Dopamine Transporter Concentration Is Reduced in Asymptomatic Machado-Joseph Disease Gene Carriers

Tzu-Chen Yen, MD, PhD[1]; Kai-Yuan Tzen, MD[1,2]; Min-Chi Chen, PhD[3]; Yah-Huei Wu Chou, PhD[4]; Rou-Shayn Chen, MD[5]; Chi-Jen Chen, MD[6]; Shiaw-Pyng Wey, PhD[7]; Gann Ting, PhD[7]; and Chin-Song Lu, MD[5]

[1]Department of Nuclear Medicine, Chang Gung Memorial Hospital at Lin-kou and Kee-long, Chang Gung University, Taiwan; [2]Department of Nuclear Medicine, Taipei Medical University, Taiwan; [3]Biostatistics Center/Department of Public Health, Chang Gung University, Taiwan; [4]Human Molecular Genetics Laboratory, Chang Gung Memorial Hospital at Lin-kou, Chang Gung University, Taiwan; [5]Department of Neurology, Chang Gung Memorial Hospital at Lin-kou, Chang Gung University, Taiwan; [6]Department of Radiology, Chang Gung Memorial Hospital at Lin-kou, Chang Gung University, Taiwan; and [7]Institute of Nuclear Energy Research, Lung-Tan, Taiwan

Dopamine transporter (DAT) binding is decreased in Machado-Joseph disease (MJD) patients. To further investigate the DAT activity in asymptomatic MJD (aMJD) gene carriers, we performed this prospective study using ^{99m}Tc-TRODAT-1 ([^{99m}Tc][2[[2-[[[3-(4-chlorophenyl)-8-methyl-8-azabicyclo[3,2,1]-oct-2-yl]-methyl](2-mercaptoethyl)amino]ethyl]amino]ethane-thiolato(3-)-*N*2,*N*2′,*S*2,*S*2]oxo-[1*R*-(exo-exo)])) brain SPECT on 5 aMJD gene carriers, 10 age-matched MJD patients, and 10 age-matched healthy control subjects. **Methods:** Brain SPECT images were acquired 4 h after intravenous injection of 925 MBq (25 mCi) ^{99m}Tc-T RODAT-1, which is known to bind specifically to the DAT on the nigrostriatal terminals. By fusing these SPECT images with a striatal atlas, obtained from MRI, binding of this tracer in the entire striatum was measured and the uptake values in bilateral striatal areas were compared between these 3 groups. **Results:** The uptake values of the aMJD gene carriers ($P < 0.001$) and MJD patients ($P < 0.001$) displayed a significant reduction compared with those of the control subjects. The reduction was more severe in the MJD patient group ($P < 0.05$). Bilateral putamen-to-caudate ratios were significantly lower in the aMJD gene carrier and MJD patient groups ($P < 0.001$). The dopamine neuronal activity, as represented by the tracer binding, was more prominently affected in the putamen in these patients and gene carriers. **Conclusion:** ^{99m}Tc-TRODAT-1 brain SPECT is capable of detecting early alteration of dopamine neurons in the striatal region. Significantly, the results suggest that this impairment of presynaptic dopamine function actually occurs at an early stage, which was previously unrecognized in these aMJD gene carriers.

Key Words: ^{99m}Tc-TRODAT-1 brain SPECT; Machado-Joseph disease; asymptomatic Machado–Joseph gene carrier; dopamine transporter

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For correspondence or reprints contact: Chin-Song Lu, MD, Department of Neurology, Chang Gung Memorial Hospital at Taipei 199, Tung Hwa North Rd., Taipei 105, Taiwan.

E-mail: c81214@adm.cgmh.org.tw

Using ^{99m}Tc-TRODAT-1 ([^{99m}Tc][2[[2-[[[3-(4-chlorophenyl)-8-methyl-8-azabicyclo[3,2,1]oct-2-yl]-methyl](2-mercaptoethyl)amino]ethyl]amino]ethane-thiolato(3-)-*N*2,*N*2′,*S*2,*S*2]oxo-[1*R*-(exo-exo)])) SPECT scanning of the brain, we have previously reported a significant decrease of dopamine transporter (DAT) binding in the striatum of most patients with Machado-Joseph disease (MJD), even though most of them do not show clinical parkinsonian signs (*1*). This high incidence of impaired nigrostriatal DAT binding could correspond to the fact that neuronal loss and gliosis in the substantia nigra and striatum were often observed on postmortem examination of MJD patients (*2–7*). ^{99m}Tc-TRODAT-1 brain SPECT has proven to have the potential to detect the nigrostriatal involvement in Parkinson's disease as well as in varied parkinsonian syndromes (*1,8–12*).

Through the identification of abnormally expanded cytidylate, adenylate, and guanylate (CAG) repeats in the MJD1 gene, one may expect to find asymptomatic MJD (aMJD) gene carriers in MJD families (*13*). When these aMJD carriers will become symptomatic is a crucial problem in genetic counseling. Similar to other degenerative diseases, such as Parkinson's disease, the onset of the degeneration process in MJD probably occurs long before the clinical presentation (*14–17*). Decreased glucose metabolism in the cerebellar hemispheres, brain stem, and occipital cortex has been reported in aMJD carriers (*18*). It is reasonable to assume that the presymptomatic involvement of the nigrostriatal system could also be found in the aMJD carriers. This early nigrostriatal alteration has been detected using ^{99m}Tc-TRODAT-1 brain SPECT (*1,8–10*). The involvement of the nigrostriatal system is also a possible indicator for progression of the disease (*11*). Because of the relentless progress of the illness, early detection of the dopamine dysfunction is important, not only in monitoring disease progression but also in future therapeutic intervention, such

as gene therapy. Therefore, we have extended our ^{99m}Tc-TRODAT-1 brain SPECT scan study to include aMJD carriers and to focus on the difference in the selective binding of ^{99m}Tc-TRODAT-1 in the putamen and caudate nucleus in terms of their distribution patterns.

MATERIALS AND METHODS

Subjects

This prospective study included 5 aMJD gene carriers, 10 MJD patients, and 10 healthy control subjects. All participants in the 3 groups were age-matched. The 5 aMJD gene carriers (from 2 MJD families) consisted of 3 women and 2 men with a mean age of 25.7 ± 4.2 y (age range, 20–30 y). None showed any clinical features of cerebellar ataxia or other neurologic signs on a thorough neurologic examination by 2 board-certified neurologists. The 10 MJD patients from 10 different families consisted of 6 women and 4 men with a mean age of 28.5 ± 5.1 y (age range, 20–35 y). All showed a dominant cerebellar ataxia, dysarthria, and pyramidal signs in association with other varied symptoms, such as orofacial fasciculations, ophthalamoplegia, muscle atrophy, and bulging eyes. Only 1 patient showed an associated rigidity and tremor on 4 limbs (patient 10 with MJD; Table 1). Genetic testing indicated that an abnormal expansion over 45 CAG repeats in 1 allele of the MJD1 gene on chromosome 14q32.1 was proven by polymerase chain reaction in the aMJD gene carrier and MJD patient groups in our Human Genetic Laboratory. Ten healthy control subjects, including 2 subjects from 1 MJD family without abnormal CAG repeats (control subjects 1 and 2; Table 1), consisted of 7 women and 3 men with a mean age of 23.5 ± 2.5 y (age range, 20–29 y). All subjects gave informed consent for the SPECT and MRI studies, which were approved by the medical ethics committee of this institution. Quantitative analyses (imaging registration using MRI and SPECT images) and qualitative comparisons (visual differences between 3 nuclear physicians) were performed. All participants in this study were free from any other neurologic or psychiatric diseases and were not taking any drugs known to affect the dopaminergic system.

^{99m}Tc-TRODAT-1 Brain SPECT

The ^{99m}Tc-TRODAT-1 was prepared from a research kit obtained from the Institute of Nuclear Energy Research (Lung-Tan,

TABLE 1
CAG Repeat Numbers, Striatal Binding, and P/C of ^{99m}Tc-TRODAT-1 in 5 aMJD Carriers, 10 MJD Patients, and 10 Healthy Control Subjects

Participant type	No.	Abnormal CAG no.	Striatum		P/C	
			Right	Left	Right	Left
aMJD	1*	79	2.08	1.97	0.74	0.57
	2*	72	1.83	1.81	0.73	0.69
	3*	68	2.17	2.22	0.75	0.77
	4†	72	2.07	1.92	0.70	0.61
	5†	72	1.79	1.82	0.74	0.66
Mean ± SD		72.6 ± 4.0‡	1.99 ± 0.17‡	1.95 ± 0.17‡	0.73 ± 0.02	0.66 ± 0.08
MJD	1†	75	1.73	1.75	0.66	0.72
	2	82	1.44	1.55	0.71	0.74
	3	79	1.50	1.63	0.59	0.75
	4	85	1.43	1.53	0.67	0.63
	5	80	1.69	1.65	0.63	0.62
	6	77	1.53	1.45	0.67	0.60
	7	79	1.53	1.43	0.89	0.83
	8	80	1.40	1.56	0.63	0.72
	9	77	1.56	1.56	0.62	0.67
	10	72	1.94	1.89	0.72	0.74
Mean ± SD		78.6 ± 2.5‡	1.58 ± 0.17‡	1.60 ± 0.14‡	0.68 ± 0.06	0.70 ± 0.07
Control	1*		2.36	2.36	0.87	0.82
	2*		2.49	2.43	0.73	0.75
	3		2.81	2.78	0.84	0.87
	4		2.80	3.00	0.78	0.83
	5		3.02	3.09	0.73	0.78
	6		2.74	2.64	0.89	0.85
	7		3.26	3.09	0.81	0.82
	8		2.85	2.73	0.76	0.76
	9		2.60	2.80	0.70	0.73
	10		2.94	2.79	0.79	0.81
Mean ± SD			2.79 ± 0.26	2.77 ± 0.25	0.79 ± 0.06	0.80 ± 0.05

*Members of same family.
†Members of same family.
‡Significant difference between aMJD and MJD ($P < 0.05$) using generalized estimating equations approach.
All comparisons between control and aMJD and between control and MJD are significant ($P < 0.001$).

Taiwan). Imaging was performed 4 h after intravenous injection of 925 MBq (25 mCi) in a volume of 2 mL. SPECT images were obtained using a MULTISPECT 3 gamma camera (Siemens Medical Systems, Hoffman Estates, IL) with fanbeam collimators and 120 equally spaced projections over 360°, taking 60 s per step and using a 128 × 128 matrix size. Individual images were reconstructed with backprojection using a ramp-Butterworth filter, with a cutoff of 0.3 cm^{-1} and an order of 10. The data were corrected for the effects of photon attenuation using Chang's first-order method with an attenuation coefficient of 0.12 and with the attenuation ellipses defined on the summed images of the entire dataset and applied individually, without modification, to all images. No attempt was made to correct for partial-volume effects. The slice thickness and in-plane size were 2.9 mm. Four reconstructed transaxial slices were summed together and reoriented to be parallel to the orbitomeatal line with the highest signal in the region of the basal ganglia as the central slice (*1,8–10*). All images were reviewed blindly by 3 nuclear physicians, and all decisions were confirmed by at least 2 of the 3 physicians.

MRI

All subjects were examined using a VISION VB33D 1.5-T MRI scanner (Siemens Medical Systems). T1-weighted axial images (repetition time [TR], 500 ms; echo time [TE], 25 ms) and T2-weighted axial images (TR, 3,500 ms; TE, 120 ms) were obtained in the transaxial plane (6-mm thickness and 0.6-mm gap). Three neuroradiologists, who did not know the clinical or genetic status of the gene carriers, performed diagnostic evaluations individually.

Data Processing

For analysis of the striatal dopaminergic pathway of ^{99m}Tc-TRODAT-1 binding, the ratio of specific to nonspecific binding was calculated by summing 4 adjacent transversal slices representing the most intense striatal DAT binding (*1,8–10*). Analyses were performed without knowledge of the clinical data. A standard region of interest (ROI) template (constructed according to a stereotactic shape from an MRI atlas and including regions for the putamen, caudate nucleus, and occipital cortex) and additional ROIs for the entire striatum were placed bilaterally on the acquired images. Subtracting occipital counts from striatal counts allowed estimates of specific striatal binding. The ratio of specific to nonspecific striatal ^{99m}Tc-TRODAT-1 binding was then calculated by dividing the specific striatal uptake by occipital binding.

Statistical Analyses

Data were entered into Excel (Microsoft Corp., Redmond, WA) and analyzed by Statistical Analysis System version 6.12 (SAS Institute, Durham, NC). $P < 0.05$ was considered statistically significant. The generalized estimating equations (*19,20*) method was used to account for possible correlation within a family and to determine whether significant differences were found among MJD, aMJD, and control groups in abnormal CAG number, striatal binding, and the putamen-to-caudate ratio (P/C) and to evaluate the associations between abnormal CAG number and striatal binding.

RESULTS

Figure 1 shows the family pedigrees and ^{99m}Tc-TRODAT-1 brain SPECT in the 2 MJD families containing 2 MJD patients, 3 aMJD gene carriers, and 2 mutation-negative subjects from our study population. Decreased ^{99m}Tc-TRODAT-1 uptake was found in the MJD patients and aMJD gene carriers but not in the mutation-negative subjects. In addition, the MJD patients had lower ^{99m}Tc-TRODAT-1 uptake in the striatum than the aMJD gene carriers. Table 1 illustrates the CAG repeat number, striatal binding, and P/C (putamen/caudate uptake) of ^{99m}Tc-TRODAT-1. The abnormal CAG repeat number in the MJD group is significantly larger than that in the aMJD gene carrier group ($P < 0.001$). This finding implies that the commencement of clinical symptoms is positively correlated with the number of CAG repeats. Table 1 also shows a significantly decreased ^{99m}Tc-TRODAT-1 uptake value bilaterally in the striatum for the MJD and aMJD groups compared with that of the healthy control subjects ($P < 0.001$). Compared with the bilateral P/C of ^{99m}Tc-TRODAT-1, a significantly lower uptake ratio was found in the MJD and aMJD groups compared with that of the healthy control subjects ($P < 0.01$). In this study, the ^{99m}Tc-TRODAT-1 uptakes were also significantly higher bilaterally in the striatum of the aMJD group compared with those of the MJD group ($P = 0.013$ for the right side and $P = 0.034$ for the left side). However, no significant difference was found in the P/C of ^{99m}Tc-TRODAT-1 in these 2 groups ($P = 0.13$ on the right and $P = 0.15$ on the left). Figure 2 shows the bilateral ^{99m}Tc-TRODAT-1 uptake ratios in the striata for the MJD, aMJD, and healthy groups. The ^{99m}Tc-TRODAT-1 uptake ratios for the aMJD carriers and the MJD patients were significantly lower bilaterally than those for the healthy control subjects. The MJD patients also had a significantly lower DAT uptake in both striata than that of the aMJD carriers. Symmetrically decreased DAT uptakes bilaterally in the striata for the aMJD carriers and the MJD patients were also noted. Figure 3 shows the mean P/C values of ^{99m}Tc-TRODAT-1 in these 3 groups. In comparison with the healthy control subjects, a significantly decreased bilateral P/C was found in the aMJD and MJD groups. However, for the MJD and aMJD groups, no significant difference was found between the uptake P/C for them on either side. Figure 4 shows a negative association between the striatal DAT binding and the abnormal CAG number in the MJD group bilaterally ($P < 0.001$ for the right side and $P = 0.006$ for the left side). A similar association was not observed in the aMJD group ($P > 0.05$).

DISCUSSION

After intravenous administration, ^{99m}Tc-TRODAT-1 was bound to the DAT located primarily on the membrane of the nigrostriatal terminals, and this may provide a marker for the functional integrity of the dopamine neuron (*21–23*). In this study, the DAT binding in the bilateral striata was decreased significantly in all 5 aMJD gene carriers and the 10 MJD patients compared with that of the age-matched healthy control subjects. Our data also showed that the DAT activity was reduced bilaterally in the putamen and caudate nuclei and was reduced more so in the former. This finding

further implies that dopamine neurons in the aMJD gene carriers have already been impacted, although the extent of reduction of uptake is smaller than that of those neurons in the MJD patients. It also suggests that the substantia nigra had suffered early damage, before commencement of any clinical manifestations of MJD. In Parkinson's disease, 40%–60% of neuronal loss is required before manifestation of clinical symptoms (*14–17*). Likewise, this phenomenon was found in our participants with aMJD. Therefore, we have shown that a study of ^{99m}Tc-TRODAT-1 binding using SPECT is capable of detecting early alteration of the nigrostriatal function in the aMJD gene carriers. In comparison with the 10 MJD patients, this striatal binding was significantly reduced less severely in the aMJD carriers (Table 1). The decreased binding was apparently more dominant in the putamen than that in the caudate nucleus; nevertheless, using the putamen results in the P/C indicated that the selective vulnerability of the striatum alone did not change between the MJD patients and the aMJD carriers groups. This similarity in P/C distribution in the aMJD gene carriers and the MJD patients may suggest that once the degenerative process began in the substantia nigra, it would maintain a similar pace in different parts of the substantia nigra. Another point worth noting is the symmetric impairment of bilateral striatal ^{99m}Tc-TRODAT-1 binding in the MJD patients, who displayed a bilateral symmetric impairment of the substantia nigra. These findings suggest that the mode of progression of MJD is essentially different from that of Parkinson's disease. In the latter, it is usually initiated from the unilateral ventrolateral tier of the substantia nigra, which has been well illustrated by ^{18}F-6-fluorodopa PET scan (*24–26*) and ^{99m}Tc-TRODAT-1 brain SPECT (*10–12*) studies. We propose that serial ^{99m}Tc-TRODAT-1 brain SPECT studies may allow understanding of the mode of progression and pathogenesis of MJD.

The numbers of CAG repeats in the MJD patients were significantly larger than those in the aMJD gene carriers ($P < 0.001$). Nevertheless, despite the limited number of subjects in our series, the mean ages of the subjects in these 2 groups were similar. This suggests that the larger the abnormal CAG number becomes, the earlier the symptoms

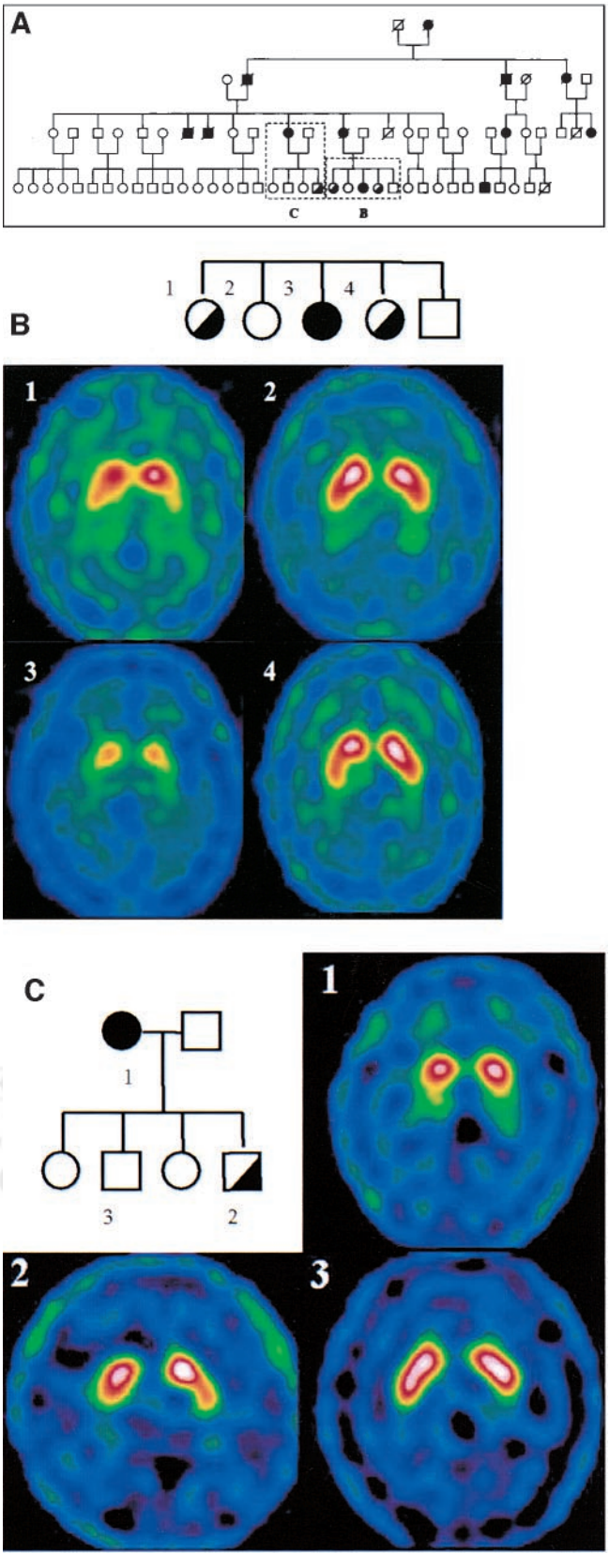

FIGURE 1. Family pedigrees (A) and ^{99m}Tc-TRODAT-1 uptake SPECT images in 2 MJD nuclear families (B and C) containing 2 MJD patients, 3 aMJD gene carriers, and 2 mutation-negative subjects. Squares denote males and circles denote females. Black squares and circles represent MJD patients, hemiblack shapes represent aMJD carriers, and white shapes denote mutation-negative subjects. Diffusely decreased uptake in bilateral putamen and caudate nucleus was noted in MJD patients. Slightly asymmetrically decreased uptakes in bilateral putamen were noted in aMJD gene carriers. These uptakes in bilateral putamen and caudate nucleus were normal in mutation-negative subjects (B and C). In family B, 1 and 4 were aMJD gene carriers, 2 was mutation-negative subject, and 3 was MJD patient. In family C, 1 was MJD patient, 2 was aMJD gene carrier, and 3 was mutation-negative subject.

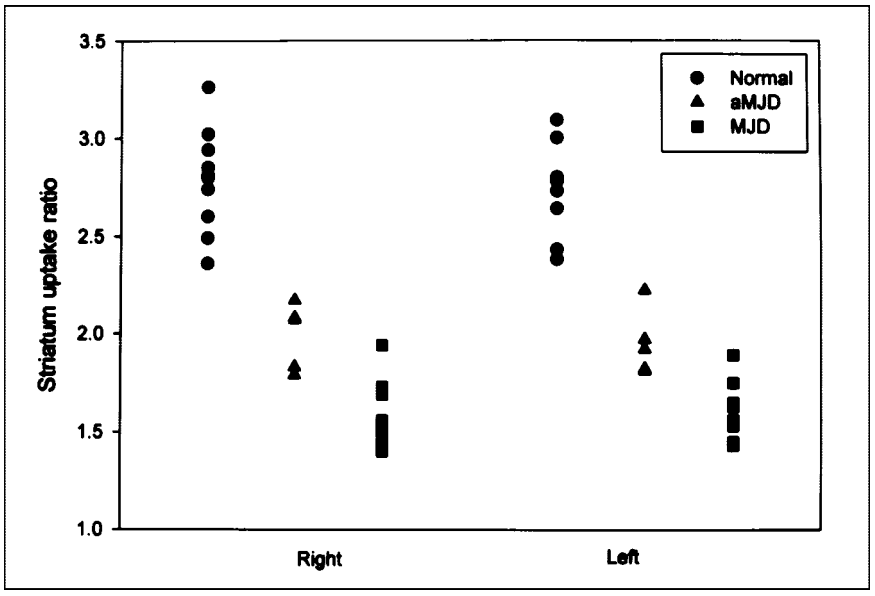

FIGURE 2. Bilateral striatal uptake ratios of ^{99m}Tc-TRODAT-1 in aMJD carriers (▲), age-matched MJD patients (■), and age-matched healthy subjects (●) are given. In aMJD carriers, striatal uptake of ^{99m}Tc-TRODAT-1 was significantly lower than that in healthy control group and was even lower in MJD patients on both sides.

develop. These findings support the general concept that a reverse correlation exists between the age of onset and the number of abnormal CAG repeats (*27*). The negative association between the striatal binding and the abnormal CAG number in the MJD group provides further biochemical evidence. However, the fact that this negative association did not hold true in the aMJD group might be attributed to the small sample size or other unknown factors. Investigation of the age at which the degeneration actually starts is worthy of further attention.

The binding of the DAT in the striatal terminals deduced from the ^{99m}Tc-TRODAT-1 investigations is our target for evaluation of the integrity of the nigrostriatal function. Binding of the striatal DAT has been used similarly in (1γ)-2β-carbomethyoxy-3β-(4-iodophenyl)tropane (β-CIT) brain SPECT (*28,29*). Our results show that the detection of decreased DAT binding in the aMJD gene carriers by SPECT imaging is effective and sensitive. Other activities, such as dopa decarboxylase and vesicular monoamine transporter, have been studied by PET to evaluate presynaptic dopamine function (*30–32*). Tedroff et al. (*31*) reported that dopa decarboxylase activity was relatively upregulated compared with DAT binding in established Parkinson's disease; this was most marked in the posterior putamen, where dopamine denervation was the greatest (*31*). Lee et al. (*33*) compared the PET findings of ^{18}F-dopa and ^{11}C-methylphenidate with ^{11}C-dihydrotetrabenazine in Parkinson's disease. It has been shown that ^{18}F-dopa uptake was relatively elevated and ^{11}C-methylphenidate binding was relatively reduced (*31,33*). Both studies suggested that the

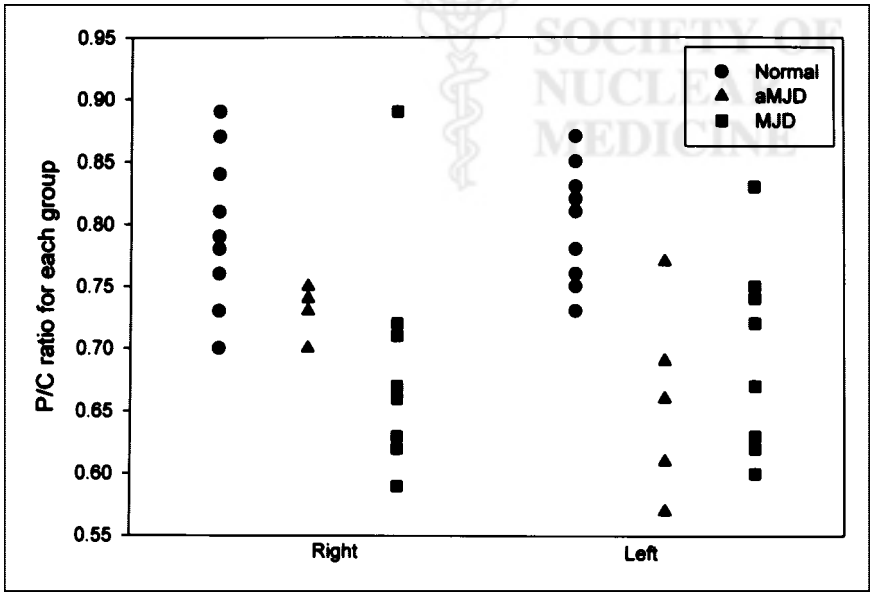

FIGURE 3. Bilateral P/C values for ^{99m}Tc-TRODAT-1 in aMJD carriers (▲), age-matched MJD patients (■), and age-matched healthy subjects (●) are given. aMJD carriers and MJD patients had significantly lower P/C values than those of control group. However, no significant difference was found between values for aMJD carriers and MJD patients.

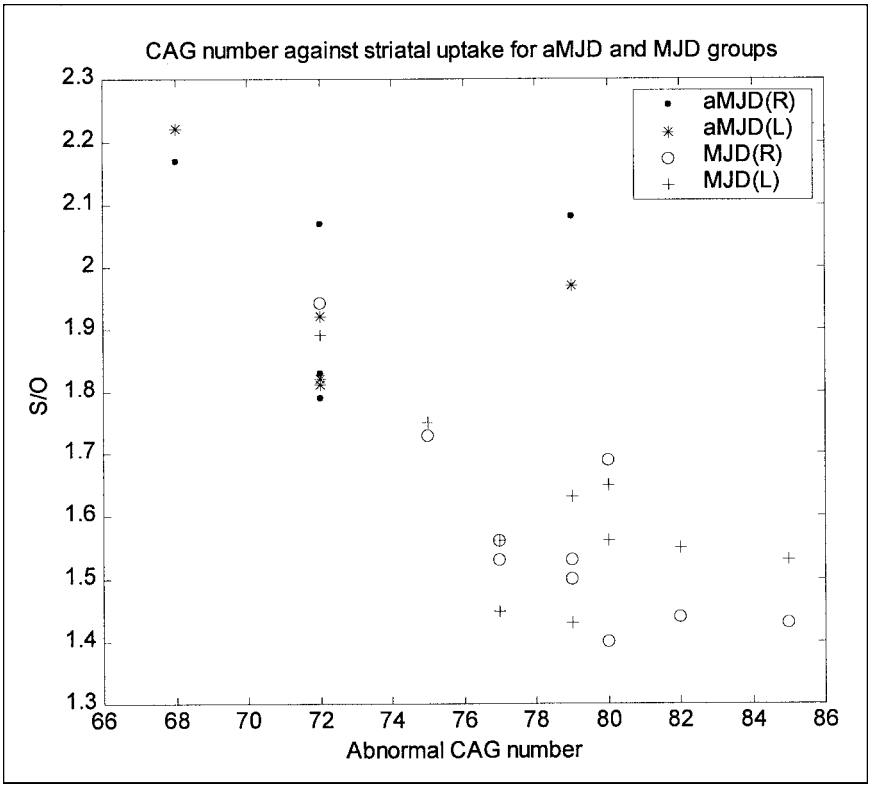

FIGURE 4. Relationship between abnormal CAG number and striatal binding was not obvious in aMJD group ($P = 0.48$ and $P = 0.44$ for right side [R] and left side [L], respectively). In contrast, negative association in MJD group was significant ($P < 0.001$ and $P = 0.006$ for right side and left side, respectively). S/O = striatal DAT binding.

DAT binding was one of the most sensitive in the detection of presynaptic dopamine function because of its downregulation in Parkinson's disease patients. Thus, we conclude that ^{99m}Tc-TRODAT-1 brain SPECT is a sensitive method to evaluate the nigrostriatal dysfunction through the binding ability with the DAT. Whether the nigrostriatal involvement is fully representative of the progression of MJD is a matter of debate. Another matter of debate is whether such nigrostriatal involvement is representative of the progression of all aspects of MJD. Besides, whether such progression is similar in the cerebellum, brain stem, and basal ganglia in MJD remains unresolved. However, at this time, the nigrostriatal integrity could be monitored with ^{99m}Tc-TRODAT-1 brain SPECT in the aMJD gene carriers.

MJD is an autosomal dominant pleiotropic disease. Although multiple phenotypes and varied pathologic findings within a family were often present, parkinsonian symptoms are rarely observed, especially for those with predominant parkinsonism, who were even more uncommon. However, the substantia nigra was frequently reported to be involved as were other structures, such as the subthalamic nucleus, pontine nuclei, dentate nuclei, Clark's column, spinocerebellar tracts, anterior horn cells, and posterior root ganglia, in autopsy cases (*2–7*). In our study, a significant reduction of striatal ^{99m}Tc-TRODAT-1 binding was found in all 5 aMJD carriers and the 10 MJD patients. Only 1 of the 10 patients showed rigidity and tremor in addition to cerebellar ataxia. The high incidence of the nigrostriatal dysfunction detected by ^{99m}Tc-TRODAT-1 brain SPECT faithfully corresponds to the high vulnerability of the dopamine neurons, as reported in pathologic studies (*2–7*). We suggest that using ^{99m}Tc-TRODAT-1 SPECT to evaluate the DAT activity may prove to be a practical method for monitoring the progression of MJD.

CONCLUSION

A significantly and symmetrically decreased DAT binding of ^{99m}Tc-TRODAT-1 is found in the striatum and particularly in the putamen of MJD patients and the aMJD gene carriers. This new radiopharmaceutical allows for early detection of this nigrostriatal derangement and provides an opportunity to be a useful and important marker for serial evaluations of idiopathic parkinsonism as well as its secondary form such as occurs in MJD.

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