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Proton magnetic resonance spectroscopy in developmentally delayed young boys with or without autism

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Summary *Objective*: The aim of the present study is to investigate whether brain metabolism of boys with autism spectrum disorder (ASD) is altered compared to boys with a developmental delay without autism if corrected for patient age and developmental level. *Study design*: 25 boys with ASD (with or without concurrent mental retardation) and 12 boys without ASD with mental retardation or language disorder underwent proton magnetic resonance spectroscopy. All analyses were performed with chronological age and developmental level as independent variables. *Results*: No metabolic differences were found between boys with ASD and without ASD. *Conclusions*: Our findings do not replicate previous reports of differences in NAA, Cho and Cr levels in ASD.

Keywords: Autism spectrum disorder, developmental delay, MRS (or proton) magnetic resonance spectroscopy

Introduction

Autism is a pervasive developmental disorder characterized by a triad of social deficits, language and communication problems and a pattern of stereotyped, repetitive and restricted behaviors and interests (American Psychiatric Association, 1994). It is a clinically heterogeneous condition that has a broad range of severity and is frequently associated with concomitant learning disability.

Imaging of patients with autism offers valuable information on anatomy and function (Akshoomoff et al., 2002; Cody et al., 2002). At present, several magnetic resonance spectroscopy (MRS) studies in patients with autism (in the age range of 2–32 years) have been performed. In general, a trend of decreased N-acetylaspartate (NAA) concentrations, decreased NAA/choline (NAA/Cho) or decreased NAA/creatine (NAA/Cr) ratios was found (Friedman et al., 2003; Filippi et al., 2002; O'Neill et al., 2002). Still, interpretation of MRS data in autism studies is complicated. In addition to differences in absolute and relative metabolic concentrations between studies, regions of interest vary widely (Rumsey and Ernst, 2000; Sokol et al., 2002; Yurgelun-Todd and Renshaw, 2000). Otsuka et al. found lower NAA levels in the amygdala-hippocampal region (Otsuka et al., 1999) whereas others made similar observations in the cerebellum (Chugani et al., 1999) or parietal cortex (Hashimoto et al., 1997). More important, in general it is difficult to elucidate which cerebral metabolic changes are related to autism itself. Approximately three-quarter of the patients with autism also suffers from learning disabilities, which in itself may give rise to metabolic abnormalities (Volkmar and Pauls, 2003). Therefore, when comparing a group of (retarded) autistic children with a group of normal developing children (Hashimoto et al., 1995; O'Neill et al., 2002), autism itself as well as mental retardation may underlie metabolic differences. Except for a study of patients with Asperger syndrome (Murphy et al., 2002), none of the MRS studies in this field used a correction for mental retardation. Moreover, the effects of age dependency on metabolic concentrations are often underestimated. Developmental studies have shown that the concentration of NAA, creatine (Cr), and glutamine and glutamate (Glx)

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increases with brain development, whereas the concentration of choline (Cho) and myo-inositol (mI) decreases with brain development (Bhakoo and Pearce, 2000; Friedman et al., 2003; Moore, 1998; Kreis et al., 1993). In this respect, it is important to keep the age range of both patient and (age matched) control group as small as possible. Even then, including age as an independent covariate in data analysis seems obligatory.

The overall purpose of the present study is to replicate the previously reported abnormalities in our group of boys with autism spectrum disorders (ASD) and a control group of boys with a developmental delay without ASD. The aim was to determine these possible metabolic cerebral changes in the amygdala-hippocampal region and in the frontal subcortical white matter. All participants were between the ages of 1 year 9 months and 6 years 7 months. To correct for the effects of age and developmental level, all analyses were performed with chronological age and developmental level as independent variables.

Material and methods

37 children participated. Participants were recruited from referrals to the Department of Child and Adolescent Psychiatry of University Medical Center Utrecht. Diagnosis was made by a team of board certified child psychiatrists (EvD, JB, HvE) according to DSM-IV criteria (American Psychiatric Association, 1994). Children younger than 42 months at the time of scanning had a final diagnosis when they reached 42 months. The diagnosis was confirmed by Autism Diagnostic Interview Revised (Lord et al., 1994) and the Autism Diagnostic Observation Schedule G (Lord et al., 1989) diagnoses.

When a child was diagnosed with autism (A) or PDD-NOS with or without concurrent developmental retardation; mental retardation (MR) (without an autism spectrum diagnosis); or language disorder (LD), parents were offered the possibility of MRI and MRS. Patients were included if a) they were 18 months to 7 years of age, b) were diagnosed with an autism spectrum disorder, a language disorder or mental retardation, and c) had no contraindication for MRI. For all four groups, children having significant motor or sensory impairment (e.g., blindness, deafness), major physical abnormalities, history of serious head injury, identifiable neurologic disorder or metal implants such as prostheses were excluded. Patients were scanned under full anesthesia with sevofluran. The study design was approved by the Medical Ethical Review Board of the University Medical Center Utrecht. All parents gave written informed consent.

Children were administered the Mullen Scales of Development (Mullen, 1995) to measure the developmental level. Several children were at the floor of the standardized scores. It was decided, therefore, to convert raw scores to a developmental level in order to allow us to look more closely at the functioning of the more impaired children. Four subtests were used; visual receptive, fine motor, receptive language, and expressive language. The developmental level was calculated as Mean Age Equivalent of the four subtests/Chronological Age $\times$ 100. For some children the Mullen was found to be too difficult. For 7 patients with A and one patient with PDD-NOS the Psychoeducational Profile – Revised (Schopler et al., 1994) was used to assess the developmental level. For 5 children either the Griffith (1986) (in one case with PDD-NOS), the Dutch Snijders-Oomen niet-verbale intelligentietest (Tellegen et al., 1996) (for one patient with LD and one with A) or the Kaufman Assessment Battery for Children

Table 1. *Descriptives of the patients*

	A	PDD-NOS	MR	LD
N	17	8	4	8
Age in months (SD)	43 (SD 7)	45 (SD 15)	40 (SD 8)	39 (SD 14)
Developmental quotient (SD)	44 (SD 8)	80 (SD 13)	67 (SD 10)	86 (SD 8)

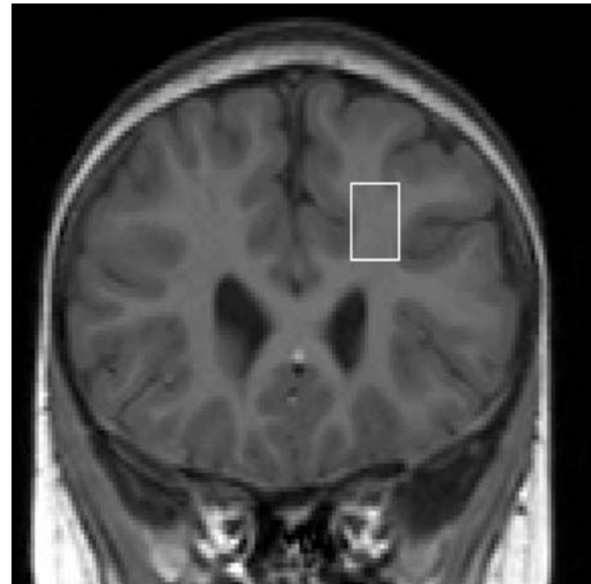

Fig. 1. The VOI in the frontal subcortical white matter

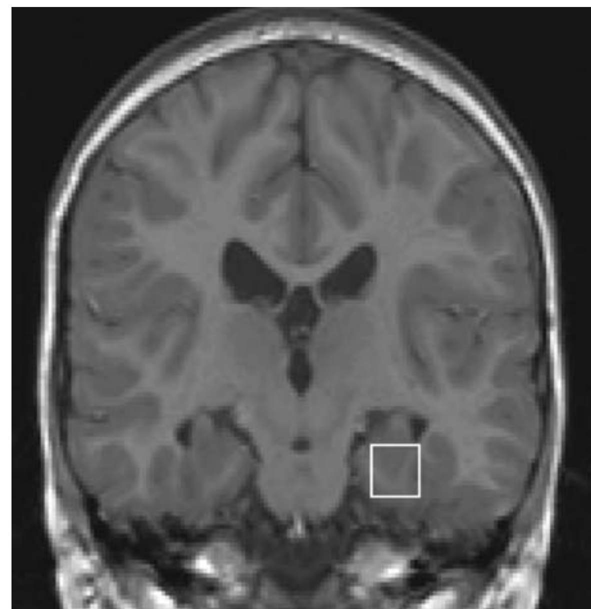

Fig. 2. The VOI in the amygdala-hippocampal complex

(Kaufmann and Kaufmann, 1983) (for two patients with PDD-NOS) was used.

The group with either autism or PDD-NOS, the ASD group, included 25 children (17 were diagnosed with A, 8 with PDD-NOS). The control group, with mental retardation or language disorder, included 12 children; (4 children were diagnosed with MR, 8 with LD). See Table 1 for further descriptives of the sample. The groups differed on mean developmental level ($p < 0.005$), but not on chronological age.

Participants had a physical examination and medical history, including assessment of perinatal circumstances and maternal illness during pregnancy, completed by a developmental paediatrician. Hearing and language evaluation was done by an audiologist and speech and language therapist. During admission on the neuro-pediatric ward of the hospital extensive blood work, including amino-acids, thyroid function, a karyogram and Fragile X testing was completed (van Daalen et al., in preparation). Two children were born prematurely at thirty and thirty-three weeks of gestation with weights accordingly. All children were born with a birth weight above fifteen hundred gram. No children were diagnosed with a metabolic disorder. All children had a karyogram as expected according to gender and no children tested positive for Fragile X or 22q11 deletion syndrome. Two children were treated with valproic acid for symptoms of epilepsy; one boy with autism, no metal retardation and one boy with mental retardation. Two children used thioridazine for excessive fear and panic attacks during which they displayed self-destructive behaviour.

All scans were acquired on a Philips NT Gyroscan scanner, operating at 1.5 Tesla. The ^{1}H-MRS investigations were performed with a single voxel technique. Two volumes-of-interest (VOI) were selected, one in the frontal subcortical white matter (Fig. 1) and another in the amygdala-hippocampus complex (Fig. 2) of each subject. These regions are indicated in neuropathological studies (Araghi-Niknam and Fatemi, 2003; Kemper and Bauman, 1998). Both VOIs were placed in the left hemisphere. This hemisphere was chosen based on studies that found a significantly greater left than right hemisphere dysfunction (Chiron et al., 1995; Dawson, 1983).

The VOIs were chosen from a 3D-FFE T1 (TR/TE 30/4.6 ms, FOV 256/70%, matrix 256×256, slice thickness 1.5 mm, flip = 30). The dimensions of the selected VOIs were typically 15 mm in anterior-posterior, 10 mm in left-right directions and 10 mm in caudo-cranial directions. Special care was taken to position the VOIs away from gray matter (in the case of the frontal subcortical white matter voxel) and CSF and blood vessels (in the case of the amygdala-hippocampal voxel) and the VOI size was reduced

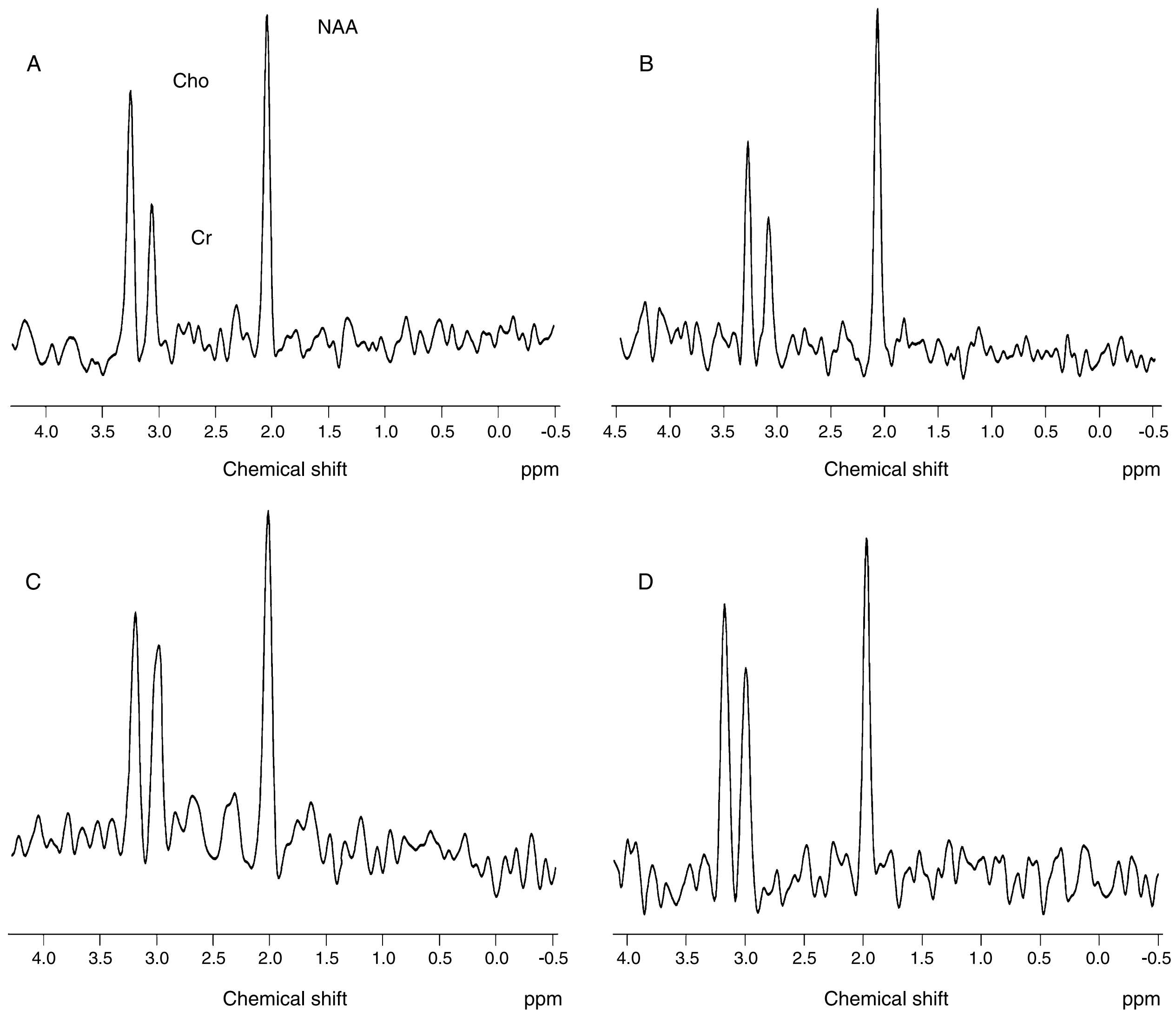

Fig. 3. Typical MR spectra. **a** ASD group frontal. **b** MR group frontal. **c** ASD group amygdala. **d** MR group amygdala

Table 2. *The metabolite concentrations in the frontal subcortical white matter, mean ± SD. No significant differences were found. The N may be smaller than 37. Due to e.g. motion artifacts some measurements were found to be unusable*

Prefrontal subcortical white matter					
	Cho	Cr	NAA	NAA/Cho	NAA/Cr
ASD (SD) (N)	2.10 (SD 0.57) (24)	5.43 (SD 1.09) (25)	9.46 (SD 1.29) (24)	1.43 (SD 0.42) (24)	2.21 (SD 0.45) (24)
MR (SD) (N)	1.75 (SD 0.24) (4)	5.01 (SD 1.81) (4)	9.21 (SD 0.49) (4)	1.58 (SD 0.27) (4)	2.54 (SD 1.12) (4)
LD (SD) (N)	1.94 (SD 0.83) (8)	5.03 (SD 1.31) (8)	8.91 (SD 1.58) (8)	1.28 (SD 0.37) (7)	2.22 (SD 0.39) (8)

Table 3. *The metabolite concentrations in the amygdala-hippocampal complex, mean ± SD. No significant differences were found. The N may be smaller than 37. Due to e.g. motion artifacts some measurements were found to be unusable*

Amygdala-hippocampal complex					
	Cho	Cr	NAA	NAA/Cho	NAA/Cr
ASD (SD) (N)	1.48 (SD 0.52) (21)	5.73 (SD 1.97) (21)	7.06 (SD 1.09) (21)	1.53 (SD 0.46) (23)	1.63 (SD 0.50) (22)
MR (SD) (N)	1.66 (SD 0.48) (2)	4.87 (SD 1.40) (2)	5.61 (SD 0.19) (2)	1.05 (SD 0.34) (2)	1.47 (SD 0.47) (2)
LD (SD) (N)	1.45 (SD 0.40) (8)	5.15 (SD 1.24) (8)	6.57 (SD 1.20) (8)	1.42 (SD 0.39) (8)	1.63 (SD 0.45) (8)

when necessary. After selection of a VOI, the 90-degree pulse length was determined. To minimize eddy currents and to maximize the water echo signal, localized spectroscopy was first performed without water suppression for adjustments of the gradients ("gradient tuning"). This was followed by localized automatic shimming of the VOI, resulting typically in a water resonance line-width of 6 Hz (full width at half maximum) or less. Water suppression was performed by selective excitation (60 Hz bandwidth), followed by a spoiler gradient. A double spin-echo PRESS (point resolved spectroscopy) sequence was used for VOI localization. Each measurement was performed with a repetition time of 2000 ms, an echo time of 144 ms, 2048 time domain data points, 4000 Hz spectral width and 64 signals acquired.

After zero-filling to 4096 data points, exponential multiplication of 2 Hz, Fourier transformation and linear baseline correction, NAA (referenced at 2.0 ppm), total choline (referenced at 3.23 ppm) and total creatine (referenced at 3.02 ppm) peaks were quantitated with the program MRUI, using prior knowledge (van der Veen et al., 1988). Additionally, a water reference spectrum was obtained in order to calculate absolute concentrations of NAA, Cho and Cr.

All analyses were conducted using the SPSS statistical package (version 9.0). We used logistic regression to compare metabolites and metabolite ratios in both VOIs in the autism and the control groups. In the logistic regression analyses we adjusted for chronological age and developmental level.

Results

Typical examples of ^{1}H MR spectra of children in the ASD group and of children in the control group in the frontal subcortical white matter and the amygdala-hippocampal complex are shown in Fig. 3. Mean VOI size in the frontal subcortical white matter was 1145 (SD = 178). Mean VOI size in the amygdala-hippocampal complex was 1091 (SD = 199). Groups did not differ in mean VOI size. No differences in Cho, Cr and NAA concentration nor in NAA/Cho and NAA/Cr ratios were found between the ASD group and the control groups in the frontal subcortical white matter (Table 2). Also, no metabolic differences were found with the MR group or with the LD in the amygdala-hippocampal complex (Table 3). Moreover, in none of the subjects lactate resonances were found in any of the regions. Analyses were repeated without adjusting for chronological age and developmental level. Results did not change.

We did however find a significant positive correlation between age and Cr ($r^2 = 0.44$, $P = 0.006$) and age and NAA ($r^2 = 0.57$, $P < 0.001$) in the frontal subcortical white matter, indicating rising levels of Cr and NAA in the frontal cortex as children get older (see Fig. 4).

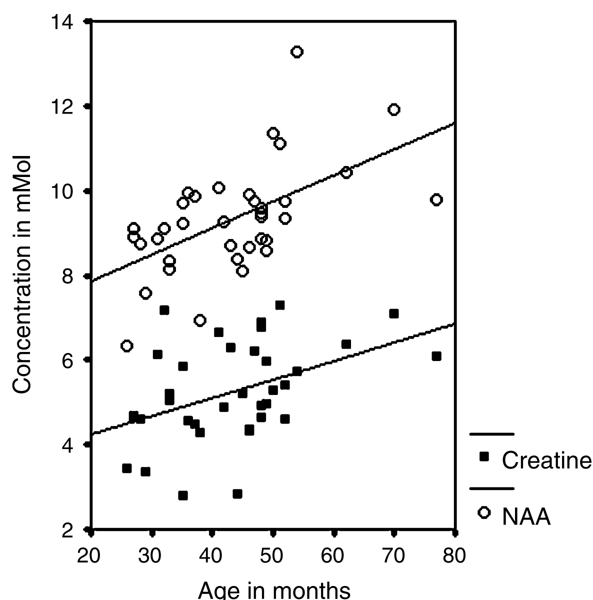

Fig. 4. The correlation of age with frontal NAA ($r^2 = 0.57$, $P < 0.001$) and frontal Cr ($r^2 = 0.44$, $P = 0.006$)

Discussion

The most important finding of this study is that, while controlling for chronological age and developmental level, we did not find any difference in NAA, Cho and Cr concentration in the frontal lobe and in the amygdala-hippocampal complex between boys with ASD and boys with MR or LD.

Previous studies have implied a decreased NAA level in some parts of the brain in autism (Filippi et al., 2002; Friedman et al., 2003; O'Neill et al., 2002; Otsuka et al., 1999; Chugani et al., 1999; Hashimoto et al., 1997; Murphy et al., 2002; Volkmar and Pauls, 2003). The NAA signal reflects tissue concentrations of both NAA and N-acetylaspartylglutamate (NAAG). It has been suggested that the former may be an acetyl-group carrier between mitochondria and cytoplasm in neuronal cells (Kok et al., 2002). A decrease of NAA level is usually interpreted as a reduction in neuronal functioning. In our study in which we corrected for chronological age and developmental level we were not able to replicate these findings, indicating that no differences in brain development (neuronal functioning) are present between children with autism and children with a developmental delay in this age window. This implicates that between the ages of two and six years children with autism or PDD-NOS do not have a different cerebral metabolism when compared to children with either mental retardation or language disorder. It can of course not be ruled out that differences may appear at a later age.

Our results confirm the results of a study by Hashimoto et al. (1998). This study compares subjects with AD with normal controls (NC). In this study the NAA/Cho, NAA/Cr and Cho/Cr ratios in the parietal lobe were not significantly different between the AD and NC. However, it should be noted that the study contained patients groups with a large age distribution (from 4 to 20 years), and data were analyzed without any correction for chronological or developmental age, which might have led to negative results. In another study by the same group (Hashimoto et al., 1997) no differences were found between the autism group and the NC. They did however report finding a lower NAA/Cho in the group with MR compared to the ASD and the NC. No correction for chronological or developmental age was done. A very recent study by Levitt et al. (2003) found no differences in NAA between the group patients with autism and the normal control group. They did, however, find differences in Cho and Cr levels, especially in the caudate. The control group was matched to the autism group on chronological age (both groups included participants between 5 and 16 years), but not on developmental level.

As mentioned previously, most studies found slightly decreased NAA concentrations (Friedman et al., 2003; O'Neill et al., 2002; Levitt et al., 2003) or NAA related metabolic ratios (Hashimoto et al., 1997) in patients with autism. At the Human Brain Mapping meeting of 2002 O'Neill et al. presented a spectroscopic imaging study comparing 28 ASD with 18 NC (O'Neill et al., 2002). They reported a decreased NAA in the frontal cortex and the left frontal-parietal white matter in the autistic group. They also reported decreased Cho levels in the left anterior cingulate gyrus and an increased concentration of Cr in the right caudate head. However, the groups were not matched for chronological or developmental age nor did the analyses correct for these factors. Hisaoka et al. (2001) found a decreased NAA concentration in the temporal region in a group of 55 patients with autism, compared with a group of healthy controls. They included subjects between 0 and 21 years of age. No correction for age or developmental level was done. A previous study by the same group (Otsuka et al., 1999) included children between the age of 2 and 18 years and again there was no correction for chronological or developmental age related metabolic changes. Chugani et al. (1999) compared 9 autistic children with 5 sibling controls without controlling for age or developmental level. Although all these studies found metabolic differences between probands and their control group, no correction for age or developmental level was done.

The effect of age on metabolite changes has been described extensively (Kreis et al., 1993). The predominant changes with brain development include an increase in concentration of NAA, Cr, and Glx, and a decreased concentration of Cho and mI (Moore, 1998). Looking at ratios, one sees an increase with age in NAA/Cho and NAA/Cr ratios; the Cho/Cr ratio decreases with age in the majority of infants (Grattan-Smith et al., 1996). These latter 2 changes are thought to be associated with myelination of the developing brain (Moore, 1998). The most rapid changes were noted during the first 3 years of life, but changes were still observed at the age of 16 years (van der Knaap et al., 1990). Our results of a correlation of Cr and NAA with age in the frontal cortex confirm these age-related changes also in children with an abnormal development either with or without autism.

In addition to age differences, stage of development has a large effect on metabolite levels as well. Filippi et al. (2002) found that children with a developmental delay older than 2 years had a decreased NAA/Cr ratio in the frontal and parieto-occipital subcortical white matter compared with pediatric controls (children who were scanned for headache, migraine, or epilepsy, but whose MRI was

considered normal). In a recent study children with ASD were compared with a group of normal controls and a group of children with a developmental delay without autism (DD) (Friedman et al., 2003). It was found that ASD subjects demonstrated reduced levels of NAA, Cr and Myo concentrations compared to NC. There were no differences in Cho and Cr between ASD and DD in this study. Nor were there differences in NAA levels in the frontal, temporal or parietal cortex. Only a slight decrease in occipital NAA was found in the ASD group.

There are several limitations in our study. Although no statistical differences between the groups were found, this could have been due to a type II error. However, a power analysis, calculated for NAA in the frontal voxel ($\alpha = 0.05$, $\beta = 0.10$) shows that at least 537 samples in each group would have been necessary to find a significant difference.

Secondly, our group of patients with ASD included a large proportion of children with a low level of functioning. It is possible that there are differences in the metabolite concentrations in high and low functioning groups. A study by Jung et al. (1999) found that in normal adults, NAA and Cho were independently associated with fullscale IQ. Moreover, the only study in the field of autism research to find a higher level of NAA in patients compared patients with Asperger syndrome with healthy comparison subjects (Murphy et al., 2002). However, we believe that correcting for developmental level assures that the results of the study can be generalized to the entire population of patients with autism. Unfortunately, several tests were used to measure the level of cognitive functioning of the boys in this study. Underlying this are problems with test taking due to low levels of functioning or anxiety, that are inherent to these populations. However, we believe that the results of this study would have been no different had the patients been tested with the same developmental scales.

It cannot be excluded that differences may have been detected had measurements been done in other anatomic areas. Still the frontal cortex and the amygdala-hippocampal region have often been implicated as involved in autism (Araghi-Niknam and Fatemi, 2003; Kemper and Bauman, 1998). Sampling was done only in the left hemisphere, which differs from the bilateral sampling done in most studies. This may account for why no differences were found. However, based on studies that found a greater left than right hemisphere dysfunction (Chiron et al., 1995; Dawson, 1983), it is not expected that right hemispheric sampling would have shown group differences.

Fourth, we did not measure relaxation times as did Friedman et al. (2003), who reported a prolonged T2 relaxation of NAA for the A group compared to the typically developing and developmental delay groups. Relaxation times are indicative of neuronal density, but they increase scanning time substantially, which in turn increases the strain on the children.

Furthermore, scans were made under full anesthesia. This could not have influenced the results of this study since all children received the same anesthesia. It might however have implications for the comparison of our results to those of other studies. The study of Friedman et al. (2003) scanned the autistic group and the mental retardation group under anesthesia, but not the normal developing children. This might account for differences reported between these groups.

We found that the outcome of our study did not change without the correction for chronological age or developmental level. This study only included children within a small age range. The effect of correcting for age is very small because of the small age range in our study. Still we believe that using both developmental level and chronological age in the calculations is essential in child psychiatric research.

To conclude, in this study we measured NAA, Cho and Cr in a group of young boys with autism and a control group of children with a developmental delay without autism. When controlling for chronological age and developmental level, we have found no differences between these groups.

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