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Cerebellar neurometabolite abnormalities in pediatric attention/deficit hyperactivity disorder: A proton MR spectroscopic study

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ABSTRACT

We designed a case–control proton magnetic resonance spectroscopic study comparing the cerebellar and prefrontal regions of a group of 17 ADHD (attention deficit/hyperactivity disorder) medicated children and a group of 17 control children matched for laterality, gender and age. As we had found decreased gray matter volume in the right prefrontal region and the left cerebellar hemisphere in a previous voxel-based morphometry study conducted on an independent ADHD sample, we tested the hypothesis that these regions should show neurometabolite abnormalities. MRI (magnetic resonance imaging) was performed with a 1.5 T system; spectral acquisition was performed with a single-voxel technique and a PRESS sequence. Two volumes of interest were selected in the right prefrontal region and the left cerebellar hemisphere. NAA (N-acetylaspartate), Cre (creatine), Cho (choline), MI (myo-inositol) and Glx (glutamate-glutamine) resonance intensities were absolutely quantified. In the left cerebellar hemisphere, ADHD children showed significant decreased MI and NAA absolute concentrations with high effect sizes ($p = 0.004$, ES = 1.184; $p = 0.001$, ES = 1.083). The diminished absolute concentration of the NAA could be related to a gray matter volume decrease in the same cerebellar region found in the previous voxel-based morphometry MRI study, while the reduced MI absolute concentration could express a decreased glial density. This is the first proton MR spectroscopic study examining the cerebellum and it provides additional support for the role of cerebellum in the ADHD neurobiology.

Although attention deficit/hyperactivity disorder (ADHD) pathophysiology remains as yet incompletely understood, magnetic resonance imaging (MRI) has revealed brain regions that display morphometric abnormalities in ADHD. The most replicated findings [26,30] are decreased total brain volume and prefrontal, caudate and cerebellum abnormalities. All these regions, with the salient exception of the cerebellum, have been investigated using proton magnetic resonance techniques, with controversial results.

There is converging evidence, including recent work by our group [8], that cerebello–thalamo-striatal-prefrontal circuit dysfunction could partially explain ADHD motor control/inhibition and executive function deficits [5].

In a voxel-based morphometry (VBM) study [9], we found decreased gray matter volume in several regions, especially in the right prefrontal cortex (including the dorsolateral prefrontal cortex) and left cerebellar hemisphere (posterior lobe). Employing a diffusion tensor imaging (DTI) technique, Ashtari et al. has reported white matter abnormalities in these regions [3].

We present here a case–control study testing the hypothesis, based on previous evidence, that the prefrontal region and the left cerebellar hemisphere in ADHD subjects should show neurometabolite abnormalities.

The research ethics boards of both participating institutions approved our study. All parents or legal tutors signed a written informed consent and a verbal assent was obtained from all participants.

We enrolled 21 ADHD and 21 control children between April 2006 and December 2007. The ADHD group was recruited from

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Table 1
Demographic and clinical data.

	n	Sex	Handedness	Type	Age (years), mean ± SD	IQ	MTF dosage (mg/kg), mean ± SD	Time (months), mean ± SD
ADHD	17	Boys = 15 Girls = 2	R = 13 L = 1 CD = 3	I = 2 H-I = 1 C = 14	10.41 ± 2.48	106.12 ± 15.33	30.29 ± 13.05	18.21 ± 19.70
Control	17	Boys = 15 Girls = 2	R = 15 L = 1 CD = 1	N/A	10.76 ± 2.80	108.71 ± 14.40	N/A	N/A

IQ, intelligence coefficient; MTF, methylphenidate; R, right-handed; L, left-handed; CD, cross-dominance; I, inattention subtype; H-I, hyperactive-impulsive subtype; C, combined subtype; N/A, not applicable.

the Barcelona's Hospital Vall d'Hebron Paidopsychiatry Unit. All ADHD children were diagnosed according to DSM-IV TR criteria for ADHD [2] by a psychologist and a psychiatrist with 6 and 27 years of expertise respectively. They were characterized by classroom teachers and parents using the Conners' Teacher and Parent's Rating Scale [12,13] and the Child Behavior Checklist (CBCL) [1]. ADHD Children were categorized into hyperactive-impulsive, inattentive and combined subtypes using DSM-IV-TR criteria at the time of diagnosis. WISC-R full-scale intelligence coefficient (IQ) was evaluated [32]. All ADHD subjects were receiving stimulant medication (methylphenidate), but no amphetamines, and were considered by their physicians (based on clinical and neuropsychological evaluations), parents, and teachers to respond positively to the medication. Exclusion criteria for the ADHD group included comorbidity of any other axis I psychiatric disorder (including oppositional disorder), neurological disorders, perinatal anoxia, and WISC-R full-scale IQ below 80. All ADHD children were taken off medication at least 24 h for the purpose of the scan.

Controls were selected from the Traumatology Department using a convenience sampling, and were age-, gender- and laterality-matched with the ADHD group. Control children were outpatients that had suffered minor physical trauma, but none had the diagnosis of head trauma. At the time of the scan all children were fully recovered. Two psychologists excluded ADHD diagnosis employing a semi-structured interview with parents (CBCL). Eventual stress related to trauma was assessed with the CBCL anxiety scale. IQ was estimated by Block Design, Digits and Vocabulary subscales of the WISC-R. Demographic and clinical data is summarized in Table 1.

Before scanning, subjects were screened for contraindications. The MRI examination was performed with a 1.5 T system (Signa, General Electric, Milwaukee, USA). We performed a FSPGR-T1 3D axial sequence (TR = 13.2 ms; TE = 4.2 ms; FA = 15; NEX = 1; 256 × 256 matrix), with 2 mm partitions, and a FSE-PD-T2 axial sequence (TR = 3980 ms; TE = 20/100 ms; NEX = 2; 512 × 512 matrix), with 5 mm sections and 2 mm gap. Spectral acquisition was performed with a single-voxel technique and a PRESS sequence (TR = 2000 ms; TE = 30 ms; NEX = 256; bandwidth = 2500 Hz), after careful shimming of the magnetic field and three chemical-shift selective water suppression pulses. Two volumes of interest of 2 cm × 2 cm × 2 cm were selected using the FSPGR-T1 3D axial sequence in the right dorsolateral prefrontal region and the left cerebellar hemisphere (posterior lobe) by a experienced neuroradiologist, encompassing gray and white matter (Figs. 1 and 2). For metabolite quantification, water signal was first modeled by a non-linear time-domain analysis procedure (AMARES) [31], fitting the spectroscopic signal to an addition of damping sinusoids (Lorentzian model function) to avoid T2* effects in the quantification. The water resonance intensity was quantified as damping-sinusoids amplitude, and suppressed using the HSVD method. NAA (N-acetylaspartate), Cre (creatine), Cho (choline), MI (myo-inositol) and Glx (glutamate-glutamine) resonance intensities were quantified in the same manner as that of water. Finally, normalized values were obtained by dividing the intensity of each peak by the water signal. All peaks were quantified as singlets, selecting the peaks around 3.56 ppm and 2.25 ppm for MI and Glx respectively. All the fittings were assessed visually, and those that were not reliably quantifiable were discarded. A representative MR spectrum of the cerebellum of an ADHD subject is depicted in Fig. 3.

The signal-to-noise ratio (SNR) was calculated as Cr signal over standard deviations of signals from 0.4 ppm to 0.9 ppm, where no significant signal from metabolites is expected, according to the procedure proposed by Okada et al. [22].

Statistical analyses were performed with SPSS (v15.0) and R (v2.8.0). Due to the violation of the normality assumption (Shapiro-Wilk tested), non-parametric statistical analyses were used (Mann–Whitney *U* for two independent samples). Effect size was determined through corresponding confidence intervals for two medians difference and a non-parametric Glass's "delta" statistic analog. Multiple comparisons Bonferroni correction was applied (p-value = 0.005).

A Student's *t*-test compared age and IQ group differences. χ^2 test compared laterality differences. Correlation analyses investigated possible relationships between deviant neurometabolites and dosage and time of methylphenidate intake.

The spectra acquisition was successful in 17 ADHD children and 20 control subjects. We analysed the 17 ADHD children and their age-, gender- and laterality-matched controls, and discarded the remaining three controls.

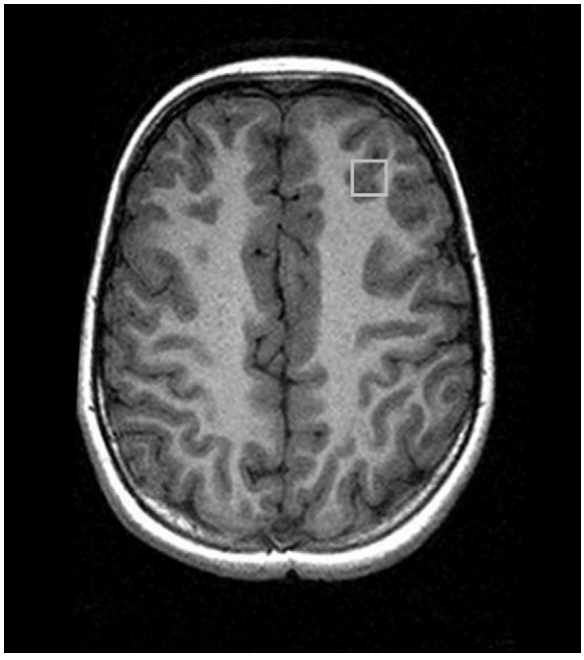

Fig. 1. Volume of interest for spectra acquisition in the right dorsolateral prefrontal region.