Cerebral Cortex Advance Access published January 29, 2008

Cerebral Cortex
doi:10.1093/cercor/bhm25-i

Sex-Linked Neuroanatomical Basis of Human Altruistic Cooperativeness

Hidenori Yamasue[1], Osamu Abe[2], Motomu Suga[1], Haruyasu Yamada[2], Mark A. Rogers[1,3], Shigeki Aoki[2], Nobumasa Kato[1] and Kiyoto Kasai[1]

[1]Department of Neuropsychiatry, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan, [2]Department of Radiology, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan and [3]Department of Psychology, Monash University, Clayton 3800, Victoria, Australia

Human altruistic cooperativeness, one of the most important components of our highly organized society, is along with a greatly enlarged brain relative to body size a spectacular outlier in the animal world. The "social-brain hypothesis" suggests that human brain expansion reflects an increased necessity for information processing to create social reciprocity and cooperation in our complex society. The present study showed that the young adult females (n = 66) showed greater Cooperativeness as well as larger relative global and regional gray matter volumes (GMVs) than the matched males (n = 89), particularly in the social-brain regions including bilateral posterior inferior frontal and left anterior medial prefrontal cortices. Moreover, in females, higher cooperativeness was tightly coupled with the larger relative total GMV and more specifically with the regional GMV in most of the regions revealing larger in female sex-dimorphism. The global and most of regional correlations between GMV and Cooperativeness were significantly specific to female. These results suggest that sexually dimorphic factors may affect the neurodevelopment of these "social-brain" regions, leading to higher cooperativeness in females. The present findings may also have an implication for the pathophysiology of autism; characterized by severe dysfunction in social reciprocity, abnormalities in social-brain, and disproportionately low probability in females.

Keywords: altruism, cooperativeness, sex difference, social brain, voxel-based morphometry

Introduction

Human altruistic cooperation is one of the most important components of our highly organized society and of what characterizes us as human (Fehr and Fischbacher 2003). The idea of reciprocity has been proposed to account for important patterns of human altruism such as that toward nonrelatives, which represent a spectacular outlier in the animal world (Gintis et al. 2003; Silk et al. 2005; Nowak and Sigmund 2005). Humans have memory systems capable of keeping complex information concerning faces and good- or bad-feelings toward people, even those who they do not encounter for years at a time. In comparison, most other species exhibit reciprocity only over very short timescales, if at all. Therefore, considerable information processing capacity is a prerequisite for reciprocal cooperativeness (Pennisi 2005).

One can postulate that the unusually large brain for our body size may be correlated with high altruistic cooperativeness in humans. In fact, evolutionary biologists have put forward the "social-brain hypothesis," which sees social complexity as a driving force for information processing capacity in brain evolution (Dunbar 1998, 2003). Although the theory was based on evidence of positive correlation between social complexity and relative neocortex volume in primates (Dunbar 1998; Reader and Laland 2002), there is no human evidence that neocortex volume correlates with indices representing human social function.

Although human altruistic cooperativeness represents a huge anomaly in the animal world, there is much individual heterogeneity in our altruistic cooperativeness. For example, although in humans an excessive deviation in this trait is observed in severe mental disorders such as autism (Soderstrom et al. 2002), one of the major factors contributing to such heterogeneity is sex difference among healthy individuals (Clutton-Brock et al. 2002). In humans, females tend to show strong cooperativeness across nations and cultures (Cloninger et al. 1993; Brandstrom et al. 2001; Farmer et al. 2003), although previous literature showed some inconsistency (Rapoport and Chammah 1965). Because human altruistic cooperativeness has recently been thought to evolve with genes (Fehr and Fischbacher 2003), relatively specific effects of X-linked genes on social cognition (Check 2005; Ross et al. 2005; Skuse 2005) may at least in part explain such sex differences in altruistic cooperativeness. Recent functional imaging studies have reported activation of posterior superior temporal gyrus (pSTG), posterior inferior frontal gyrus (pIFG), anterior medial prefrontal cortex (amPFC), anterior insula, and fusiform gyrus as neural correlates of human altruistic cooperativeness and related factors such as empathy, understanding other's emotion, and interpersonal interaction (McCabe et al. 2001; Rilling et al. 2002; Carr et al. 2003; Decety et al. 2004; Singer et al. 2004; Iacoboni et al. 2005). Furthermore, a few studies have suggested sex differences in these activations (Leibenluft et al. 2004; Azim et al. 2005; Platek et al. 2005; Singer et al. 2006). The above findings suggest it is necessary to consider the effects of sex in attempting to uncover the neuroanatomical underpinnings of human altruistic cooperativeness.

Another line of evidence has suggested that neuroanatomy is a highly heritable trait marker as estimated from magnetic resonance imaging (MRI) gray matter volume (GMV) data of twins (Baare et al. 2001; Thompson et al. 2001; Geschwind et al. 2002; Wright et al. 2002), and many studies have shown sex differences in the neuroanatomy (Gur et al. 1999; Good et al. 2001a; Luders et al. 2004). Genetic factors, which include X-linked genes, promote sex-dimorphism in brain anatomy directly by modulating early gonadal secretions (Carrer and Cambiasso 2002; Simerly 2002). Therefore, sex differences of neuroanatomy are thought to be one of the major phenotypes of X-linked genes (Arnold 2004). Furthermore, sex-dimorphism in brain anatomy may provide clues for understanding the

neural background of sex-biased probability of mental illness (Andreasen 2005; Baron-Cohen et al. 2005), whereas the behavioral correlates of sex-dimorphism in brain morphology as yet remain unclear.

Taken together, sex differences in altruistic cooperativeness and neuroanatomy might at least in part share a genetic background. Furthermore, the shared factors might influence the altruism-neuroanatomy relationship. Therefore, there are reasonable grounds to predict a sex-linked correlation between individual differences in altruistic cooperativeness and regional GMV of the social brain regions. In addition, based on the "social-brain hypothesis" (Dunbar 1998), altruistic cooperativeness is expected to correlate even with total neocortex volume. Because recent research in genetics has suggested that within-species variation precedes between species variation (Insel 2006), between-species differences in social behavior and social-brain development might suggest a link between within human individual differences in social-brain morphology and social behavior. However, little is known about the neuroanatomical basis of altruism, although a few recent studies (Moll et al. 2006; Harbaugh et al. 2007; Tankersley et al. 2007) have studied neural correlates of human altruism. For example, Tankersley et al. (2007) argued that the individual differences in activation of superior temporal sulcus associated with social learning predict individual's differences in altruism. Furthermore, the relationship between gender and neural correlates of altruism has never been studied.

The use of self-report questionnaires such as Temperament and Character Inventory (TCI) has been well-established as a means to assess individual differences in behavioral traits (Cloninger 1987; Cloninger et al. 1993). In the *Cooperativeness* subscale of TCI (C), cooperative individuals are described as socially tolerant, empathic, helpful, and compassionate (Cloninger et al. 1993). According to the original concept of Cloninger et al. (1993), temperament is independently heritable, manifest early in life, and involves preconceptual biases in perceptual memory and habit formation, whereas Character mature in adulthood and influence personal and social effectiveness by insight learning about self concepts. However, they also assumed that genetic factors are as important for 3 dimensions of character development in TCI, including C, as they are for temperament. In accordance with the theory, previous studies have reported a familiality of C (Farmer et al. 2003; Ando et al. 2004). Previous studies have reported that individuals who have less social reciprocity, such as subjects with autism-spectrum disorder (Soderstrom et al. 2002; Anckarsater et al. 2006) and with antisocial behavior (Tremblay et al. 1994; Ball et al. 1998), scored low in C. Therefore, the index seems to be suitable for a probe to index individual differences in altruistic cooperativeness.

The present study explored the neuroanatomical basis of human altruistic cooperativeness and its relationship to sex using voxel-based morphometry (VBM) (Good et al. 2001b) throughout the entire brain in healthy young adults. Therefore, the study was designed 1) to replicate the sex-dimorphism of global and regional brain morphology, 2) to identify the correlation between individual differences in C indexed by TCI and global and regional GMVs, and 3) to examine whether or not sex differences exist in the association between global and regional GMV and human altruistic cooperativeness.

Materials and Methods

Participants and Clinical Evaluation

A total of 155 Japanese right-handed (Oldfield 1971) subjects (89 male/66 female), mainly college students, hospital staff, and their acquaintances, participated in the study. Because the present study was concerned with trait aspects of brain morphology and personality, the age of subjects was restricted to the 3rd and 4th decades of life to minimize aging and menopausal effect on brain morphology. The participants were interviewed by a trained psychiatrist (H.Y. or M.S.) to be screened for the presence or absence of neuropsychiatric disorders through the Structured Clinical Interview for DSM-IV Axis I Disorder (American Psychiatric Association 1994), Non-patient Edition (First et al. 1997). Self- and parental-socio-economic state (SES) were assessed using the Hollingshead scale (1965). These interviews were performed on the same day as MR-scanning. The ethical committee of the University of Tokyo Hospital approved this study. After a complete explanation of the study, written informed consent was obtained from all participants. None of the subjects had a history of neuropsychiatric disorder, serious head trauma with any known cognitive consequences or loss of consciousness for more than 5 min, alcohol/substance abuse, or dependence. All participants had to have IQ greater than 75. Each subject completed a valid Japanese translation (Takeuchi et al. 1993; Kijima et al. 2000) of 240-item TCI (Cloninger 1987; Cloninger et al. 1993) within 3 months before or after MR scan. The present study focused on C subscale in TCI.

MRI Acquisition

The method of MRI acquisition was the same as that in our previous studies (Yamasue et al. 2003, 2007). Briefly, the MRI data were obtained using a 1.5-Tesla scanner (General Electric Signa Horizon Lx version 8.2, GE Medical Systems, Milwaukee, WI). Three-dimensional Fourier-transform spoiled gradient recalled acquisition with steady state was used. The repetition time was 35 ms, the echo time 7 ms with 1 repetition, the nutation angle 30°, the field of view 24 cm, and the matrix 256 × 256 (192) × 124. A trained neuroradiologist (Ha.Ya. or O.A.) evaluated the MRI scans and found no gross abnormalities in any of the subjects.

Image Processing for VBM

The same image processing for VBM as our previous study (Yamasue et al. 2007) was conducted using SPM 2 (Ashburner and Friston 2000; Good et al. 2001b) (Institute of Neurology, London, UK) running in MATLAB 7.1 (Mathworks, Sherborn, MA) in the current study. The spatial normalization to standard anatomical space was performed in a 2-stage process. In the 1st step, each image was registered to the International Consortium for Brain Mapping template (Montreal Neurological Institute, Montreal, Canada). This step applied a 12-parameter affine transformation to correct for image size and position. The normalized images of all participants were averaged and smoothed with a Gaussian kernel of 8-mm full-width at half-maximum (FWHM) and then used as a new template with reduced scanner- and population-specific bias. In the 2nd step, each image of was locally deformed to the new study-specific template using a nonlinear spatial transformation. Finally, using a modified mixture model cluster analysis, normalized images were corrected for nonuniformities in signal intensity and partitioned using a study-specific customized prior probability map into gray and white matter, cerebrospinal fluids (CSF), and background. To remove unconnected nonbrain voxels, a series of morphological erosions and dilations to the segmented images were applied (Good et al. 2001a, 2001b). In the intensity modulation step, voxel values of the segmented images were multiplied by the measure of warped and unwarped structures derived from the nolinear step of the spatial normalization. The resulting modulated gray and white matter images were smoothed with a Gaussian kernel of 12-mm FWHM.

Statistical Analysis for Global Brain Volume

Sex differences in absolute and relative volume, adjusted for individual differences in intracranial volume (ICV), of total gray matter, white

matter, CSF, and ICV calculated from optimized VBM procedure were tested using Mann-Whitney Test. Then, to test the "social-brain hypothesis," the correlation between relative total GMV and the score of C was tested using Spearman's rank-order correlation in each sex separately, because sex differences in the correlation were predicted in advance. Furthermore, sex difference in the calculated correlation was examined employing Fisher's r to z transformation. Significance level was defined at $P < 0.05$.

Statistical Analysis for Regional Brain Volume

Statistical comparison between males ($N = 89$) and females ($N = 66$) was performed using an analysis of covariance model (Friston et al. 1990). To account for global anatomical variations, ICV was treated as a confounding covariate. To test hypotheses with respect to regionally specific sex differences, the estimates were compared using 2 linear contrasts (Friston et al. 1995). The resulting set of voxel values for each contrast constituted a statistical parametric map of the t-statistic (SPM{t}). The SPM{t} values were transformed to the normal distribution (SPM{z}) and with a threshold at $P < 0.001$. The significance of each region was corrected for multiple comparisons using false discovery rate (FDR), because previous literature suggests that multiple hypothesis testing (Bonferroni type) family-wise error correction tends to wipe out both false and true positives when applied to the entire data in neuroimaging (Genovese et al. 2002). Thus, the significance level was set at FDR-corrected $P < 0.05$.

To detect the neuroanatomical correlates of individual differences in altruism, statistical analysis was performed with ICV as a confounding covariate, and the score of C as the covariate of interest within each sex separately. To test the specificity of correlation, the correlation was also examined in the sex-combined sample. The threshold for statistical significance was also set at FDR-corrected $P < 0.05$.

Sex difference in the correlation between altruism and regional brain volume was tested for using the condition by covariates interaction analysis. The interaction analysis treated sex as condition, the score of C as covariate of interest, and ICV as the confounding covariate. Because this analysis was employed as a post hoc analysis to examine the significance of interaction between sex and altruism on the single peak coordinate showing a significant correlation with altruism, the threshold for statistical significance was set at $P < 0.05$ without correction for multiple comparisons.

Post hoc partial correlational analyses were further added for evaluating to what degree the regional correlations between C and specific areas account for the global correlation between C and relative total GMV in females, or whether the regional correlations exist beyond the global correlation. The values of regional GMV were extracted from the peak coordinates showing the regional correlation (Table 3) to test for specific effects of regional GMV after controlling for the relative total GMV effects, and for the relative total GMV effect after controlling for the regional effects.

To test whether the different areas capture independent sources of individual differences, the additional regression analyses were conducted using individual's score of C as dependent value and regional GMVs in each brain region as independent values.

Results

Demographic Characteristics

There were no significant sex differences in age, handedness, self-, or parental-SES (Hollingshead 1965). The scores of C ($P = 0.026$), reward dependence ($P = 0.007$), and self-transcendence ($P = 0.041$) were significantly higher in females than in males. (Table 1). The correlations between C and the other subscales of TCI were further examined in each sex. The Spearman's correlations between C and other items of the TCI were as follows: novelty seeking (rho = 0.004; $P = 0.98$), harm avoidance (rho = -0.257; $P = 0.038$), reward dependence (rho = 0.426, $P < 0.001$), persistence (rho = 0.0; $P = 0.99$), self-directedness (rho = 0.511, $P < 0.001$), and self-transcendence (rho = 0.281; $P = 0.022$), in female, and novelty seeking (rho = -0.037; $P = 0.73$), harm avoidance (rho = -0.413, $P < 0.001$), reward dependence (rho = 0.489, $P < 0.001$), persistence (rho = 0.384, $P < 0.001$), self-directedness (rho = 0.434, $P < 0.001$), and self-transcendence (rho = 0.347, $P = 0.001$) in male. Next, we conducted VBM analysis for exploring correlations between regional GMV and items of the TCI showing significant correlations with C ($P < 0.05$). However, the VBM analysis revealed no regions that show significant correlations with TCI scores for any items (FDR-corrected $P > 0.05$). Therefore, we did not conduct the analysis employing these TCI indices as confounding covariates.

Table 1
Subject characteristics

	Male (n = 89)		Female (n = 66)		Mann-Whitney	
Variable	Mean	SD	Mean	SD	Z value	P
Demographic variables						
Age (range)	28.9 (21-40)	4.2	28.0 (22-40)	4.3	−1.56	0.12
Handedness (range)[a]	96.0 (25-100)	11.1	96.5 (50-100)	9.0	−0.94	0.35
Socioeconomic status[b]	1.58	0.5	1.77	0.7	−1.4	0.15
Parental socioeconomic status[b]	2.15	0.6	2.11	0.6	−0.36	0.72
Temperament and Character Inventory						
Harm avoidance	15.8	7.2	16.5	6.4	−0.86	0.39
Novelty seeking	22.8	5.7	22.3	5.2	−0.65	0.51
Reward dependence	14.9	3.4	16.5	3.1	−2.68	0.007
Persistence	4.7	1.6	4.5	1.6	−0.47	0.63
Self-directedness	29.0	6.9	31.3	6.3	−1.9	0.051
Cooperativeness	28.4	5.5	30.3	5.4	−2.23	0.026
Self-transcendence	9.0	4.8	11.3	6.2	−2.05	0.041
Global brain measures (Fig. 1)						
Total GMV (cc)	782	57	705	47	−7.47	< 0.001
Total white matter volume (cc)	473	42	411	33	−7.99	< 0.001
Total cerebrospinal fluid volume (cc)	402	53	319	41	−8.47	< 0.001
ICV (cc)	1659	126	1436	103	−8.76	< 0.001
Gray matter/ICV	0.472	0.02	0.491	0.02	−6.26	< 0.001
White matter/ICV	0.285	0.014	0.286	0.013	−0.49	0.625
Cerebrospinal fluid/ICV	0.242	0.02	0.221	0.018	−5.80	< 0.001

[a]Determined using Edinburgh Inventory (Oldfield 1971): Scores greater than 0 indicate right-handedness. A score of 100 indicates strong right-handedness.
[b]Assessed using the Hollingshead scale (Hollingshead 1965). Higher scores indicate lower educational and/or occupational status.

Sex Difference in Global Brain Morphology

The absolute volumes of ICV, total gray matter, white matter, and CSF were significantly larger in males ($P < 0.001$). Relative total gray matter, however, was significantly larger in females ($P < 0.001$). By contrast, males had significantly larger relative total CSF volume ratio to ICV ($P < 0.001$). (Fig. 1*a–c*, Table 1).

Global GMV Associated with Altruistic Cooperativeness

A significant positive correlation between higher scores of C and larger relative total GMV was found in females (rho = 0.256, $P = 0.038$), whereas a homologous correlation was not found in males (rho = −0.131, $P = 0.222$). These correlations showed a significant sex difference ($Z = 2.37$, $P = 0.018$), indicating that the correlation was specific to females (Fig. 1*d*).

Sex Difference in Regional Brain Volumes

The VBM showed significantly larger regional GMV in females in several clusters including bilateral STG, IFG, insular cortices, occipitotemporal cortices, anterior cingulate cortices, thalamus, left parahippocampal gyrus, and medial and lateral PFC (FDR-corrected $P < 0.05$). In contrast, no voxel in the contrast showing regions larger in males than in females reached the statistical threshold, FDR-corrected $P < 0.05$. Small volume correction for multiple comparisons were then used for regions that had been predicted in advance, because previous studies have reported larger amygdala and cerebellum in males than in females (Rhyu et al. 1999; Good et al. 2001a; Goldstein et al. 2001). The regional GMVs in bilateral cerebellum and amygdala were significantly larger in males (Fig. 2 and Table 2).

Regional GMV Associated with C

In females, significant positive correlations between the score of C and regional GMV were found in 5 clusters (corrected $P < 0.05$) (Fig. 3 and Table 3). Furthermore, 3 of the 5 clusters showed significant sex and C interactions as well as significant correlation with C. Of note, all of the 3 clusters, including bilateral pIFG and left amPFC, showed a female > male sexual dimorphism as well as the female-specific correlation with C (Table 3). The regional GMV in left fusiform gyrus also showed a trend level significant female-specific correlation with C revealed by both the correlational and interaction analyses ($P = 0.072$), whereas the regional volume did not show any significant sex dimorphism. No regions showed a significantly negative correlation with scores of C in females. In contrast to the correlations in females, there was no suprathreshold gray matter voxel showing a correlation with C in males, even when a liberal threshold (uncorrected $P < 0.001$) was utilized. When both sexes combined, the correlation between C and regional GMV in right pIFG was found (peak coordinate = [54 8 8], *Z*-score = 3.90, uncorrected $P < 0.001$), although it did not remain statistically significant after correction for multiple comparisons (FDR-corrected $P = 0.209$).

The partial correlational analysis between C and regional GMVs remained statistically significant even after controlling for relative total GMV effect ($r > 0.335$, $P < 0.006$, df = 63). In contrast, the global correlations between C and relative total GMV did not reach the statistically significant level after controlling for regional GMV effects ($r = 0.232$, $P = 0.072$, df = 59).

The regression analyses showed positive regression coefficients for the all brain regions ($r^2 = 0.243$, $F_{5,60} = 3.85$, $P = 0.004$, coefficients = 8.9–20.8), suggesting that the each area might capture similar sources of individual differences.

Discussion

The crucial finding of the current study is that individual variability in altruistic cooperativeness showed significant

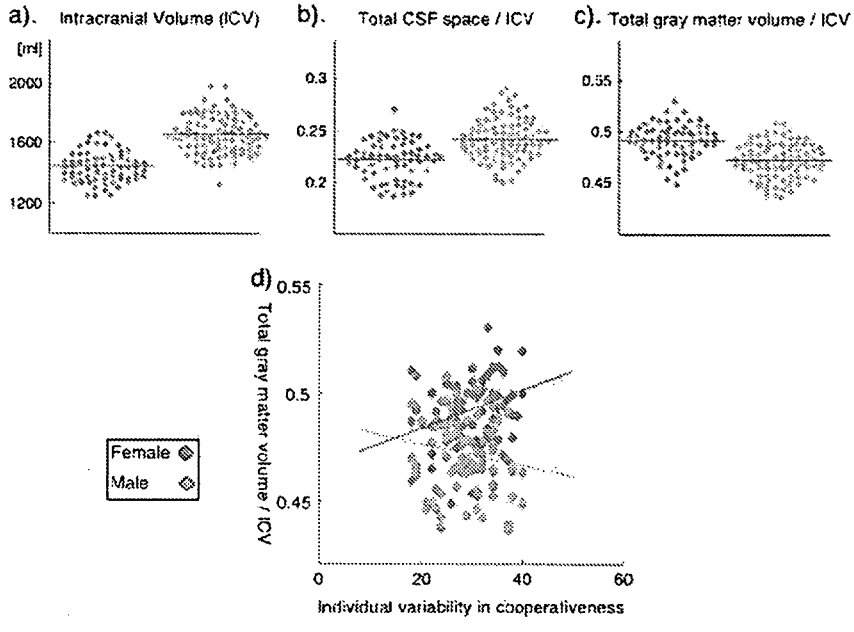

Figure 1. Sex dimorphism in global brain measures and *Cooperativeness*. Means and distributions of ICV (*a*) and total CSF space ratio to ICV (*b*) in which male participants exhibited a significantly large ratio compared to female participants ($P < 0.001$). In contrast, total gray matter ratio to ICV (*c*) is significantly larger in females ($P < 0.001$). Means are represented by solid horizontal lines drawn on each group's distribution. (*d*) Scatterplots depicting correlations between total GMV/ICV ratio and individual variability in cooperativeness in males and females. The sex difference of these correlations was statistically significant (Fisher's *r* to *z* transformation; $Z = 2.37$, $P = 0.018$).

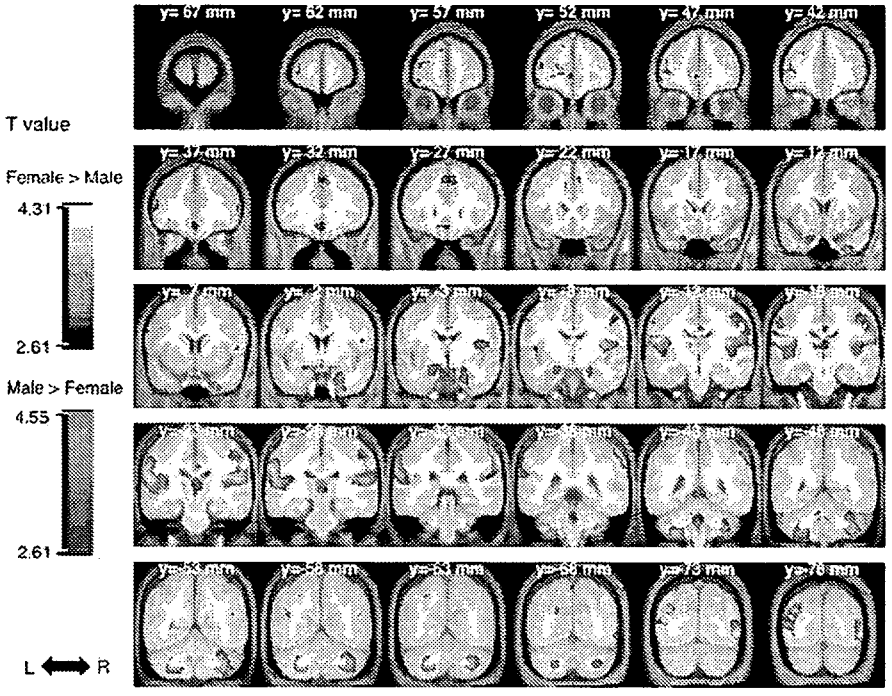

Figure 2. Sex dimorphism in regional GMV. The gray matter regions showing significant sex-dimorphism were rendered onto the averaged coronal images of the whole sample ($N = 155$) (Voxel threshold: uncorrected $P < 0.005$). The gray matter regions for which females are larger than males are presented in red ~ yellow. Conversely, the gray matter regions for which males are larger are presented in blue ~ green. The *y*-coordinate for each coronal slice in the Montreal Neurological Institute space is given in millimeters. L: left, R: right (see Table 2).

Table 2
Gender difference in regional brain volume (Fig. 2)

Anatomical location	Peak coordinate			*Z* score	FDR-corrected *P*	Cluster size (mm^3) (voxel threshold: $P < 0.001$)
	x	*y*	*z*			
Larger in female ($n = 155$)						
Left STG, IFG, and insula	−56	−20	16	4.04	0.03	9160
Left occipitotemporal cortex	−36	−86	−8	4.03	0.03	984
Right occipitotemporal cortex	48	−78	−4	3.95	0.03	3384
Right STG, IFG, and insula	52	−20	20	3.89	0.03	11616
Left lateral prefrontal cortex	−45	45	12	3.78	0.03	400
Left anterior medial prefrontal	−14	52	4	3.33	0.03	64
Anterior cingulate (pregenual)	−14	52	4	3.33	0.03	64
Bilateral thalamus	2	−22	10	3.3	0.03	128
Anterior cingulate (dorsal)	8	28	42	3.23	0.03	96
Larger in male ($n = 155$)						
Left cerebellar hemisphere	−22	−64	−44	4.4	< 0.001 (SV 30cc[a])	3664
Right cerebellar hemisphere	30	−62	−40	4.33	0.001 (SV 30cc[a])	4976
Right amygdala	22	0	−30	3.57	0.003 (SV 3cc[a])	2848
Left amygdala	−20	−4	−20	3.54	0.004 (SV 3cc[a])	392

Note: SV: searched volume.
[a]The definitions of searched volumes were based on previous studies.

female-specific correlations with total gray matter/ICV ratio (Fig. 1*d*) and more specifically with the regional GMV in bilateral pIFG and left amPFC (Figs 1 and 3 and Tables 3). In addition, the present study replicated findings of significantly larger global and regional brain volumes in all of these regions (Good et al. 2001a) (Figs 1 and 2 and Tables 1 and 2) and higher C in females (Farmer et al. 2003). Because the other subscale of TCI showed no significant global or regional correlations with the GMV, the relationships are thought to be specific to C. Thus, the present findings demonstrated the 1st evidence of sex-linked neuroanatomical background of human altruism.

The present study replicates previous findings of sexual dimorphism in brain structure. Although larger ICV as well as whole brain volume in males has been consistently reported (Filipek et al. 1994; Goldstein et al. 2001), larger total gray matter compositions, STG, cingulate, IFG, PFC, and thalamus in females and larger amygdala and cerebellum volumes in males have also been consistently reported when sex differences in

Table 3
Neural coorelates of Cooperativeness

Anatomical location	Peak coordinate			Z score	Correlation coefficient	FDR-corrected P	Cluster size (mm^3) (voxel threshold: $P < 0.001$)	Gender × Cooperativeness interaction[a] ($n = 155$)		Gender difference	
	x	y	z					Z score	P	Z score	P
Positive correlation in female ($n = 66$) (Figs 2 and 3)										Female > Male	
Right pIFG	54	6	10	4.36	0.512	0.037	11 144	2.33	0.01	2.57	0.005
Left amPFC	−16	58	8	3.89	0.464	0.037	1112	3.23	0.001	2.17	0.015
Right medial parietal cortex	10	−40	62	3.81	0.455	0.037	440	n.s.	n.s.	n.s.	n.s.
Left pIFG	−56	14	0	3.56	0.429	0.046	296	3.36	< 0.001	1.96	0.025
Right precentral gyrus	56	−2	44	3.56	0.428	0.046	1904	n.s.	n.s.	n.s.	n.s.
Left fusiform gyrus	−40	−22	−22	3.14	0.382	0.072	24	3.22	0.001	n.s.	n.s.

Note: Correlation in male ($n = 89$) and negative correlation in female ($n = 66$). No suprathreshold cluster. SV: searched volume.
[a]The interactions indicate significantly greater positive correlation in females.

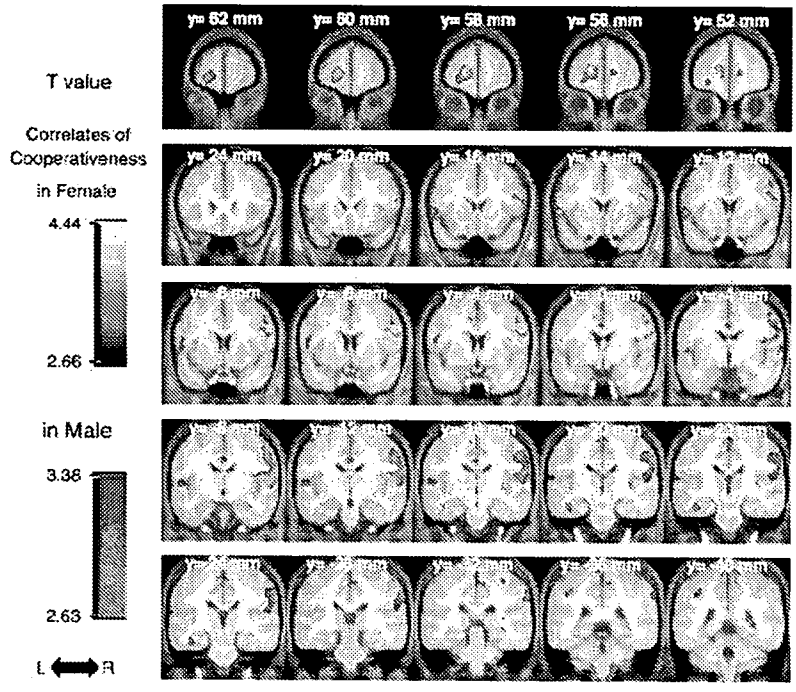

Figure 3. Localized neuroanatomical correlates of *Cooperativeness*. The gray matter regions showing correlations with the individual variability of *Cooperativeness* were rendered onto the averaged images of the whole sample ($N = 155$) (voxel threshold: uncorrected $P < 0.005$). The gray matter regions where the correlation was found in females are presented in red ~ yellow. Conversely, the gray matter regions where the correlation was found in males are presented in blue ~ green. The *y*-coordinate for each coronal slice in the Montreal Neurological Institute space is given in millimeters. L: left, R: right (see Table 3).

global brain measures is taken into account (Jacobs et al. 1993; Schlaepfer et al. 1995; Giedd et al. 1996; Murphy et al. 1996; Paus et al. 1996; Harasty et al. 1997; Gur et al. 1999; Nopoulos et al. 2000; Good et al. 2001a). The present findings are also consistent with the suggestion that greater gyrification in females implies more cortical surface area, which may offset sex differences in global brain size (Luders et al. 2004).

In accordance with the "social-brain hypothesis" (Dunbar 1998, 2003), the current study demonstrated that larger relative total GMV showed a significant correlation with higher C in females. Although there is some evidence for a link between neural measures and various lifestyles in mammals (Harvey and Krebs 1990; Joffe and Dunbar 1997; White and Byrne 1997; De Winter and Oxnard 2001; Byrne and Corp 2004), the present study provides the 1st human evidence supporting the social-brain hypothesis. The original social-brain hypothesis argued for the extreme development of the human brain compared with the other animals, however, the current study was conducted only with human individuals and consequently cannot conclude human superiority relative to other animals.

The voxel-by-voxel analysis further revealed localizations of neuroanatomical correlates of altruistic cooperativeness; larger regional volume in pIFG, and amPFC showed significant intensive correlations with higher altruistic cooperativeness. The location of peak coordinate in pIFG is close to those in previous studies showing neural correlates of observation and imitation of other's action (Nishitani et al. 2005), grasping intention of others (Iacoboni et al. 2005, Dapretto et al. 2006), and empathy (Carr et al. 2003; Adolphs 2003; Leslie et al. 2004). Evolution of cooperativeness occurs through social learning based on action observation and imitation of others, which

contributes to creating reciprocal altruism (Fehr and Fischbacher 2003). Therefore, it is reasonable to find neural substrates of altruistic cooperativeness in the regions known to be implicated in observation and imitation of others. Because previous studies have reported activations around the currently identified peak location of amPFC during tasks such as the attribution of emotion to self and others close to the self (Ochsner et al. 2004) and mentalizing tasks such as predicting the behavior of others (Harris et al. 2005), the region is thought to be one of the core areas for social cognition (Amodio and Frith 2006). The localization in neuroanatomical correlates of altruistic cooperativeness revealed by the present VBM is consistent with the previous functional neuroimaging studies overviewed above. In addition, the current correlations between C and neocortical regions are consistent with the "social-brain hypothesis" arguing that enlarged human neocortex volume reflects our social complexity.

Of note, all 3 clusters, including bilateral pIFG and left amPFC, demonstrating significant female-specific correlations with altruistic cooperativeness also revealed a significant female > male sex-dimorphism. The findings are in line with a previous functional imaging study, which showed greater left PFC including pIFG activation elicited by humor appreciation in females (Azim et al. 2005). A candidate for a biological mechanism shaping the sex-linked neuroanatomy–cooperativeness relationship is genetic factors, because previous studies have reported that a significant part of individual differences (e.g. 82% (33)) in GMV in healthy adults derives from genetic factors (Baare et al. 2001; Thompson et al. 2001; Geschwind et al. 2002; Wright et al. 2002). Therefore, the present findings suggest that genetic factors coding development of the social-brain influence altruistic cooperativeness more directly in females. Recent studies reported that a number of X-linked genes code mental development and social intelligence (Check 2005; Skuse 2005). Genes on sex chromosomes determine the sex of the brain in 2 ways: by acting on the gonads to induce sex differences in levels of gonadal secretions that have sex-specific effects on brain, and by acting in the brain itself to differentiate XX and XY brain cells (Arnold 2004). Thus, action of gonadal hormones has also been suggested as a key biological mechanism shaping sex differences in brain and mental development (Simerly 2002). Sexually dimorphic brain regions are rich in sex-hormone receptors, and their development may therefore be rather directly affected by sex hormones (Goldstein et al. 2001). Therefore, early gonadal-hormonal exposure interacting with neurotrophic factors (Carrer and Cambiasso 2002) might contribute to promoting a relationship between sexually dimorphic brain anatomy and sex difference in altruistic cooperativeness. Taking the above evidence into account, it seems likely that females are more likely to have more highly developed social-brain regions and altruistic cooperativeness as a phenotype of genetic factors such as X-linked genes coding gonadal secretions and social-brain development. Consistent with this notion, structural abnormalities related to X-chromosome abnormalities are found in PFC of patients with turner syndrome (Reiss et al. 1993; Good et al. 2003; Kesler et al. 2003; Molko et al. 2004; Rae et al. 2004). By contrast, it has also been suggested that current gene-based evolutionary theories cannot totally explain important patterns of human altruism, pointing to the importance of both theories of cultural evolution as well as gene-culture coevolution (Fehr and Fischbacher 2003). Therefore, altruistic cooperativeness in males, having only 1 X-chromosome, may be more likely to be associated with nongenetic factors, such as social learning and cultural evolution.

The present findings may also contribute to uncovering the neural background of autism, a pervasive developmental disorder characterized by severe social and interpersonal dysfunction, abnormalities in social-brain, and disproportionately low probability in females (Folstein and Rosen-Sheidley 2001). Baron-Cohen (2002) proposed the extreme-male brain theory of autism that the male brain is a defined psychometrically as those individuals in whom systemizing is performed significantly better than empathizing or friendship, and that the female brain is defined as the opposite cognitive profile (Brandstrom et al. 2001; Baron-Cohen 2002; Soderstrom et al. 2002; Baron-Cohen and Wheelwright 2003, 2004; Baron-Cohen et al. 2003, 2005; Farmer et al. 2003; Lawson et al. 2004). Using these definitions, autism can be considered as an extreme of the normal male profile. Recently, the hypothesis further suggests that specific aspects of autistic neuroanatomy may also be extremes of typical male neuroanatomy (Baron-Cohen et al. 2005). The current results are consistent with this hypothesis. Previous studies using structural MRI demonstrated smaller anterior cingulate (Haznedar et al. 1997, 2000), STG, PFC (Boddaert et al. 2004; De Fosse et al. 2004; Waiter et al. 2004; McAlonan et al. 2005; Yamasue et al. 2005; Hadjikhani et al. 2006), thalamus (Tsatsanis et al. 2003), pIFG (Hadjikhani et al. 2006) and enlarged amygdala, cerebellum (Howard et al. 2000; Sparks et al. 2002) in subjects with autism-spectrum disorders. The present study revealed sex-dimorphism in brain anatomy at a similar location and in the same direction as these previous studies of individuals with autism. Thus, the current study may add supportive evidence for Baron-Cohen's extreme-male brain theory of autism at the level of brain structure. In addition, the results from partial correlational analyses support for the case that the regional correlations exist beyond the global correlation. Although the current results are inconclusive, the regionally specific association might indicate specific extreme-male brain theory of autism against very general social brain hypothesis.

Here we address the methodological considerations and limitations of our study. First, the present study includes only Japanese participants. It is possible, however, that the pure ethnicity of the present sample might contribute to the clarity of findings. Because previous studies have reported significant ethnic differences in brain morphology (Zilles et al. 2001), future replication in other ethnicities is necessary to generalize the findings. Second, the present study employed a self-report questionnaire as an index for individual variability in altruistic cooperativeness. Although the validity of questionnaires to study biological aspects of personality has been demonstrated (Cloninger 1987), future studies should confirm the findings using other indices of altruism. Third, the present study participants, who were mainly college students, hospital staff, and their acquaintances, might not be representative of the average Japanese population. Therefore, the future study should replicate the present findings in the general population recruited in a different way such as advertisements.

The present study demonstrates that females had higher altruistic cooperativeness and larger global and regional GMV than males, and that these were tightly interrelated in females only. These regions included pIFG, and amPFC, participating in the social-brain regions and/or human mirror neuron system.

The findings at least partly support the "social-brain hypothesis" (Dunbar 1998, 2003) and suggest an important role of X-linked genes on social cognition (Skuse 2005). Moreover, the present results may also be consistent with the extreme-male brain theory of autism (Baron-Cohen 2002).

Funding

Japan Society for the Promotion of Science and the Ministry of Education, Culture, Sports, Science and Technology, Japan, grants-in-aid for scientific research (No. 16790675 and 17025015) to K.K. (No. 17-5234) to M.A.R.

Notes

Conflict of Interest: None declared.

Address correspondence to Hidenori Yamasue, MD, PhD, Department of Neuropsychiatry, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. Email: yamasue-tky@umin.ac.jp.

References

Adolphs R. 2003. Cognitive neuroscience of human social behaviour. Nat Rev Neurosci. 4:165-178.

American Psychiatric Association. 1994. Diagnostic and statistical manual of mental disorders. 4th ed. Washington (DC): American Psychiatric Press.

Amodio DM, Frith CD. 2006. Meeting of minds: the medial frontal cortex and social cognition. Nat Rev Neurosci. 7:268-277.

Anckarsater H, Stahlberg O, Larson T, Hakansson C, Jutblad SB, Niklasson L, Nyden A, Wentz E, Westergren S, Cloninger CR, et al. 2006. The impact of ADHD and autism spectrum disorders on temperament, character, and personality development. Am J Psychiatry. 163:1239-1244.

Ando J, Suzuki A, Yamagata S, Kijima N, Maekawa H, Ono Y, Jang KL. 2004. Genetic and environmental structure of Cloninger's temperament and character dimensions. J Personal Disord. 18:379-393.

Andreasen NC. 2005. Vulnerability to mental illnesses: gender makes a difference, and so does providing good psychiatric care. Am J Psychiatry. 162:211-213.

Arnold AP. 2004. Sex chromosomes and brain gender. Nat Rev Neurosci. 5:701-708.

Ashburner J, Friston KJ. 2000. Voxel-based morphometry—the methods. Neuroimage. 11:805-821.

Azim E, Mobbs D, Jo B, Menon V, Reiss AL. 2005. Sex differences in brain activation elicited by humor. Proc Natl Acad Sci USA. 102:16496-16501.

Baare WF, Hulshoff Pol HE, Boomsma DI, Posthuma D, de Geus EJ, Schnack HG, van Haren NE, van Oel CJ, Kahn RS. 2001. Quantitative genetic modeling of variation in human brain morphology. Cereb Cortex. 11:816-824.

Ball SA, Kranzler HR, Tennen H, Poling JC, Rounsaville BJ. 1998. Personality disorder and dimension differences between type A and type B substance abusers. J Personal Disord. 12:1-12.

Baron-Cohen S. 2002. The extreme male brain theory of autism. Trends Cogn Sci. 6:248-254.

Baron-Cohen S, Knickmeyer RC, Belmonte MK. 2005. Sex differences in the brain: implications for explaining autism. Science. 310:819-823.

Baron-Cohen S, Richler J, Bisarya D, Gurunathan N, Wheelwright S. 2003. The systemizing quotient: an investigation of adults with Asperger syndrome or high-functioning autism, and normal sex differences. Philos Trans R Soc Lond B Biol Sci. 358:361-374.

Baron-Cohen S, Wheelwright S. 2003. The Friendship Questionnaire: an investigation of adults with Asperger syndrome or high-functioning autism, and normal sex differences. J Autism Dev Disord. 33:509-517.

Baron-Cohen S, Wheelwright S. 2004. The empathy quotient: an investigation of adults with Asperger syndrome or high functioning autism, and normal sex differences. J Autism Dev Disord. 34:163-175.

Boddaert N, Chabane N, Gervais H, Good CD, Bourgeois M, Plumet MH, Barthelemy C, Mouren MC, Artiges E, Samson Y, et al. 2004. Superior temporal sulcus anatomical abnormalities in childhood autism: a voxel-based morphometry MRI study. Neuroimage. 23:364-369.

Brandstrom S, Richter J, Przybeck T. 2001. Distributions by age and sex of the dimensions of Temperament and Character Inventory in a cross-cultural perspective among Sweden, Germany, and the USA. Psychol Rep. 89:747-758.

Byrne RW, Corp N. 2004. Neocortex size predicts deception rate in primates. Proc Biol Sci. 271:1693-1699.

Carr L, Iacoboni M, Dubeau MC, Mazziotta JC, Lenzi GL. 2003. Neural mechanisms of empathy in humans: a relay from neural systems for imitation to limbic areas. Proc Natl Acad Sci USA. 100:5497-5502.

Carrer HF, Cambiasso MJ. 2002. Sexual differentiation of the brain: genes, estrogen, and neurotrophic factors. Cell Mol Neurobiol. 22:479-500.

Check E. 2005. Genetics: the X factor. Nature. 434:266-267.

Cloninger CR. 1987. A systematic method for clinical description and classification of personality variants. A proposal. Arch Gen Psychiatry. 44:573-588.

Cloninger CR, Svrakic DM, Przybeck TR. 1993. A psychobiological model of temperament and character. Arch Gen Psychiatry. 50:975-990.

Clutton-Brock TH, Russell AF, Sharpe LL, Young AJ, Mellrath GM. 2002. Evolution and development of sex differences in cooperative behavior in meerkats. Science. 297:253-256.

Dapretto M, Davies MS, Pfeifer JH, Scott AA, Sigman M, Bookheimer SY. 2006. Understanding emotions in others: mirror neuron dysfunction in children with autism spectrum disorders. Nat Neurosci. 9: 28-30.

De Fosse L, Hodge SM, Makris N, Kennedy DN, Caviness VS Jr, McGrath L, Steele S, Ziegler DA, Herbert MR, Frazier JA, et al. 2004. Language-association cortex asymmetry in autism and specific language impairment. Ann Neurol. 56:757-766.

De Winter W, Oxnard CE. 2001. Evolutionary radiations and convergences in the structural organization of mammalian brains. Nature. 409:710-714.

Decety J, Jackson PL, Sommerville JA, Chaminade T, Meltzoff AN. 2004. The neural bases of cooperation and competition: an fMRI investigation. Neuroimage. 23:744-751.

Dunbar RI. 1998. The social brain hypothesis. Evol Anthropol. 6:178-190.

Dunbar RI. 2003. Psychology. Evolution of the social brain. Science. 302:1160-1161.

Farmer A, Mahmood A, Redman K, Harris T, Sadler S, McGuffin P. 2003. A sib-pair study of the Temperament and Character Inventory scales in major depression. Arch Gen Psychiatry. 60:490-496.

Fehr E, Fischbacher U. 2003. The nature of human altruism. Nature. 425:785-791.

Filipek PA, Richelme C, Kennedy DN, Caviness VS Jr. 1994. The young adult human brain: an MRI-based morphometric analysis. Cereb Cortex. 4:344-360.

First MB, Spitzer RL, Gibbon M, Williams JBW. 1997. Structured clinical interview for DSM-IV axis I disorders, non-patient ed. New York: Biometrics Research Department, New York State Psychiatric Institute.

Folstein SE, Rosen-Sheidley B. 2001. Genetics of autism: complex aetiology for a heterogeneous disorder. Nat Rev Genet. 2:943-955.

Friston KJ, Frith CD, Liddle PF, Dolan RJ, Lammertsma AA, Frackowiak RS. 1990. The relationship between global and local changes in PET scans. J Cereb Blood Flow Metab. 10:458-466.

Friston KJ, Holmes AP, Worsley KJ, Poline JB, Frith CD, Frackowiak RS. 1995. Statistical parametric maps in functional imaging: a general linear approach. Hum Brain Mapp. 2:189-210.

Genovese CR, Lazar NA, Nichols T. 2002. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. Neuroimage. 15:870-888.

Geschwind DH, Miller BL, DeCarli C, Carmelli D. 2002. Heritability of lobar brain volumes in twins supports genetic models of cerebral laterality and handedness. Proc Natl Acad Sci USA. 99:3176-3181.

Giedd JN, Snell JW, Lange N, Rajapakse JC, Casey BJ, Kozuch PL, Vaituzis AC, Vauss YC, Hamburger SD, Kaysen D, et al. 1996.

Quantitative magnetic resonance imaging of human brain development: ages 4–18. Cereb Cortex. 6:551–560.
Gintis H, Bowles S, Boyd R, Fehr E. 2003. Explaining altruistic behavior in humans. Evol Hum Behav. 24:153–172.
Goldstein JM, Seidman LJ, Horton NJ, Makris N, Kennedy DN, Caviness VS, Jr, Faraone SV, Tsuang MT. 2001. Normal sexual dimorphism of the adult human brain assessed by in vivo magnetic resonance imaging. Cereb Cortex. 11:490–497.
Good CD, Johnsrude I, Ashburner J, Henson RN, Friston KJ, Frackowiak RS. 2001a. Cerebral asymmetry and the effects of sex and handedness on brain structure: a voxel-based morphometric analysis of 465 normal adult human brains. Neuroimage. 14:685–700.
Good CD, Johnsrude IS, Ashburner J, Henson RN, Friston KJ, Frackowiak RS. 2001b. A voxel-based morphometric study of ageing in 465 normal adult human brains. Neuroimage. 14:21–36.
Good CD, Lawrence K, Thomas NS, Price CJ, Ashburner J, Friston KJ, Frackowiak RS, Oreland L, Skuse DH. 2003. Dosage-sensitive X-linked locus influences the development of amygdala and orbitofrontal cortex, and fear recognition in humans. Brain. 126:2431–2446.
Gur RC, Turetsky BI, Matsui M, Yan M, Bilker W, Hughett P, Gur RE. 1999. Sex differences in brain gray and white matter in healthy young adults: correlations with cognitive performance. J Neurosci. 19:4065–4072.
Hadjikhani N, Joseph RM, Snyder J, Tager-Flusberg H. 2006. Anatomical differences in the mirror neuron system and social cognition network in autism. Cereb Cortex. 16:1276–1282.
Harasty J, Double KL, Halliday GM, Kril JJ, McRitchie DA. 1997. Language-associated cortical regions are proportionally larger in the female brain. Arch Neurol. 54:171–176.
Harbaugh WT, Mayr U, Burghart DR. 2007. Neural responses to taxation and voluntary giving reveal motives for charitable donations. Science. 316:1622–1625.
Harris LT, Todorov A, Fiske ST. 2005. Attributions on the brain: neuroimaging dispositional inferences, beyond theory of mind. Neuroimage. 28:763–769.
Harvey PH, Krebs JR. 1990. Comparing brains. Science. 249:140–146.
Haznedar MM, Buchsbaum MS, Metzger M, Solimando A, Spiegel-Cohen J, Hollander E. 1997. Anterior cingulate gyrus volume and glucose metabolism in autistic disorder. Am J Psychiatry. 154:1047–1050.
Haznedar MM, Buchsbaum MS, Wei TC, Hof PR, Cartwright C, Bienstock CA, Hollander E. 2000. Limbic circuitry in patients with autism spectrum disorders studied with positron emission tomography and magnetic resonance imaging. Am J Psychiatry. 157:1994–2001.
Hollingshead AB. 1965. Two-factor index of social position. New Haven: Yale University Press.
Howard MA, Cowell PE, Boucher J, Broks P, Mayes A, Farrant A, Roberts N. 2000. Convergent neuroanatomical and behavioural evidence of an amygdala hypothesis of autism. Neuroreport. 11:2931–2935.
Iacoboni M, Molnar-Szakacs I, Gallese V, Buccino G, Mazziotta JC, Rizzolatti G. 2005. Grasping the intentions of others with one's own mirror neuron system. PLoS Biol. 3:e79.
Insel TR. 2006. From species differences to individual differences. Mol Psychiatry. 11:424.
Jacobs B, Schall M, Scheibel AB. 1993. A quantitative dendritic analysis of Wernicke's area in humans. II. Gender, hemispheric, and environmental factors. J Comp Neurol. 327:97–111.
Joffe TH, Dunbar RI. 1997. Visual and socio-cognitive information processing in primate brain evolution. Proc Biol Sci. 264:1303–1307.
Kesler SR, Blasey CM, Brown WE, Yankowitz J, Zeng SM, Bender BG, Reiss AL. 2003. Effects of X-monosomy and X-linked imprinting on superior temporal gyrus morphology in Turner syndrome. Biol Psychiatry. 54:636–646.
Kijima N, Tanaka E, Suzuki N, Higuchi H, Kitamura T. 2000. Reliability and validity of the Japanese version of the Temperament and Character Inventory. Psychol Rep. 86:1050–1058.
Lawson J, Baron-Cohen S, Wheelwright S. 2004. Empathising and systemising in adults with and without Asperger Syndrome. J Autism Dev Disord. 34:301–310.
Leibenluft E, Gobbini MI, Harrison T, Haxby JV. 2004. Mothers' neural activation in response to pictures of their children and other children. Biol Psychiatry. 56:225–232.
Leslie KR, Johnson-Frey SH, Grafton ST. 2004. Functional imaging of face and hand imitation: towards a motor theory of empathy. Neuroimage. 21:601–607.
Luders E, Narr KL, Thompson PM, Rex DE, Jancke L, Steinmetz H, Toga AW. 2004. Gender differences in cortical complexity. Nat Neurosci. 7:799–800.
McAlonan GM, Cheung V, Cheung C, Suckling J, Lam GY, Tai KS, Yip L, Murphy DG, Chua SE. 2005. Mapping the brain in autism. A voxel-based MRI study of volumetric differences and intercorrelations in autism. Brain. 128:268–276.
McCabe K, Houser D, Ryan L, Smith V, Trouard T. 2001. A functional imaging study of cooperation in two-person reciprocal exchange. Proc Natl Acad Sci USA. 98:11832–11835.
Molko N, Cachia A, Riviere D, Mangin JF, Bruandet M, LeBihan D, Cohen L, Dehaene S. 2004. Brain anatomy in Turner syndrome: evidence for impaired social and spatial-numerical networks. Cereb Cortex. 14:840–850.
Moll J, Krueger F, Zahn R, Pardini M, de Oliveira-Souza R, Grafman J. 2006. Human fronto-mesolimbic networks guide decisions about charitable donation. Proc Natl Acad Sci USA. 103:15623–15628.
Murphy DG, DeCarli C, McIntosh AR, Daly E, Mentis MJ, Pietrini P, Szczepanik J, Schapiro MB, Grady CL, Horwitz B, et al. 1996. Sex differences in human brain morphometry and metabolism: an in vivo quantitative magnetic resonance imaging and positron emission tomography study on the effect of aging. Arch Gen Psychiatry. 53:585–594.
Nishitani N, Schurmann M, Amunts K, Hari R. 2005. Broca's region: from action to language. Physiology. 20:60–69.
Nopoulos P, Flaum M, O'Leary D, Andreasen NC. 2000. Sexual dimorphism in the human brain: evaluation of tissue volume, tissue composition and surface anatomy using magnetic resonance imaging. Psychiatry Res. 98:1–13.
Nowak MA, Sigmund K. 2005. Evolution of indirect reciprocity. Nature. 437:1291–1298.
Ochsner KN, Knierim K, Ludlow DH, Hanelin J, Ramachandran T, Glover G, Mackey SC. 2004. Reflecting upon feelings: an fMRI study of neural systems supporting the attribution of emotion to self and other. J Cogn Neurosci. 16:1746–1772.
Oldfield RC. 1971. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia. 9:97–113.
Paus T, Otaky N, Caramanos Z, MacDonald D, Zijdenbos A, D'Avirro D, Gutmans D, Holmes C, Tomaiuolo F, Evans AC. 1996. In vivo morphometry of the intrasulcal gray matter in the human cingulate, paracingulate, and superior-rostral sulci: hemispheric asymmetries, gender differences and probability maps. J Comp Neurol. 376:664–673.
Pennisi E. 2005. How did cooperative behavior evolve? Science. 309:93.
Platek SM, Keenan JP, Mohamed FB. 2005. Sex differences in the neural correlates of child facial resemblance: an event-related fMRI study. Neuroimage. 25:1336–1344.
Rae C, Joy P, Harasty J, Kemp A, Kuan S, Christodoulou J, Cowell CT, Coltheart M. 2004. Enlarged temporal lobes in Turner syndrome: an X-chromosome effect? Cereb Cortex. 14:156–164.
Rapoport A, Chammah AM. 1965. Sex differences in factors contributing to the level of cooperation in the prisoner's dilemma game. J Pers Soc Psychol. 2:831–838.
Reader SM, Laland KN. 2002. Social intelligence, innovation, and enhanced brain size in primates. Proc Natl Acad Sci USA. 99:4436–4441.
Reiss AL, Freund L, Plotnick L, Baumgardner T, Green K, Sozer AC, Reader M, Bochm C, Denckla MB. 1993. The effects of X monosomy on brain development: monozygotic twins discordant for Turner's syndrome. Ann Neurol. 34:95–107.
Rhyu IJ, Cho TH, Lee NJ, Uhm CS, Kim H, Suh YS. 1999. Magnetic resonance image-based cerebellar volumetry in healthy Korean adults. Neurosci Lett. 270:149–152.
Rilling J, Gutman D, Zeh T, Pagnoni G, Berns G, Kilts C. 2002. A neural basis for social cooperation. Neuron. 35:395–405.

Ross MT, Grafham DV, Coffey AJ, Scherer S, McLay K, Muzny D, Platzer M, Howell GR, Burraus C, Bird CP, et al. 2005. The DNA sequence of the human X chromosome. Nature. 434:325-337.

Schlaepfer TE, Harris GJ, Tien AY, Peng L, Lee S, Pearlson GD. 1995. Structural differences in the cerebral cortex of healthy female and male subjects: a magnetic resonance imaging study. Psychiatry Res. 61:129-135.

Silk JB, Brosnan SF, Vonk J, Henrich J, Povinelli DJ, Richardson AS, Lambeth SP, Mascaro J, Schapiro SJ. 2005. Chimpanzees are indifferent to the welfare of unrelated group members. Nature. 437:1357-1359.

Simerly RB. 2002. Wired for reproduction: organization and development of sexually dimorphic circuits in the mammalian forebrain. Annu Rev Neurosci. 25:507-536.

Singer T, Kiebel SJ, Winston JS, Dolan RJ, Frith CD. 2004. Brain responses to the acquired moral status of faces. Neuron. 41:653-662.

Singer T, Seymour B, O'Doherty JP, Stephan KE, Dolan RJ, Frith CD. 2006. Empathic neural responses are modulated by the perceived fairness of others. Nature. 439:466-469.

Skuse DH. 2005. X-linked genes and mental functioning. Hum Mol Genet. 14:R27-32.

Soderstrom H, Rastam M, Gillberg C. 2002. Temperament and character in adults with Asperger syndrome. Autism. 6:287-297.

Sparks BF, Friedman SD, Shaw DW, Aylward EH, Echelard D, Artru AA, Maravilla KR, Giedd JN, Munson J, Dawson G, et al. 2002. Brain structural abnormalities in young children with autism spectrum disorder. Neurology. 59:184-192.

Takeuchi M, Yoshino A, Kato M, Ono Y, Kitamura T. 1993. Reliability and validity of the Japanese version of the Tridimensional Personality Questionnaire among university students. Compr Psychiatry. 34:273-279.

Tankersley D, Stowe CJ, Huettel SA. 2007. Altruism is associated with an increased neural response to agency. Nat Neurosci. 10:150-151.

Thompson PM, Cannon TD, Narr KL, van Erp T, Poutanen VP, Huttunen M, Lonnqvist J, Standertskjold-Nordenstam CG, Kaprio J, Khaledy M, et al. 2001. Genetic influences on brain structure. Nat Neurosci. 4:1253-1258.

Tremblay RE, Pihl RO, Vitaro F, Dobkin PL. 1994. Predicting early onset of male antisocial behavior from preschool behavior. Arch Gen Psychiatry. 51:732-739.

Tsatsanis KD, Rourke BP, Klin A, Volkmar FR, Cicchetti D, Schultz RT. 2003. Reduced thalamic volume in high-functioning individuals with autism. Biol Psychiatry. 53:121-129.

Waiter GD, Williams JH, Murray AD, Gilchrist A, Perrett DI, Whiten A. 2004. A voxel-based investigation of brain structure in male adolescents with autistic spectrum disorder. Neuroimage. 22: 619-625.

White A, Byrne RW. 1997. Machiavellian Intelligence II: extensions and evaluations. Cambridge: Cambridge University Press.

Wright IC, Sham P, Murray RM, Weinberger DR, Bullmore ET. 2002. Genetic contributions to regional variability in human brain structure: methods and preliminary results. Neuroimage. 17:256-271.

Yamasue H, Abe O, Kasai K, Suga M, Iwanami A, Yamada H, Tochigi M, Ohtani T, Rogers MA, Sasaki T, et al. 2007. Human brain structural change related to acute single exposure to sarin. Ann Neurol. 61:37-46.

Yamasue H, Ishijima M, Abe O, Sasaki T, Yamada H, Suga M, Rogers M, Minowa I, Someya R, Kurita H, et al. 2005. Neuroanatomy in monozygotic twins with Asperger disorder discordant for comorbid depression. Neurology. 65:491-492.

Yamasue H, Kasai K, Iwanami A, Ohtani T, Yamada H, Abe O, Kuroki N, Fukuda R, Tochigi M, Furukawa S, et al. 2003. Voxel-based analysis of MRI reveals anterior cingulate gray-matter volume reduction in posttraumatic stress disorder due to terrorism. Proc Natl Acad Sci USA. 100:9039-9043.

Zilles K, Kawashima R, Dabringhaus A, Fukuda H, Schormann T. 2001. Hemispheric shape of European and Japanese brains: 3-D MRI analysis of intersubject variability, ethnical, and gender differences. Neuroimage. 13:262-271.

ELSEVIER

Brain & Development xxx (2007) xxx–xxx

BRAIN & DEVELOPMENT
Official Journal of the Japanese Society of Child Neurology

www.elsevier.com/locate/braindev

Case report

Mental retardation, spasticity, basal ganglia calcification, cerebral white matter lesions, multiple endocrine defects, telangiectasia and atrophic skin: A new syndrome?

Yoshiaki Saito [a,*], Mitsuo Toyoshima [a], Akira Oka [a], Luan Zhuo [a], Shin-ichi Moriwaki [b], Osamu Yamamoto [c], Susumu Kanzaki [d], Kei-ichi Hanaki [d], Haruaki Ninomiya [e], Eiji Nanba [f], Akiko Kondo [a], Yoshihiro Maegaki [a], Kousaku Ohno [a]

[a] *Division of Child Neurology, Institute of Neurological Sciences, Faculty of Medicine, Tottori University, 36-1 Nishi-cho, Yonago 683-8504, Japan*
[b] *Department of Dermatology, Osaka Medical College, 2-7 Daigaku-cho, Takatsuki 569-8686, Japan*
[c] *Division of Dermatology, Faculty of Medicine, Tottori University, 36-1 Nishi-cho, Yonago 683-8504, Japan*
[d] *Division of Pediatrics and Perinatology, Faculty of Medicine, Tottori University, 36-1 Nishi-cho, Yonago 683-8504, Japan*
[e] *Department of Neurobiology, Faculty of Medicine, Tottori University, 36-1 Nishi-cho, Yonago 683-8504, Japan*
[f] *Division of Functional Genomics, Research Center for Bioscience and Technology, Tottori University, 36-1 Nishi-cho, Yonago 683-8504, Japan*

Received 1 May 2007; received in revised form 2 August 2007; accepted 7 August 2007

Abstract

We report on an 8-year-old boy with mental retardation and spastic tetraparesis associated with atrophic skin on the face and extremities, telangiectasia, and severe dental caries. Basal ganglia calcification and multiple lesions in the subcortical white matter have been present since infancy. The patient has complications of liver dysfunction, multiple endocrine defects, and elevation of blood/cerebrospinal fluid lactate. Extensive laboratory examinations, including skin and muscle biopsies, and UV- and mitomycin C-sensitivity tests on fibroblasts, provided no evidence of a specific disease entity. No deterioration was noted, and supplementation of riboflavin and other vitamins had no apparent effect on the neurodevelopmental status of this patient. This patient may represent a novel disease entity, with unclear pathogenesis.

Keywords: Neurocutaneous syndrome; DNA repair; Riboflavin; Cockayne syndrome; Rothmund-Thomson syndrome

1. Introduction

Cutaneous lesions accompany neurological symptoms in various conditions. These include hereditary metabolic disorders, nutritional deficiencies, autoimmune diseases, neurocutaneous syndromes, and disorders of DNA repair. Here, we report a boy with unusual presentation of neurological symptoms, multiple endocrine defects, and skin lesions at sites exposed to sunlight. Since the manifestation of this patient was similar to the neurological symptoms in the Cockayne syndrome and the cutaneous symptoms of the Rothmund–Thomson syndrome, differential diagnoses are discussed with a focus on the disorders of DNA repair.

2. Case report

The patient was born uneventfully to non-consanguineous parents at 39 gestational weeks. His family history was unremarkable. He had a birth weight of 2264 g (−2.5 SD), body length of 46 cm (−2.0 SD), and OFD of 32 cm (−1.0 SD). He was referred to our clinic at

* Corresponding author. Tel.: +81 859 38 6777; fax: +81 859 38 6779.
E-mail address: saitoyo@grape.med.tottori-u.ac.jp (Y. Saito).

0387-7604/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.braindev.2007.08.004

Please cite this article in press as: Saito Y et al., Mental retardation, spasticity, basal ganglia calcification, ..., Brain Dev (2007), doi:10.1016/j.braindev.2007.08.004

age 9 months due to mild psychomotor developmental delay. His nose was small with anteverted nostrils, and his skin was firm in texture. White coloring changes were also noted on the tip of his nose, cheeks, and ears. There was no hepatosplenomegaly. Mild hypertonia was noted in the lower extremities. Ophthalmological examination was unremarkable. Brain CT revealed small, high-density puncta in the bilateral putamen (Fig. 1A). Biochemical analysis revealed a mild elevation of liver enzymes and thyroid-stimulating hormone, but normal serum parathyroid hormone and calcium levels. The patient suffered from febrile convulsions at age 11 months, when MRI revealed multiple high-signal lesions in the cerebral white matter bilaterally, over the frontal, temporal, and parietal areas (Fig. 1B and C), and cerebrospinal fluid (CSF) levels of lactate (24.9 mg/dl) and pyruvate (1.65 mg/dl) were elevated. A metabolic disorder was suspected, prompting the following diagnostic tests: blood and urine amino acids, urine organic acids, lymphocyte PDHC enzymatic activity, plasma very long chain fatty acids, lymphocyte lysosomal enzyme activities including arylsulfatase A, and α-B crystalline in the CSF. All tests were within the normal range. There were no signs of skeletal dysplasia on a bone survey. Serum and CSF titers for viruses with a potential for congenital infection were unremarkable other than positive serum IgG against cytomegalovirus. Muscle biopsy revealed type 2 fiber atrophy, but without ragged red fibers or abnormal cytochrome C oxidase staining. Respiratory chain enzyme activities in muscle tissue were normal. Mitochondrial DNA analysis showed negative results for common mutations at amino acids 3243, 8344, and six other residues. G-banding chromosomal analysis showed a normal male karyotype. We further examined blood levels of vitamins related to mitochondrial energy production, and found a mildly decreased level of vitamin B2 (flavin adenine dinucleotide (FAD) 38.4 ng/ml, normal 43–75; flavin mononucleotide (FMN) 0.6 ng/ml, normal 1–10). Glutathione reductase in red blood cells showed an activity coefficient of 1.12 (normal <1.2). Although this result did not support vitamin B2 deficiency in the tissues, we still administrated this and other vitamins in the hope of ameliorating symptoms of a suspected progressive disease. Thereafter, the patient gained the ability to walk without assistance at three years. He could utter meaningful words at age 17 months, and speak in sentences at 2 6/12 years. Administration of vitamins was cautiously tapered by 8 years age, after observation of neurodevelopmental progress without deterioration.

The hypothyroidism was treated with levothyroxin sodium from the age 2 6/12 years. By age 3 years, dry skin and mild depigmentation on the cheeks appeared (Fig. 2A). Darkening of the skin was noted at the periphery of fingers, while erythema and telangiectasia with diameters of 1–2 mm appeared on the fingers, palms, soles, and ears (Fig. 2B). A small penis and testes was noted at this time. The patient had a short stature of −3.5 SD by the age 4 years, when growth hormone therapy was initiated. Bone age was estimated as 1 6/12 years when he was four years old. Anterior lobe of the pituitary gland was mildly hypoplastic on T1-weighted MR imaging. Follicle-stimulating hormone was also elevated, and the patient showed a hyperresponse to the lutenizing hormone-releasing hormone loading test.

Vessels on the conjunctiva became prominent at age 6 years (Fig. 2C) and sporadic photosensitivity appeared after exposure to sunlight at 7 years. At this age, we also observed telangiectasia on the hands, lower legs, and soles, skin atrophy with mottled depigmentation and pigmentation with telangiectasia over the cheeks, lichenoid changes on the dorsum of hands, tapering of the fingers (Fig. 2B), white nailbeds, and dry skin with follicular keratosis over the forearm. Severe dental caries was also present. A skin biopsy revealed mild vascular dilatation at the dermis. Suspecting DNA repair deficiency, we performed the following tests: (1) a UVB-exposure test on the chest skin, (2) colony-formation or cell-growth assay

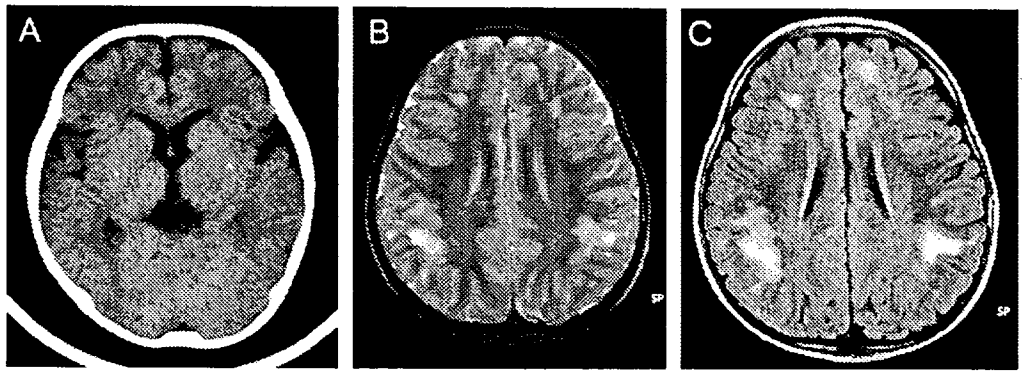

Fig. 1. Neuroimaging. (A) Brain CT at age 9 months. (B and C) Magnetic resonance imaging at age 4 years (B, T2-weighted image; C, fluid-attenuated inversion recovery image). Multiple high-signal lesions with vague contour distribute in the subcortical, and adjacent deep white matter bilaterally, at the frontal, temporal and parietal areas. No cortical lesion has been identified on neuroimaging.

Please cite this article in press as: Saito Y et al., Mental retardation, spasticity, basal ganglia calcification, ..., Brain Dev (2007), doi:10.1016/j.braindev.2007.08.004

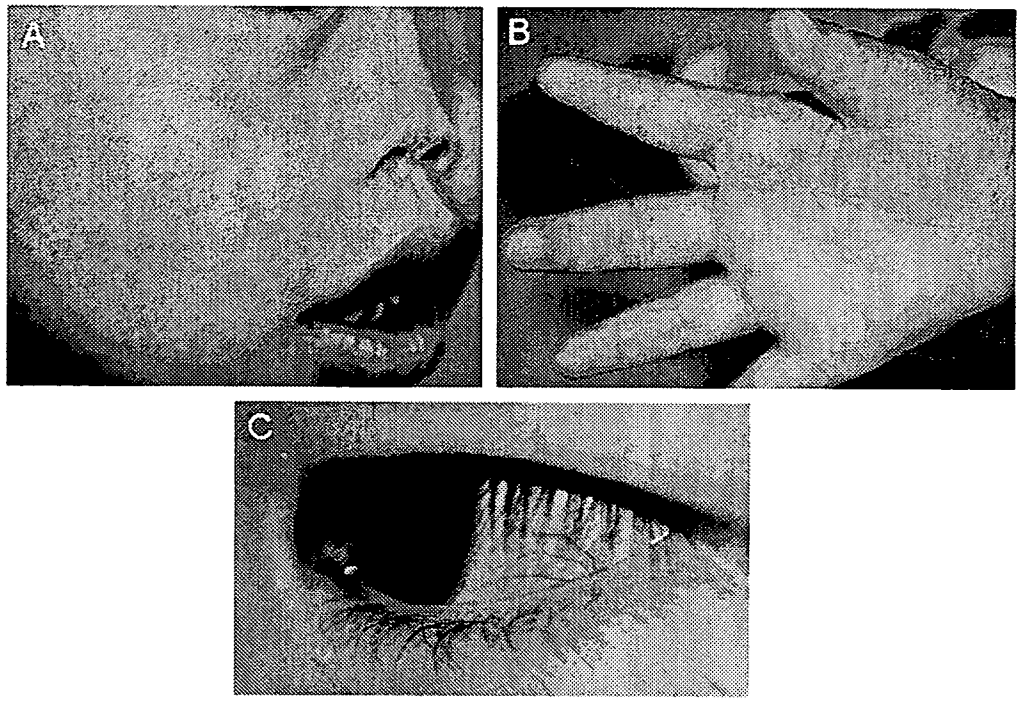

Fig. 2. Cutaneous findings of the patient at the age of seven years. (A) Depigmentation with atrophic changes on the cheek. White-colored changes on the tip of the nose, and the presence of caries can be seen. (B) Short, tapering fingers are dark-colored at the periphery. Small telangiectasia are scattered on the palm and fingers. (C) Prominent vessels on the conjunctiva. Permission for the presentation of these pictures was obtained from the parents of the patient.

of fibroblasts after UV irradiation [1] and mitomycin-C treatment [2], (3) unscheduled DNA synthesis after UV irradiation [1], (4) DNA repair ability of the fibroblasts, estimated by plasmid host cell reactivation assay using UV-irradiated luciferase expression vector, and (5) mutation analysis of CSA and CSB genes in white blood cells. However, these tests yielded normal results, and there have been no further episodes suggesting the presence of immunodeficiency. White blood cell count, lymphocyte subsets, immunoglobulin M, G and A, α-fetoprotein, and antinuclear antibody, were all normal.

The intelligence quotient of this patient was estimated as 60 at 7 years. Spasticity of all extremities persisted, but has not worsened. There is no amyotrophy or attenuation of deep tendon reflex. Although no ataxia or tremor is present in the upper extremities, his gait is wide-based. Follow-up brain CT and MRI at age two and four years showed the same abnormalities revealed during infancy, with no evidence of progressive brain atrophy and no change in the distribution or extent of white matter lesions. Electrophysiological tests at 1, 2, 4, and 7 years of age showed normal findings in electroencephalography, brainstem auditory evoked potentials, and flash-visual evoked potentials. Motor conduction velocity of median nerve has developed from 37.7 m/s (−3.2 SD; amplitude 4.3 mV) at age 1 year to 49.8 m/s (−3.6 SD; amplitude 5.7 mV) at 7 years. Sensory nerve conduction velocity of median nerve between the wrist and elbow was 31.2 m/s (−1.7 SD; amplitude 17 μV) at 1 year, and 44.7 m/s (−1.4SD; amplitude 22 μV) at 7 years of age. Short-latency somatosensory evoked potentials the age of 4 years showed delayed latency of N9 (5.85 ms, +3.5 SD) and N11 (7.86 ms, +5.0SD), but the latency of N20 (17.19 ms, −0.65SD) was normal.

3. Discussion

Despite the extensive examinations to identify his primary disorder, none of the data was consistent with a specific disease. Congenital cytomegalovirus infection can cause basal ganglia calcification and non-progressive white matter lesions, but it cannot explain the emergence of cutaneous lesions, peripheral hypothyroidism/hypogonadism, and elevated CSF lactate. Electrophysiological and laboratory investigations have not shown findings suggestive of demyelinating disorders of the central nervous system. Riboflavin deficiency can cause cutaneous lesions including facial dermatitis and conjunctival changes [3], and can involve the central and peripheral nervous systems [4]. However, the normal glutathione reductase levels in red blood cells weaken the possibility of riboflavin dysfunction in the present patient. We concluded that vitamin deficiency was not the primary, critical defect in the present patient, after the on–off trial of certain vitamins.

Please cite this article in press as: Saito Y et al., Mental retardation, spasticity, basal ganglia calcification, ... Brain Dev (2007), doi:10.1016/j.braindev.2007.08.004

Disorders of DNA repair deficits, including the Cockayne syndrome (CS) [5,6] and the Rothmund–Thomson syndrome (RTS) [7], are complicated by sunlight sensitivity, psychomotor retardation, growth failure, hypogonadism, and dental caries. Spastic tetraplegia, basal ganglia calcification, white matter lesions, demyelination of peripheral nerves, and possibly an elevated brain lactate [8], also accompany CS. Meanwhile, atrophic skin change with pigmentation and depigmentation on the face and extremities, short fingers, and hypothyroidism can be observed in RTS. However, normal sensitivity of fibroblasts to UV irradiation and mitomycin C are inconsistent to these diagnoses [1,6,7]. The diagnosis of CS was further ruled out by normal results of the normal host cell reactivation assay and the mutation analysis of CSA and CSB genes. Sparse hair and eyebrows, and erythematous lesions before development of skin atrophy, both typical in the RTS, were not present in the present patient. There are rare cases of poikiloderma with neurological symptoms [9], but the presence of brain lesions and lack of bullae are not consistent with these conditions. Xeroderma pigmentosum (XP) is a disorder of DNA repair with neurological deterioration. However, the neuroimaging shows non-specific atrophy in this disorder, and the cutaneous lesions are characterized by the presence of freckles, dry and scaling skin, and ocular involvement with keratitis/conjunctivitis [10]. Decreased unscheduled DNA synthesis and cell growth after UV irradiation is obligatory for this diagnosis. Dyskeratosis congenita, a hereditary disorder with defects in the maintenance of chromosomal structure, can be associated with intracerebral calcification [11]. However, the present patient did not show any of the mucocutaneous symptoms in this disorder (Table 1). The neurological and the endocrine problems do not fit to this entity. Prominent conjunctival vessels were

Table 1
Clinical features of the patient and differential diagnosis of disorders with DNA repair deficits

	Present patient	Cockayne syndrome [5,6]	Rothmund–Thomson syndrome [7]	Dyskeratosis congenita [11]	Xeroderma pigmentosum [10,12]	Ataxia–telangiectasia [13]
Cutaneous						
Atrophic skin with pigmentation/depigmentation	+	−	+*1	+*1	+*1	−
Photosensitivity	+*2	+	+	−	+	−
Dental caries	+	+	+	−	+	−
Telangiectasia	+	+	+	+	+	+
Blistering	−	−	+ (Occasional)	−	+	−
Hyperpigmentation	−	−	+ (Reticular)	+ (Reticular)	+ (Freckles)	−
Nail dystrophy	−	−	−	+	−	−
Leukoplakia	−	−	−	+	−	−
Xerosis and scaling	−	−	−	−	+	−
Café-au-lait spots	−	−	−	−	−	+
Atopic/seborrheic dermatitis	−	−	−	−	−	+
Fibroblast UV sensitivity	−	+	−	NE	+	+ (NE)
Mitomycin-C sensitivity	−	−	+ (NE)	−	+ (XPA, XPE, XPF)	NE
X-irradiation sensitivity	Not examined	+ (NE)	NE	−	+ (NE)	+
Neurological						
Mental retardation	+	+	+ (Rare)	+ (Rare)	+ (XPA, XPD)	−
Convulsion	+	+	−	−	+	−
Intracranial calcification	+	+	−	+ (Rare)	−	−
Cerebral white matter lesions	+	+	−	−	−	+ (Rare)
Spastic tetraplegia	+	+	−	−	+	−
Peripheral neuropathy	+	+	−	+ (Rare)	+	+
Intellectual/motor deterioration	−	+	−	−	+ (XPA, XPD)	+
Endocrinological						
Short stature	+	+	+	−	+	+
Hypothyroidism	+	+	−	−	+ (Rare)	+ (Rare)
Hypogonadism	+	+	+	+	+	+
Ophthalmological						
Cataract	−	+	+	+	−	−
Retinopathy	−	+	−	−	−	−
Conjunctivitis	−	−	−	−	+	−

*1 Atrophic skin on the face and extremities in Rothmund–Thomson syndrome and Xeroderma pigmentosum, and on the trunk and neck in Dyskeratosis congenita.
*2 Transiently appeared at 6 years of age.
NE: not established.

Please cite this article in press as: Saito Y et al., Mental retardation, spasticity, basal ganglia calcification, ..., Brain Dev (2007), doi:10.1016/j.braindev.2007.08.004

reminiscent of the telangiectasia in ataxia–telangiectasia (AT), but they were not dilated as in AT. The patient showed poikiloderma-like cutaneous lesions, while dermatitis is the predominating feature in AT. Normal ocular movement, absence of cerebellar findings, normal serum α-fetoprotein, and no signs of immunodeficiency, are also not consistent with this diagnosis [13,14].

Thus, we could not explain the mixture of cutaneous, endocrine, and neurological findings in this patient by any known etiology (Table 1). However, it is interesting that a single patient showed so many findings common to different DNA-repair disorders. Reporting of such patients with atypical presentations is important for recognizing novel disease entities, as was the case for AT-like diseases [14].

Acknowledgements

We thank Dr. Yuichi Goto for analysis of mitochondrial DNA/enzymatic activity, Dr. Ikuya Nonaka for examining the muscle biopsies, Dr. Ochi for measurements of α-B crystalline in the cerebrospinal fluid, and Dr. Hironobu Nakane for comments on differential diagnosis.

References

[1] Shinya A, Nishigori C, Moriwaki S, Takebe H, Kubota M, Ogino A, et al. A case of Rothmund–Thomson syndrome with reduced DNA repair capacity. Arch Dermatol 1993;129:332–6.

[2] Ohno K, Takeshita K. Patients with tuberous sclerosis have fibroblasts with normal limits for growth characteristics and sensitivities to DNA alkylating agents. Jpn J Hum Genet 1984;29:359–69.

[3] Roe DA. Riboflavin deficiency: mucocutaneous signs of acute and chronic deficiency. Semin Dermatol 1991;10:293–5.

[4] Powers HJ. Riboflavin (vitamin B-2) and health. Am J Clin Nutr 2003;77:1352–60.

[5] Soffer D, Grotsky HW, Rapin I, Suzuki K. Cockayne syndrome: unusual neuropathological findings and review of the literature. Ann Neurol 1979;6:340–8.

[6] Nance MA, Berry SA. Cockayne syndrome: review of 140 cases. Am J Med Genet 1992;42:68–84.

[7] Vennos EM, James WD. Rothmund–Thomson syndrome. Dermatol Clin 1995;13:143–50.

[8] Grodd W, Krageloh-Mann I, Klose U, Sauter R. Metabolic and destructive brain disorders in children: findings with localized proton MR spectroscopy. Radiology 1991;181:173–81.

[9] Antinolo G, Nieto M, Borrego S, Sierra J, Rufi M, Siljeström ML. Familial spastic paraplegia with neuropathy and poikiloderma. A new syndrome? Clin Genet 1992;41:281–4.

[10] Bootsma D, Kraemer KH, Cleaver JE, Hoeijmakers JHJ. Nucleotide excision repair syndromes : xeroderma pigmentosum, Cockayne syndrome, and trichothiodystrophy. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. The metabolic and molecular bases of inherited disease. New York: McGraw-Hill; 2001. p. 677–703.

[11] Vulliamy T, Dokal I. Dyskeratosis congenita. Semin Hematol 2006;43:157–66.

[12] Kraemer KH, Lee MM, Scotto J. Xeroderma pigmentosum: cutaneous, ocular and neurologic abnormalities in 830 published cases. Arch Dermatol 1987;123:241–50.

[13] Gatti RA. Ataxia–telangiectasia. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. The metabolic and molecular bases of inherited disease. New York: McGraw-Hill; 2001. p. 705–32.

[14] Chun HH, Gatti RA. Ataxia–telangiectasia, an evolving phenotype. DNA Repair 2004;3:1187–96.

Please cite this article in press as: Saito Y et al., Mental retardation, spasticity, basal ganglia calcification, ..., Brain Dev (2007), doi:10.1016/j.braindev.2007.08.004

J Hum Genet (2007) 52:985–989
DOI 10.1007/s10038-007-0207-5

ORIGINAL ARTICLE

No evidence for significant association between GABA receptor genes in chromosome 15q11–q13 and autism in a Japanese population

Mamoru Tochigi · Chieko Kato · Shinko Koishi · Yuki Kawakubo · Kenji Yamamoto · Hideo Matsumoto · Ohiko Hashimoto · Soo-Yung Kim · Keiichiro Watanabe · Yukiko Kano · Eiji Nanba · Nobumasa Kato · Tsukasa Sasaki

Received: 22 August 2007 / Accepted: 26 September 2007 / Published online: 24 October 2007

Abstract The γ-aminobutyric acid (GABA) receptor genes *GABRB3*, *GABRA5*, and *GABRG3* located on chromosome 15q11–q13 have been major candidates for susceptibility genes for autism, a neurodevelopmental disorder with a complex genetic etiology. In this study, we first investigated the association between the GABA receptor genes and autism in a Japanese population by analyzing 11 single nucleotide polymorphisms (SNPs). Intron 3 of *GABRB3* was densely mapped because the previous studies observed the association of the microsatellite 155CA-2 located in the region. We observed no significant difference in allelic frequencies or genotypic distributions of the 11 SNPs between patients and controls. A permutation test showed no significant global differences in estimated haplotype frequencies between patients and controls. Analysis after confining the subjects to males showed similar results. Thus, this study provides no positive evidence of an association between the GABA receptor genes and autism in a Japanese population. However, in a SNP (rs3212337) located near the microsatellite 155CA-2, a significant deviation from the Hardy–Weinberg equilibrium was observed in patients ($p = 0.029$, corrected for multiple testing). This finding may suggest further studies around the markers for more definitive conclusions.

Keywords Autism · Chromosome 15 · GABA · *GABRB3* · SNP

M. Tochigi · C. Kato · Y. Kawakubo · S.-Y. Kim · K. Watanabe · Y. Kano · N. Kato · T. Sasaki
Department of Neuropsychiatry, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo, Tokyo 113-8655, Japan

S. Koishi · K. Yamamoto · H. Matsumoto
Department of Psychiatry, Tokai University School of Medicine, 143 Shimokasuya, Isehara, Kanagawa 259-1193, Japan

O. Hashimoto
Department of Medical Technology, Aino University, 4-5-4 Higashi-Ohta, Ibaraki, Osaka 567-0012, Japan

S.-Y. Kim · K. Watanabe · Y. Kano
Department of Child Psychiatry, University of Tokyo Hospital, 7-3-1 Hongo, Bunkyo, Tokyo 113-8655, Japan

E. Nanba
Gene Research Center, Tottori University, 36-1 Nishi-machi, Yonago, Tottori 683-0826, Japan

T. Sasaki (✉)
Health Service Center, University of Tokyo, 7-3-1 Hongo, Bunkyo, Tokyo 113-8655, Japan
e-mail: psytokyo@yahoo.co.jp

Introduction

Autism is a developmental disorder characterized by three areas of abnormality: impairment in social interaction, impairment in communication, and restricted and stereotyped pattern of interest or behavior. Impairment in all three areas is observed before age 3 years, and disrupted brain growth of unknown mechanism is implicated in the etiology. Twin and family studies have indicated a robust role of genetic factors in autism development, whereas few susceptibility genes have been elucidated (Freitag 2007). Chromosome 15q11–q13 has been a focus of genetic studies of autism susceptibility due to the presence of cytogenic abnormalities of this region in autistic patients. The γ-aminobutyric acid (GABA) receptor genes *GABRB3*, *GABRA5*, and *GABRG3* located on chromosome 15q11–q13 have received considerable attention because a

decreased GABA receptor density was observed in the hippocampus of autism, and a suppressed GABAergic inhibition has been implicated in autism etiology (Blatt et al. 2001; Hussman 2001).

To our knowledge, 12 studies to date have investigated the genetic association between GABA receptor genes and autism. Two of the studies (Cook et al. 1998; Buxbaum et al. 2002) observed the association of a microsatellite located in intron 3 of *GABRB3* (155CA-2), whereas the other four studies did not replicate the association (Salmon et al. 1999; Maestrini et al. 1999; Martin et al. 2000; Curran et al. 2005). Except for 155CA-2, only nominal significant associations were observed with respect to markers located in or around these three GABA receptor genes (Martin et al. 2000; Menold et al. 2001; McCauley et al. 2004; Ashley-Koch et al. 2006). In another three studies, no significant association was observed between the genes and autism (Nurmi et al. 2001; Ma et al. 2005; Kim et al. 2006). Thus, the results were inconclusive, and further investigation may be needed to elucidate the problem. In the study reported here, we investigated the association between GABA receptor genes in chromosome 15q11–q13 and autism in Japanese case-control subjects. To our knowledge, this is the first study to investigate the association in a Japanese population.

Subjects and methods

In this study, Japanese patients and control subjects around Tokyo, Japan, were recruited: 166 unrelated patients (147 males and 19 females; age 19.9 ± 9.8 years, mean ± SD) with autistic disorder diagnosed by the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV) criteria and 412 unrelated healthy volunteers (136 males and 276 females; age 36.0 ± 11.5 years). Patient diagnosis was confirmed by two experienced child psychiatrists independently through semistructured behavior observation and parent interview. At the interview, the Child Behavior Questionnaire Revised (Izutsu et al. 2001) was used to assist the evaluation of autism-specific behaviors and symptoms. After the initial observation and interview, patients were followed up for 6 months to confirm the diagnosis. In order to exclude other genetic syndromes, we performed standard karyotyping and fragile X testing for the trinucleotide repeat expansion in the *FMR-1* gene (Chong et al. 1994). The objective of our study was clearly explained, and written informed consent was obtained from all parents. The consent was also obtained from the patients when they were able to follow the explanation. The study was approved by the Ethical Committee of the Faculty of Medicine, the University of Tokyo.

Genomic DNA was extracted from leukocytes by using the standard phenol-chloroform method. We genotyped 11 single nucleotide polymorphisms (SNPs), as detailed in Table 1. Intron 3 of *GABRB3* was densely mapped because the previous studies observed the association of the microsatellite 155CA-2 located in the region (Cook et al. 1998; Buxbaum et al. 2002). The 155CA-2 is located between SNP4 and SNP5 in our study at chromosome position 5170956. All SNPs were analyzed using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, CA, USA). The chi-square test was used to compare SNP frequencies between patients and controls. Lewontin's D' was used to analyze pairwise linkage disequilibrium (LD) (Lewontin 1964). Haplotype block analysis was conducted with the Gabriel and the Four Gamete methods (Gabriel et al. 2002; Wang et al. 2002). SNP haplotypes and their frequencies were estimated by

Table 1 Allelic frequencies of 11 single nucleotide polymorphisms (SNPs) in the *GABR* genes

SNPs	db SNP ID	Location	Alleles (major/minor)	Minor allele frequency			Chromosome position (bp)
				Autism[a]	Control[a]	*p* value	
SNP1	rs11637141	*GABRB3* (3′-UTR)	C/T	0.042 (166)	0.055 (397)	0.36	4954325
SNP2	rs890317	*GABRB3* (intron 3)	A/C	0.49 (164)	0.47 (409)	0.60	5084462
SNP3	rs2059574	*GABRB3* (intron 3)	T/A	0.38 (166)	0.37 (407)	0.95	5159328
SNP4	rs11161335	*GABRB3* (intron 3)	A/T	0.35 (160)	0.34 (358)	0.74	5166381
SNP5	rs3212337	*GABRB3* (intron 3)	C/T	0.39 (166)	0.38 (399)	0.98	5173373
SNP6	rs8179184	*GABRB3* (5′ upstream)	C/T	0.38 (165)	0.38 (407)	0.89	5181451
SNP7	rs140682	*GABRA5* (exon 8)	C/T	0.31 (166)	0.35 (406)	0.19	5343538
SNP8	rs140685	*GABRA5* (exon 10)	C/T	0.32 (166)	0.37 (409)	0.14	5349640
SNP9	rs4887536	*GABRG3* (intron 3)	A/C	0.42 (166)	0.48 (411)	0.073	5508122
SNP10	rs28564251	*GABRG3* (intron 5)	G/A	0.40 (165)	0.35 (348)	0.16	5738611
SNP11	rs4778109	*GABRG3* (intron 5)	G/A	0.38 (164)	0.36 (409)	0.62	5884510

[a] Number of genotyped individuals for each SNP is given in parenthesis

the maximum likelihood method with an expectation-maximization algorithm (Excoffier and Slatkin 1995). Permutation p values were calculated in comparison with haplotype frequencies between patients and controls (Fallin et al. 2001). The SNPAlyze 5.1 standard software (DYNACOM, Japan) was used to conduct LD, haplotype block, and haplotype analyses.

Results

Table 1 shows allelic frequencies of the 11 SNPs in patients and controls. The distributions of all 11 SNPs follow the Hardy–Weinberg equilibrium in controls. In patients, however, distributions of SNPs 5, 6, and 9 significantly deviated from the Hardy–Weinberg equilibrium (p = 0.0026, 0.018, and 0.035, respectively), whereas distributions of the other eight polymorphisms were within the values expected from the Hardy–Weinberg equilibrium. No significant difference was observed in allelic frequencies of the 11 SNPs between the patients and controls. In genotypic distributions, there were significant differences between patients and controls in SNP5 (major homo/hetero/minor homo = 0.32/0.59/0.09 vs. 0.38/0.47/0.15, respectively, $\chi^2 = 6.65$, $df = 2$, $p = 0.036$ in codominant model) and SNP9 (0.30/0.57/0.13 vs. 0.26/0.52/0.22, respectively, $\chi^2 = 5.37$, $df = 1$, $p = 0.021$ in dominant model for major allele). No significant difference was observed in genotypic distributions of the other nine SNPs between patients and controls. Analysis after confining the subjects to males showed no significant difference between patients and controls in allelic frequencies or genotypic distributions of the 11 SNPs (data not shown).

LD strength denoted as D' between pairs of the SNPs is shown in Table 2. Two haplotype blocks, SNPs 3–4 and 7–8, were suggested by the Gabriel and Four Gamete methods of haplotype-block analysis (Gabriel et al. 2002; Wang et al. 2002). In those analyses, no significant difference was observed in frequencies of any estimated haplotype or in distributions of all estimated haplotypes between patients and controls. Similar results were obtained in LD, haplotype block, and haplotype analyses confining the subjects to males (data not shown).

Discussion

In this study, we first investigated the association between the three GABA receptor genes, *GABRB3*, *GABRA5*, and *GABRG3*, and autism in a Japanese population. Nominal significant differences were observed between patients and controls in genotypic distributions of SNPs 5 and 9, although the statistical levels became insignificant after Bonferroni correction. A permutation test showed no significant global difference in estimated haplotype frequencies between patients and controls. Analysis after confining the subjects to males showed similar results. Thus, this study provides no positive evidence of the association between GABA receptor genes and autism in a Japanese population.

Distributions of SNPs 5, 6, and 9 significantly deviated from Hardy–Weinberg equilibrium in patients but not in controls. Statistical levels of deviations in SNPs 6 and 9 became insignificant after Bonferroni correction; however, that in SNP5 was significant (corrected p = 0.029). This could suggest an association of SNP5 with autism, although it might be a chance observation. Also, a possibility of population stratification in the sample may not be completely ruled out. SNP5 is located in intron 3 of *GABRB3*, 2.4 kb telomeric from the microsatellite 155CA-2, which was suggested to be associated with autism in previous studies (Cook et al. 1998; Buxbaum et al. 2002). It

Table 2 Linkage disequilibrium (LD) strength between single nucleotide polymorphisms (SNP) pairs in patients and controls

	SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	SNP7	SNP8	SNP9	SNP10	SNP11
SNP1		0.49	0.51	0.22	1.00	0.51	0.38	0.47	0.04	0.43	0.09
SNP2	0.42		0.05	0.08	0.13	0.05	0.14	0.15	0.08	0.04	0.02
SNP3	0.25	0.03		0.89	0.88	0.92	0.09	0.05	0.14	0.15	0.10
SNP4	0.07	0.22	0.94		0.93	0.96	0.05	0.07	0.12	0.29	0.17
SNP5	0.21	0.01	0.88	0.97		0.96	0.13	0.14	0.12	0.07	0.06
SNP6	0.23	0.03	0.94	0.98	0.93		0.07	0.08	0.14	0.13	0.07
SNP7	0.15	0.10	0.11	0.02	0.09	0.10		0.97	0.30	0.19	0.04
SNP8	0.11	0.11	0.11	0.04	0.07	0.07	0.99		0.24	0.16	0.09
SNP9	0.15	0.07	0.02	0.09	0.04	0.06	0.12	0.10		0.06	0.09
SNP10	0.29	0.06	0.11	0.05	0.09	0.08	0.07	0.07	0.05		0.12
SNP11	0.14	0.03	0.13	0.01	0.08	0.09	0.03	0.06	0.06	0.01	

LD strength is denoted as D'. D' values for patients are shown in the upper diagonal, and those for controls are shown in the lower diagonal

may be interesting to further investigate the region around SNP5 or the microsatellite, although no evidence for a significant association was obtained in our study.

Statistical power of this study was 0.77 ($\alpha = 0.05$) when assuming that the prevalence of autism is 0.21% in a Japanese population (Honda et al. 1996), genotypic relative risk is 1.8 (dominant model), and marker allele frequency is 0.1. Thus, our results might have adequate statistical power to detect the effect of the gene, with odds ratios of approximately 1.8 or more, although smaller effects might not have been detected. Caution may be advised for controls because they were not age-matched to patients. However, this likely may not significantly affect the results, considering no major effect of environmental factors in autism (Folstein and Rosen-Sheidley 2001). Imbalance in sex ratio between patients and controls may be overcome by analysis confining subjects to males, considering autism's higher prevalence in males than in females.

In conclusion, no significant association was observed between GABA receptor genes on chromosome 15q11–q13 and autism in the Japanese subjects. However, the significant deviation from Hardy–Weinberg equilibrium in SNP5 might suggest further search for a susceptibility variant around microsatellite 155CA-2.

References

Ashley-Koch AE, Mei H, Jaworski J, Ma DQ, Ritchie MD, Menold MM, Delong GR, Abramson RK, Wright HH, Hussman JP, Cuccaro ML, Gilbert JR, Martin ER, Pericak-Vance MA (2006) An analysis paradigm for investigating multi-locus effects in complex disease: examination of three GABA receptor subunit genes on 15q11–q13 as risk factors for autistic disorder. Ann Hum Genet 70:281–292

Blatt GJ, Fitzgerald GM, Guptill JT, Booker AB, Kemper TL, Bauman ML (2001) Density and distribution of hippocampal neurotransmitter receptors in autism: an autoradiographic study. J Autism Dev Disord 31:537–543

Buxbaum JD, Silverman JM, Smith CJ, Greenberg DA, Kilifarski M, Reichert J, Cook EH Jr, Fang Y, Song CY, Vitale R (2002) Association between a *GABRB3* polymorphism and autism. Mol Psychiatry 7:311–316

Chong SS, Eichler EE, Nelson DL, Hughes MR (1994) Robust amplification and ethidium-visible detection of the fragile X syndrome CGG repeat using Pfu polymerase. Am J Med Genet 51:522–526

Cook EH Jr, Courchesne RY, Cox NJ, Lord C, Gonen D, Guter SJ, Lincoln A, Nix K, Haas R, Leventhal BL, Courchesne E (1998) Linkage-disequilibrium mapping of autistic disorder, with 15q11–13 markers. Am J Hum Genet 62:1077–1083

Curran S, Roberts S, Thomas S, Veltman M, Browne J, Medda E, Pickles A, Sham P, Bolton PF (2005) An association analysis of microsatellite markers across the Prader-Willi/Angelman critical region on chromosome 15 (q11–13) and autism spectrum disorder. Am J Med Genet 137B:25–28

Excoffier L, Slatkin M (1995) Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. Mol Biol Evol 12:921–927

Fallin D, Cohen A, Essioux L, Chumakov I, Blumenfeld M, Cohen D, Schork NJ (2001) Genetic analysis of case/control data using estimated haplotype frequencies: application to APOE locus variation and Alzheimer's disease. Genome Res 11:143–151

Folstein SE, Rosen-Sheidley B (2001) Genetics of autism: complex aetiology for a heterogeneous disorder. Nat Rev Genet 2:943–955

Freitag CM (2007) The genetics of autistic disorders and its clinical relevance: a review of the literature. Mol Psychiatry 12:2–22

Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D (2002) The structure of haplotype blocks in the human genome. Science 296:2225–2229

Honda H, Shimizu Y, Misumi K, Niimi M, Ohashi Y (1996) Cumulative incidence and prevalence of childhood autism in children in Japan. Br J Psychiatry 169:228–235

Hussman JP (2001) Suppressed GABAergic inhibition as a common factor in suspected etiologies of autism. J Autism Dev Disord 31:247–248

Izutsu T, Osada H, Tachimori H, Naganuma Y, Kato S, Kurita H (2001) The usefulness of the child behavior questionnaire revised (CBQ-R) as a supplementary scale for diagnosis of pervasive developmental disorders. Rinsyo-Seishin Igaku 30:525–532 (Japanese)

Kim SA, Kim JH, Park M, Cho IH, Yoo HJ (2006) Association of *GABRB3* polymorphisms with autism spectrum disorders in Korean trios. Neuropsychobiology 54:160–165

Lewontin RC (1964) The interaction of selection and linkage. I. General considerations; heterotic models. Genetics 120:849–852

Ma DQ, Whitehead PL, Menold MM, Martin ER, Ashley-Koch AE, Mei H, Ritchie MD, DeLong GR, Abramson RK, Wright HH, Cuccaro ML, Hussman JP, Gilbert JR, Pericak-Vance MA (2005) Identification of significant association and gene-gene interaction of GABA receptor subunit genes in autism. Am J Hum Genet 77:377–388

Maestrini E, Lai C, Marlow A, Matthews N, Wallace S, Bailey A, Cook EH, Weeks DE, Monaco AP, the International Molecular Genetics Study of Autism (IMGSA) Consortium (1999) Serotonin transporter (*5-HTT*) and γ-aminobutyric acid receptor subunit β3 (*GABRB3*) gene polymorphisms are not associated with autism in the IMGSA families. Am J Med Genet 88:492–496

Martin ER, Menold MM, Wolpert CM, Bass MP, Donnelly SL, Ravan SA, Zimmerman A, Gilbert JR, Vance JM, Maddox LO, Wright HH, Abramson RK, DeLong GR, Cuccaro ML, Pericak-Vance MA (2000) Analysis of linkage disequilibrium in γ-aminobutyric acid receptor subunit genes in autistic disorder. Am J Med Genet 96:43–48

McCauley JL, Olson LM, Delahanty R, Amin T, Nurmi EL, Organ EL, Jacobs MM, Folstein SE, Haines JL, Sutcliffe JS (2004) A linkage disequilibrium map of the 1-Mb 15q12 $GABA_A$ receptor subunit cluster and association to autism. Am J Med Genet 131B:51–59

Menold MM, Shao Y, Wolpert CM, Donnelly SL, Raiford KL, Martin ER, Ravan SA, Abramson RK, Wright HH, Delong GR, Cuccaro ML, Pericak-Vance MA, Gilbert JR (2001) Association analysis of chromosome 15 GABAA receptor subunit genes in autistic disorder. J Neurogenet 15:245–259

Nurmi EL, Bradford Y, Chen YH, Hall J, Arnone B, Gardiner MB, Hutcheson HB, Gilbert JR, Pericak-Vance MA, Copeland-Yates SA, Michaelis RC, Wassink TH, Santangelo SL, Sheffield VC, Piven J, Folstein SE, Haines JL, Sutcliffe JS (2001) Linkage disequilibrium at the Angelman syndrome gene *UBE3A* in autism families. Genomics 77:105–113

Salmon B, Hallmayer J, Rogers T, Kalaydjieva L, Petersen PB, Nicholas P, Pingree C, McMahon W, Spiker D, Lotspeich L,

Kraemer H, McCague P, Dimiceli S, Nouri N, Pitts T, Yang J, Hinds D, Myers RM, Risch N (1999) Absence of linkage and linkage disequilibrium to chromosome 15q11–q13 markers in 139 multiplex families with autism. Am J Med Genet 88:551–556

Wang N, Akey JM, Zhang K, Chakraborty R, Jin L (2002) Distribution of recombination crossovers and the origin of haplotype blocks: the interplay of population history, recombination, and mutation. Am J Hum Genet 71:1227–1234