

Proton Magnetic Resonance Spectroscopy in Asperger's Syndrome: Correlations with Neuropsychological Test Scores

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Abstract

Objectives: It has been shown that autistic spectrum patients have impaired theory of mind (ToM) performance; however, no study has investigated the relationship between ToM performance and brain neurochemistry in these patients. The present study aimed to investigate the correlations between dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC) N-acetyl-aspartate (NAA)/choline (Cho), NAA/creatine (Cr), and Cho/Cr values based on ¹H magnetic resonance spectroscopy and ToM tests.

Method: The study sample included 13 adult, right-handed, Caucasian males with Asperger's syndrome (AS) (age range: 17-37 years) and 20 controls matched by age, gender, handedness, and Wechsler Adult Intelligence Scale, Revised (WAIS-R) full-scale IQ scores.

Results: AS cases had significantly lower ToM performance. DLPFC NAA/Cho levels were inversely correlated to ToM scores ($r = -0.738$, $P = 0.004$). On the other hand, ToM performance improved as DLPFC Cho/Cr increased ($r = 0.656$, $P = 0.015$). ACC MRS variables were not significantly correlated with ToM performance in the AS group. No significant correlation was observed between ACC or DLPFC MRS variables and ToM performance in the control group. **Discussion:** Because NAA/Cho was inversely correlated with ToM performance and Cho/Cr was correlated with ToM performance, it can be suggested that the Cho level was related to better ToM test performance in the AS group. An increase in the Cho peak was associated with an increase in membrane breakdown or turnover. The Cho peak was also thought to reflect cellular density and astrocytosis. It is suggested that membrane turnover and astrocytosis might affect cognitive functioning.

Key Words: Asperger's syndrome, magnetic resonance spectroscopy, cingulate gyrus, prefrontal cortex

INTRODUCTION

Theory of mind (ToM), the ability to represent one's own or someone else's mental states, is an outgrowth of social intelligence (Abu-Akel, 2003). ToM impairments have been studied in relation to social interaction problems, such as those observed in Asperger's syndrome (AS), as well as in other pervasive developmental disorders. Whereas some studies have reported that AS patients performed worse than controls on ToM tests (Baron-Cohen et al., 1997; Jolliffe and Baron-Cohen, 1999), others have not (Dahlgren and Trillingsgaard, 1996).

Neuroimaging has been widely used to study the neural basis of ToM. Functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) studies indicate that healthy individuals activate a wide brain region, including the medial frontal cortex, anterior cingulate, inferior frontal cortex, temporal cortex, and cerebellum, when performing ToM tests, whereas autism and AS patients have atypical activation patterns (Mundy, 2003). It has been shown that when performing ToM tests, autism spectrum patients have dorsal-medial frontal cortical activity instead of medial frontal gyrus activity (Happé et al., 1996), less dorsal-medial

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activity compared to controls (Castelli et al., 2002), and no right medial frontal activation (Baron-Cohen et al., 1999). These results suggest that autistic patients have abnormal brain activation during ToM tests.

^1H magnetic resonance spectroscopy (MRS) has been used to measure the level of many metabolites, including N-acetyl-aspartate (NAA), choline (Cho), and creatine (Cr) (Passe et al., 1995). ^1H MRS has also been extensively used in studies of neurological and psychiatric disorders in order to investigate pathological mechanisms, monitor long-term changes, and, more recently, to investigate the relationships between cognitive variables and neurometabolism (Ross and Sachdev, 2004). Studies have shown that the anterior cingulate cortex is part of the “emotional brain” and that this brain region might be related to ToM impairments in autistic patients (Mundy, 2003). MRI and PET studies conducted with autistic patients reported reduced volume and activity in the anterior cingulate cortex (Haznedar et al., 2000). Studies have show that patients with ventromedial prefrontal cortex lesions have impaired ToM functioning (Dawson et al., 2002).

To date, there have been very few ^1H MRS studies conducted with AS patients. One such study (Murphy et al., 2002) used ^1H MRS to compare prefrontal and parietal NAA, Cho, Cr, and phosphocreatine levels in 14 AS patients to those of 18 normal controls. The researchers used the Wechsler Adult Intelligence Scale-Revised (WAIS-R) to measure full-scale IQ. The study’s results indicated that prefrontal cortex NAA, Cho, Cr, and phosphocreatine levels in the AS patients were significantly higher than those in the controls. The study also reported a significant correlation between social behavior and prefrontal NAA levels. Supporting the results of that study, we found that male AS patients had higher anterior cingulate cortex (ACC) NAA/Cho than healthy controls (Oner et al., 2007) and that higher anterior cingulate NAA/Cho levels were correlated with more severe obsessive/repetitive behaviors. Another study that investigated the relationships between MRS variables and cognitive performance using ^{31}P MRS in high-functioning autistic patients observed that dorsal prefrontal cortex phosphomonoester levels were correlated and phosphodiester levels were inversely correlated with neuropsychological test scores (Minshew et al., 1993).

Understanding the relationship between neurochemistry and neuropsychological variables is important, because the resulting convergence of data would lead to more comprehensive analysis and interpretation of the

results obtained with different techniques. The present study aimed to investigate the relationship between dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC) NAA/Cho, NAA/Cr, and Cho/Cr values (measured with ^1H MRS) and ToM performance in adult AS patients and controls matched for age, gender, handedness, and WAIS-R full-scale IQ score.

METHODS

Subjects

The sample included patients that presented to the outpatient clinic at Ankara University School of Medicine, Psychiatry Department and were evaluated by a team of 2 researchers to meet DSM-IV criteria for AS. The parents of all patients were interviewed to establish each patient’s developmental history, and symptom onset and offset.

Inclusion criteria included age between 5 and 45 years, graduation from middle school, right-handedness, diagnosis of AS based on DSM-IV criteria, diagnosis by 2 researchers, and informed consent. For patients younger than 18 years of age and those unable to provide consent, informed consent was obtained from their parents.

Exclusion criteria were comorbid psychosis or a mood disorder, a history of neurological disorder or head trauma resulting in unconsciousness, and WAIS-R total IQ score < 70 . In all, 18 patients were diagnosed with AS, but 5 were excluded from the study, as they did not fulfill the inclusion criteria or did not provide informed consent. All the patients were screened for additional DSM-IV diagnoses using the Structural Clinical Interview for DSM-IV Diagnosis (SCID-I); all the results were negative.

Controls were also screened for DSM-IV Axis I diagnoses using SCID-I. Inclusion criteria for the controls included being male and right-handed. Exclusion criteria for the controls were the presence of psychiatric and neurological disorders.

MRS Imaging

The MRS imaging methods used in the present study were previously described (Oner et al., in press). Briefly, all scans were made with a 1.5 T scanner (Magnetom Symphony, version Syngo VA21C, Siemens Medical Systems, Erlangen, Germany) equipped with high performance gradients (maximum gradient strength: 40 mT m^{-1} ; maximum slew rate: $200\text{ mT m}^{-1}\cdot\text{ms}$) using a standard head

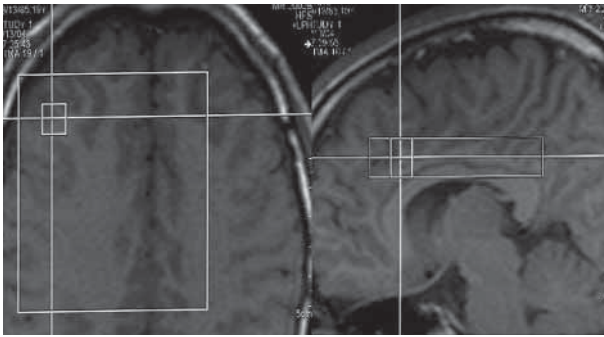


Figure 1. (A): Right anterior cingulate; (B): Right dorsolateral prefrontal regions of interest in MRS imaging.

coil. Prior to performing ^1H MRS, GRE T1-weighted images in the coronal, axial, and sagittal planes of the entire brain were acquired using a gradient echo pulse sequence (slice thickness: 3 mm; FOV: 23 cm; TR: 585 ms; TE: 12 ms; flip angle: 70° , matrix: 101×192) in order to have an anatomic image for selecting a volume of interest to perform localized ^1H MRS. PRESS (point resolved selective spectroscopy) 2D-CSI (two-dimensional chemical shift imaging) was performed to measure the metabolite ratios in the right prefrontal cortex, anterior cingulate cortex, and amygdala with the following scan parameters: TR: 1500 ms; TE: 270 ms; volume of interest (VOI): $1 \times 1 \times 1 \text{ cm}^3$. We tried to minimize sinus-induced MR susceptibility artifacts in the temporal lobe that concerned the amygdala region by positioning each subject in a hyper-extended position. The CSI sequence produced a $16 \times 16 \text{ cm}^2$ transversely oriented matrix that was defined by phase encoding with a FOV of varying dimensions to allow optimal measurement in the areas of concern. The field inhomogeneity achieved in automated non-localized multiple angle protection (MAP) shimming resulted in water peak line widths of less than 8 Hz in the VOI. The 2D-CSI raw data were filtered with Hanning and Gaussian filters on the spatial and chemical shift domains, respectively, before Fourier transformation. Frequency domain curve fitting was subsequently used for quantification with the assumption of Gaussian line shapes, using the standard spectroscopic evaluation SYNGO software package provided with the MR system. We used areas under the curve to compute NAA, Cr, and Cho values.

VOIs were located in the right anterior cingulate cortex and right dorsolateral prefrontal cortex. The voxels in the anterior cingulate cortex corresponded to Brodman area (BA) 24 and 32, and in the dorsolateral prefrontal cortex to BA 9 and 46, based on the Talairach atlas (Talairach and Tournoux, 1998) (Figures 1 and 2). We selected right-sided VOIs for two reasons. First, previous studies suggest that patients with autism spectrum disorders might have

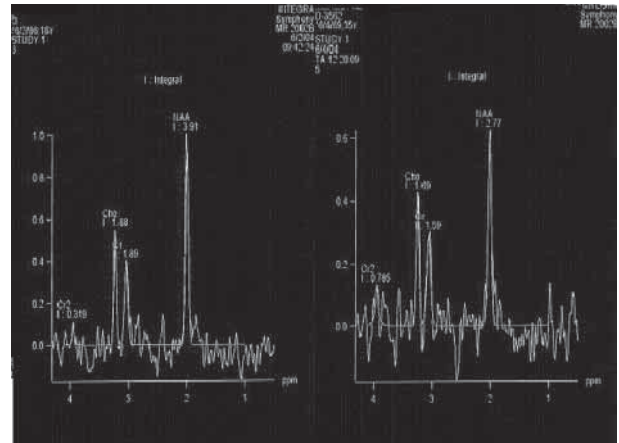


Figure 2. MRS spectra obtained from AS patients and controls.

decreased activity in the right cortical regions (Mundy, 2003). Second, Murphy et al. (2002) investigated right frontal and parietal regions, and we wanted to know if we could replicate their findings. All AS and control subjects cooperated fully during the procedure.

ToM Testing

ToM Test

Second-order ToM, which is acquired by 6-7 years of age and refers to the ability to understand that one can have a false belief about the belief of someone else. The second order task scenarios were based upon those developed by Bowler (1992) and were adapted to Turkish characters and places. The task was about John, Mary, and an ice cream man.

After reading the scenarios, each subject was asked a naming question, prompt questions, a reality question, a memory question, and a belief question. The naming and prompt questions can control the memory affect on the main response. Each of the tasks was comprised of a short scenario followed by a set of questions. Scenarios and/or questions were repeated upon the subject's request. Subjects' responses were scored according to ToM test performance as pass or fail.

Data Analysis

ToM testing and MRS imaging were performed during the same week. In order to compare ToM test scores between groups we used analysis of variance. In order to compute the correlations between the neuropsychological test scores and MRS variables we used the non-parametric Spearman's rank correlation test. We computed correlations for AS cases and controls separately. Two-tailed significance tests ($P < 0.05$) were reported throughout.

RESULTS

Subject Characteristics and ToM Scores

Thirteen AS patients aged 17-38 years (mean: 24.5 ± 7.4 years) were included in the study. All subjects were right handed, Caucasian, and male. While 8 of the subjects were non-medicated, 4 subjects were on long-term risperidone treatment and 1 was on olanzapine treatment. There were no demographic differences between the medicated and non-medicated subjects. Twenty right-handed, Caucasian male controls aged 19-37 years (mean: 26.5 ± 4.9 years) were also included in the study. WAIS-R full-scale IQ scores were similar in the AS cases (88.4 ± 11.1) and controls (90.1 ± 11.7). There were no significant differences in age or WAIS-R full-scale IQ scores between the 2 groups. Mean ToM score was lower in the AS group (2.85 ± 0.80) than in the control group (3.80 ± 0.52) ($F(1, 32) = 17.2, P < 0.001$).

Intragroup Correlations with MRS

AS Subjects

DLPFC NAA/Cho levels were inversely correlated with ToM scores ($r = -0.738, P = 0.004$). On the other hand, ToM performance increased as the DLPFC Cho/Cr level increased ($r = 0.656, P = 0.015$). ACC MRS variables were not significantly correlated with ToM performance in the AS group (Table 1).

Controls

There were no significant correlations between MRS data and ToM performance in the control group (Table 1).

DISCUSSION

Our goals in this study were to evaluate the relation-

ship between brain neurochemistry and ToM performance in AS patients. The main findings were as follows: 1) AS patients had impaired ToM performance; 2) Significant relationships were observed between DLPFC NAA/Cho and Cho/Cr, and ToM performance in the AS patients. The first finding was consistent with previous studies that reported significant differences (Baron-Cohen et al., 1997; Jolliffe and Baron-Cohen, (1999). To the best of our knowledge this is the first report of a relationship between ToM test performance and Cho, Cr, and NAA ratios.

Our results show that dorsolateral prefrontal cortex Cho/Cre levels were correlated and that NAA/Cho levels were inversely correlated with ToM performance in the AS patients. In the present study the dorsal prefrontal VOI included BA9, which has been indicated as a part of the ventral neural network and includes the dorsal-medial prefrontal cortex, anterior cingulate, amygdala, and temporal cortex, which is believed to be more closely related to ToM ability (Abu-Akel, 2003; Mundy, 2003). Nonetheless, we did not observe a significant correlation between ACC variables and ToM performance. Another important point was that we did not observe any significant differences in DLPFC metabolite ratios between the AS and control subjects (Oner et al., 2007). Thus, the reported correlation might not indicate a causal relationship.

Previous analysis indicated that Daha önce yapılan analizlerde AS olgularında, NAA/Cho is higher and Cho/Cr is lower in AS patients than in controls; however, the difference was not statistically significant (Öner et al., 2007). Since NAA/Cho was inversely correlated and Cho/Cr was correlated with ToM performance, it can be suggested that the Cho level that was related to better ToM test performance in the AS group. These results are not completely consistent with those of Murphy et al. (2002), who reported higher NAA, Cho, Cr, and phosphocreatinine levels in the prefrontal cortex. Nonetheless, in that study absolute metabolite levels were reported and a correlation between the metabolites and ToM performance was not investigated. The Cho peak includes soluble membrane phospholipids, such as phosphorylcholine (PCho), glycerophosphocholine (GPCho), and a relatively negligible amount of free choline (Miller et al., 1996). An increase in the Cho peak is associated with an increase in membrane breakdown or turnover. The Cho peak is also thought to reflect cellular density and astrocytosis (Miller et al., 1996). It has been suggested that membrane turnover and astrocytosis might affect cognitive function; this could be via an indirect mechanism,

TABLE 1. Correlation between ToM test scores and MRS variables. Spearman's rho. NAA: N acetylaspartate; Cho; choline; Cr; creatine.

	Asperger's Syndrome	Controls
Anterior cingulate cortex		
NAA/Cho	$r = -0.393; P = 0.184$	$r = 0.079; P = 0.742$
Cho/Cr	$r = 0.117; P = 0.703$	$r = -0.114; P = 0.633$
NAA/Cr	$r = -0.229; P = 0.453$	$r = -0.064; P = 0.788$
Dorsolateral prefrontal cortex		
NAA/Cho	$r = -0.738; P = 0.004$	$r = -0.368; P = 0.110$
Cho/Cr	$r = 0.656; P = 0.015$	$r = 0.071; P = 0.765$
NAA/Cr	$r = -0.340; P = 0.256$	$r = -0.134; P = 0.572$

such as cholinergic pathway dysfunction, as the observed relationships are weak (Ross and Sachdev, 2004).

Nonetheless, the increase in the metabolites associated with cell membranes can also be interpreted as an increase in the processes related to plasticity. Exton (1994) reported that Cho resonance was affected by intracellular signal transmission changes; therefore, it can be suggested that AS patients with increased plasticity might have better ToM performance. Our results also suggested that changes in the Cho peak might be differentially associated with ToM performance. A previous study that investigated the correlation between neuropsychological measures, including full-scale IQ, Wisconsin Card Sorting Test (WCST) perseverative error score, and Delayed Free Recall score from the California Verbal Learning Test (CVLT) with 31P MRS variables showed that only the high-functioning autistic subjects, not the controls, had significant correlations (Minshew et al., 1993). These authors reported that dorsal prefrontal phosphocreatine levels decreased with decreasing WCST, full-scale IQ, and CVLT scores, reflecting the biological significance of the hypermetabolic energy state in autism. Our results were somewhat consistent with the results of that study, as changes in PME and PDE were also related to membrane turnover and function (Ross and Sachdev, 2004). The discrepancy between our results and Minshew et al.'s might be due the fact that we did not analyze absolute metabolite levels, but metabolite ratios.

Similarly to Minshew et al. (1993), we did not observe significant correlations between neuropsychologi-

cal measures and MRS variables in the control group. The lack of significant correlations between neurometabolites and cognitive variables in the control group is just what one would expect in view of the small amount of variation in the performance of that group on the relevant neuropsychological tests; correlation coefficients decrease when the variation in a sample is restricted. Thus, one explanation is that the controls simply performed better and, because of a possible ceiling effect, with lower variation. Another possibility is that there may indeed be a threshold before significant correlations with cognitive functioning occurs, or a rate-limiting reduction in membrane functions that may cause the variance to be smaller in controls (Ross and Sachdev, 2004). Ross and Sachdev (2004) concluded that these possibilities are consistent with studies that reported significant correlations between neurometabolite levels and cognitive variables in patient groups, but not in control groups. If this is the case, controls can be expected to have higher "reserve" than AS patients, making the correlations less significant.

The small sample size used in the present study is an obvious limitation, especially because of the multiple comparisons that were made. We used non-parametric tests in order to decrease the effects of outliers on the results. Nevertheless, our results should be considered preliminary and in need of replication with larger studies. As is the case with many AS patients, our study sample was restricted only to males; the results, therefore, cannot be generalized to all AS patients.

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