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Brief report

5-HTTLPR polymorphism influences prefrontal neurochemical metabolites in autism spectrum disorder

Taro Endo*, Hideaki Kitamura, Ryu Tamura, Jun Egawa, Takuro Sugai, Naoki Fukui, Yutaro Suzuki, Toshiyuki Someya

Department of Psychiatry, Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan

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ABSTRACT

We investigated whether the promoter region of the serotonin transporter gene (*5-HTTLPR*) polymorphism influenced neurochemical metabolism in 26 individuals with autism spectrum disorder. Individuals with the *S/S* genotype of the *5-HTTLPR* polymorphism showed significantly lower levels of *N*-acetylaspartate/creatine in the right medial prefrontal cortex compared with those with the *S/L* genotype.

1. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by repetitive and stereotyped behaviors, and qualitative impairments in reciprocal social interaction and communication. ASD has been found to have a strong heritable component, and a number of neuroimaging studies have suggested the involvement of structural and functional abnormalities in brain regions including the medial prefrontal lobe, amygdala, hippocampus, and cerebellum (Amaral et al., 2008).

Proton magnetic resonance spectroscopy (^{1}H-MRS) is a noninvasive method for investigating cellular neurochemistry *in vivo* and provides spectra that can be used to measure *N*-acetylaspartate (NAA), choline-containing compounds (Cho), and creatine and phosphocreatine (Cr). Past ^{1}H-MRS studies, although inconsistent, have indicated that ASD is associated with altered chemical metabolism in various brain regions. Reduced NAA concentration has been reported in frontal areas (Chugani et al., 1999a,b), lateral temporal lobes (Hisaoka et al., 2001), the amygdala–hippocampus region (Otsuka et al., 1999; Gabis et al., 2008), the cerebellar hemispheres (Chugani et al., 1999a,b; Otsuka et al., 1999), and gray matter (Friedman et al., 2006; DeVito et al., 2007). In contrast, increased NAA concentration has been reported in the medial prefrontal lobe in adults with Asperger's disorder (Murphy et al., 2002). In addition, an increased Cho to Cr ratio has been reported in the left amygdala–hippocampus region in children with autism (Sokol et al., 2002; Gabis et al., 2008). A previous study in our laboratory revealed a relative decrease of NAA in the right amygdala–hippocampus region in individuals with ASD (Endo et al., 2007). In this study, marked heterogeneity in NAA among individuals with ASD appeared to be related to disease severity. However, the role of other contributing factors, such as the effect of genetic variation, remains unclear.

Serotonergic neurons are generated early in brain development, and serotonin regulates growth cone motility, synaptogenesis, synaptic plasticity, and the development of multiple neuronal subtypes (Sodhi and Sanders-Bush, 2004). The polymorphism of the promoter region of the serotonin transporter gene (*5-HTTLPR*) is located in the *SLC6A4* promoter region and has short (*S*) and long (*L*) alleles that differ in size by 44 nucleotides. The polymorphism has been shown to influence *SLC6A4* expression (Bradley et al., 2005) and an association with autism has been reported (Huang and Santangelo, 2008). Several previous studies have indicated abnormalities of serotonin neurotransmission in ASD (e.g. Chugani et al., 1999b). Wassink et al. (2007) recently reported that *5-HTTLPR* influenced the total cortical and frontal lobe gray matter volumes of participants with autism. Moreover, Brune et al. (2006) found that this polymorphism was related to variation in autistic symptoms.

Based on these reports, we hypothesized that these functional polymorphisms may have a subtle but significant impact on the concentration of brain metabolites measured by *in vivo* ^{1}H-MRS in individuals with ASD. We conducted this study to evaluate whether the polymorphism of *5-HTTLPR* influences neurochemical metabolism in individuals with ASD.

* Corresponding author. Department of Psychiatry, Graduate School of Medical and Dental Sciences, Niigata University, 1-757 Asahimachi-dori, Niigata, 951-8510, Japan. Tel.: +81 25 227 2213; fax: +81 25 227 0777.
E-mail address: tarou@med.niigata-u.ac.jp (T. Endo).

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2. Methods

2.1. Participants

Participants in the present study included 26 individuals with ASD (5 females, 21 males) aged 8–20 years (mean age = 13.4 ± 3.7 years). These participants included some participants that have been previously described (Endo et al., 2007). All participants met the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria for pervasive developmental disorder, including six with autistic disorder, 13 with Asperger's disorder, and seven with pervasive developmental disorder not otherwise specified. The autistic symptoms of all individuals with ASD were assessed with the Childhood Autistic Rating Scale—Tokyo Version (CARS-TV; Kurita et al., 1989). Standardized cognitive testing using the Wechsler Intelligence Scale for Children—Third Edition or the Wechsler Adult Intelligence Scale—Revised was administered to all participants. Handedness was assessed for all participants using the Edinburgh Handedness Inventory (Oldfield, 1971). No participants received medication before the study.

This study was approved by the ethics committee of the Niigata University Graduate School of Medical and Dental Science. After a complete explanation of the study was given to the participants, written informed consent was obtained from all participants and their parents.

2.2. ^{1}H-MRS protocol

Individuals with ASD were scanned using the Signa Excite EchoSpeed Plus 1.5T excite version 11.0 system (General Electric) at the Niigata Neurosurgical Hospital, Japan. A standard head coil, 8-channel Neurovascular Phased Array Coil 1.5T, was used for both MRI and ^{1}H-MRS. Imaging was performed according to a previously described protocol (Endo et al., 2007). The axial T-2 weighted imaging (echo time, 102 ms; repetition time, 4000 ms; field of view, 240 × 240 mm; matrix, 256 × 192; slices, 20) was performed before ^{1}H-MRS to define the voxels of interest (VOIs). The 8.0-ml (2 × 2 × 2 cm^3) VOIs were positioned in the right medial prefrontal cortex (MPFC), right medial temporal lobe (MTL), and cerebellar vermis (CV). The field homogeneity achieved in local shimming resulted in a water peak line width of 3–5 Hz, and a chemical shift selective excitation pulse at the unwanted water resonance suppressed the water signal using the PROBE/SV algorithm with the SAGE spectroscopy package (General Electric). The spectra were obtained with a point-resolved spectroscopic sequence with the following parameters: echo time, 35 ms; repetition time, 2000 ms. Quantitative analysis of spectra was confined to Cho (chemical shift 3.2 ppm), Cr (3.0 ppm), and NAA (2.0 ppm) with automatic integration. In the present study, the relative concentrations of NAA and Cho to Cr (NAA/Cr and Cho/Cr, respectively) were used as the surrogate indices of absolute measures.

2.3. Genotyping

DNA samples were obtained from blood or saliva of all participants. We examined the polymorphisms of *5-HTTLPR*, and the *S* and *L* alleles were screened according to Kim et al. (2002).

2.4. Data analysis

Data analysis was carried out using SPSS software. We compared differences in sex and handedness using Fisher's exact test. Student's *t* tests were used for analyzing parametric variables including age, full-scale IQ, and CARS-TV scores. Analyses of covariance (ANCOVAs) were used for relationships between genotypes and neurochemical metabolites. Neurochemical metabolites such as NAA/Cr and Cho/Cr were used as dependent measures, genotype was the independent predictor, and we included age at the time of image acquisition as a covariate. Prior to this analysis, we investigated the dependent measures using Levene's test, and confirmed that these variables were normally distributed. We considered a *P* value of <0.05 to indicate statistical significance.

3. Results

3.1. Participants and genotype

The demographics and characteristics of the study participants are shown in Table 1. The mean CARS-TV total scores were 35.8 ± 3.5 in the autism group, 28.7 ± 3.0 in the Asperger's disorder group, and 28.1 ± 3.4 in the pervasive developmental disorder not otherwise specified group, respectively. For *5-HTTLPR*, 16 probands were homozygous for the *S* allele (*S*/*S*), 10 were heterozygous (*S*/*L*), and none were homozygous for the *L* allele. These genotype distributions were consistent with Hardy–Weinberg equilibrium ($\chi^2 = 1.47$, $df = 1$, $P = 0.54$). There were no significant differences in age, sex, full-scale IQ, handedness, and CARS-TV total scores between genotype groups.

3.2. Effect of genotype–phenotype relations on neurochemical metabolites

ANCOVA revealed that autistic individuals with the *S*/*S* genotype of *5-HTTLPR* exhibited significantly lower levels of NAA/Cr in the right MPFC compared with those with the *S*/*L* genotype ($F = 4.77$, $df = 1,23$, $P = 0.039$, Table 1). *5-HTTLPR* genotype did not have a significant effect on the NAA/Cr ratio in the right MTL and CV or on the Cho/Cr ratio in the right MPFC, MTL, and CV.

4. Discussion

This is the first study to investigate the influences of serotonin-related gene polymorphisms on neurochemical metabolite levels in individuals with ASD. We found that the *S*/*S* genotype of *5-HTTLPR* was associated with a significantly reduced concentration of NAA/Cr, exclusively in the right MPFC. In accord with a report by Wassink et al. (2007), we found that this effect was specific to the MPFC, and did not occur in other brain regions such as the CV or MTL.

The NAA signal is present at high concentrations in mature neurons and is often used as a chemical marker of neuronal integrity (Birken and Oldendorf, 1989; Urenjak et al., 1993; Clark, 1998). The Cr signal might reflect glial or overall cellular density (Urenjak et al., 1993) and Cr has

Table 1
Participants' demographics and clinical characteristics.

	Genotype of autism spectrum disorder		
	S/*S* group ($n = 16$)	*S*/*L* group ($n = 10$)	*P* value
Age	13.2 ± 4.0	13.9 ± 3.4	0.67
Sex (F:M)	2:14	3:7	0.34
Full-scale IQ	87.9 ± 18.5	95.5 ± 17.2	0.29
Handedness (R:L)	13:3	10:0	0.26
CARS-TV total score	30.9 ± 4.4	29.1 ± 4.5	0.32
Medial prefrontal voxel			
NAA/Cr	1.59 ± 0.17	1.75 ± 0.20	0.04
Cho/Cr	1.01 ± 0.19	1.08 ± 0.19	0.43
Medial temporal voxel			
NAA/Cr	1.02 ± 0.01	1.08 ± 0.11	0.98
Cho/Cr	0.85 ± 0.09	0.87 ± 0.19	0.58
Cerebellar vermis voxel			
NAA/Cr	1.30 ± 0.25	1.31 ± 0.24	0.12
Cho/Cr	1.07 ± 0.20	1.01 ± 0.25	0.77

All continuous data are presented as mean ± S.D. CARS-TV: Childhood Autistic Rating Scale—Tokyo Version; NAA, *N*-acetylaspartate; Cr, creatine and phosphocreatine; Cho, choline-containing compounds.