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ORIGINAL ARTICLE

Age-related effects and gender differences in Japanese healthy controls for [^{123}I] FP-CIT SPECT

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Abstract
Objective Dopamine transporter (DAT) imaging with [^{123}I]FP-CIT (DaTSCAN) is a widely used diagnostic tool for Parkinsonism and dementia. Since it was approved by the Japanese Ministry of Health, Labor, and Welfare in 2013, there have been no articles focusing on a Japanese normal population. The aim of this study was to examine the effect of aging and gender on DAT availability in Japanese people.
Methods SPECT imaging of 30 healthy Japanese controls (17 males, 13 females; range 50–86 years, mean 70 years) was performed. SPECT images were reconstructed using a three-dimensional order subset expectation maximization (OSEM) algorithm with correction of the point spread function and scatter correction, without attenuation correction. The specific binding ratio (SBR) was calculated by DATview software. Statistical analyses were performed using linear regression analysis, analysis of variance, and multiple comparison analysis.
Results A strong correlation between the SBR and age was observed. The correlation coefficient in males and females were −0.566 and −0.502, respectively. The analysis of variance revealed that aging led to a decline of the SBR, and a significant difference ($p=0.005$) was observed among generations. Gender also affected the SBR, and there was a significant difference between males and females ($p=0.036$). The SBR in females was higher than in males. Consequently, the multiple comparison revealed a significant difference between 50s and 70s ($p=0.015$) and 50s and 80s ($p=0.006$).
Conclusions This is the first [^{123}I]FP-CIT SPECT study on subjects with normal dopamine function in Asian countries. This study provides a database of [^{123}I]FP-CIT SPECT in Japanese healthy controls. Higher DAT availability was found in women than in men. An average age-related decline in DAT availability of 8.9% was found in both genders. The data collected in this study would be helpful for Japanese physicians to make a differential diagnosis in Parkinsonian syndrome.
The registration identification number for this study is UMIN000018045.

Keywords [^{123}I]FP-CIT SPECT · Japan · Normal database · Dopamine transporter

Introduction

Single photon emission computed tomography (SPECT) imaging of the dopamine transporter (DAT) is widely used to assess presynaptic dopamine neuronal dysfunction in Parkinsonian syndrome and dementia with Lewy bodies (DLB). The European Medicines Agency (EMA) approved the agent [^{123}I]FP-CIT under the name DaTSCAN in

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2000 [1]. The US Food and Drug Administration (FDA) approved it under the trade name DaTscan in 2011 [2]. In Asia, it was first approved by the Japanese Ministry of Health, Labor, and Welfare in 2013.

In European countries, several studies on [^{123}I]FP-CIT SPECT in normal human populations have been reported [3–11]. All of them showed a progressive decline of DAT expression with aging. Some of them reported a gender difference, with higher striatal binding in women compared with men, while others did not find this difference. As far as we know, there have been no reports of this kind in Asian countries. In this study, we sought to evaluate the effect of both age and gender on [^{123}I]FP-CIT binding to dopamine transporters in a Japanese population.

Materials and methods

Subjects

Subjects were 30 healthy controls (17 males, 13 females; range 50–86 years, mean 70 years) who underwent [^{123}I]FP-CIT SPECT imaging. The study obtained the approval of ethical review board of our institution and was performed on the basis of informed consent from the subjects. All subjects were Japanese, applied for participation voluntarily, and understood the purpose of this study. Eligibility criteria included: healthy male and female Japanese volunteers (range 40–89 years); subjects required to attend examinations at an appointed date; a total score of the Unified Parkinson's Disease Rating Scale (UPDRS) Part III, which was performed 3 months before and after agreement was 0 (60 years or younger) or less than 5 (61 years or over); magnetic resonance imaging (MRI) images, which were performed 3 months before and after agreement, were normal for the age (allowed atrophy for age and small lacunar infarction); consent for the study was provided in a document. On the other hand, exclusion criteria included: alcoholism or a history of alcoholism; pregnancy, possible pregnancy, or lactating woman; epilepsy or history of epilepsy; an education history of 6 years or below; a history of neuropsychiatric disorder affecting cognitive brain function; severe complications (hepatopathy, nephropathy, and endocrine diseases); the use the drugs that influence the accumulation of [^{123}I]FP-CIT in the brain, antidepressants, psychotropic agents, sedatives (including an antianxiety drugs), cocaine hydrochloride, mazindol, methylphenidate hydrochloride, sertraline hydrochloride, and serotonin reuptake inhibitors.

Smoking data

All subjects were interviewed about smoking history and classified into three categories: non-smokers ($n=23$), ex-smokers ($n=4$) and active-smokers ($n=3$), as shown in the Table 1. The number of cigarettes smoked per day for active-smokers and packs in lifetime for active and ex-smokers were registered.

SPECT imaging

[^{123}I]FP-CIT (167 MBq) was injected intravenously as a bolus in a volume of 2 ml. Scans were obtained at 3 h after tracer injection, and the total scan time was 30 min. [^{123}I]FP-CIT SPECT imaging was performed using Siemens SYMBIA E with a low-middle energy general purpose collimator (LMEGP). The acquisition parameters were as follows: rotational radius fixed for all SPECT studies 15 cm; matrix 128 × 128; angular sampling 3° (360° rotation), and a pixel size of 3.3 mm. The photo-peak imaging window (158 keV ± 10%) and 2 scatter energy windows (below 142 keV −7%, above 173 keV +7%) were acquired.

As reported previously, reconstruction parameters effect image quality and quantitative value in [^{123}I]FP-CIT SPECT imaging [7, 12], although Varrone et al. described that the effect of attenuation correction on the SBR was small [11]. Matsumoto et al. reported that the optimal reconstruction parameters for order subset expectation maximization (OSEM) with resolution recovery, scatter, and attenuation correction have the potential to improve the performance and the image quality of [^{123}I]FP-CIT SPECT in comparison with the FBP reconstruction [13]. Therefore, we confirmed the optimal reconstruction parameters for OSEM, and validated the effects of three OSEM reconstruction methods: without attenuation correction and scatter correction (NOACSC), with only attenuation correction (AC) and with attenuation correction and scatter correction

Table 1 Age and smoking habits for 30 healthy volunteers

	Number	Mean age ± SD (range)	Current average number of cigarettes per day ± SD (range)	Total number of packages in lifetime mean (range)
Non-smokers	23 (12 males)	71.3 ± 11.7 (51–86)	0	0
Ex-smokers	4 (2 males)	71.3 ± 9.7 (58–84)	0	4084 (457–10,950)
Active smokers	3 (3 males)	60.3 ± 7.3 (50–66)	14.3 (10–20)	9156 (5475–11,315)

(ACSC). The count converged with an update number over 60 and the coefficient of variance (CV) continuously increased with the update number in each method. In the update number of 60, the striatum-background count ratio showed the approximate truth value for NOACSC. However, those indicated were 1.5 times higher than the truth value for ACSC and slightly higher for AC. Accordingly, SPECT images were reconstructed using an OSEM algorithm with 6 iterations and 10 subsets of 120 projections. Three-dimensional OSEM reconstructions were performed with correction of point spread function and NOACSC. Post-filtering was applied using Gaussian filter at 6.6 mm.

The specific binding ratio (SBR) was calculated using the ratio of specific binding to nonspecific accumulation of [^{123}I]FP-CIT in the striatum [14]. The SBRs were calculated using DATview software (AZE, Tokyo). The striatal region was defined from trans axial images within 44 mm thickness of the template volume of interest (VOI), and the reference region was semi-automatically defined from the non-specific accumulation in the whole brain, excluding the striatal region, by count threshold. The SBRs were calculated as follows:

$$\mathrm{SBR} = \left(\frac{C_{\mathrm{StrVOI}} - C_{\text{per pixel BG VOI}} \times \text{Number of pixel}_{\mathrm{StrVOI}}}{\mathrm{Vol}_{\mathrm{str}}} \right) \frac{1}{C_{\text{per pixel BG VOI}}}$$

where C_{StrVOI} is the count of the striatum VOI, $C_{\text{per pixel BG VOI}}$ is the count of the reference VOI per pixel, number of $\mathrm{pixel}_{\mathrm{StrVOI}}$ is number of pixels in the striatum VOI, and $\mathrm{Vol}_{\mathrm{Str}}$ is the volume of the striatum that is 11.2 ml const. The absolute value of asymmetry index (AI) was obtained using the following formula: $[(R-L)/(R+L)] \times 2 \times 100$, where R and L represent right and left SBR, respectively.

Statistical analysis

The differences in the SBR were evaluated using a one-way analysis of variance (ANOVA) to validate the effects of three categories between non-smokers, ex-smokers and active-smokers. A correlation coefficient was calculated between the SBR and the number of cigarettes per day. Linear regression analysis was performed to investigate the relationship between age and the SBR in both genders. The Bartlett test was performed to confirm homogeneity of variance, which was required for analysis of variance. The differences in the SBR were evaluated a using a two-way factorial ANOVA to validate the effects of 2 variables, age and gender, which affect the SBR. In addition, a multiple comparison using Tukey's honestly significant difference (HSD) test was performed to investigate the effects of age. Differences between the right and left SBR were evaluated with a paired *t*-test. Statistical analyses were conducted using R version 3.3.0 (R Core Team 2016).

Results

Representative SPECT images of healthy controls aged from 50 to 86 years are shown in Fig. 1. These images show that [^{123}I]FP-CIT binding in the caudate nucleus

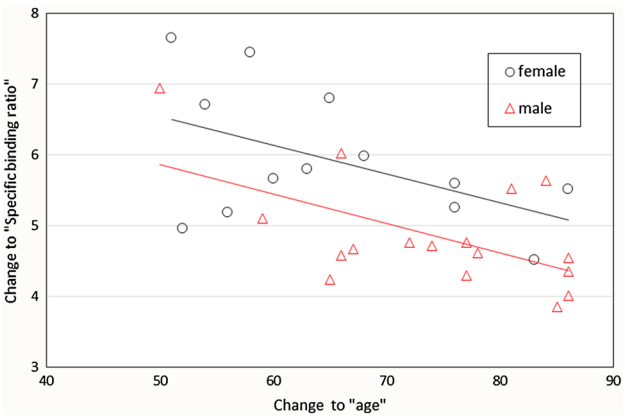

Fig. 2 Linear regression analysis of the SBR values obtained in males and females as a function of age

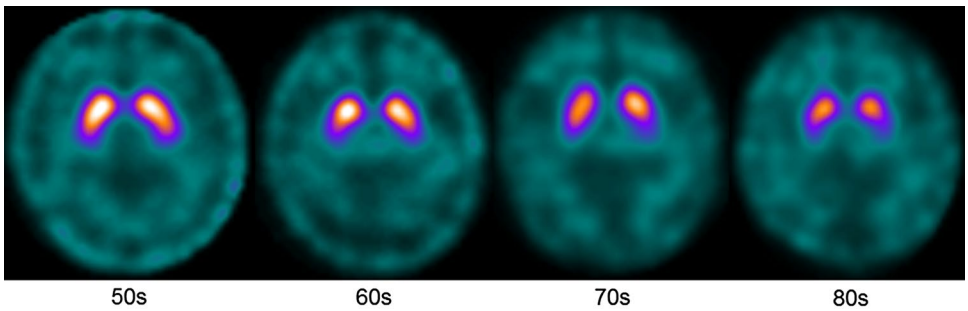

Fig. 1 Representative SPECT images of ^{123}I-FP-CIT in subjects in their 50s, 60s, 70s, and 80s. The pixel intensity of the scaled SPECT image was normalized by the SBR. The SBR image was displayed with predefined *color table* and *upper* threshold fixed at SBR = 6

and putamen is likely to decrease with aging. Linear regression analysis between age and SBR is shown in Fig. 2. A strong correlation between the SBR and age was observed. The correlation coefficient in males and females was −0.566 and −0.502, respectively. The relationship between age and the SBR in males and females is shown in Fig. 3. The average SBR in males was lower than that in females in each generation. The SBRs declined between generations: 13.1% (50–60s), 11.2% (60–70s), and 2.4% (70–80s). An average age-related decline in DAT binding of 8.9% per decade was found. When we assume the cutoff score for the SBR equals the average minus the standard deviation, it was 5.14 in 50s, 4.65 in 60s, 4.63 in 70s, and 4.17 in 80s. *p*-values of age and gender were greater than 0.05 in the Bartlett-test, therefore variance in each group was equal. Two-way factorial analysis of variance (ANOVA) showed that aging led to a decline in the SBR, and a significant difference ($p=0.005$) was observed among generations. Gender also affected the SBR in the ANOVA, and there was a significant difference between males and females ($p=0.036$). However, an interaction between age and gender was not confirmed ($p=0.740$). From these results, a multiple comparison was conducted (supplementary Fig. 1). Consequently, a significant difference between 50s and 70s ($p=0.015$), and 50s and 80s ($p=0.006$) was confirmed. In contrast, there was no difference in the SBR ($p=0.364$) between the right and left striatal regions in a paired *t*-test. The correlation coefficients of the AI, ranging from 0.5 to 19.3, in males and females were −0.0895 and 0.0783, respectively. Moreover, no significant difference was observed among generations ($p=0.811$), between each gender ($p=0.973$) and an interaction between age and gender ($p=0.286$) in the ANOVA (Fig. 4).

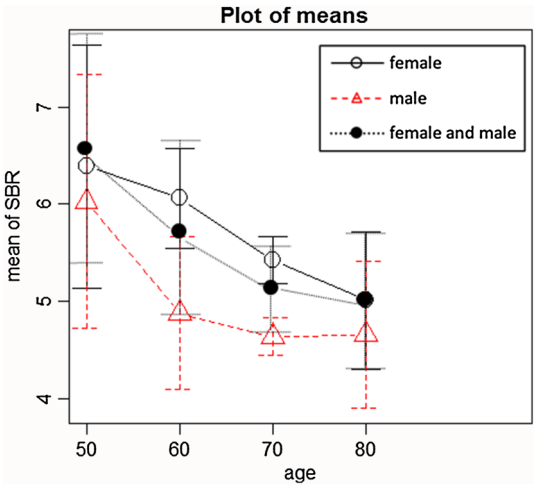

Fig. 3 Mean of the SBRs in males and females as a function of age

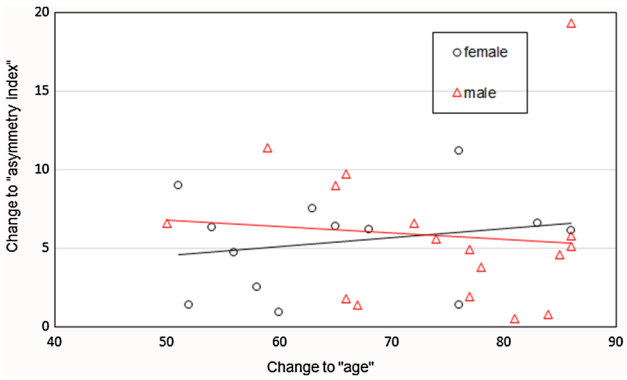

Fig. 4 Linear regression analysis of the AI values obtained in males and females as a function of age

There was no significant difference ($p=0.46$) between non-smokers, ex-smokers and active-smokers (Fig. 5). Moreover, the correlation was not observed between the number of cigarettes per day and SBR ($r=0.156$) (Supplementary Fig. 2).

Discussion

This is the first [^{123}I]FP-CIT SPECT study on subjects with normal dopamine function in Asian countries. Our study of a Japanese population demonstrated that women had significantly higher DAT availability than men, and that there was an age-related decrease. An average age-related decline in DAT binding of 8.9% per decade was found between people in their 1950s and 1980s.

The higher age-related decline rate (8.9%) in our study compared with 6.5% in western countries [11] may be associated with the racial difference in the polymorphism of the dopamine transporter gene, *hDAT1*(SLC6A3). A

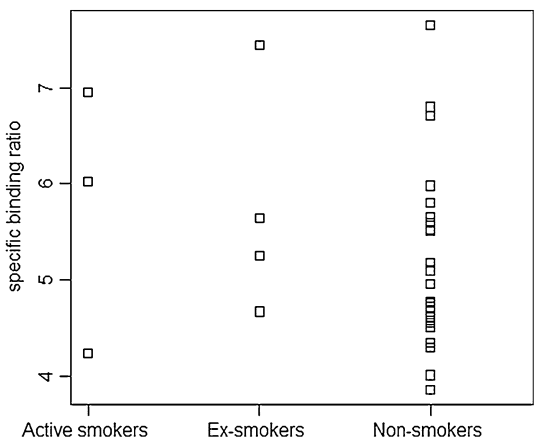

Fig. 5 Relationship between SBR and number of cigarettes per day

polymorphism involving a variable number of tandem repeats has been reported in the 3′ untranslated region of SLC6A3 gene coding for the DAT. This polymorphism has 2 common alleles, designated as 10-repeat and 9-repeat. The frequency of a 10-repeat allele, a dominant allele, was higher in the Japanese than in the Caucasian population while a 9-repeat allele decreased in Japanese participants compared with the Caucasian controls [15]. It was reported that the expressions of DAT mRNA and protein were decreased in the 9-repat carriers [16]. Therefore, the amount of striatal DAT protein in the Japanese population may be higher than in European countries. DAT knockout mice displayed reduction of age-related locomotor effects and dopamine metabolites in the striatum [17], suggesting that high level of DAT protein could alter aging of dopaminergic systems.

DAT is expressed in the presynaptic axonal terminals of nigrostriatal pathways, and striatal DAT may be associated with nigral dopamine neuronal loss. In a group of autopsy-confirmed cases with Alzheimer's disease, DLB, and Parkinson's disease dementia, [^{123}I]FP-CIT uptake was associated with nigral dopaminergic density [18]. Our analysis of a normal population aged between 50 and 80 years demonstrated that the average age-related decline in the SBR was 8.9% per decade. Previous studies in healthy controls aged between 20 and 80 years reported a 3.6–7.5% decrease in [^{123}I]FP-CIT binding [3–5, 7, 10, 11, 19]. An analysis of autopsied brains from subjects aged from 21 to 91 years revealed that there was a decrease of pigmented neurons in the pars compacta of the substantia nigra at a rate of 4.7% [20]. Therefore, the aging effect of [^{123}I]FP-CIT uptake in the basal ganglia is likely to be attributed to dopaminergic cell number in the substantia nigra.

Our study reported that women had significantly higher DAT availability than men. Studies in western countries have shown higher [^{123}I]FP-CIT binding in women than in men [7, 11, 19], although 2 studies have shown negative findings [8, 19]. The binding of striatal DAT labeled with [^{3}H]GBR 12,935 and substantia nigra DAT mRNA were higher in female rats compared with males [21]. Laboratory studies show that estrogen may play a neuroprotective role. A subcutaneous injection of 17β-estradiol into male C57Bl/6 male mice increased the number of tylosine hydroxylase-immunoreactive neurons in the substantia nigra pars compacta [22]. Estradiol modulates dopamine release via membrane estradiol receptors [23]. Parkinson's disease incidence has been shown to be 1.5 times higher in men than women [24]. The gender difference in DAT availability may reflect a protective effect of estrogen on dopaminergic cells.

The SBR could be estimated too low in patients with enlargement of ventricles or atrophic brains, and can differ with the use different cameras. Quantification of [^{123}I] FP-CIT SPECT has been shown to be affected by reconstruction strategies, AC, and SC [13, 25, 26]. However, we could minimize the influence of these various factors that affect [^{123}I]FP-CIT SPECT imaging, because all images were able to be obtained with identical imaging parameters in a single institutional study. Another weakness of this study is that the number of subjects was relatively small. Especially, the ratio of females to males was deviated in some generations, including only 2 males in 1950s and 2 females in 1970s and 1980s. Further study using a larger population in multiple centers is needed to establish a Japanese database for clinical trials.

In conclusion, we performed [^{123}I] FP-CIT SPECT analysis for the first time in Japanese healthy controls. The average value minus a standard deviation of the SBR between 50 and 80 years of age was 4.64, which is almost same (4.5) as the cutoff originally proposed [14]. The data collected in this study would be helpful for Japanese physicians to make a differential diagnosis of Parkinsonian syndrome or dementia with Lewy bodies.

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Compliance with ethical standards

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

Conflict of interest This research was supported by Nihon Medi-Physics CO., Ltd.

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