3 metabolite ratios $\times$ 4 SNAP-IV domains = 72 tests). Associations with FDR $q < 0.05$ were subsequently examined in age- and sex-adjusted linear regression models to assess whether the observed associations persisted after covariate adjustment; these models were considered secondary and were not treated as independent confirmation.

Robustness was assessed using complementary sensitivity analyses. Spearman rank correlations were calculated for the four FDR-significant left hippocampal head associations. Pearson correlations were also repeated after post hoc exclusion of observations with unusually high left hippocampal head NAA/Cr or NAA/(Cho + Cr) ratios (> 3.0). Because this threshold was selected after inspection of the data, these exclusion analyses were considered exploratory. Regression influence was examined using Cook's distance, with $4/n$ used as a heuristic screening threshold, together with leverage values. To assess whether availability of usable spectral data was associated with symptom severity, SNAP-IV scores were compared between children with and without a usable left hippocampal head spectrum using the Mann–Whitney U test.

QCD and CPRS-48 analyses were exploratory because these measures were available only in smaller subsets. FDR correction was applied across the complete functional-outcome correlation matrix (6 subregions $\times$ 3 metabolite ratios $\times$ 7 measures [QCD total plus six CPRS-48 scores] = 126 tests). Available-case analyses were used, and missing data were not imputed. Analyses were performed in Python using pandas, SciPy and statsmodels. All tests were two-sided. $P < 0.05$ before multiple-comparison correction was considered nominally significant, whereas FDR-adjusted $q < 0.05$ defined statistical significance for the prespecified correlation matrices. Complete correlation matrices are provided in the Supplementary material.

Results

Participant characteristics

The analytic cohort included 72 medication-naïve children with ADHD, comprising 65 boys (90.3%) and 7 girls (9.7%), with a mean age of 8.93 ± 1.55 years. Complete SNAP-IV data were available for 70 children. QCD total scores were available for 21 children, and CPRS-48 hyperactivity-index scores were available for 27 children (Table 1).

Table 1
Baseline characteristics of the study population.

Characteristic	N	Mean $\pm$ SD	Median (IQR)
Age, years	72	8.93 ± 1.55	9.00 (8.00–10.00)
Height, cm	71	138.52 ± 11.69	135.70 (130.20–145.35)
Weight, kg	72	36.39 ± 10.84	33.80 (27.78–43.30)
BMI, kg/m ²	71	18.67 ± 3.25	17.84 (16.16–20.66)
SNAP-IV inattention	70	2.24 ± 0.40	2.30 (2.02–2.40)
SNAP-IV hyperactivity/impulsivity	70	1.79 ± 0.60	1.80 (1.40–2.18)
SNAP-IV oppositional defiant	70	1.13 ± 0.51	1.00 (0.80–1.48)
SNAP-IV mean score	70	1.73 ± 0.37	1.75 (1.50–2.00)
QCD total score	21	37.10 ± 10.40	37.00 (26.00–45.00)
Conners hyperactivity index	27	1.20 ± 0.36	1.20 (1.00–1.40)

BMI, body mass index; IQR, interquartile range; QCD, Questionnaire-Children with Difficulties; SD, standard deviation; SNAP-IV, Swanson, Nolan and Pelham-IV Rating Scale.

Availability and distribution of hippocampal MRS data

MRS data availability varied across hippocampal subregions. Left hippocampal head NAA/Cr data were available for 57 children, left hippocampal body data for 33, left hippocampal tail data for 28, right hippocampal head data for 45, right hippocampal body data for 31, and right hippocampal tail data for 29. Metabolite-ratio distributions by subregion are summarized in Table 2.

Descriptively, NAA-based ratios appeared lowest in the hippocampal head and higher in the body and tail in both hemispheres. These values were not formally compared across subregions because sample availability differed and spectral quality was not quantitatively characterized.

Table 2
Availability and distributions of hippocampal MRS metabolite ratios.

Region	Available NAA/Cr, N	NAA/Cr, median (IQR)	NAA/Cho, median (IQR)	NAA/(Cho + Cr), median (IQR)
Left hippocampal head	57	1.19 (0.88–1.50)	0.85 (0.68–1.27)	0.48 (0.37–0.64)
Left hippocampal body	33	1.36 (1.20–1.55)	1.24 (1.01–1.47)	0.65 (0.56–0.72)
Left hippocampal tail	28	1.73 (1.48–2.19)	1.78 (1.39–2.05)	0.87 (0.73–0.99)
Right hippocampal head	45	1.13 (0.89–1.58)	0.93 (0.79–1.21)	0.50 (0.43–0.65)
Right hippocampal body	31	1.32 (1.08–1.66)	1.21 (1.10–1.63)	0.65 (0.54–0.76)
Right hippocampal tail	29	1.69 (1.45–1.77)	1.70 (1.48–1.86)	0.82 (0.75–0.90)

Values are median (interquartile range). Cho, choline; Cr, creatine; MRS, magnetic resonance spectroscopy; NAA, N-acetylaspartate.

Associations between hippocampal MRS ratios and SNAP-IV scores

In the initial Pearson correlation analysis, four associations remained statistically significant after FDR correction across the 72-test SNAP-IV matrix, all involving metabolite ratios in the left hippocampal head. Lower NAA/Cr was associated with higher SNAP-IV inattention ($r = -0.521$, $q = 0.003$) and mean scores ($r = -0.455$, $q = 0.012$). Lower NAA/(Cho + Cr) was similarly associated with higher inattention ($r = -0.467$, $q = 0.012$) and mean scores ($r = -0.437$, $q = 0.016$) (Table 3; Supplementary Table S1). No other association survived FDR correction.

Table 3
SNAP-IV associations surviving false-discovery-rate correction.

Region/metabolite	Variable	N	r	P	FDR q
Left hippocampal head NAA/Cr	SNAP-IV inattention	55	-0.521	< 0.001	0.003
Left hippocampal head NAA/Cr	SNAP-IV mean score	55	-0.455	< 0.001	0.012
Left hippocampal head NAA/(Cho + Cr)	SNAP-IV inattention	55	-0.467	< 0.001	0.012
Left hippocampal head NAA/(Cho + Cr)	SNAP-IV mean score	55	-0.437	< 0.001	0.016

Only associations with FDR $q < 0.05$ are shown. FDR, false discovery rate; NAA, N-acetylaspartate; SNAP-IV, Swanson, Nolan and Pelham-IV Rating Scale.

The four associations remained statistically significant in age- and sex-adjusted linear regression models (Table 4). These models were considered secondary covariate-adjusted analyses rather than independent confirmation because they were based on the same observations and remained sensitive to influential data points.

Table 4
Age- and sex-adjusted left hippocampal head associations.

Metabolite	Outcome	N	β	95% CI	P	Adjusted R ²
Left hippocampal head NAA/Cr	SNAP-IV inattention	55	-0.181	-0.262 to -0.100	< 0.001	0.240
Left hippocampal head NAA/Cr	SNAP-IV mean score	55	-0.153	-0.228 to -0.077	< 0.001	0.232
Left hippocampal head NAA/(Cho + Cr)	SNAP-IV mean score	55	-0.305	-0.464 to -0.146	< 0.001	0.214
Left hippocampal head NAA/(Cho + Cr)	SNAP-IV inattention	55	-0.336	-0.512 to -0.160	< 0.001	0.180

Linear regression models included age and sex as covariates. β is the change in clinical score per one-unit increase in metabolite ratio. CI, confidence interval; Cr, creatine; MRS, magnetic resonance spectroscopy; NAA, N-acetylaspartate; SNAP-IV, Swanson, Nolan and Pelham-IV Rating Scale.

Sensitivity and influence analyses

None of the four FDR-significant Pearson associations remained statistically significant in Spearman rank analyses. The correlations were also attenuated and no longer statistically significant after post hoc exclusion of observations with metabolite ratios > 3.0 (Table 5). These findings suggested that the parametric estimates were sensitive to a small number of influential observations.

Cook's distance identified one to three observations per regression model exceeding the heuristic 4/n screening threshold (approximately 0.07). The largest Cook's distance was 1.21 in the NAA/(Cho + Cr)-inattention model and corresponded to a child with an NAA/(Cho + Cr) ratio of 3.93. Such unusually high ratios may reflect measurement or spectral-quality limitations, although this could not be determined because quantitative spectral-quality metrics were unavailable.

SNAP-IV scores did not differ significantly between children with and without a usable left hippocampal head spectrum (inattention medians, 2.30 vs 2.30; $P = 0.68$; mean score, 1.80 vs 1.60; $P = 0.38$). The two children contributing ratios > 3.0 had comparatively low inattention and mean scores; however, because this subgroup was very small, defined post hoc, and comprised observations that substantially influenced the fitted associations, these values were considered descriptive and were not interpreted as evidence of a distinct biological subgroup.

Table 5
Sensitivity analyses for left hippocampal head associations.

Association	Initial Pearson analysis	Spearman sensitivity analysis	Pearson analysis after post hoc ratio exclusion	Interpretation
Left hippocampal head NAA/Cr vs SNAP-IV inattention	N = 55; $r = -0.521$; $P < 0.001$; FDR $q = 0.003$	$\rho = -0.230$; $P = 0.091$	N = 53; $r = -0.170$; $P = 0.223$	Not statistically significant
Left hippocampal head NAA/Cr vs SNAP-IV mean score	N = 55; $r = -0.455$; $P < 0.001$; FDR $q = 0.012$	$\rho = -0.118$; $P = 0.389$	N = 53; $r = -0.069$; $P = 0.621$	Not statistically significant
Left hippocampal head NAA/(Cho + Cr) vs SNAP-IV inattention	N = 55; $r = -0.467$; $P < 0.001$; FDR $q = 0.012$	$\rho = -0.131$; $P = 0.342$	N = 54; $r = -0.208$; $P = 0.131$	Not statistically significant
Left hippocampal head NAA/(Cho + Cr) vs SNAP-IV mean score	N = 55; $r = -0.437$; $P < 0.001$; FDR $q = 0.016$	$\rho = -0.047$; $P = 0.734$	N = 54; $r = -0.225$; $P = 0.102$	Not statistically significant

High-ratio-exclusion analyses used post hoc thresholds of left hippocampal head NAA/Cr ≤ 3.0 and NAA/(Cho + Cr) ≤ 3.0 and were exploratory. Cr, creatine; NAA, N-acetylaspartate; SNAP-IV, Swanson, Nolan and Pelham-IV Rating Scale.

Functional measures

No correlations between hippocampal MRS ratios and QCD or CPRS-48 scores remained statistically significant after FDR correction. Complete results are provided in Supplementary Tables S2a and S2b.

Discussion

In this retrospective cross-sectional study of medication-naïve children with ADHD, the initial Pearson analysis identified four FDR-significant associations between lower NAA-based ratios in the left hippocampal head and greater inattention or overall SNAP-IV symptom severity. These associations remained statistically significant after adjustment for age and sex. However, none remained significant in Spearman rank analyses, and all were attenuated after post hoc exclusion of unusually high-ratio observations. Influence diagnostics further showed that a small number of observations had a disproportionate effect on the regression estimates.

These findings indicate that the initial left hippocampal head associations were sensitive to influential observations. The available data cannot determine whether the unusually high metabolite ratios reflected biological variation, acquisition-related effects, or limitations of vendor-based peak-area estimation. Quantitative spectral-quality measures, including SNR, linewidth and CRLB, were unavailable,

preventing direct assessment of spectral reliability. This limitation is particularly relevant to hippocampal spectroscopy, where small voxel size and susceptibility-related field inhomogeneity can compromise spectral quality.

In-scanner motion represents an additional potential source of measurement error in pediatric MRS. Motion may impair shimming and spectral quality, and behavioral symptom severity could plausibly covary with motion during acquisition. Motion estimates were not retained in the archived dataset and therefore could not be examined directly. SNAP-IV scores did not differ between children with and without a usable left hippocampal head spectrum, which argues against marked symptom-related differences in spectral availability, although it does not exclude motion-related effects within retained acquisitions.

The localization of the initial associations to the left hippocampal head should not be interpreted as evidence of regional specificity. Although the anterior hippocampus interacts with prefrontal and limbic systems involved in contextual processing, attention and emotional regulation, no typically developing comparison group was available, and the observed associations were not robust in sensitivity analyses. The present study therefore cannot establish that hippocampal neurochemistry, or the left hippocampal head specifically, is abnormal in ADHD.

NAA is commonly interpreted in relation to neuronal integrity and mitochondrial metabolism, but ratio-based MRS measures depend on the relative stability of the denominator metabolites. Because absolute metabolite concentrations were unavailable, the present data cannot distinguish changes in NAA from changes in Cr, Cho, or both. Mechanistic interpretation is therefore limited. This caution is consistent with the broader ADHD MRS literature, in which findings vary across brain regions, age groups, treatment exposure, acquisition protocols and quantification methods [7–14].

The initial associations were strongest for inattention, which is biologically plausible given hippocampal-prefrontal interactions, but this observation remains hypothesis-generating because it was not reproduced in the sensitivity analyses. Similarly, no association involving QCD or CPRS-48 measures survived FDR correction. Because these measures were available only in small subsets, the analyses were imprecise and do not support strong conclusions regarding the absence of functional associations.

Several limitations should be considered. The study was retrospective and single-center, and no typically developing comparison group was available. IQ and comorbidity were incompletely characterized, spectral availability differed across hippocampal subregions, and quantitative spectral-quality and motion metrics were unavailable. Metabolite concentrations were expressed only as relative ratios, the > 3.0 sensitivity threshold was selected post hoc, the sample was markedly imbalanced by sex, and the functional-outcome subsets were small. In addition, the exact interval between rating-scale assessment and MRS acquisition could not be uniformly recovered from the retrospective records. These limitations constrain causal, mechanistic and region-specific interpretation.

Overall, the present findings illustrate how apparently significant symptom–MRS associations in pediatric datasets may depend on a small number of influential observations when quantitative spectral-quality information is unavailable. Future studies should prospectively retain SNR, FWHM and CRLB measures, prespecify spectral-quality exclusion criteria and robustness analyses, quantify in-scanner motion, use absolute or water-referenced metabolite estimates where feasible, include typically developing comparison groups, and recruit samples with more balanced sex representation.

Conclusions

In medication-naïve children with ADHD, initial parametric analyses suggested associations between lower NAA-based ratios in the left hippocampal head and greater inattention and overall symptom severity. These associations were not reproduced in rank-based analyses and were attenuated after exclusion of a small number of unusually high-ratio observations. In the absence of quantitative spectral-quality metrics and a typically developing comparison group, the findings remain inconclusive and should be considered hypothesis-generating. Prospective controlled pediatric MRS studies incorporating quantitative spectral-quality assessment and motion monitoring are needed to determine the clinical relevance of hippocampal metabolite ratios in ADHD.

Abbreviations

ADHD
attention-deficit/hyperactivity disorder
Cho
choline
CI
confidence interval
CPRS-48
48-item Conners Parent Rating Scale
Cr
creatine
CRLB
Cramér-Rao lower bound
DSM-5
Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
FDR
false discovery rate
FWHM
full width at half maximum
MRS
magnetic resonance spectroscopy
NAA

N-acetylaspartate
QCD
Questionnaire-Children with Difficulties
SNAP-IV
Swanson, Nolan and Pelham-IV Rating Scale
SNR
signal-to-noise ratio
STROBE
Strengthening the Reporting of Observational Studies in Epidemiology
TR/TE
repetition time/echo time

Declarations

Ethics approval and consent to participate

This study involved secondary analysis of pre-existing clinical and neuroimaging data collected during routine clinical care in 2021. The study was approved by the Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (approval number: TJ-IRB202608015). The requirement for informed consent to participate was waived by the Ethics Committee because the study used pre-existing data and all analyses were performed on de-identified records.

Consent for publication

Written informed consent for publication of the anonymized images shown in Figs. 4 and 5 was obtained from the participant's parent or legal guardian.

Competing interests

The authors declare that they have no competing interests.

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Author Contribution

LY conceived the study, curated the analytic dataset, performed the statistical analyses and drafted the manuscript. YL, WC, HL and XH contributed to acquisition, verification and interpretation of the clinical

and neuroimaging data. MC supervised the study and critically revised the manuscript for important intellectual content. All authors reviewed and approved the final manuscript and agree to be accountable for the work.

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Not applicable.

Data Availability

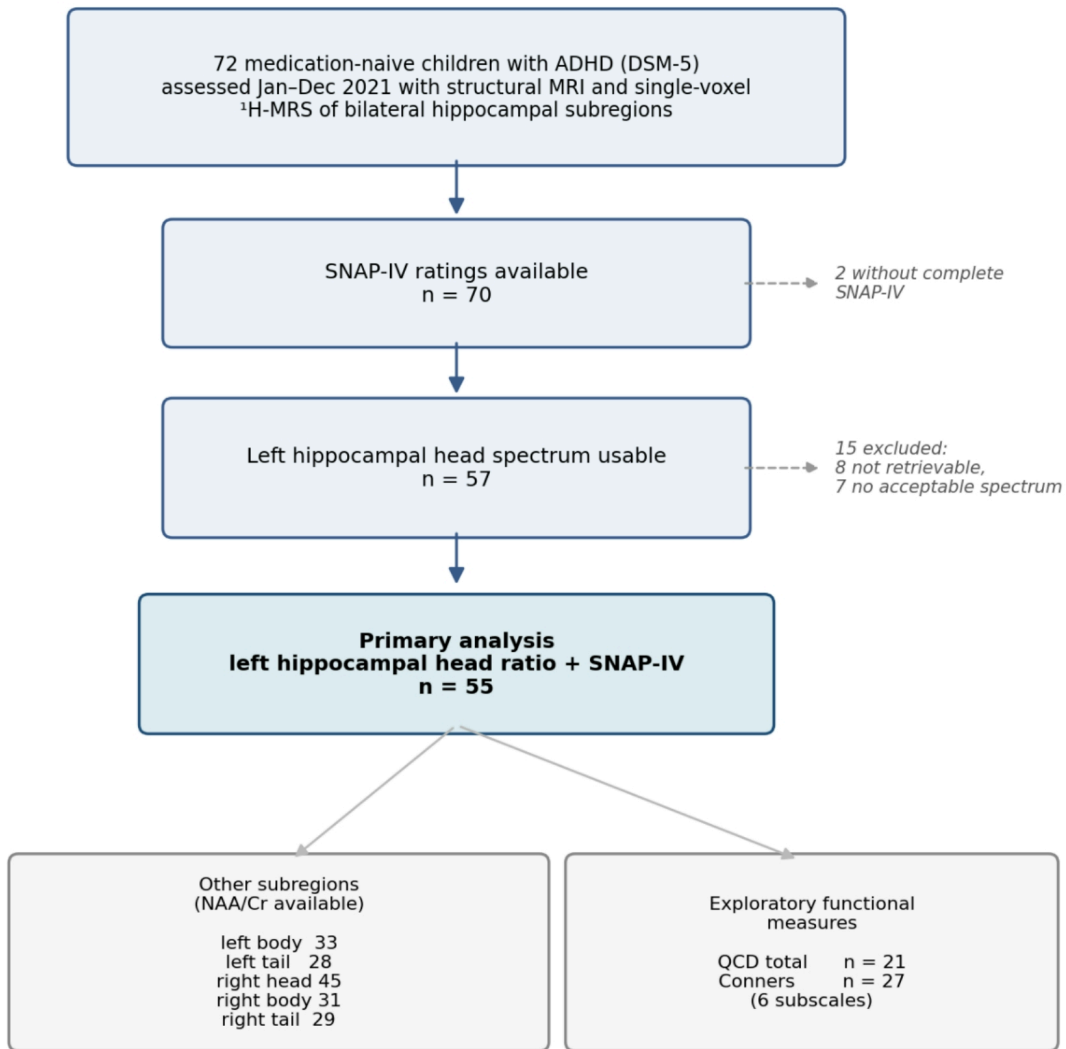
The datasets used and/or analysed during the current study are not publicly available because they contain pediatric clinical and neuroimaging data. They are available from the corresponding author on reasonable request, subject to applicable institutional and ethical approvals.

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Figures



*Single-voxel ¹H-MRS; spectra screened by visual inspection of baseline and lineshape.
MRS data availability varied by subregion (informative missingness).*

Figure 1

Participant and data flow. Of 72 medication-naive children with ADHD, SNAP-IV data were available for 70 children and usable left hippocampal head MRS data for 57; 55 children had both measures available for the principal left hippocampal head analyses. Availability varied across hippocampal subregions and clinical measures. ADHD, attention-deficit/hyperactivity disorder; CPRS-48, 48-item Conners Parent

Rating Scale; MRS, magnetic resonance spectroscopy; QCD, Questionnaire-Children with Difficulties; SNAP-IV, Swanson, Nolan and Pelham-IV Rating Scale.

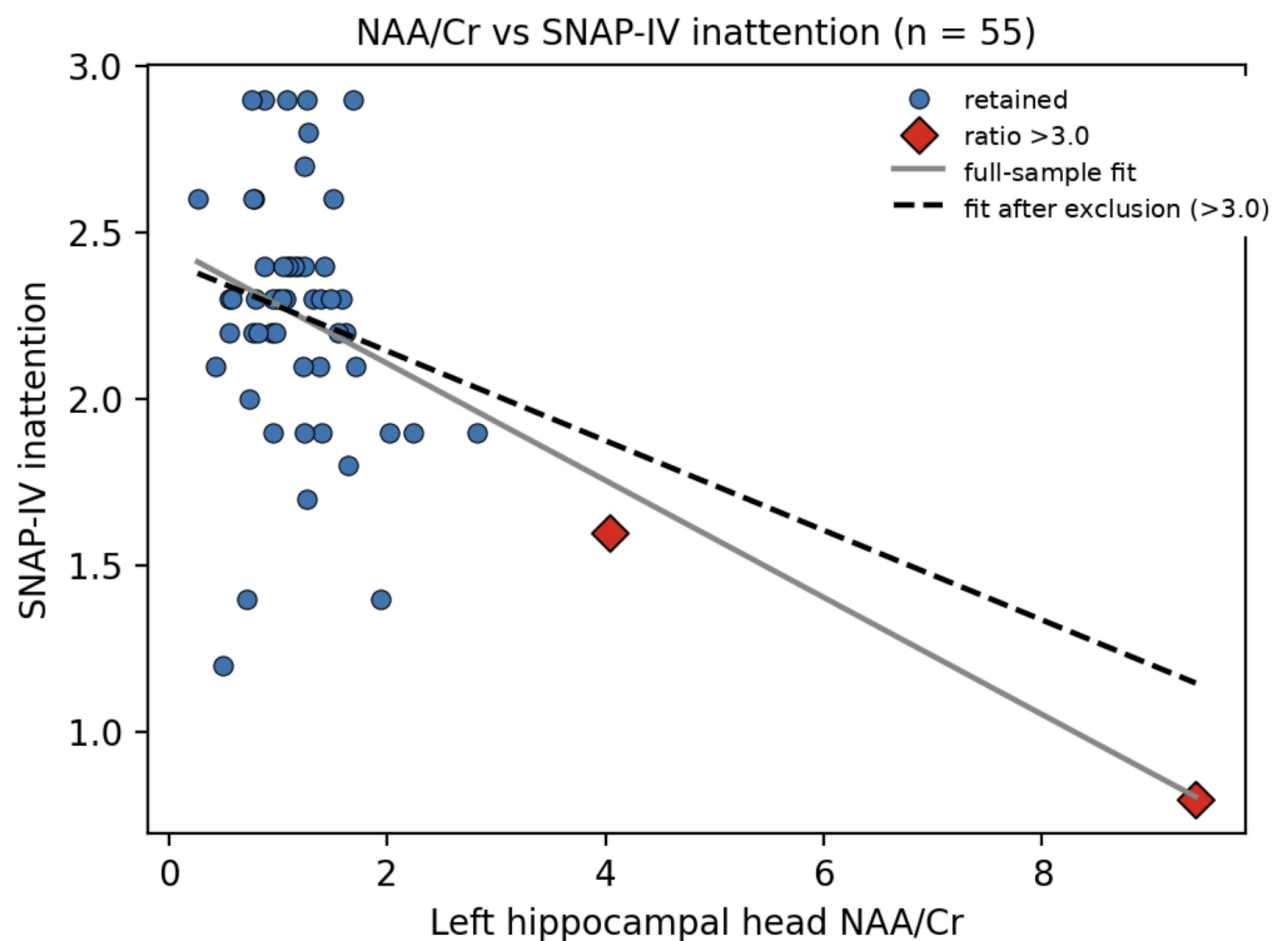


Figure 2

Left hippocampal head NAA/Cr and SNAP-IV inattention score. Each point represents one child (n = 55). Diamonds indicate observations with NAA/Cr >3.0. The solid line represents the least-squares fit for the complete sample, and the dashed line represents the fit after post hoc exclusion of observations with ratios >3.0. Cr, creatine; NAA, N-acetylaspartate; SNAP-IV, Swanson, Nolan and Pelham-IV Rating Scale.

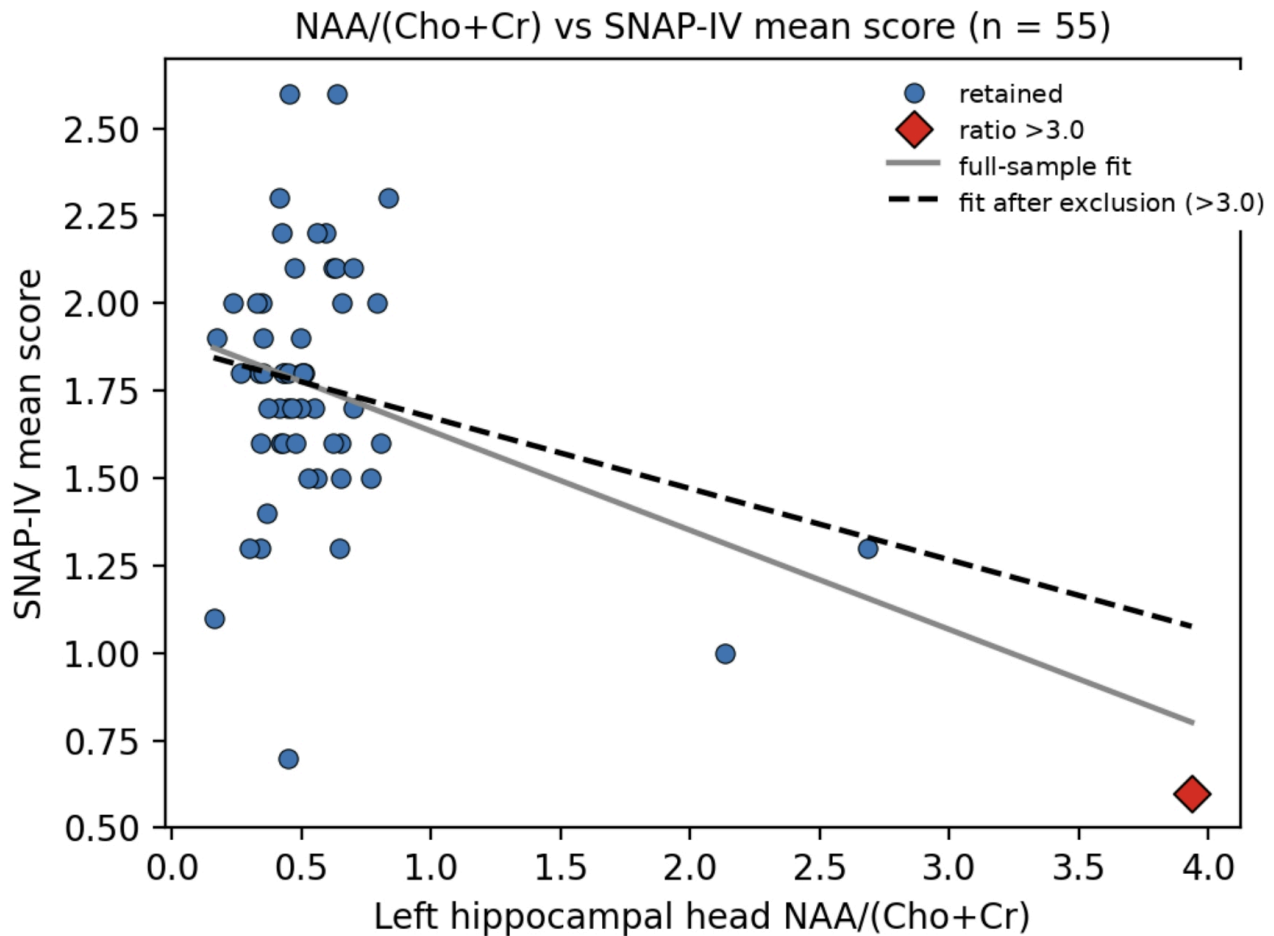


Figure 3

Left hippocampal head NAA/(Cho+Cr) and SNAP-IV mean score. Each point represents one child (n = 55). The diamond indicates the observation with NAA/(Cho+Cr) >3.0. The solid line represents the least-squares fit for the complete sample, and the dashed line represents the fit after post hoc exclusion of the high-ratio observation. Cho, choline-containing compounds; Cr, creatine; NAA, N-acetylaspartate; SNAP-IV, Swanson, Nolan and Pelham-IV Rating Scale.

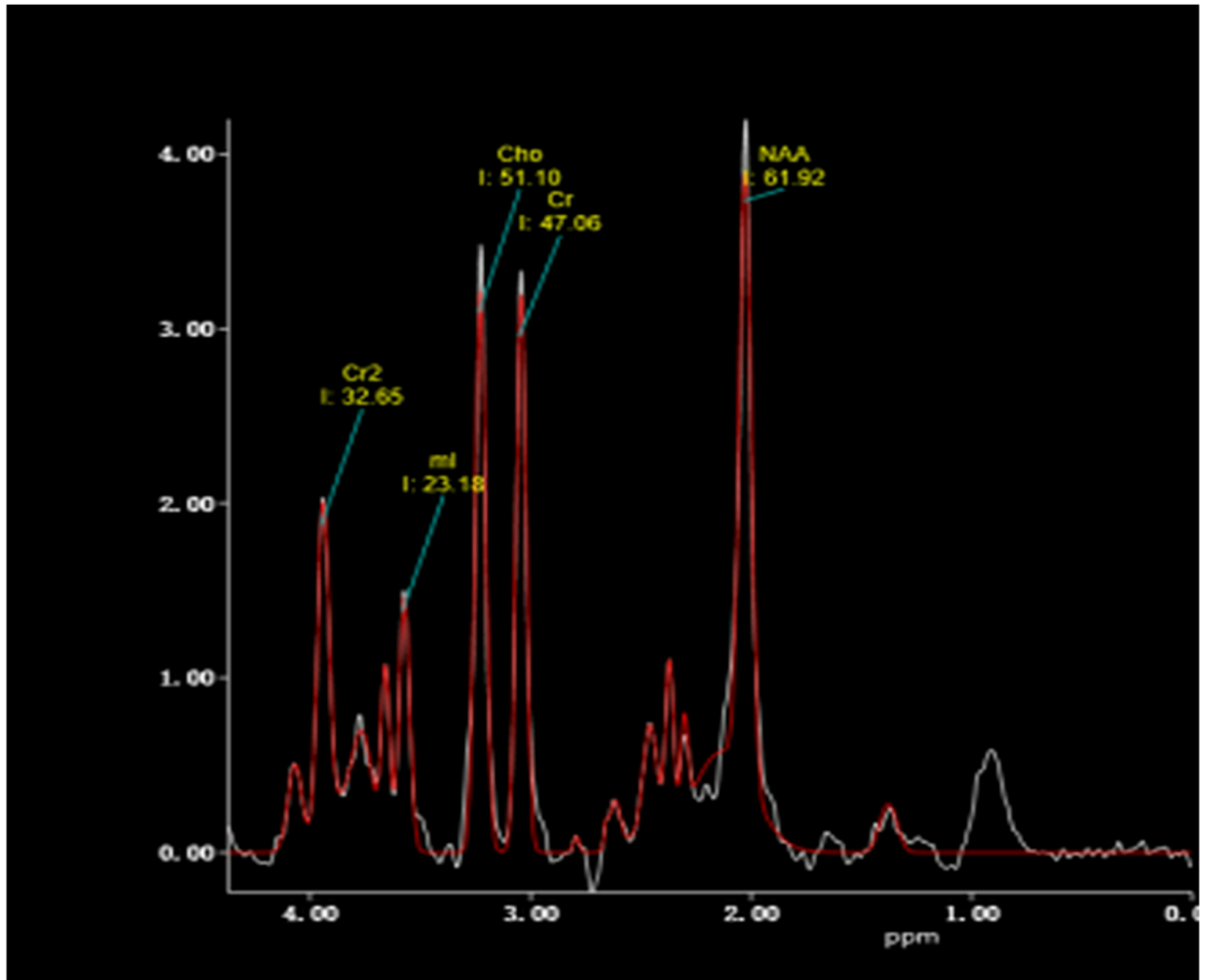
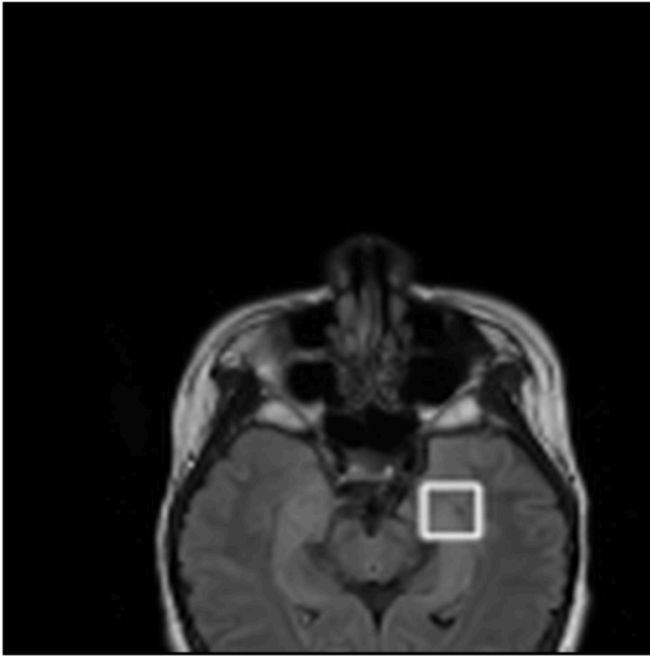


Figure 4

Representative hippocampal ^1H -MRS spectrum. The spectrum shows the principal metabolite resonances, including N-acetylaspartate (NAA) at approximately 2.0 ppm, creatine (Cr) at 3.0 ppm, choline-containing compounds (Cho) at 3.2 ppm and myo-inositol (ml) at 3.5 ppm.

Axial



Coronal

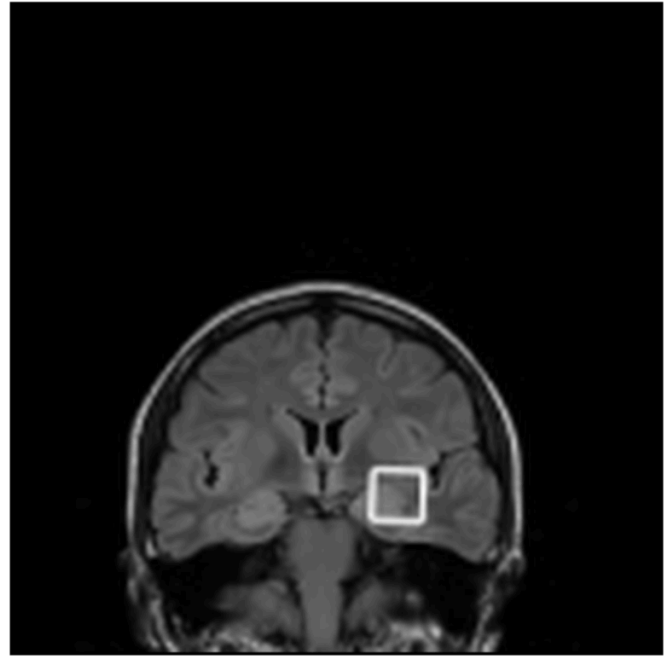


Figure 5

Representative hippocampal voxel placement for single-voxel ^1H -MRS. Axial and coronal structural images show the spectroscopy voxel positioned within the hippocampal region. ^1H -MRS, proton magnetic resonance spectroscopy.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [03SupplementaryMaterial.pdf](#)