

## A longitudinal study of auditory evoked field and language development in young children



Yuko Yoshimura<sup>a</sup>, Mitsuru Kikuchi<sup>a,\*</sup>, Sanae Ueno<sup>a</sup>, Kiyomi Shitamichi<sup>b</sup>, Gerard B. Remijn<sup>c</sup>, Hirotooshi Hiraishi<sup>a</sup>, Chiaki Hasegawa<sup>a</sup>, Naoki Furutani<sup>b</sup>, Manabu Oi<sup>a</sup>, Toshio Munesue<sup>a</sup>, Tsunehisa Tsubokawa<sup>d</sup>, Haruhiro Higashida<sup>a</sup>, Yoshio Minabe<sup>b</sup>

<sup>a</sup> Research Center for Child Mental Development, Kanazawa University, Kanazawa, Japan

<sup>b</sup> Department of Psychiatry and Neurobiology, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan

<sup>c</sup> International Education Center, Kyushu University, Fukuoka, Japan

<sup>d</sup> Department of Anesthesiology, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan

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### ABSTRACT

The relationship between language development in early childhood and the maturation of brain functions related to the human voice remains unclear. Because the development of the auditory system likely correlates with language development in young children, we investigated the relationship between the auditory evoked field (AEF) and language development using non-invasive child-customized magnetoencephalography (MEG) in a longitudinal design.

Twenty typically developing children were recruited (aged 36–75 months old at the first measurement). These children were re-investigated 11–25 months after the first measurement. The AEF component P1m was examined to investigate the developmental changes in each participant's neural brain response to vocal stimuli. In addition, we examined the relationships between brain responses and language performance. P1m peak amplitude in response to vocal stimuli significantly increased in both hemispheres in the second measurement compared to the first measurement. However, no differences were observed in P1m latency. Notably, our results reveal that children with greater increases in P1m amplitude in the left hemisphere performed better on linguistic tests. Thus, our results indicate that P1m evoked by vocal stimuli is a neurophysiological marker for language development in young children. Additionally, MEG is a technique that can be used to investigate the maturation of the auditory cortex based on auditory evoked fields in young children. This study is the first to demonstrate a significant relationship between the development of the auditory processing system and the development of language abilities in young children.

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### Introduction

Language acquisition in early childhood is one of the most fundamental human traits. Dramatic developmental changes occur in the brains of young children in concert with this ability (Sakai, 2005). Our previous magnetoencephalography (MEG) study reported that the auditory evoked brain response (i.e., P50m) to vocal stimuli in the left hemisphere was significantly correlated with language conceptual inference ability in normal 2- to 5-year-old children (Yoshimura et al., 2012) and 3- to 7-year-old children (Yoshimura et al., 2013). Although the relationships between longitudinal changes in the brain response to

vocal stimuli and language development remain unknown, Choudhury and Benasich (2011) examined auditory responses to tone stimuli using electroencephalography (EEG) in longitudinal samples of typically developing children and children at higher risk for language disorders between 6 and 48 months of age. Their results demonstrated that infants with larger responses and shorter peak latencies from 6 to 9 months old had better language and cognitive skills at 3 and 4 years old. Moreover, other studies have reported that brain responses to auditory stimuli in infancy and childhood are associated with subsequent language-related skills (Espy et al., 2004) or language impairments such as dyslexia (Leppänen et al., 2010, 2012). However, to date, there has been no study that has followed this change in the brain response evoked by vocal stimuli using a longitudinal design or that has investigated its relationship to language development in young, typically developing children without family risk factors for language-related impairment (e.g., dyslexia).

\* Corresponding author at: Research Center for Child Mental Development, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-8641, Japan.  
E-mail address: [mitsuru@zc4.so-net.ne.jp](mailto:mitsuru@zc4.so-net.ne.jp) (M. Kikuchi).

The purpose of this study was to investigate the relationship between longitudinal changes in the auditory evoked response (P1m) to vocal stimuli and language development in early childhood in typically developing children using MEG. A previous study demonstrated that the maturational patterns in auditory processing differ depending on the type of stimulus (e.g., speech or non-speech stimuli) (Pang and Taylor, 2000). In the present study, we focused on human voice-evoked responses. Understanding the normal maturation pattern of AEFs evoked by the human voice may aid in the development of neurophysiological techniques for evaluating the central auditory maturation that coincides with language development in young children.

P1(m) is a prominent component in 1- to 10-year-old children (Gilley et al., 2005; Oram Cardy et al., 2004; Ponton et al., 2002; Sharma et al., 1997) and provides insight into the development of auditory processing. P1m is thought to be a suitable metric for measuring changes in auditory input for speech-like signals (Chait et al., 2004; Hertrich et al., 2000). A recent study indicated that P1m is sensitive to the place-of-articulation features of speech and their co-articulatory processes (Tavabi et al., 2007). In previous MEG studies, this component has been alternatively labeled M50 (Oram Cardy et al., 2004) or P100m (Orekhova et al., 2012, 2013). In our previous study, this component was labeled P50m (Yoshimura et al., 2012). Some EEG studies have also labeled this component P1 (Gilley et al., 2005; Ponton et al., 2002). According to Orekhova et al. (2012), the P1m component at approximately 100 ms after stimulation is the most prominent component of the auditory evoked magnetic field response in children, and the equivalent current dipoles (ECD) of P1m have a predominantly anterosuperior direction. This component can be reliably identified in the majority of children because of its large amplitude (Oram Cardy et al., 2004). This component, which we analyzed in our previous study, was identical to the results of previous MEG studies (Oram Cardy et al., 2004; Orekhova et al., 2012; Pihko et al., 2007). To avoid confusion, we call this component P1m in the present study.

We hypothesize that changes in P1m amplitude/latency are correlated with language development in early childhood. The aim of this study was to investigate the brain response to human vocal stimuli measured using a longitudinal design in 3- to 7-year-old typically developing children and to investigate the relationship between language development and the changes in brain response.

## Methods

### Participants

Twenty children (3 females and 17 males) participated and were 36–75 months old at the first measurement. All children participated in additional measurements at 11- to 25-month intervals for 3 years. All participants were native Japanese and had no previous developmental, learning, or behavioral problems according to information obtained from their caregivers by questionnaire. All participants confirmed (through an interview with their caregivers) that they had no diagnosed hearing problems (mass screening at age 3) and that there were no hearing problems in their daily lives. Left- or right-hand dominance was determined based on the participants' preferences when handling objects, and all children were right-handed. All children participated in cognitive tasks and MEG measurements separately over 2 days. On the first day, the participants performed cognitive tests and were introduced to the environment used for the MEG measurements. The actual MEG measurements were performed on the second day. The caregivers consented to their child's participation in the study with full knowledge of the experimental nature of the research. Written informed consent was obtained from the caregivers prior to participation in this study. The Ethics Committee of Kanazawa University Hospital approved the methods and procedures, all of which were performed in accordance with the Declaration of Helsinki. The demographic data for all participants are presented in Table 1.

**Table 1**  
Demographic characteristics of all participants.

	First measurement	Second measurement	t	P
Number of subjects	20			
Gender (male/female)	17/3			
Chronological age (months)	51.0 ( $\pm$ 9.7)	69.0 ( $\pm$ 8.9)	22.20	<.0001
Head size (cm)	50.7 ( $\pm$ 1.8)	51.6 ( $\pm$ 1.8)	5.37	<.0001
K-ABC				
Mental processing scale	98.2 ( $\pm$ 7.8)	99.5 ( $\pm$ 11.5)	0.13	n.s.
Achievement scale	99.7 ( $\pm$ 17.1)	100.1 ( $\pm$ 12.7)	0.11	n.s.
Language conceptual inference task (riddles)	96.6 ( $\pm$ 17.2)	97.2 ( $\pm$ 12.9)	-0.19	n.s.

K-ABC, Kaufman Assessment Battery for Children.

The values are the mean (and standard deviation) for chronological age, head size and standardized scores for the mental processing scale, achievement scale and language conceptual inference task in the K-ABC. n.s., not significant.

### Cognitive and language performance measurements

The children were assessed using the Japanese adaptation of the Kaufman Assessment Battery for Children (K-ABC) (Kaufman and Kaufman, 1983), which is typically used to assess the cognitive skills of 30- to 155-month-old children. To confirm the standardized scores of the mental processing and achievement scales in children, subtests from this battery that are appropriate for the ages of the children were used. In this study, the potential correlation between one of the components of the AEF (i.e., P1m amplitude and latency) and performance on a single language-related task (i.e., a subset of K-ABC 'riddles') in a previous study (Yoshimura et al., 2012) was assessed. In the riddle task, children must respond to the examiner's question, such as "Which fruit has a rounded shape with a depression at the top where the stem is attached? The color of the skin can be red, green, yellow, or a combination of these colors." In this case, the answer is "an apple." The riddle task consists of 32 questions, and the questions are presented in ascending order of difficulty. The linguistic level is defined by the child's degree of achievement. The K-ABC 'riddle' subtest reflects conceptual language inference abilities (Kaufman and Kaufman, 1983).

### Magnetoencephalography recordings

The conditions in the first and second MEG recordings were completely identical to those detailed in our previous study (Yoshimura et al., 2012). MEG data were recorded using a 151-channel SQUID (Superconducting Quantum Interference Device), whole-head coaxial gradiometer MEG system for children (PQ 1151R; Yokogawa/KIT, Kanazawa, Japan) in a magnetically shielded room (Daido Steel, Nagoya, Japan) installed at the MEG Center of Yokogawa Electric Corporation (Kanazawa, Japan). The custom child-sized MEG system facilitates the measurement of brain responses in young children, which would otherwise be difficult using conventional adult-sized MEG systems. The child MEG system ensures that sensors are easily and effectively positioned for the child's brain and that head movements are constrained (Johnson et al., 2010). We determined the position of the head within the helmet by measuring the magnetic fields after passing currents through coils attached at 3 locations on the surface of the head, which served as fiducial points relative to specific landmarks (the bilateral mastoid processes and nasion). Although we could not account for the effect of individual head shape on the accuracy of dipole estimation, to calculate ECD without magnetic resonance imaging anatomical data, a sphere (as a spherical model of the volume) conductor was fitted to the center of the helmet after confirming that each participant's head was located in the center of the MEG helmet by measuring the above-described three locations on the surface of the head (Yoshimura et al., 2012). An examiner remained in the room to encourage the children and to prevent movement throughout the analysis. Stimuli were presented while the

child was in a supine position on the bed and viewed silent video programs projected onto a screen.

#### Auditory evoked field stimuli and procedures

The stimuli and procedure were based on our previous study (Yoshimura et al., 2012). MEG recordings were obtained from all participants during auditory syllable sound stimulation consisting of the Japanese syllable /ne/ (Yoshimura et al., 2012). We employed this syllable because /ne/ is one of the Japanese final sentence particles, which convey prosodic information (Anderson et al., 2007; Cook, 1990). The syllable /ne/ is often used in Japanese mother–child conversations and expresses a speaker's request for acknowledgment or empathy from the listener (Kajikawa et al., 2004; Squires, 2009). In the present study, we used typical oddball sequences consisting of standard stimuli (456 times, 83%) and deviant stimuli (90 times, 17%). In the standard stimulus, /ne/ was pronounced with a steady pitch contour, whereas in the deviant condition, /ne/ was pronounced with a falling pitch. Eventually, we adopted only standard stimuli for subsequent ECD estimations because a sufficient number of periods to calculate ECD remained after artifact rejection in all children. A female native Japanese speaker produced the /ne/ sounds, which were recorded using a condenser microphone (NT1-A; Rode, Silverwater, NSW, Australia) and a personal computer. The duration of the stimulus was 342 ms, and the duration of the consonant /n/ was 65 ms. In this study, the beginning of the vowel sound /e/ was defined as the onset time. The interstimulus interval (ISI) was 818 ms. Both stimuli had an intensity level of approximately 65 dB (A-weighted) at the head position against a background noise of 43 dB. Intensity was measured using an integrating sound level meter (LY20; Yokogawa, Tokyo, Japan). The stimulus was presented to participants binaurally through a hole in the MEG chamber using speakers (HK195 Speakers; Harman Kardon, Stamford, CT) placed outside the shielded room. The speaker was placed facing the participant's head from the direction of the foot, and the distance between the speaker and MEG helmet was 3.3 m. The recording was 12 min long.

#### AEF acquisition and analysis

The procedures for the AEF acquisition and analysis were identical to those in our previous study (Yoshimura et al., 2012). The bandpass-filtered MEG data (0.16–200 Hz) were collected at a sampling rate of 1000 Hz. The time series from the onset of the syllable stimulus at –150 to 1000 ms and subsequent segments (at least 300 for standard stimuli) were averaged for each sensor after baseline correction (–50 to 0 ms). Segments contaminated with artifacts (eye-blink and eye and body movements, typically more than  $\pm 4$  pT) were excluded from the analysis. A single ECD model was used to estimate current sources in the activated cerebral cortex using more than 30 sensors for each hemisphere (left and right) (Elberling et al., 1982). MegLaboratory 160 software (Yokogawa/KIT, Kanazawa, Japan) was used to estimate the localization of the current sources.

To identify P1m, we accepted estimated ECDs when (i) the goodness of fit (GOF) exceeded 80%; (ii) the location of estimated dipoles using a single ECD model was stabilized within  $\pm 5$  mm of each coordinate for at least 6 ms during the P1m response; (iii) the dipole amplitudes were  $\leq 80$  nA m; and (iv) ECDs predominantly had an anterosuperior direction. The latency time point was defined as the maximum estimated dipole amplitude value obtained in accordance with the above criteria within a time window of 40 to 150 ms. Regarding the P1m component coordinates, the center of a sphere as a spherical model of the volume conductor for the ECD estimation was defined as the origin, and the x-, y-, and z-coordinates represented the leftward direction, occipital direction, and vertex, respectively.

#### Data analysis

Each physiological variable of P1m (amplitude and latency) was analyzed using a two-way repeated measures analysis of variance (ANOVA). The two within-subjects variables were Measurement time (first vs. second measurement) and Hemisphere (left vs. right). Significance was established at  $P < 0.05$ .

To compare the dipole source location between the first and second measurements, we employed a principal component analysis (PCA) because brain activations were not necessary along one of the Cartesian coordinates (i.e., x-, y-, and z-coordinates) of the source space. PCA identifies a coordinate system whose coordinates follow the most prominent spread of the data distribution. PCA allows us to replace the 3D statistical analysis of the locations with a 1D analysis of the representative coordinates for each hemisphere (Herrmann et al., 2011). The location (i.e., principal component score) differences for each hemisphere were compared between the first and second measurements using a paired *t*-test.

Pearson correlation coefficients were used to investigate the relationships between maturational changes in P1m (amplitude and latency) and language development. For the maturational change in the P1m amplitude variable, the values from the second measurement were divided by the values from the first measurement. For the maturational change in the P1m latency variable, the value was determined by subtracting the first measurement value from the second measurement value. The percent change in the standardized scores of the riddles in the K-ABC was used as the developmental change in the language variables. Statistical significance was defined as  $P < 0.05$ .

To evaluate possible confounding effects (i.e., age at the first measurement time, the time interval between the two measurements, and change in total cognitive ability) on the relationship between the change in P1m (amplitude and latency) and language development, we employed a multiple linear regression analysis to predict the change in P1m (i.e., dependent variable) using age at first measurement, the time interval between the two measurements, change in total cognitive ability and change in language ability as predictors (i.e., four independent variables). Statistical significance was defined as  $P < 0.05$ .

#### Results

##### P1m amplitude and latency (Figs. 1, 2, and 3)

We were able to detect P1m at both measurement times (i.e., first and second measurements) in the right and left hemispheres of 17/20 and 18/20 participants, respectively. Fig. 1 shows the typical auditory evoked fields and typical P1m component in one participant. Fig. 2 shows that the P1m amplitude tends to increase with age, whereas the P1m latency tends to decrease with age (Fig. 3) in both hemispheres.

##### The changes in P1m amplitude and latency (Table 2)

In 80% (16/20) of the participants, we were able to detect P1m in both hemispheres in both measurements. Therefore, those 16 participants were analyzed with a repeated measures two-way ANOVA. For the P1m amplitude, the repeated measures ANOVA indicated a main effect of Measurement time ( $F(1,15) = 12.724, P = 0.003$ ) and Hemisphere ( $F(1,15) = 18.935, P = 0.001$ ). However, there was no significant interaction (Measurement time  $\times$  Hemisphere) ( $F(1,15) = 4.414, P = 0.053$ ). These results indicate that the P1m amplitude was significantly higher in the left hemisphere compared to the right hemisphere, and the P1m amplitude was significantly higher in the second measurement compared to the first measurement, regardless of the hemisphere. Although there was no significant interaction, there was a trend suggesting that the increase in P1m amplitude in the left hemisphere between the first and second measurements was larger than that in the right hemisphere (left: first measurement, mean ( $\pm$  standard

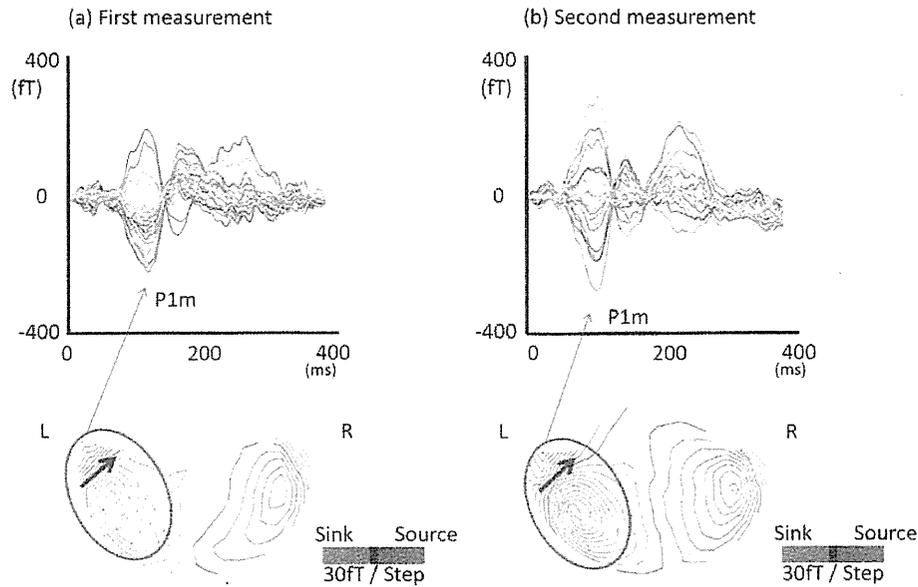


Fig. 1. Auditory evoked fields and P1m component in one subject.

deviation) = 18.3 ( $\pm$ 7.2), second measurement, mean ( $\pm$ standard deviation) = 26.7 ( $\pm$ 10.8); right: first measurement, mean ( $\pm$ standard deviation) = 10.8 ( $\pm$ 4.9); second measurement, mean ( $\pm$ standard deviation) = 15.0 ( $\pm$ 7.5).

For the P1m latency, the repeated measures ANOVA indicated no significant main effect of Measurement time ( $F(1,15) = 2.243, P = 0.155$ ) or Hemisphere ( $F(1,15) = 4.192, P = 0.059$ ). There was no significant interaction (Measurement time  $\times$  Hemisphere) ( $F(1,15) = 0.466, P = 0.505$ ). Although there was no significant main effect, we observed that P1m latency in the right hemisphere tended to be faster than in the left hemisphere for both measurements, and P1m latency tended to be faster for the second measurement than the first measurement in both hemispheres (first measurement: left,

mean ( $\pm$ standard deviation) = 89.0 ( $\pm$ 22.4), right, mean ( $\pm$ standard deviation) = 85.0 ( $\pm$ 21.5); second measurement: left, mean ( $\pm$ standard deviation) = 78.1 ( $\pm$ 15.3), right, mean ( $\pm$ standard deviation) = 75.5 ( $\pm$ 12.6)).

*The change in location of the estimated P1m dipole sources (Table 3)*

We were able to detect P1m for both measurements (i.e., first and second measurements) in the left and right hemispheres of 17/20 and 18/20 participants, respectively. Therefore, in conjunction with these two measurements, 34 and 36 dipole locations were used for the PCA of the left and right hemispheres, respectively. The PCA indicated that in both hemispheres, one component (i.e., coordinate) had an

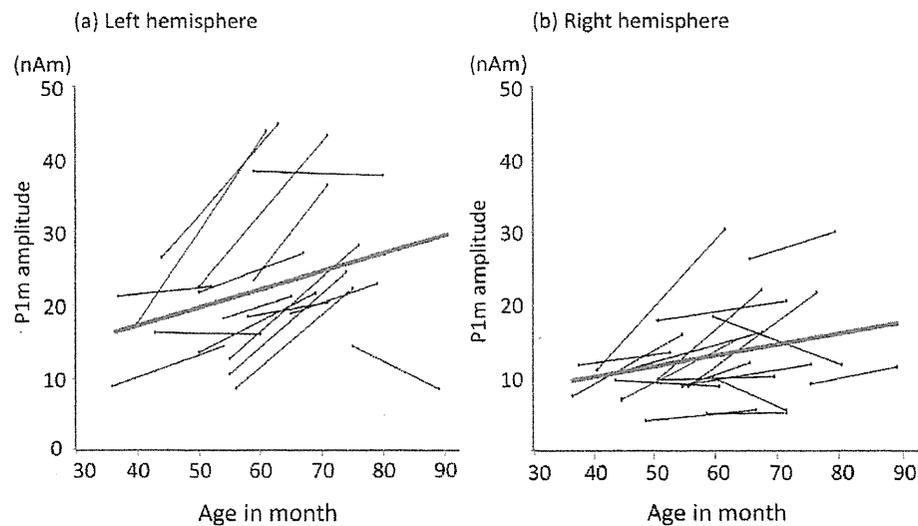
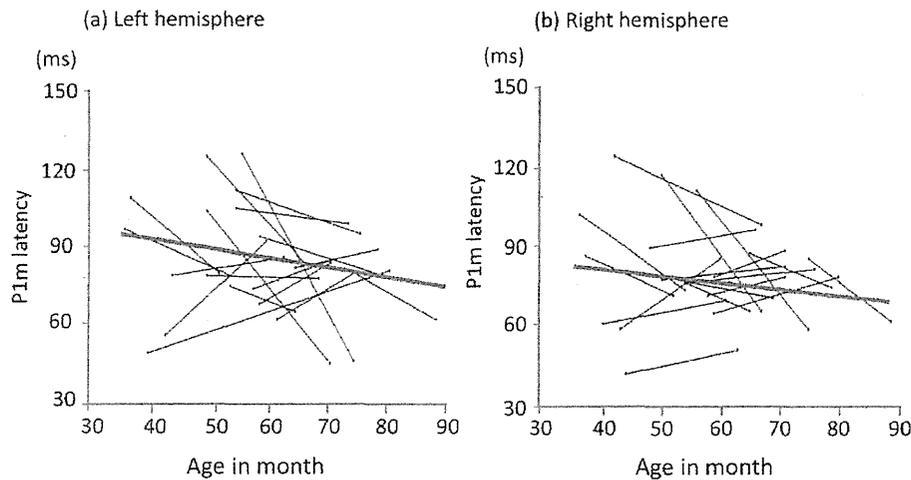


Fig. 2. Trajectories of the P1m amplitude in the left (a) and right (b) hemispheres. In the left and right hemispheres, P1m was detected during both measurements (i.e., first and second measurements) in 17/20 and 18/20 participants, respectively. The bold red line is the regression line that corresponds to all data (i.e., the combination of the first and second measurements).



**Fig. 3.** Trajectories of the P1m latency in the left (a) and right hemispheres (b). In the left and right hemispheres, P1m was detected for both measurements (i.e., first and second measurements) in 17/20 and 18/20 participants, respectively. The bold red line is the regression line that corresponds to all data (i.e., the combination of the first and second measurements).

eigenvalue greater than one. In both hemispheres, positive values of the first component (coordinate) occasionally corresponded to the medial-vertex direction. A significant difference was observed for the PCA score of the right hemisphere between the first and second measurements ( $df = 17$ ,  $t = -3.58$ ,  $P = 0.002$ ) (Table 3). This result indicates that the location of the P1m dipole source in the right hemisphere shifted toward the medial-vertex direction between the first and second measurements. In the left hemisphere, there was no significant difference between the first and second measurements ( $df = 16$ ,  $t = 0.31$ ,  $P > 0.05$ ) (Table 3).

*The relationships between changes in P1m amplitude and latency and language development (Figs. 4 and 5)*

We detected P1m for both measurements (i.e., first and second measurements) in the right and left hemispheres of 17/20 and 18/20 participants, respectively. Therefore, 17 and 18 participants were used to examine the right and left hemispheres, respectively, in the correlation analysis for longitudinal changes. A significant positive correlation was observed between the P1m amplitude change in the left hemisphere and the percent change in the language conceptual inference task (i.e., riddles) ( $n = 17$ ,  $r = 0.526$ ,  $P = 0.028$ ). However, this correlation was not significant for the right hemisphere ( $n = 18$ ,  $r = 0.088$ ,  $P > 0.05$ ) (Fig. 4). The change in P1m latency was not significantly correlated with the percent change in the language conceptual inference task (i.e., riddles) for either hemisphere (Fig. 5).

In the multiple regression model shown in Table 4, the time interval between the two measurements ( $n = 17$ ,  $\beta = 0.476$ ,  $P = 0.030$ ) and the percent change in the language conceptual inference task ( $n = 17$ ,  $\beta = 0.473$ ,  $P = 0.027$ ) were significant predictors of the P1m amplitude change in the left hemisphere. By contrast, age at the first measurement ( $n = 17$ ,  $\beta = 0.027$ ,  $P > 0.05$ ) and change in total cognitive ability

(i.e., change in the mental processing scale according to the K-ABC) ( $n = 17$ ,  $\beta = 0.277$ ,  $P > 0.05$ ) showed no significant effects. In the right hemisphere ( $n = 18$ ), the coefficient of multiple determination for multiple regression (i.e.,  $R^2 = 0.260$ ) was not significant ( $P = 0.380$ ), and no independent variables reached statistical significance, as indicated by the multiple linear regression (Table 4). For the change in P1m latency, no variables reached statistical significance for either hemisphere, as indicated by the multiple linear regression. There was no significant correlation among independent variables ( $r$  values ranged from  $-0.407$  to  $0.171$ ). The correlation coefficients between independent variables were as follows: 'change in language conceptual inference tasks' and 'change in mental processing scale' ( $r = 0.171$ ), 'change in language conceptual inference tasks' and 'age at first measurement' ( $r = -0.126$ ), 'change in language conceptual inference tasks' and 'time interval' ( $r = 0.001$ ), 'change in mental processing scale' and 'age at first measurement' ( $r = 0.112$ ), 'change in mental processing scale' and 'time interval' ( $r = -0.011$ ) and 'age at first measurement' and 'time interval' ( $r = -0.407$ ).

## Discussion

This study demonstrated a relationship between longitudinal change in P1m elicited by vocal stimuli and language development in young children. Our results indicate that the maturation of the auditory cortex in the left hemisphere is associated with language development. In this study, we also demonstrated that our child-customized MEG device allowed us to acquire bilateral (i.e., left and right hemispheres) simultaneous brain responses in conscious young children. MEG is currently a suitable technology for measuring the absolute value of brain auditory responses in young children. Because it shows a good signal-to-noise ratio, excellent temporal resolution, and a long history of EEG studies (to aid study interpretations), is safe and non-invasive, and is supported

**Table 2**  
Repeated measures two-way ANOVA for the P1m amplitude and latency.

	P1m amplitude		P1m latency	
	F (1,15)	P	F (1,15)	P
Measurement time	12.724	0.003	2.243	n.s.
Hemisphere	18.935	0.001	4.192	n.s.
Interaction	4.414	n.s.	0.466	n.s.

Interaction: interaction between Measurement time and Hemisphere. n.s., not significant.

**Table 3**  
Location of the P1m source.

	Left	Right
EV in the first measurement	0.047 (1.11)	-0.236 (1.00)
EV in the second measurement	-0.047 (0.91)	0.262 (0.96)
N	17	18
t (first vs. second)	0.31	-3.58
P (first vs. second)	n.s.	0.002

In both hemispheres, positive EV indicates locations toward the medial-vertex direction. EV, eigenvalue.

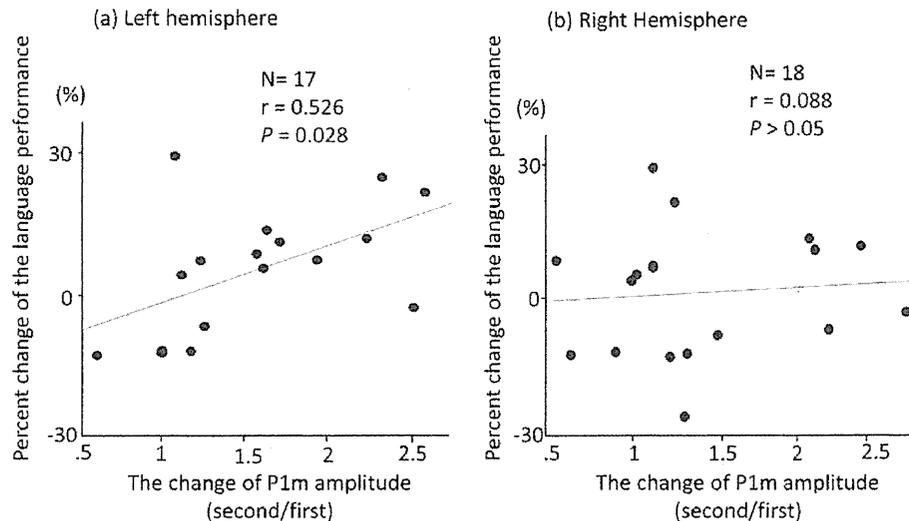


Fig. 4. The correlation between the percent change in the standardized score of the language conceptual inference task in K-ABC and the P1m amplitude change in the left (a) and right (b) hemispheres. In the left and right hemispheres, P1m was detected for both measurements (i.e., first and second measurements) in 17/20 and 18/20 participants, respectively.

by recent technical advancements (e.g., child custom-sized MEG), MEG is an excellent tool for investigating the spatio-temporal dynamics of neural activation associated with sensory perception in early childhood (Huotilainen et al., 2008; Pang, 2011).

The component analyzed in our previous study was identical to P100m, M50 and P1m responses, which have been demonstrated in previous MEG studies (Oram Cardy et al., 2004; Orekhova et al., 2012; Pihko et al., 2007). To avoid confusion, we called this component P1m in the present study.

We noted a significant relationship between language development and increased P1m amplitude in the left hemisphere. This result supports our previous cross-sectional finding that P1m amplitude in the left hemisphere is significantly correlated with language conceptual ability in 2- to 5-year-old children (Yoshimura et al., 2012). In terms of the relationship between the auditory brain response and language development, Oram Cardy et al. (2008) also reported that M50 latency to tone stimuli in the right hemisphere is a predictor of language impairment

(i.e., specific language impairment (SLI) and autism spectrum disorder) in 7- to 18-year-old children and adolescents (Oram Cardy et al., 2008). In addition, Choudhury and Benasich (2011) demonstrated that significant age-related changes in the latency and amplitude of P1 and later components in response to tone stimuli (70-ms ISI) predicted language abilities at 36 and 48 months of age. However, in typically developing children, the relationships between longitudinal language development and earlier components (e.g., P1m) have remained controversial. Our child-customized MEG system allows us to provide new evidence that developmental changes in P1m amplitude in response to vocal stimuli reflect the maturation of language-related brain regions.

From the first to the second measurement, we observed a significant increase in P1m amplitude in both hemispheres. Furthermore, P1m amplitude in the left hemisphere was significantly larger than in the right hemisphere at both measurements. This P1m left hemispheric dominance is consistent with other AEF studies (Fujioka et al., 2006; Heim et al., 2003; Tavabi et al., 2007; Yoshimura et al., 2012). However, no

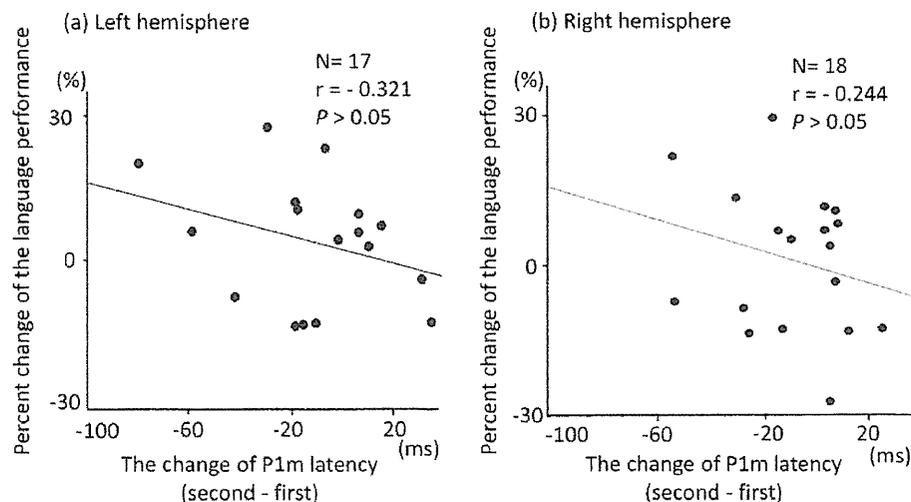


Fig. 5. The correlation between the percent change in the standardized score of the language conceptual inference task in K-ABC and the change in P1m latency in the left (a) and right (b) hemispheres. In the left and right hemispheres, P1m was detected for both measurements (i.e., first and second measurements) in 17/20 and 18/20 participants, respectively.

**Table 4**

Values of the standardized regression coefficient  $\beta$  and  $t$  values for the multiple linear regression models using the change in P1m amplitude as the dependent variable. The age at first measurement, time interval, percent change in the standardized score of the mental processing scale on the K-ABC and percent change in the language conceptual inference task were independent variables.

	Left hemisphere		Right hemisphere	
	$\beta$	$t$	$\beta$	$t$
N	17		18	
$R^2$	0.620*		0.260	
Age (months) at first measurement	0.027	0.139	-0.301	-1.109
Time interval	0.476	2.457*	0.262	0.988
Change in mental processing scale	0.277	1.524	0.199	0.832
Change in language conceptual inference task	0.473	2.520*	0.033	0.135

\*  $P < 0.05$ .

previous study has examined longitudinal change in the auditory evoked field (i.e., P1m) to vocal stimuli. Only Fujioka et al. (2006) have examined changes in P1m amplitude to non-vocal sounds (i.e., noise burst and violin tones) in 4- to 6-year-old typically developing children. These authors investigated the effects of 1 year of music lessons on the P100m (P1m) component. Over four repeated MEG measurements, a significantly greater response to violin sounds in the left hemisphere compared to the right hemisphere was observed. In our results, left-hemispheric dominance in P1m was enhanced for vocal sounds. In our study, the participants did not receive structured intervention (e.g., musical training) during the interval; however, these participants would have been affected by language input and would have had daily exposure to language from their parents and other children. We speculate that the same brain development mechanisms may be associated with the perception of both the violin and human voice. The identical developmental changes in the auditory cortex in the left hemisphere were observed after continuous exposure to violin sound and human voice. Pihko et al. (2007) also reported an effect of language intervention on P1m amplitude and latency for children with SLI. The authors observed that after language training, P1m amplitudes increased in response to auditory stimuli, although this result was not significant (Pihko et al., 2007).

It appears that P1m amplitude is a dynamic factor that is influenced by longitudinal auditory input. However, in this study, we investigated P1m only using one type of auditory stimulus (i.e., the human voice / ne/). Therefore, we cannot determine whether our results are specific to human vocal stimuli.

Increases in P1 amplitude with age have been reported in several previous developmental studies (Gilley et al., 2005; Ponton et al., 2002; Sussman et al., 2008), as was observed in our study. Sussman et al. (2008) revealed that P1 amplitudes were larger for 8-year-old children than for older age groups with short ISI (i.e., <1 s). Gilley et al. (2005) reported that the increase in the P1 amplitude was not significant for three groups (3–4 years old, 5–6 years old and 7–8 years old); however, there was a tendency for P1 amplitudes to increase with age until approximately 7–8 years of age. Ponton et al. (2002) also reported that P1 peak amplitudes increased from 5–6 to 10 years of age. These results are consistent with our findings, in which P1m amplitudes increased from 3 to 7 years of age. Using a longitudinal design, our results provide new evidence in young children (including 3–4-year-olds).

In the present study, we did not observe a significant P1m latency change in either hemisphere within our 11–25 month interval, although we observed a tendency toward decline (Fig. 3). Previous cross-sectional studies in children older than 5 have reported a change in P1m latency with age (Cunningham et al., 2000; Fujioka et al., 2006; Kotilahti et al., 2010; Ponton et al., 2002). Cunningham et al. (2000) and Ponton et al. (2002) reported that P1 latencies in response to brief click trains decreased with age; however, these changes were not significant during early school-age years (i.e., from 5 to 12 years old). Significant changes

appear to occur later in childhood, as these authors also reported that P1 latencies in children older than 13 were significantly shorter than those of 5- to 10-year-old children. In addition, in a previous study with a longitudinal design, no significant decrease in P100m (P1m) latency was observed in a cross-section of 4- to 6-year-old children (Fujioka et al., 2006), consistent with our result.

Our results indicated that the location of the P1m dipole source in the right hemisphere shifted in the medial-vertex direction between the first and second measurements. Ponton et al. (2002) also reported a lateral-to-medial shift in the P1m source location and suggested that this source location shift is only observed in P1m (Ponton et al., 2002). Our results partially support this previous result for the right hemisphere (i.e., the location of P1m shifted medially).

The present study had some general limitations. First, we cannot discuss the detailed location of the magnetic field source as would be possible with anatomical images such as MRI. Future studies using child-friendly, open-type MRI devices and/or brain models that match the child brain (Fonov et al., 2011; Wilke et al., 2008) are necessary to reduce the uncertainties in the source level estimation. Second, the nominally lower correlation between P1m amplitude in the right hemisphere and language performance may be explained by the lower amplitude of P1m in the right hemisphere (i.e., lower signal/noise ratio). Further studies with larger sample sizes may reveal significant relationships for both hemispheres. Despite some limitations, we demonstrate a relationship between language development and longitudinal changes in P1m. Our results revealed that P1m reflects the maturation of brain functions related to language development.

#### Abbreviations

AEF	auditory evoked field
ECD	equivalent current dipole
EEG	electroencephalography
ISI	interstimulus interval
K-ABC	Kaufman Assessment Battery for Children
MEG	magnetoencephalography
ANOVA	analyzed using a two-way repeated measures analysis of variance
PCA	principal component analysis
SLI	specific language impairment.

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#### Conflict of interest

The authors report no biomedical financial interests or potential conflicts of interest.

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## Regular Article

# Unusual developmental pattern of brain lateralization in young boys with autism spectrum disorder: Power analysis with child-sized magnetoencephalography

Hirotoishi Hiraishi, MS,<sup>1</sup> Mitsuru Kikuchi, MD,<sup>1,2\*</sup> Yuko Yoshimura, PhD,<sup>1</sup> Sachiko Kitagawa, AA,<sup>1</sup> Chiaki Hasegawa, MS,<sup>1</sup> Toshio Munesue, MD,<sup>1,2</sup> Natsumi Takesaki, MD,<sup>2</sup> Yasuki Ono, MD,<sup>2</sup> Tsutomu Takahashi, MD,<sup>3</sup> Michio Suzuki, MD,<sup>3</sup> Haruhiro Higashida, MD,<sup>1</sup> Minoru Asada, PhD<sup>4</sup> and Yoshio Minabe, MD<sup>1,2</sup>

<sup>1</sup>Research Center for Child Mental Development, <sup>2</sup>Department of Psychiatry and Neurobiology, Graduate School of Medical Science, Kanazawa University, Kanazawa, <sup>3</sup>Department of Neuropsychiatry, University of Toyama, Toyama, and <sup>4</sup>Department of Adaptive Machine Systems, Graduate School of Engineering, Osaka University, Osaka, Japan

**Aims:** Autism spectrum disorder (ASD) is often described as comprising an unusual brain growth pattern and aberrant brain lateralization. Although it is important to study the pathophysiology of the developing ASD cortex, examples of physiological brain lateralization in young children with ASD have yet to be well examined.

**Methods:** Thirty-eight boys with ASD (aged 3–7 years) and 38 typically developing (TD) boys (aged 3–8 years) concentrated on video programs and their brain activities were measured non-invasively. We employed a customized child-sized magnetoencephalography system in which the sensors were located as close to the brain as possible for optimal recording in young children. To produce a credible laterality index of the brain oscillations, we defined two clusters

of sensors corresponding to the right and left hemispheres. We focused on the laterality index ( $[(\text{left} - \text{right})/(\text{left} + \text{right})]$ ) of the relative power band in seven frequency bands.

**Results:** The TD group displayed significantly rightward lateralized brain oscillations in the theta-1 frequency bands compared to the ASD group.

**Conclusions:** This is the first study to demonstrate unusual brain lateralization of brain oscillations measured by magnetoencephalography in young children with ASD.

**Key words:** autism, brain lateralization, magnetoencephalography, spontaneous brain activity, young children.

**A**UTISM SPECTRUM DISORDER (ASD) appears in infancy and early childhood and causes a restricted range of interests, as well as delays or impairments in social interaction and communication. The evidence gathered from recent developments in neuroimaging methodology suggests that

an aberrant brain development pattern<sup>1–3</sup> and/or aberrant hemispheric lateralization reflect important aspects of neurologic dysfunction in ASD.<sup>4–9</sup>

Oscillations at various neural frequencies are a prominent feature of the spontaneous brain activities recorded by electroencephalography (EEG) and magnetoencephalography (MEG).<sup>10,11</sup> These oscillations arise from the coordinated activity of a vast number of neurons<sup>12–14</sup> and are believed to reflect the functional states of the brain.<sup>13,15,16</sup> Measurement of neural oscillations is a suitable method to investigate ASD pathophysiology because it has been reported that ASD is a disorder of neural synchrony. In fact,

\*Correspondence: Mitsuru Kikuchi, MD, Research Center for Child Mental Development, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-8641, Japan. Email: mitsuru@zvc1.so-net.ne.jp  
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multiple studies have demonstrated altered spontaneous brain oscillations in patients with ASD.<sup>17–23</sup>

To gain insight into the development of this dysfunction, it is necessary to study the pathophysiology of ASD in young children because aberrant development of the white matter pathways may occur during infancy.<sup>24</sup> We have recently reported aberrant brain connectivity in pre-school children with ASD under conscious conditions.<sup>7,25</sup> For these preliminary studies, we developed a customized child-sized MEG system in which the sensors are located as close to the head as possible for optimal recording, even in young children. This unique and effective technique can provide measures of cortical activity on a millisecond timescale.

In our study, we used MEG because MEG is superior to EEG with respect to its spatial resolution of ongoing brain activity in sensor space.<sup>26</sup> Magnetic fields generated in the temporal lobe tend to be reflected by sensors in the ipsilateral hemisphere, whereas electrical potentials recorded by scalp EEG tend to spread to both hemispheres. This spatial property of MEG makes it particularly advantageous in studies investigating the laterality of neural oscillations. Additionally, the customized child-sized MEG system utilized in the present study places all of the MEG sensors spanning the entire head as close to the cranium as possible, which optimizes recording in young children and allows simultaneous recording of both hemispheres, even in young children,<sup>25,27,28</sup> which is a problem when using conventional adult-sized MEG systems.

Autism spectrum disorder (ASD) is often described as comprising an unusual brain growth pattern<sup>1–3</sup> and aberrant brain lateralization.<sup>4–9</sup> Although it is important to study the pathophysiology of the developing ASD cortex, examples of physiological brain lateralization in young children with ASD have yet to be well examined. In our study, we hypothesized that the spontaneous MEG oscillations of ASD children would exhibit altered hemispheric asymmetry.

## METHODS

### Participants

The clinical group included 38 young boys with ASD aged 38–92 months who were recruited from the Kanazawa University Hospital and prefectural hospitals in Toyama. The patients were diagnosed by a clinical psychiatrist and a clinical psychologist with

over 5 years of experience using the Autism Diagnostic Observational Schedule-Generic (ADOS-G),<sup>29</sup> the Diagnostic Interview for Social and Communication Disorders (DISCO),<sup>30</sup> and the DSM-IV criteria at the time of MEG. The Kaufman Assessment Battery for Children (K-ABC)<sup>31</sup> was used for data acquisition. All ASD children included in this study met the DISCO criteria for a diagnosis of childhood autism ( $n = 24$ ), atypical autism ( $n = 8$ ) or Asperger's syndrome ( $n = 6$ ). Children below the ADOS thresholds were included in the present study if they met both the DSM-IV and DISCO criteria for ASD (10 out of 38 children). The controls were 38 typically developing (TD) young boys aged 36–97 months with no reported behavioral or language deficits. All TD children were native Japanese and had no previous or existing developmental, learning, or behavioral problems according to information obtained from a questionnaire completed by their parents. Based on preferences displayed while handling spoons, the children's hand dominance was as follows: TD children (right = 33, left = 4, both = 1), ASD children (right = 30, left = 2, both = 6). As shown in Table 1, the two groups did not exhibit any significant difference in age, K-ABC mental processing scores or head circumference. Parents agreed to their child's participation in the study with full knowledge of the experimental nature of the research. Written informed consent was obtained prior to participation. The Ethics Committee of Kanazawa University Hospital approved the methods used in this study, and all procedures were performed in accordance with the Declaration of Helsinki.

### Recording and analysis

All children participated in cognitive tasks and MEG measurements separately on two different days. On the first day, children participated in cognitive tests and were introduced to the MEG measurement environment. On the second day, participants were given instructions regarding the MEG measurement. MEG data were recorded using a multichannel (151 channels) superconducting quantum interference device (SQUID) whole-head coaxial gradiometer MEG system for children (PQ 1151R; Yokogawa/KIT, Kanazawa, Japan) in a magnetically shielded room (Daido Steel, Nagoya, Japan). Band-pass filtered MEG data (0.16–200 Hz) were collected with a sampling rate of 1000 Hz. During MEG recording, children lay supine on a bed and viewed a video program

**Table 1.** Demographic characteristics of all participants

Group	ASD children	TD children	t-value
Number of subjects	38	38	
Age in months (range)	67.2 (38–92)	67.4 (36–97)	0.10 (NS <sup>†</sup> )
K-ABC mental processing scale (±SD)	99.4 (±18.2)	97.3 (±13.2)	−0.59 (NS <sup>†</sup> )
K-ABC achievement scale (±SD)	96.6 (±18.1)	98.8 (±12.3)	0.61 (NS <sup>†</sup> )
Head circumference (±SD)	51.3 (±0.234)	51.4 (±0.232)	0.26 (NS <sup>†</sup> )

<sup>†</sup>Unpaired *t*-test between two groups, *P* > 0.05.  
ASD, autism spectrum disorder; K-ABC, Kaufman Assessment Battery for Children; NS, no significant difference; TD, typically developing.

projected onto a screen. Each MEG session lasted 6 min. Prior to recording, we prepared a set of video programs with stories that were particularly attractive to young children. Each participant selected his or her favorite video. Before recording, we asked the children to confirm whether they were content with the video program. During MEG recording, the participants were monitored using a video camera. We analyzed MEG data periods when participants were concentrating on video programs, which were confirmed by the monitoring system. Offline analysis of the MEG data was performed using a BrainVision Analyzer (Brain Products GmbH, Gilching, Germany) and Matlab (MathWorks, Natick, MA). MEG data were re-sampled at 500 Hz. Data were segmented into 2-s increments. Selection of artifact-free segments was based on visual inspection. The process of eliminating contaminated data was performed while blind to personal data. At least 40 artifact-free segments (80-s period) were accepted for each participant. The average number of available epochs per subject was 43.3 (range: 40–47) and 43.4 (range: 40–48) for the TD and ASD children, respectively. MEG spectra were calculated using a fast Fourier transform (FFT) with a spectral resolution of 1 Hz and divided into the following nine bands to calculate the relative power values for each channel, according to our previous study on TD and ASD young children:<sup>7,25</sup> delta (1.0–3.0 Hz), theta-1 (4.0–5.0 Hz), theta-2 (6.0–7.0 Hz), alpha-1 (8.0–10.0 Hz), alpha-2 (11.0–12.0 Hz), beta-1 (13.0–20.0 Hz), beta-2 (21.0–30.0 Hz), gamma-1 (31.0–59.0 Hz), and gamma-2 (62.0–80.0 Hz).

To test our hypothesis, we first selected eight sensors in each band for the corresponding right and left temporal regions (Fig. 1). The distance between the right and left sensors (i.e., the distance between

the center points of the eight sensors in each hemisphere) was 19.72 cm, which can minimize field-spread effects over the right and left hemispheres and is a potentially significant advantage of MEG over EEG methodology at this distance.<sup>32</sup> We averaged the relative power value of the selected eight sensors for each hemisphere, and we regarded the average values as the representative values for the right and left hemisphere. Laterality indices (LI) were calculated for each relative power value using the following formula:  $LI = (L - R) / (L + R)$ , where L and R are the relative power values of the left and right hemispheres, respectively.

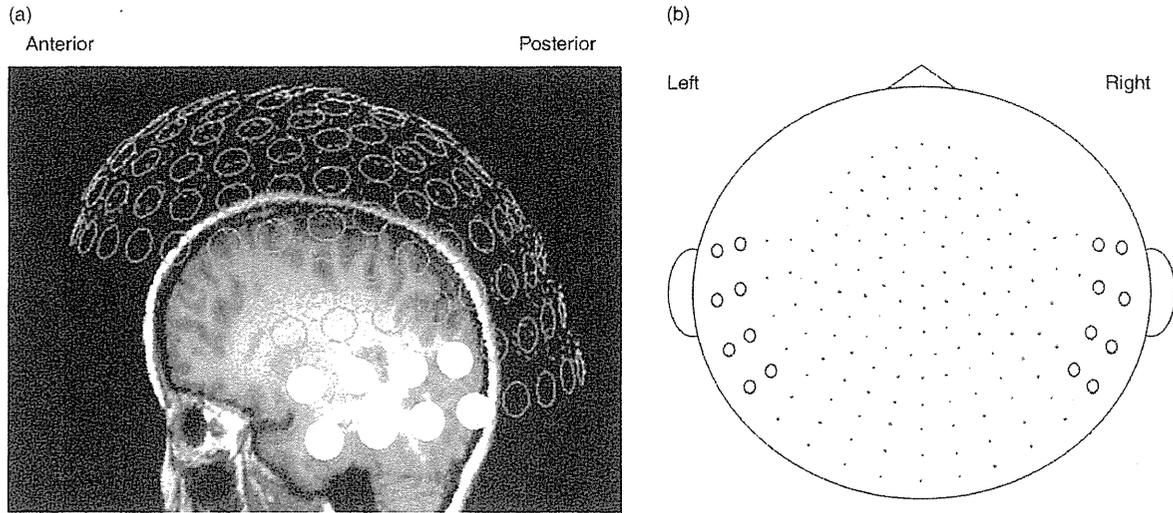
### Statistics

Mann–Whitney *U*-tests were used to compare the LI between the ASD and TD groups for each frequency band. We evaluated the correlation between the LI and age in months for the TD and ASD group using Spearman's rank correlation. To compensate for the multiple comparisons for nine frequencies of interest, the alpha level was adjusted to  $0.05/9 = 0.0056$ . As a complementary approach, an alpha level of 0.05 was also employed, with the risk of increasing the chance of Type I error, to explore the differences in physiological measures (i.e., LI) between the ASD and TD groups and to explore the correlation between the LI and age in months for the TD and ASD groups.

## RESULTS

### LI in ASD and TD groups

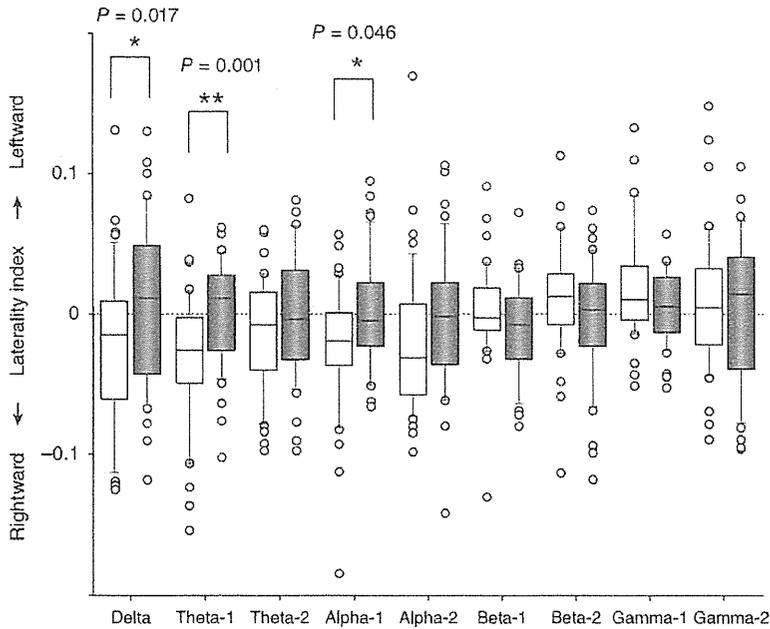
As shown in Figure 2, after Bonferroni correction, Mann–Whitney *U*-tests identified significantly



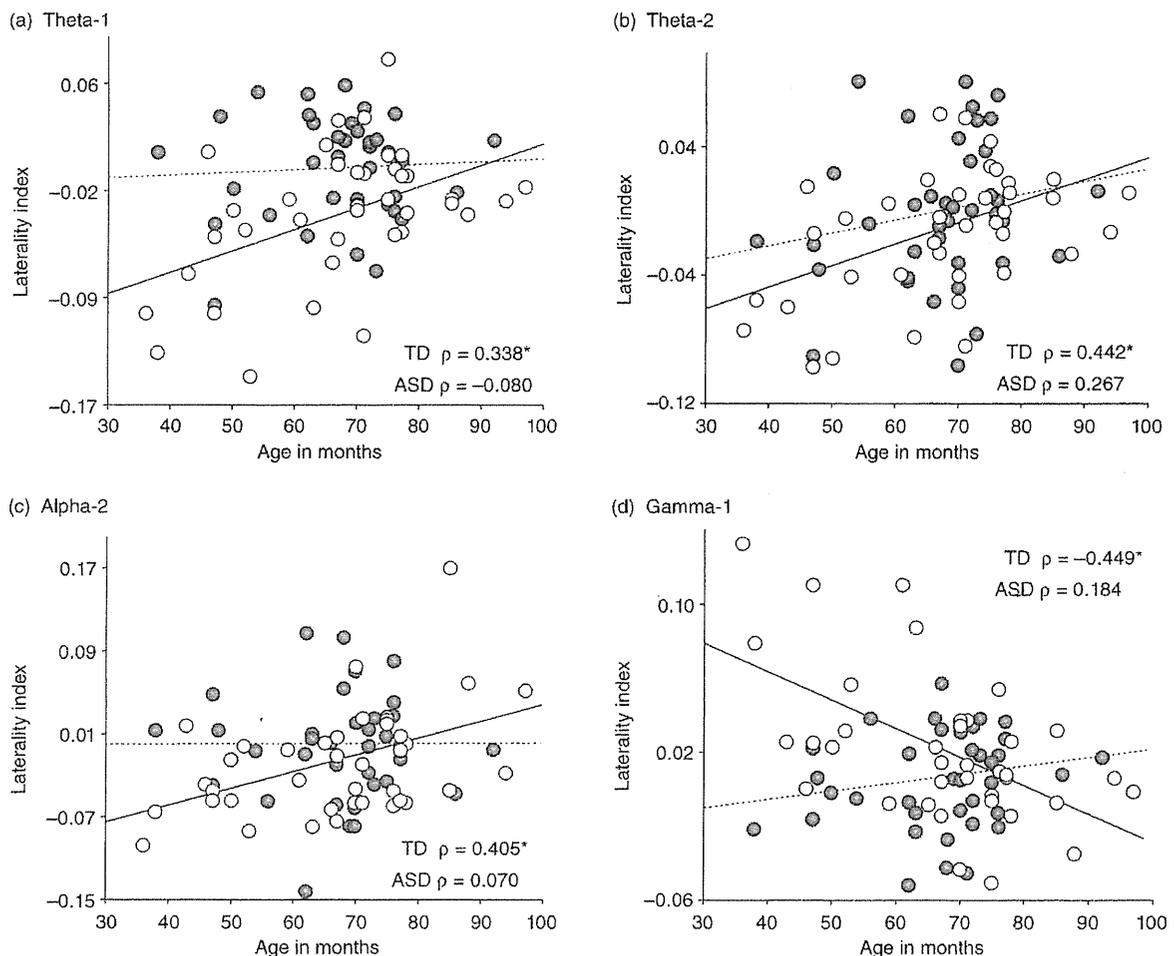
**Figure 1.** Selected sensors in both hemispheres. (a) The 8 selected sensor positions corresponding to the left hemisphere (white circles). (b) The 16 selected sensor positions in a two-dimensional map (black circles).

rightward-lateralized brain oscillations in the TD group for the theta-1 ( $z = -3.29, P \leq 0.0056$ ) compared to the ASD group. When we employed an alpha level of 0.05, with the increased risk of Type I errors,

some significant differences arose between the two groups. Significant rightward-lateralized brain oscillations in the TD group were observed for the delta ( $z = -2.39, P \leq 0.05$ ), theta-1 ( $z = -3.29, P \leq 0.05$ )



**Figure 2.** Laterality indices for all 9 frequency bands in (□) typically developing children and (■) autism spectrum disorder children. The upper edge of the box indicates the 75th percentile of the dataset, and the lower edge indicates the 25th percentile. The line in the box indicates the median value of the data. The upper ends of the vertical line indicate the 90th percentile of the dataset, and the lower ends indicate the 10th percentile of the dataset. \* $P < 0.05$ ; \*\* $P < 0.0056$  (i.e., Bonferroni correction).



**Figure 3.** Scatter plot of laterality indices (LI) values and age in months of (●) autism spectrum disorder (ASD) children and (○) typically developing (TD) children in four frequency bands. When we accepted an alpha level of 0.05, at the risk of increased Type I errors, significant correlations were detected in TD children between the LI and age in four frequency bands, (a) theta-1, (b) theta-2, (c) alpha-2 and (d) gamma-1, but not in ASD children in any frequency band. Solid line, regression line for TD children; broken line, regression line for ASD children. \* $P < 0.05$ .

and alpha-1 bands ( $z = -2.00$ ,  $P \leq 0.05$ ) compared to the ASD group. No significant differences were detected in any other frequency band between the two groups.

#### Correlations between LI and age

After Bonferroni correction, Spearman's rank correlation coefficient did not reveal any significant relations between the LI and age. When we employed an alpha level of 0.05, with the increased risk of Type I

errors, some significant correlations arose. As shown in Figure 3, TD children displayed significant age-dependent increases in the leftward lateralization for the theta-1 ( $\rho = 0.338$ ,  $P < 0.05$ ), theta-2 ( $\rho = 0.442$ ,  $P < 0.05$ ) and alpha-2 ( $\rho = 0.405$ ,  $P < 0.05$ ) bands, as well as a significant age-dependent decrease in the leftward lateralization for the gamma-1 band ( $\rho = -0.449$ ,  $P < 0.05$ ). No significant correlation was detected in any other frequency bands in the TD group. In the ASD group, there were no significant developmental changes in any frequency band.

## DISCUSSION

In the present study, as we hypothesized, we demonstrated aberrant lateralization in young boys with ASD in the theta-1 band using a custom child-sized MEG device. When we accepted an alpha level of 0.05, at the risk of increased Type I errors, aberrant lateralization in young boys with ASD was also found in the delta and alpha-1 bands. Our results were consistent with a previous EEG study (i.e., samples from Russia and Sweden) under similar experimental conditions, which was sustained visual attention in children of a similar age range (preschool children) and the same sex.<sup>22</sup> In the present study, there was no significant relation between the LI and age. When we accepted an alpha level of 0.05, at the risk of increased Type I errors, this study demonstrated the aberrant developmental pattern of LI in children with ASD relative to TD children. These results were demonstrated by MEG sensors spaced 20 cm apart, which is a simple and highly compelling method of MEG analysis.

To investigate ASD pathophysiology, measurement of neural oscillations appeared to be a suitable method because it has been reported that ASD is a disorder of neural synchrony (i.e., the functional disconnections within and among regions of the brain),<sup>33–35</sup> and several previous studies detected altered spontaneous brain oscillations in patients with ASD.<sup>17–23</sup> However, the results of these studies using EEG power analysis were not consistent. The ambiguity of the published EEG results in autism may stem from several sources, as described previously.<sup>22</sup> The first probable source of these discrepancies is the wide age range (from child to adolescent) of the participants examined in previous studies.<sup>19,36</sup> One crucial difference of ASD compared to many other psychiatric diseases is that ASD appears in infancy and early childhood. Young children with ASD follow atypical developmental trajectories in terms of their brain and head sizes,<sup>1–3,37</sup> which strongly suggests the presence of age-specific abnormalities of brain function in autism. Therefore, averaging over a wide age range may blur or even cancel out age-specific EEG abnormalities in autism. The second probable source of the discrepancies is differences in experimental conditions during EEG or MEG recording across previous studies. Although spontaneous EEG and MEG oscillations are strongly dependent on the present behavioral state, the experimental conditions of the previous studies widely

varied. Such divergent experimental conditions in previous studies are attributable to the nature of young children; e.g., it is quite a challenge to keep young children (<5 years old) in an eyes-closed resting condition because it is difficult to maintain their cooperation under such conditions. In fact, there are no previous EEG or MEG studies of young children (<5 years old) in an eyes-closed condition, whereas a few EEG studies of school-age children with ASD in eyes-closed resting conditions have been reported.<sup>17,18</sup> Instead, in young children (<5 years old) with the aid of sustained visual attention, a few EEG studies have successfully measured aberrant spontaneous brain oscillations, including in 3–5-year-old children with ASD.<sup>21,22,36</sup> Such diverse experimental conditions may lead to the inconclusive results found in previous studies using EEG and MEG power analysis.

Intriguingly, as mentioned in the first paragraph of the Discussion, one remarkable study examined the spontaneous brain oscillations of young children with ASD in two independent samples from different countries (i.e., Russia and Sweden); both of these groups were the same sex (i.e., male), were nearly the same age (3–8 years old) and were examined under similar experimental conditions of sustained visual attention.<sup>22</sup> The authors of this study reported that the most distinctive feature of spontaneous EEG oscillations in ASD children was an abnormal hemispheric asymmetry in the delta, theta and alpha bands at the temporal and some adjacent regions; these findings were replicated in the two independent samples from different countries. While the results of a number of these previous EEG studies of ASD participants have not been consistent,<sup>17–22</sup> three independent samples, including our sample (i.e., samples from Russia, Sweden and Japan), displayed relatively consistent results. It is notable that the experimental parameters (e.g., age range, sex and experimental condition) are similar between the three studies, which might contribute to the consistency of the results from heterogeneous groups of ASD participants.

The present study had several limitations. First, we eliminated any contaminated MEG data, such as data temporally coincident with clear ocular movements. However, differences in fine ocular movements between children with ASD and TD children may have confounded the results when frequent saccades occurred, which could have transpired while viewing the video program. Second, we recorded the head

positions of the participants using video monitors during the MEG recordings, and based on visual inspection, we eliminated any MEG data from the relative power analysis if the head position of the participant was clearly different from the starting position. Future studies using a quantification algorithm for the head movement will provide more reliable evidence. Third, we did not evaluate the degree to which the subjects focused on video programs that they chose. Further studies that employ attention-controlled conditions will provide more reliable evidence, although these conditions will likely be difficult to achieve in conscious preschool-aged young children.

### Conclusions

Using a customized child-sized MEG, this study extends the findings of previous EEG studies demonstrating altered functional lateralization of brain oscillations in young boys with ASD,<sup>22</sup> and this is the first study to demonstrate an unusual developmental trajectory of functional hemispheric lateralization, as reflected in brain oscillations in young children with ASD.

### ACKNOWLEDGMENT

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## Regular Article

## Broader autism phenotype in mothers predicts social responsiveness in young children with autism spectrum disorders

Chiaki Hasegawa, MA,<sup>1</sup> Mitsuru Kikuchi, MD, PhD,<sup>1,2\*</sup> Yuko Yoshimura, PhD,<sup>1</sup> Hirotoishi Hiraishi, MD,<sup>1</sup> Toshio Munesue, MD, PhD,<sup>1,2</sup> Hideo Nakatani, MD, PhD,<sup>2</sup> Haruhiro Higashida, MD, PhD,<sup>1</sup> Minoru Asada, PhD,<sup>3</sup> Manabu Oi, PhD<sup>1</sup> and Yoshio Minabe, MD, PhD<sup>1,2</sup>

<sup>1</sup>Research Center for Child Mental Development, <sup>2</sup>Department of Psychiatry and Neurobiology, Graduate School of Medical Science, Kanazawa University, Kanazawa, and <sup>3</sup>Department of Adaptive Machine Systems, Graduate School of Engineering, Osaka University, Suita, Japan

**Aims:** The aim of this study was to identify phenotypes in mothers and fathers that are specifically associated with disturbances in reciprocal social interactions and communication in their young children with autism spectrum disorder (ASD) in a Japanese sample.

**Methods:** Autistic traits in parents were evaluated using the Autism-spectrum Quotient (AQ), the Empathy Quotient (EQ) and the Systemizing Quotient (SQ) in 88 parents (44 mothers and corresponding fathers) of children with ASD and in 60 parents (30 mothers and corresponding fathers) of typically developing (TD) children. For the measurement of autistic traits in children, we employed the Social Responsiveness Scale (SRS).

**Results:** In two of the five AQ subscales (social skills and communication), the parents of ASD children scored significantly higher than did the parents of TD children, regardless of whether the parent was a

mother or a father. In addition, in mothers of ASD children, there were significant positive correlations between two of the five AQ subscales (attention-switching and communication) and the SRS T-score in their children.

**Conclusions:** This is the first study to demonstrate that the social skills and communication subscales in the AQ are more sensitive as autism traits in a Japanese sample and to demonstrate that some autistic traits in mothers are specifically associated with disturbances in the social ability of their young children with ASD, as measured by the SRS score. Further study is necessary to determine whether these results were caused by genetic or environmental factors.

**Key words:** autism spectrum disorder, Autism-spectrum Quotient, Empathy Quotient, parents, Social Responsiveness Scale.

**A**UTISM SPECTRUM DISORDERS (ASD) appear in early childhood, causing delays or impairments in social interactions and communication, as well as a restricted range of interests.<sup>1</sup> Initially, the

evidence for the genetic basis of ASD was provided by twin studies of classic autism<sup>2,3</sup> and, more recently, twin studies of autistic traits.<sup>4-6</sup> A strong genetic component was indicated by a concordance of 60% versus 3-5% in monozygotic and dizygotic twins, respectively,<sup>2</sup> and by many molecular genetic associations.<sup>7</sup> However, identifying specific DNA sequence variations that cause ASD has been difficult; replicating the results has been hampered by methodological issues, such as limited power and varying designs,

\*Correspondence: Mitsuru Kikuchi, MD, PhD, Research Center for Child Mental Development, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-8641, Japan. Email: mitsuru@zcd.so-net.ne.jp  
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along with imprecise phenotypic definitions.<sup>8</sup> ASD seems to be both genetically and phenotypically heterogeneous. The behavioral traits of ASD suggest a continuous distribution across the population;<sup>9</sup> therefore, a categorical approach to identifying causative factors may not be the best approach to identifying the specific DNA sequence and/or neurophysiological variations that cause ASD.

In first-degree relatives of individuals with ASD, a phenotype that is milder but echoes a similar profile to the defining features of ASD is often observed<sup>10–13</sup> and is referred to as the broader autism phenotype (BAP). The BAP refers to heritable, sub-threshold autism-related traits and may be related to different genetic loading in families with autism.<sup>14</sup> The BAP is generally considered to be a subclinical set of characteristics or traits that index familiarity and/or genetic liability to autism. This conception holds that the BAP is milder but qualitatively similar to the diagnosed autism phenotype. Therefore, the importance of measuring the BAP is sharply rising not only in genetic studies but also across autism research (for example, in neuroimaging and cognitive studies).<sup>15,16</sup>

For adults, there are instruments available for the assessment of the BAP. One of the instruments used extensively is the Autism-spectrum Quotient (AQ).<sup>17</sup> The AQ was developed to assess where an individual lies on the autism spectrum (that is, how many autistic traits an individual exhibits). The AQ for adults has the format of a self-report, forced-choice questionnaire and is at the reading level of a typical 10-year-old. It can be used by adults with an IQ in the average range, who can read and understand at least at this level. There are 50 items assessing behaviors across five domains: communication, social skills, attention-switching, imagination, and attention to detail. In addition to the AQ, the traits of ASD in adults have been characterized using the following two dimensions: the Empathy Quotient (EQ)<sup>18</sup> and the Systemizing Quotient (SQ).<sup>19</sup> These three dimensions can be used to assess milder variants of autistic-like traits (i.e., high AQ, low EQ, and high SQ) in typically developing individuals.<sup>6,20,21</sup> Empathy is an essential part of normal social functioning that allows us to understand the intentions of others, predict their behavior, and experience an emotion triggered by their emotions. The EQ is a self-report questionnaire for use with adults of normal intelligence that focuses purely on this domain.<sup>18</sup> Systemizing is the drive to analyze the variables in a system and derives the underlying rules that govern the

behavior of a system. The SQ is a self-report questionnaire for use with adults of normal intelligence that focuses purely on this domain across the range of different system classes.<sup>19</sup>

For children, one instrument available for the assessment of the BAP in Japan is the Social Responsiveness Scale (SRS).<sup>22</sup> The SRS is a 65-item questionnaire that is completed by an adult informant. It focuses on the ability of the subject of the questionnaire to engage in emotionally appropriate reciprocal social interactions and communication. Using the SRS, a higher number of autistic traits have been observed in the siblings of children with autism.<sup>23</sup>

The aims of this study were to determine the specific subtypes of the BAP in parents (i.e., the AQ, EQ and SQ scores) that are specifically associated with disturbances in reciprocal social interactions and communication in their young children with ASD (i.e., the SRS score) in a Japanese sample. Viewing the effects of the BAP on their offspring in this way sheds new light on existing and emerging data and has crucial implications for genetically identifying the BAP in adults.

## METHODS

### Participants

All participants were recruited from public nursery schools in Kanazawa city, Kanazawa University's Hospital and prefectural hospitals in Toyama. At first, 96 children and their parents voluntarily participated in this study. Clinically recruited children were diagnosed by a clinical psychiatrist and a clinical psychologist with more than 5 years of experience in ASD using the Autism Diagnostic Observational Schedule–Generic (ADOS),<sup>24</sup> the Diagnostic Interview for Social and Communication Disorders (DISCO),<sup>25</sup> and the DSM-IV<sup>1</sup> criteria at the time that they participated in this study. ASD children were included in this study when they fulfilled the diagnosis of childhood autism, atypical autism or Asperger's syndrome with DISCO, or the ADOS criteria for the autism spectrum. Exclusion criteria for children included known hearing loss or a central nervous system involvement other than autism. The final clinical group consisted of 44 children with ASD (35 boys, nine girls) aged 38–93 months and their parents (44 mothers and the corresponding fathers) (Table 1). The controls were 30 typically developing (TD) children (23 boys, seven girls) aged 37–95

**Table 1.** Demographic characteristics of the study participants

	TD children	ASD children	t	P
Number of subjects	30	44		
Sex (male/female)	23/7	35/9		
Chronological age (months)	66.4 (37–95)	65.4 (38–93)	0.29	NS
SRS total T-score	48.2 (5.9)	72.8 (10.4)	12.9	$P < 0.01$
K-ABC Mental Processing Scale	99.2 (11.5)	94.7 (24.3)	-1.1	NS
K-ABC Achievement Scale	100.5 (13.7)	96.4 (20.9)	-1.0	NS
<b>Parents</b>				
Age of mother (years)	36.3 (22–47)	36.6 (26–48)	0.26	NS
Age of father (years)	39.0 (24–54)	37.7 (25–48)	-0.98	NS

Values are mean (range or SD) for chronological age, father's age, mother's age and scales on the K-ABC and the SRS. ASD, autism spectrum disorder; K-ABC, Kaufman Assessment Battery for Children; NS, not significant; SRS, Social Responsiveness Scale; TD, typically developing.

months and their parents (30 mothers and the corresponding fathers) (Table 1). All TD children and their parents had no prior or current developmental, learning, or behavioral problems, as reported on a questionnaire completed by their parents. If the parents reported difficulties in daily life because of their intelligence level, we excluded them from this experiment. The parents agreed to their child's participation in the study with full knowledge of the experimental nature of the research. Written informed consent was obtained prior to participation. The Ethics Committee of Kanazawa University Hospital approved the methods, and all procedures were performed in accordance with the Declaration of Helsinki.

### Psychological tasks for autistic traits

Quantitative autistic traits in children were assessed by parents using the Japanese version<sup>22</sup> of the SRS.<sup>26</sup> Higher scores on the SRS indicate a higher degree of social impairment. The raw scores of the SRS were converted to T-scores (with a mean of 50 and a standard deviation of 10) for sex. All T-scores in the SRS were significantly higher in the ASD group compared to the controls (Table 1). The Kaufman Assessment Battery for Children (K-ABC)<sup>27</sup> was employed to estimate the intelligence level in the children.

Traits of ASD in parents were assessed by the AQ,<sup>17</sup> EQ<sup>18</sup> and SQ scores,<sup>19</sup> which consisted of self-report measures of autistic traits. These three dimensions can be used to assess milder variants of autistic-like traits (i.e., low EQ, high AQ and high SQ) in typically developing individuals.<sup>6,20,21</sup>

### Statistical analysis

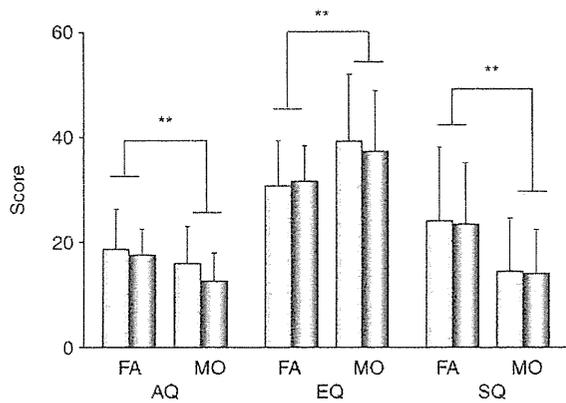
For the AQ, the EQ and the SQ total scores, a two-way ANOVA was performed (parent  $\times$  diagnosis). The within-subjects factor was the parent effect (mother vs father), whereas the between-subjects factor was the diagnosis effect (ASD vs TD).

For the AQ subscales (i.e. social skills, attention-switching, attention to detail, communication and imagination), a two-way ANOVA was performed (parent  $\times$  diagnosis) in the same manner.

In the present study, our main concern was to evaluate how autistic traits in young children are constrained by autistic traits in their mothers and fathers. We performed multiple linear regressions to predict the autistic traits in young children measured by the SRS T-score using the AQ, EQ, and SQ total scores in their mothers and fathers as predictors (i.e., six independent variables) in the ASD ( $n = 44$ ), TD ( $n = 30$ ) and mixed groups ( $n = 74$ ).

Because a multiple regression analysis demonstrated that the AQ total score in the mother was the significant predictor for the SRS score in their children in the ASD group, a Pearson's correlation was used to find significant correlations between the AQ subscales in mothers (i.e., social skills, attention-switching, attention to detail, communication and imagination) and the SRS subscales in their children (i.e., awareness, cognition, communication, motivation and mannerisms).

The significance level was set at  $P < 0.05$  for all statistical analyses.



**Figure 1.** Parents' scores on the Autism-spectrum Quotient (AQ), the Empathy Quotient (EQ) and the Systemizing Quotient (SQ). (□) Parents who have autism spectrum disorder children ( $n = 44$ ). (■) Parents who have typically developing children ( $n = 30$ ). A two-way ANOVA revealed a significant effect between the father (FA) and the mother (MO) on the AQ, the EQ and the SQ. The values indicate the mean. Error bars represent the SD. \*\* $P < 0.01$ .

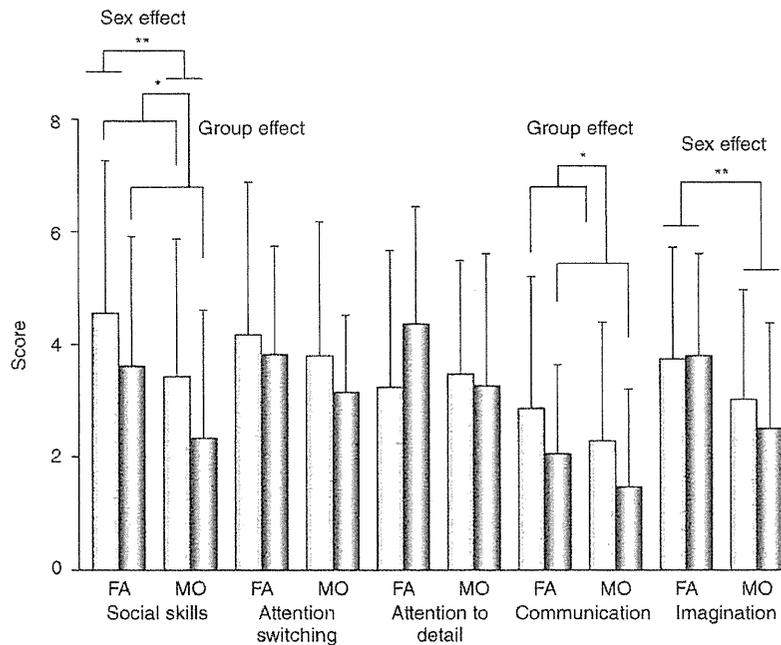
**RESULTS**

As shown in Table 1, 74 trios satisfied the inclusion criteria for the present study. There were no significant differences in the children's chronological age (months), scores on the K-ABC or parents' ages (father and mother) between the TD and ASD groups.

For the AQ, EQ and SQ scores, two-way ANOVA failed to demonstrate a significant main effect of group (i.e. ASD vs TD) or a significant interaction (i.e. group  $\times$  sex in parent). As shown in Figure 1, significant main effects of sex in parents were observed in the total AQ ( $F = 12.72, P < 0.01$ ), the EQ ( $F = 17.39, P < 0.01$ ) and the SQ ( $F = 25.50, P < 0.01$ ).

For the AQ subscales, two-way ANOVA revealed significant main effects of the group, with the ASD parents scoring higher than the TD parents on two of the five AQ subscales: social skills ( $F = 5.87, P < 0.05$ ) and communication ( $F = 5.27, P < 0.05$ ) (Fig. 2). There were significant main effects of sex in parents on two of the five AQ subscales: social skills ( $F = 8.73, P < 0.01$ ) and imagination ( $F = 9.19, P < 0.01$ ). There were no significant interactions (group  $\times$  sex in parent) in any AQ subscales (Fig. 2).

We performed multiple linear regressions separately for the ASD ( $n = 44$ ), TD ( $n = 30$ ) and mixed groups



**Figure 2.** Subscales of the Autism-spectrum Quotient in parents. (□) Parents who have autism spectrum disorder (ASD) children ( $n = 44$ ). (■) Parents who have typically developing (TD) children ( $n = 30$ ). Two-way ANOVA revealed significant group (ASD vs TD) effects in the subscales of social skills ( $P < 0.05$ ) and communication ( $P < 0.05$ ). Significant effects between the father (FA) and mother (MO) were also observed in the subscales of social skills ( $P < 0.01$ ) and imagination ( $P < 0.01$ ). The values indicate the mean. Error bars represent the SD. \* $P < 0.05$  and \*\* $P < 0.01$ .