[^{11}C]Flumazenil Positron Emission Tomography Analyses of Brain Gamma-Aminobutyric Acid Type A Receptors in Angelman Syndrome

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Objective To evaluate the role of the gamma-aminobutyric acid type A ($GABA_A$) receptor in Angelman syndrome (AS).

Study design We performed [^{11}C]flumazenil positron emission tomography (PET) and examined $GABA_A$ receptor expression in 7 patients with AS of various genotypes (5 with the deletion, 1 with an imprinting defect [ID], and 1 with a *UBE3A* mutation) and 4 normal control healthy volunteers.

Results Relative to the control subjects, the [^{11}C]flumazenil binding potentials (BPs) were significantly higher in the cerebral cortex and cerebellum in the 5 patients with the deletion and in the 1 patient with a *UBE3A* mutation, and were less frequently or barely increased in adult patients with the deletion and in the patient with IDs.

Conclusions Total $GABA_A$ receptor expression was increased in patients with AS with various genotypes. We suggest that a developmental dysregulation of the $GABA_A$ receptor subunits occurs in patients with AS. *(J Pediatr 2008;152:546-9)*

Angelman syndrome (AS) is a neurodevelopmental disorder[1] caused by a functional deficit of the E6−AP ubiquitin-protein ligase gene (*UBE3A*), the product of which is believed to function in protein ubiquitination.[2] In the brain, the *UBE3A* gene is imprinted and is expressed only from the maternal allele. Many details regarding the function of the *UBE3A* gene product and the pathogenesis of AS remain to be elucidated.

Most patients with AS have a 4-megabase pair maternal deletion involving *UBE3A* in chromosome region 15q11-q13; a minority have paternal uniparental disomy of chromosome 15, imprinting defects (IDs), or *UBE3A* mutations.[3] The deletion in 15q11-q13 contains several nonimprinted genes, including *GABRB3*, *GABRA5*, and *GABRG3*, which encode the gamma-aminobutyric acid type A ($GABA_A$) receptor subunits β3, α5, and γ3, respectively, along with maternally expressed *UBE3A* and a few paternally expressed imprinted genes. These nonimprinted genes should be normally expressed in AS without the deletion, but their expression levels should be decreased in AS with the deletion. Phenotypic analyses have demonstrated that patients with the deletion have a higher incidence of seizures, microcephaly, and hypopigmentation, greater delays in motor milestones, and absent speech compared with patients with other genotypes.[4-6] Decreased expression of the nonimprinted $GABA_A$ receptor subunit genes has been considered a cause of phenotypic severity in patients with AS with the deletion.[7-10]

$GABA_A$ receptors belong to the superfamily of ligand-gated ion channels.[11] The $GABA_A$ receptor complex comprises 5 polypeptide subunits that together form a chloride channel.[12] Recently, it has been shown that GABAergic systems play an essential role in synaptic plasticity during early postnatal life.[13] Therefore, GABAergic dysfunction may play a role in the development of neurodevelopmental disorders in patients with AS, through abnormal receptor density or the effects of subunit composition on binding affinity.

Holopainen et al[14] demonstrated a decreased benzodiazepine binding affinity to the $GABA_A$ receptor in 3 patients with AS with the deletion but not in a patient with a *UBE3A* mutation, using [^{11}C]flumazenil ([^{11}C]FMZ) positron emission tomography (PET). They related these findings to putative GABAergic dysfunction in patients with AS with the deletion. We investigated $GABA_A$ receptor distribution with PET in a series of patients with AS and controls.

AS	Angelman syndrome	ID	Imprinting defect
BP	Binding potential	MRI	Magnetic resonance imaging
FMZ	Flumazenil	PET	Positron emission tomography
$GABA_A$	Gamma-aminobutyric acid type A		

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Table I. Clinical characteristics and molecular genetic findings in the 7 patients with AS

Patient	Age/sex	Type of genetic abnormality	Seizure type	Seizure frequency or last attack	Anticonvulsant	Walking impairment	Language
1	6/M	15q11-q13 deletion	GTC	Monthly	VPA	Mild	Simple words
2	9/F	15q11-q13 deletion	CPS, pGTC	Yearly	VPA	Severe	No words
3	10/F	15q11-q13 deletion	GTC	2 years	VPA	Severe	No words
4	27/F	15q11-q13 deletion	GTC	6 years	VPA	Moderate	No words
5	28/M	15q11-q13 deletion	GTC	4 years	VPA	Severe	No words
6	10/F	ID	(-)	(-)	(-)	Mild	Gestural
7	30/M	*UBE3A* mutation	GTC, atonic seizure	15 years	VPA, ESM	Mild	Simple words

GTC, generalized tonic-clonic seizure, *CPS*, complex partial seizure, *pGTC*, partial seizure evolved GTC; *VPA*, sodium valproate; *ESM*, ethosuximide.
Classification of walking impairment: severe, impossible to walk without help; moderate, possible to walk without help using a wheelchair; mild, not using a wheelchair.

METHODS

Subjects

This study included 7 patients with genetically confirmed AS. Five patients had the deletion (patients 1 to 5; 2 males and 3 females; mean age, 16 years; range, 6 to 28 years). Patient 6 had IDs, and patient 7 had a *UBE3A* mutation due to 1 base (G) deletion in exon 9 (910delG). The patients' clinical characteristics (seizures, anticonvulsants, degree of walking impairment, and language ability) and molecular genetic findings are given in Table I.

Four normal control subjects also were enrolled in this study. They were healthy male volunteers age 22 to 29 years. All were right-handed, and none had any psychiatric or neurologic disorders. T2 weighted image, fluid attenuated inversion recovery, and T2 star weighted image were obtained to confirm that the absence of brain abnormalities.

Informed consent was obtained from each subject's parent or guardian, and from each normal volunteer. The study design was approved by the Internal Review Board of Hokkaido University Graduate School of Medicine.

Positron Emission Tomography

PET was performed using an ECAT EXACT HR+ scanner (Asahi-Siemens, Tokyo, Japan) with an in-plane resolution of 4.5 mm and an axial resolution of 3.71 mm. Photon attenuation was corrected with a 5-minute transmission scan. FMZ PET procedures were as described previously.[15,16]

For analysis of the function of GABA/benzodiazepine receptors, head PET images were obtained after injection of [^{11}C]FMZ. In the patient studies, images were acquired under anesthesia with thiopental (a short-acting barbiturate anesthetic). The injected dose of [^{11}C]FMZ was 6 MBq/kg for each subject. The specific activity for normal volunteers was 33.66 $\pm$ 4.9 GBq/μmol, and that for patients was 33.62 $\pm$ 2.1 GBq/μmol; the difference in these values was not statistically significant ($P = .6$). A total of 27 sequential PET frames of increasing duration were obtained over 60 minutes after [^{11}C]FMZ injection, according to the following protocol: 1 frame of 40 seconds, 10 frames of 20 seconds, 4 frames of 60 seconds, 4 frames of 180 seconds, and 8 frames of 300 seconds. The reference tissue compartment model was used for a noninvasive estimation of binding potential (BP), with a time–activity curve in the pons used as an indirect input function.[17,18] The 3-dimensional sinograms thus acquired were converted into 2-dimensional sinograms using a Fourier rebinning algorithm. The images were reconstructed using the direct inversion Fourier transformation method with the HR+ scanner in the brain mode. The reconstruction used a Hanning filter with a full-width half-maximum of 4 mm. The reconstruction matrix was 256 $\times$ 256, the field of view was 33 cm in diameter, and the full-width half-maximum was 6.4 mm after reconstruction.

Magnetic Resonance Imaging

We also performed a magnetic resonance imaging (MRI) study for diagnostic purposes and to illustrate our findings. High-resolution scans were performed at 1.5 T, using a Magnetom Vision MRI system (Siemens AG, Erlangen, Germany). Structural abnormalities were interpreted by an independent board-certified radiologist at Hokkaido University Hospital. MRI data for each patient's head consisted of 128 sequential 1.8-mm-thick axial slices, with a resolution of 256 $\times$ 256 pixels in a field of view of 300 mm.

Positron Emission Tomography Data Analysis

The PET and MRI data were registered using a fully automatic multimodality image registration algorithm on a Unix-based workstation (Sun Ultra 30; Sun Microsystems), as described previously.[19-21] This allowed positioning of the regions of interest, which were round and 10 mm in diameter, in the appropriate brain areas. The cortical regions examined included the following Brodmann areas: frontal (8, 9, 10, 11, 44, 45, 46, 47), temporal (20, 21, 22, 38, 42), parietal (7, 39, 40), occipital (17, 18, 19, 37), and cingulate (23, 24). These PET studies were interpreted by one of the authors (T.S.).

Statistics

Data are represented as mean $\pm$ standard deviation. The BP values from the 7 patients and normal control subjects were compared by analysis of variance for each brain region independently. We confirmed the results of analysis of variance with the

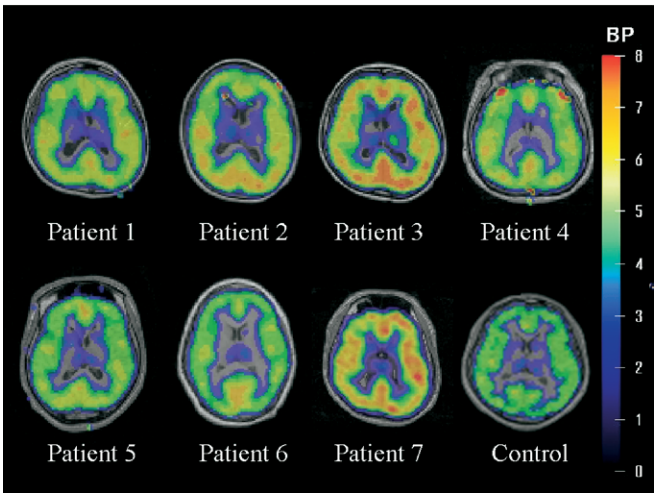

Figure. Registration images of 3-dimensional MRI and PET in patients 1 to 7 and a control subject. The PET images illustrate the BPs. Relative to controls, the BPs were higher in the frontal, parietal, and temporal cortices in patient 1; in the frontal, parietal, and occipital cortices in patient 2; in the frontal, parietal, temporal, and occipital cortices and the cerebellar hemisphere and vermis in patients 3 and 7; and in the frontal and parietal cortices in patient 4. No significant differences in any of the brain regions were demonstrated in patients 5 and 6.

nonparametric Kruskal-Wallis test and Dunn's posttest. The level of significance in all evaluations was set at $P < .05$.

RESULTS

The [^{11}C]FMZ BPs of the patients with AS were mostly greater than those of the normal control subjects (Figure; Tables II and III; available at www.jpeds.com). In patient 1 (age 6 years), the BPs were significantly greater than those of the controls in the frontal and parietal cortices (both $P < .001$) and the temporal cortex ($P < .05$). In patient 2 (age 9 years), the BPs were significantly greater in the frontal, parietal, and occipital cortices (all $P < .001$). In patients 3 (age 10 years) and 7 (age 30 years), the BPs were significantly greater in the frontal, parietal, temporal, and occipital cortices (all $P < .001$) and in the cerebellar hemisphere and vermis (both $P < .05$). In patient 4 (age 27 years), the BPs were significantly greater in the frontal and parietal cortices (both $P < .05$). In patients 5 (age 28 years) and 6 (age 10 years), the BPs were not significantly different from those of the controls in any of the brain regions examined. Notably, in no patient were there any brain regions in which the BPs were significantly lower than those of the controls. Almost all of the significantly elevated BPs were found in the cerebral cortex and cerebellum of children with the deletion (patients 1 to 3) or in the patient with a *UBE3A* mutation (patient 7). BPs greater than those of controls were found in only 1 of the 2 adult patients with the deletion (ie, patient 4, but not in patient 5), and were not found in the patient with IDs (patient 6).

DISCUSSION

In this study, we found no evidence for a significant reduction in BPs in any of the patients with AS compared with the normal control subjects. We did find significantly higher BPs in some brain regions in the children with the deletion and in a patient with a *UBE3A* mutation compared with those in normal control subjects.

The [^{11}C]FMZ BP can be influenced by the subject's age, with higher values in children compared with adults.[22,23] Therefore, the higher BPs in the children with the deletion may have been related to their age. However, the higher BPs in the patient with a *UBE3A* mutation cannot be explained by age, given that he was 30 years old.

Anticonvulsants and sedative agents may strongly influence the BP in GABA receptor studies. In previous investigations,[24-27] thiopental sodium was found to antagonize the effect of FMZ[24] or to have no significant effect on FMZ.[25] Sodium valproate therapy was found to be associated with a global reduction in BP[26] or to have no significant effect on BP.[27] Because of its pharmacologic mechanism, an affect of ethosuximide on BP is not considered likely.[28] Therefore, the drugs used in this study should either decrease or maintain the BP. Despite the administration of these drugs, however, we did not find significantly lower BP values in any of the patients compared with the control subjects.

The [^{11}C]FMZ BP is a composite factor determined by both the number of $GABA_A$ receptors available for binding and the affinity of the remaining $GABA_A$ receptor subtypes for the ligand. It is possible that either of these mechanisms could have influenced our results, but because the β subunit does not affect the affinity of the benzodiazepine site,[29] the latter possibility is unlikely.

The use of the pons as a reference tissue for calculating the BP in FMZ-PET scanning remains controversial. However, Millet et al[18] reported a strong correlation between the BP values estimated by conventional methods using the arterial input function and those estimated with the simple reference tissue model used in our study.

Our results are inconsistent with a previous study by Holopainen et al,[14] in which BPs in patients with AS with the deletion was below the normal range and significantly lower than that of a patient with a *UBE3A* mutation. Those authors argued that a decreased number of available $GABA_A$ receptors in patients with AS with the deletion was caused by hemizygosity of *GABRB3*. But in heterozygous *Gabrb3* knock-out mice ($\beta3^{+/-}$), total $GABA_A$ receptor binding is not significantly reduced, although in cultured $\beta3^{+/-}$ neurons, $\beta3$ mRNA is significantly decreased and GABA responses are reduced by 25%.[7] Therefore, we speculate that the number of available $GABA_A$ receptors, as estimated by [^{11}C]FMZ PET in our study, is not reduced in patients with AS with the deletion, and $\beta3$ subunit expression may be decreased as in the $\beta3^{+/-}$ mice. The reduced expression of the $\beta3$ subunit could be compensated for by up-regulation of the other subunits.

GABAergic systems play essential roles in the regulation of brain function during development, by acting as trophic factors during the embryonic and early postnatal periods.[30] In humans, suitable experiences during the critical period, in which plasticity is high, produce good performance

later.[31] GABAergic systems are indispensable for the introduction of the critical period.[13] During critical developmental stages, precise spatial and temporal control of the genes for each subunit of the $GABA_A$ receptor is likely to be crucially important.[29,32] Given the diversity of GABAergic systems, any GABAergic dysfunction present in patients with AS with the deletion might not be apparent simply as a reduction of total $GABA_A$ receptor expression; rather, we suggest that GABAergic dysfunction manifesting during developmental periods or later could be induced by abnormalities of $GABA_A$ receptor construction.

The patient with AS with a *UBE3A* mutation carried intact $GABA_A$ receptor subunit genes in 15q11-q13, and thus involvement of these genes in pathogenesis of the phenotype is unlikely. Nevertheless, *UBE3A* was recently demonstrated to interact with ubiquitin-like Plic proteins.[33,34] This is of particular interest because Plic-1 selectively binds to $GABA_A$ receptors containing the β3 subunit and regulates the number of these receptors in the cell membrane.[33] This process is critical for the regulation of GABAergic synaptic strength.[34] Maternal *Ube3a*-deficient adult mice demonstrated normal levels of β3 mRNA,[10] but it might have been developmentally dysregulated. In our study, the BPs were significantly higher in several cortical and cerebellar regions in the patient with a *UBE3A* mutation, suggesting that this patient had some GABAergic dysfunction. In contrast, BPs were not higher in our patient with AS with IDs, despite her young age (10 years). This finding does not exclude the possibility that IDs also might be associated with abnormal regulation of $GABA_A$ receptor expression.

In conclusion, this study provides evidence that some patients with AS have an increased, not a reduced, number of $GABA_A$ receptors available for binding. These results are inconsistent with conventional concepts, but we support our findings with the novel proposal that a developmental dysregulation of $GABA_A$ receptor subunits occurs in patients with AS. The present study also has important implications for the interpretation of [^{11}C]FMZ PET in the study of $GABA_A$ receptor function.

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Table II. [^{11}C]FMZ BPs in the 4 normal control subjects and the 7 patients with AS

Brain region	Normal control subjects (n = 4)	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Frontal cortex	3.78 ± 0.43	4.65 ± 0.31**	4.52 ± 0.37**	5.7 ± 0.55**	4.37 ± 0.71*	4.16 ± 0.34	3.78 ± 0.36	5.42 ± 0.56**
Parietal cortex	3.41 ± 0.51	4.19 ± 0.37**	4.54 ± 0.69**	5.45 ± 0.87**	3.95 ± 0.51*	3.72 ± 0.56	3.61 ± 0.43	4.66 ± 0.57**
Temporal cortex	3.88 ± 0.44	4.45 ± 0.41*	4.34 ± 0.42	5.64 ± 0.59**	4.28 ± 0.57	4.15 ± 0.54	3.61 ± 0.32	5.47 ± 0.62**
Occipital cortex	4.45 ± 0.6	5.07 ± 0.57	5.91 ± 0.63**	6.66 ± 0.82**	4.97 ± 0.52	4.95 ± 0.84	4.41 ± 0.77	6.15 ± 0.73**
Cingulate cortex	2.97 ± 0.2	2.65 ± 0.73	2.89 ± 0.58	2.84 ± 0.62	3.41 ± 0.68	2.68 ± 0.4	2.6 ± 0.37	3.15 ± 0.35
Insula	0.83 ± 0.21	1.88 ± 0.03	0.69 ± 0.1	1.43 ± 0.61	1.02 ± 0.03	0.69 ± 0.1	0.83 ± 0.24	1.56 ± 0.01
Hippocampus	1.52 ± 0.23	1.95 ± 0.27	1.6 ± 0.51	1.98 ± 0.1	1.43 ± 0.2	1.79 ± 0.24	1.31 ± 0.03	1.67 ± 0
Caudate nucleus	1.54 ± 0.37	1.1 ± 0.07	1.71 ± 0.34	2.26 ± 0.71	0.95 ± 0.07	1.5 ± 0.17	1.52 ± 0.13	0.98 ± 0.12
Putamen	2.87 ± 0.47	2.43 ± 0.2	2.17 ± 0.1	2.9 ± 0.2	2.62 ± 0.13	2.1 ± 0.07	2.52 ± 0.4	3.26 ± 0.22
Thalamus	3.07 ± 0.34	3.29 ± 0.34	2.83 ± 0.24	3.4 ± 0.24	3 ± 0	2.45 ± 0.24	1.88 ± 0.03	3.94 ± 0.06
Cerebellar hemisphere	3 ± 0.29	3.05 ± 0.07	3.29 ± 0.22	3.98 ± 0.5*	3.08 ± 0.29	2.95 ± 0.05	2 ± 0	3.84 ± 0.46*
Cerebellar vermis	2.01 ± 0.02	2.5 ± 0.71	3 ± 0	3.67 ± 0.4*	2.98 ± 0.03	2.81 ± 0	2.14 ± 0.2	3.5 ± 0.27*
White matter	1.09 ± 0.18	1.33 ± 0.17	1.15 ± 0.06	1.09 ± 0.28	1.29 ± 0.21	1.29 ± 0.43	1.18 ± 0.12	1.04 ± 0.09

*Value is significantly ($P < .05$) greater than in normal control subjects.
**Value is significantly ($P < .001$) greater than in normal control subjects.

Table III. Data for all [^{11}C]FMZ BPs in each brain region

Brain region	Right or Left	Control 1	Control 2	Control 3	Control 4	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Frontal cortex	R 1	3	3.62	3.95	3.9	4.95	5	5.29	3.71	4.33	3.05	4.41
	L 1	3.29	3.57	4.19	4.24	4.57	4	5.81	3.81	3.86	3.48	4.49
	R 2	3	4	4	4.43	4.86	4.19	5.71	4.9	4.14	3.86	5.41
	L 2	3	3.43	4.43	4.24	4.9	4.62	6.05	4.86	4.76	4.62	5.68
	R 3	3	3.76	4	3.86	4.71	4.52	5.9	3.86	4.14	3.43	4.39
	L 3	3	3	3.86	3.86	4.95	5.14	6.71	4.52	3.76	3.48	5.44
	R 4	3.81	3.57	3.81	4	4.81	4.29	5	3.95	4.1	3.52	5.73
	L 4	4	4	3.95	4	4.67	4.05	5.33	3.48	4.1	3.62	5.82
	R 3	3.95	4	3.76	3.57	4.95	4.86	6	4.62	4.76	4.33	5.1
	L 3	3.95	3.9	3	3.67	4.76	4.95	6	4.9	4.67	3.62	5.6
	R 4	3.81	3.95	3.95	4	4.19	4.05	6	4.48	3.76	3.81	5.52
	L 4	4	4.19	4	4	4.57	4.9	5	4.43	3.71	4.05	5.3
	R 5	3.43	4	4.14	3.86	3.9	4.1	5	3.24	4.43	3.95	5.79
	L 5	3.57	3.95	4.14	4.1	4.14	4.24	5.14	3.38	4.43	3.57	6.45
	R 6	3.57	5	4	3	4.95	4.67	5.62	5.57	4	3.9	5.09
	L 6	3.29	4.57	3.71	3.62	4.52	4.86	5.19	5.67	4.14	3.9	6.06
	R 7	3	3.67	4.1	3	4.48	4.38	6	4.76	4	3.9	5.55
	L 7	3.67	4	3.95	3	4.86	4.48	6.81	4.57	3.76	3.9	5.81
Parietal cortex	R 1	3	3	3	3.95	4.62	3.95	4.86	3.43	3	3.95	4.57
	L 1	3.29	3	3	3.9	4.81	4	5	3.95	3	3.71	4.75
	R 2	3.29	3	3	3.71	3.95	3.48	3.86	3.33	3.43	3.1	3.92
	L 2	3	2.95	3.86	3.86	3.81	3.43	4.38	3.81	3.24	3.1	4.14
	R 3	3	3	3.67	3.9	4	3.48	3.81	3.57	3	2.81	3.35
	L 3	3	3	3.33	4.05	3.95	3.81	4.57	3.38	3.1	2.95	3.75
	R 4	2.71	3.43	3	3	4.62	4.95	6	4.14	3.86	3.71	5.37
	L 4	2.71	3	3.24	3.05	5	5	5.48	4	3.9	3.1	4.72
	R 3	2.43	4	3	3	3.86	5.05	5.62	3.48	4.38	3.86	4.67
	L 3	2.81	4.62	3.86	3	3.95	5.48	6.29	3.19	4.76	3.57	4.21
	R 4	2.52	3.9	3.48	3.95	4	5.19	6	3.95	4.05	4.1	4.73
	L 4	2.86	4	4	3.86	4	5.57	6.29	3.81	4.1	4.19	5
	R 5	3	3.95	3.1	4.1	4	4.57	6	4.43	4.38	3.95	5.24
	L 5	3	4	3	3.86	4.1	4.86	6.11	4.33	4.1	3.43	5.25
	R 6	3	3.86	3.9	3.67	3.9	4.67	6.44	4.67	3.76	3.81	5.07
	L 6	3	3.86	4	3.62	4	4.57	6	3.95	3	4	4.94
	R 7	3	3.62	4.81	3.24	4.1	4.81	6.33	5	3.86	3.9	5.23
	L 7	3	4	4.05	3.81	4.67	4.9	5	4.62	4.1	3.81	5.02
Temporal cortex	R 1	4	3.9	4	3.48	4.95	4	5.33	4.19	4.38	3.33	5.82
	L 1	4	3.9	4	3.62	4.24	4.52	5.43	4.1	3.71	3.38	5.82
	R 2	4.71	4	4.14	3.71	5	4.86	6	4.57	4.57	4	5.58
	L 2	4	4	3.86	3.86	4.29	4.38	5.38	4	4.62	2.9	5.68
	R 3	4.24	3.86	4.14	3.81	4.9	4.9	6.24	5.38	4.43	3.57	6.03
	L 3	4.1	4.33	4	3.52	4.76	4.95	5.71	4.76	5.14	3.67	6.4
	R 4	3.9	4	3.81	3	3.86	4	5.05	3.38	3.48	3.57	4.15
	L 4	4	4	3.71	3.05	3.9	3.81	4.48	3.52	3.57	3.62	4.91
	R 3	4.29	4	3.9	3.19	4.67	4.1	5.95	4.57	3.48	4	5.32
	L 3	5	3.24	3.86	3.57	4.38	3.81	5.57	4	4	3.48	4.79
	R 4	4.71	4	4	3	4	4.57	6.76	4.86	3.81	3.95	5.91
	L 4	4.76	3.24	3.67	3.14	4.43	4.19	5.81	4	4.57	3.86	5.27
Occipital cortex	R 1	4.67	4.71	4.95	4	4.81	4.95	7	5.33	4.71	4.14	5.6
	L 1	4.71	4.14	4.76	3.95	5	4.86	5.67	5	4	3.43	6.07
	R 2	4.67	4.76	6	3.95	4.57	5.48	6.33	4.71	4.71	4.52	5.66
	L 2	4	4.81	5.81	4	4.76	5.76	6.78	4.1	4.48	4.57	5.89
	R 3	4	4.9	4	4.95	5	6	5.43	4.81	4.95	4.29	5.69
	L 3	4	3.95	4.38	5.05	4.95	5.95	6.1	4.29	4.48	3.9	5.8
	R 4	4	5.19	3.29	4.81	4.81	5.71	6.38	4.95	4.67	3.19	6.19

Table III. Continued

Brain region	Right or Left	Control 1	Control 2	Control 3	Control 4	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
	L 4	3.76	4.9	3.48	4	4.33	6.05	6.81	4.9	4.43	3.9	5.47
	R 3	3.81	4.71	4.62	4.95	4.9	6	6.43	4.9	5.05	4.86	5.89
	L 3	3.62	4.9	4.48	5	5	6.1	6.76	4.38	4.9	4.76	6.92
	R 4	4.52	4	5	4.86	5.38	5.86	6.38	5.48	5.19	5.86	5.87
	L 4	4.57	5	4.9	5	5	5.86	6.62	5.05	4.57	5.71	5.77
	R 5	3.95	3.71	4	5	6.52	7.05	8.24	5.86	7.52	4.86	7.48
	L 5	3	3.95	4	5	6	7.14	8.38	5.81	5.67	3.81	7.84
Cingulate cortex	R 1	3.24	2.81	2.81	2.67	3.81	3	3.33	3.81	3.24	2.86	3.49
	L 1	3.33	2.95	2.95	3.05	3.71	3.9	3.52	4.67	3.29	3.38	3.86
	R 2	2.95	2.81	3	3.14	2.05	2.05	2.11	3.67	2.24	2.38	3.07
	L 2	3.19	2.95	3.14	3.14	2.05	2.86	2.11	3.38	2.86	2.33	3.07
	R 3	2.86	3	2.71	3.29	2.19	2.24	2.44	2.86	2.43	2.62	2.97
	L 3	3	3.1	2.62	3	2.14	2.9	2.44	2.43	2.38	2.24	2.96
	R 4	3.05	2.86	2.67	2.86	2.71	2.86	3.48	3.05	2.52	2.43	2.94
	L 4	3.29	3	2.95	2.62	2.52	3.29	3.29	3.43	2.52	2.57	2.84
Insula	R 1	3	3	2	3	2.57	2.24	3.05	2.71	2.14	2.81	3.41
	L 1	3	3.57	2.38	3	2.29	2.1	2.76	2.52	2.05	2.24	3.1
Hippocampus	R 1	3	3.76	3.24	2.76	3.05	3	3.24	3	2.29	1.86	3.99
	L 1	3	3.24	2.86	2.71	3.52	2.67	3.57	3	2.62	1.9	3.9
Caudate nucleus	R 1	0.81	1	0.33	0.86	1.86	0.76	1.86	1	0.76	0.67	1.55
	L 1	0.86	1	0.86	0.9	1.9	0.62	1	1.05	0.62	1	1.57
Putamen	R 1	1.62	1.76	1.43	1.38	2.14	1.95	2.05	1.57	1.62	1.29	1.67
	L 1	1.76	1.76	1.24	1.24	1.76	1.24	1.9	1.29	1.95	1.33	1.67
Thalamus	R 1	1	1.81	1.19	1.62	1.14	1.95	1.76	0.9	1.38	1.43	0.9
	L 1	1.95	1.81	1.76	1.14	1.05	1.48	2.76	1	1.62	1.62	1.07
Cerebellar hemisphere	R 1	2.95	3	2.71	2.62	3.14	3.57	4.48	3.48	2.9	2	4.32
	L 1	2.86	3	2.81	3	3.05	3.33	3.86	3.1	3	2	4.12
	R 2	3	3	3	3	3	3.1	4.24	2.95	2.9	2	3.31
	L 2	3.11	4	3	3	3	3.14	3.33	2.81	3	2	3.62
Cerebellar vermis	R 1	2.05	2	2	2	3	3	3.95	3	2.81	2.29	3.69
	L 1	2	2	2	2	2	3	3.38	2.95	2.81	2	3.3
White matter	R 1	1.14	1.14	1	0.95	1.48	1.14	0.78	1.52	0.9	1	0.91
	L 1	1.19	1.05	1.29	1.33	1.38	1.24	1.44	1.48	0.62	1.33	1.03
	R 2	1.14	1.19	1.05	1.38	1.43	1.19	1.44	1.1	1.38	1.29	1
	L 2	1.19	1.05	1.33	0.95	1.05	1.05	1	1.29	1.52	1.1	1.16
	R 3	0.86	1.1	1.19	0.71	1.43	1.14	0.89	1	1.67	1.19	0.99
	L 3	0.9	1.29	1	0.71	1.19	1.14	1	1.33	1.67	1.19	1.13