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*　　*　　*

# Seven Cases of Late-Life Depression Treated With Cilostazol-Augmented Therapy

***To the Editors:***

Recent progress in diagnostic imaging has revealed significantly high prevalence of diffuse white matter lesions and asymptomatic lacunar infarction in late-life depression.[1] Based on the results of previous research on late-life depression using magnetic resonance imaging (MRI), in 1997, Alexopoulos et al[2] defined the depression complicated with cerebrovascular lesion, as discovered through image diagnosis, and so forth, as "vascular depression." Vascular depression is poorly responsive to antidepressants or electroconvulsive therapy, leading to frequent recurrence.

Meanwhile, the efficacy of an antiplatelet agent, cilostazol, for prevention of recurrence of cerebral infarction has recently been demonstrated.[3] Cilostazol has attracted attention because it not only inhibits platelet aggregation but also promotes vasodilation, as shown in many reports on its effect for improvement of blood flow in the brain, offering hope for improvement of various symptoms associated with cerebral infarction.[4,5]

We had 7 cases of elderly patients diagnosed with major depressive disorder that had insufficiently responded to antidepressant treatment yet responded markedly to additional treatment with cilostazol. All patients were older than 65 years (2 men and 5 women), with a mean age of 74.0 years (SD, 6.5 years). All met *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* criteria for major depressive disorder. All had deep white matter hyperintensity (DWMH) and/or periventricular hyperintensity (PVH) on MRI, while multiple lacunar infarctions were also found in 3 cases. Before the start of cilostazol treatment, antidepressants were administered for 4 weeks. Clinical features were evaluated using Hamilton Depression Scale. The severity of DWMH and PVH was evaluated using rating scales of DWMH and PVH. Cilostazol was discontinued before 10 weeks in 3 cases because of headache or palpitation of mild severity without remarkable changes in brain computed tomography, electroencephalogram, or electrocardiogram. These side effects disappeared immediately after discontinuation of cilostazol (Table 1).

Although all cases did not adequately respond to 4-week treatment with antidepressants, the symptoms were remarkably improved in 6 of the 7 cases by administration of cilostazol, with percentages of improvement correlated with severity of DWMH ($R = 0.85$, $P < 0.05$). This result suggests

**TABLE 1.** Patient Characteristics and Course of Cilostazol-Augmented Therapy

| | | | Brain MRI | | | Cilostazol | | | HAM-D Score | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Case | Age, yr | Sex | DWMH | PVH | Lacunar | Antidepressant, mg | Initial Dose, mg | Final Dose, mg | Baseline | 1 wk | 2 wk | 3 wk | 4 wk | 8 wk | 10 wk | Improvement, % |
| 1 | 78 | M | 1 | 1 | + | Mianserin, 30 | 50 | 100 | 11 | 2 | | 3 | 2 | 2 | 2 | 82 |
| 2 | 66 | F | 0 | 2 | – | Paroxetine 40 | 50 | 100 | 23 | | | | | 15 | 13 | 43 |
| 3 | 67 | F | 0 | 1 | – | Maprotiline, 50 | 50 | 50 | 15 | | | * | 15 | | | 0 |
| 4 | 73 | F | 2 | 2 | – | Maprotiline, 75 | 50 | 200 | 29 | 26 | 19 | 13 | 11 | 12 | 11 | 62 |
| 5 | 85 | F | 1 | 3 | – | Milnacipran, 60 | 50 | 150 | 16 | 8 | | | | * | | 50 |
| 6 | 74 | F | 1 | 1 | + | Milnacipran, 60 | 100 | 100 | 11 | 5 | 5* | | | | | 55 |
| 7 | 75 | M | 2 | 2 | + | Paroxetine, 20 | 100 | 100 | 21 | 12 | | | 10 | 3 | 2 | 90 |

*Discontinued because of headache or palpitation of mild severity (both are reported to be major side effects of cilostazol).[3]
HAM-D indicates Hamilton Depression Scale.

that the mechanism of the depression-improving effect observed in our cases is attributable to the cerebral vasodilation and consequent cerebral blood flow improvement promoted by cilostazol, and also that the severity of DWMH may be an estimated marker for response to cilostazol-augmented therapy. This experience raises the possibility that cilostazol augmentation could be a therapeutic strategy for late-life depression.

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# Event-Related Desynchronization of Frontal-Midline Theta Rhythm During Preconscious Auditory Oddball Processing

**Masaru Kawamata, Eiji Kirino, Reiichi Inoue and Heii Arai**

Key Words

Event-Related Desynchronization
Event-Related Potential
Frontal-Midline Theta Rhythm
Videogame

**ABSTRACT**

The goal of this study was to explore the frontal-midline theta rhythm (Fm theta) generation mechanism employing event-related desynchronization/synchronization (ERD/ERS) analysis in relation to task-irrelevant external stimuli. A dual paradigm was employed: a videogame and the simultaneous presentation of passive auditory oddball stimuli. We analyzed the data concerning ERD/ERS using both Fast Fourier Transformation (FFT) and wavelet transform (WT).

In the FFT data, during the periods with appearance of Fm theta, apparent ERD of the theta band was observed at Fz and Cz. ERD when Fm theta was present was much more prominent than when Fm theta was absent. In the WT data, as in the FFT data, ERD was seen again, but in this case the ERD was preceded by ERS during both the periods with and without Fm theta. Furthermore, the WT analysis indicated that ERD was followed by ERS during the periods without Fm theta. However, during Fm theta, no apparent ERS following ERD was seen.

In our study, Fm theta was desynchronized by the auditory stimuli that were independent of the video game task used to evoke the Fm theta. The ERD of Fm theta might be reflecting the mechanism of "positive suppression" to process external auditory stimuli automatically and preventing attentional resources from being unnecessarily allocated to those stimuli. Another possibility is that Fm theta induced by our dual paradigm may reflect information processing modeled by multi-item working memory requirements for playing the videogame and the simultaneous auditory processing using a memory trace. ERS in the WT data without Fm theta might indicate further processing of the auditory information free from "positive suppression" control reflected by Fm theta.

**INTRODUCTION**

Conditions requiring continuous attention or concentration in order to perform a task have been shown to evoke a particular rhythm at midline Cz and Fz leads, i.e., the frontal-midline theta rhythm (Fm theta).[1,2] Fm theta is defined as a train of rhythmic waves, observed in the frequency band of 5.5-7.5 Hz, and having a focal distribution with a maximum around the frontal midline in the electroencephalogram (EEG) of normal subjects.[1,3,4] The train usually lasts for several seconds and tends to wax and wane.[1,3]

Magnetoencephalogram (MEG) studies modeled the generator sources of Fm theta in various parts of the lateral frontal cortex of both hemispheres or in a large area of the medial prefrontal cortex including the anterior cingulated cortex (ACC).[5-8] Despite these findings, the neuronal substrates underlying the Fm theta generation mechanism have not been fully elucidated. One way to assess the stimulus-related responses of different EEG frequency bands is event-related desynchronization (ERD) and synchronization (ERS) analysis.[9,10] ERD is defined as a phasic relative amplitude decrease, whereas ERS denotes a phasic relative amplitude increase in a defined frequency band occurring in relation to an event.[11] It has been documented that distributed theta, alpha, and gamma oscillatory systems might act as communication networks with functional relations to memory and integrative functions.[12-14]

It has been indicated that the ERD of theta and alpha rhythms during an auditory memory task could be closely associated with higher cortical processes such as memory functions, rather than with auditory stimulus processing per se.[15] Theta synchronization is related to episodic memory performance,[16-18] or interpreted to reflect visuo-spatial working memory processes.[19] Since theta long-term potentiation (LTP) is closely linked to the synchronous activity of the hippocampal theta rhythm,[20,21] theta synchronization may be related to the encoding of new information.[16,18] The hippocampal theta may be transmitted to the cortex via hippocampal cortical feedback loops.[16,18] The authors suggested that the initiation and maintenance of stimulus-

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locked hippocampal theta observed in neuromagnetic responses may facilitate processing of potentially salient and/or novel input with respect to a context established by the contents of working memory. This may be essential for orientation to perturbations in the sensory environment, a function requiring the use of a context established by a constellation of stimuli.[12] Furthermore, the induced band power (IBP),[22] a variant method of ERD, in a visuo-spatial working memory task revealed that the sustained theta decreased more over the frontal electrodes for memory trials than for the sensory trials.

A Sternberg working memory task evoked a phenomenon termed "cognitive gating of theta oscillations" in which the amplitude of theta oscillations increased dramatically at the start of a trial, continued through all phases of the trial, including the delay period, and decreased sharply at the end.[23] These results suggest that theta oscillations could have an important role in organizing working memory. These findings are compatible with the finding that theta or Fm theta during working memory tasks increased and overt performance improved after practice on the tasks and that theta increased with increased task difficulty.[6,7]

Therefore, ERD/ERS may serve to assess cognitive processes specifically associated with a specific EEG rhythm such as Fm theta. The goal of this study was to explore the generation mechanism of Fm theta employing ERD/ERS analysis for the theta rhythm in relation to task-irrelevant external stimuli. We employed a dual-task paradigm to explore reactivity to cognitive events not involved in the evoking of Fm theta. The primary task was a videogame (designed to evoke Fm theta) and a passive auditory oddball task with stimuli independent of the videogame.

ERD/ERS are different phenomena from phase-locked EEG activity including all types of event-related potentials (ERPs). For the quantification of phase-locked EEG activity the technique of signal averaging is employed, whereby the non-phase-locked EEG such as ERP/ERS is attenuated. Phase-locked and non-phase-locked EEG activity cannot be distinguished when both types of activity are within the same frequency band. This means an ERP can mask ERD/ERS.[24] With the classical ERD methods, event-related EEG data are first bandpass filtered, the samples squared and then averaged over trials. Bandpass filtering of each trial, squaring of samples and averaging across all trials, results in a time course of instantaneous band power (power method). Bandpass filtering of each trial and calculating the intertrial variance by averaging all the trials also results in a time course of instantaneous band power (intertrial variance method). The difference between the two methods is that in the power method both phase-locked and non-phase-locked EEG activities contribute to the band power changes, while in the intertrial variance method only the non-phase-locked activity is quantified.[24]

The intertrial variance method attenuates the phase-locked activity, whereas the presentation of a stimulus may lead to a resetting of the phase of an EEG/MEG oscillation.[25,26] Since the phase resetting is evoked by the stimulus, the induced activity "becomes" evoked activity for a short period of time. Specifically, P300, evoked by an "oddball" paradigm, may be a result of a transient event-related phase-resetting of induced rhythmic activity.[27,28] Thus, the averaged ERP may be partly composed of synchronized background activity.[29]

Furthermore, a disadvantage of the classical ERD/ERS including the intertrial variance method technique using band pass filtering such a short-term Fast Fourier Transformation (FFT) is that it has a relatively poor temporal resolution.[30] On the other hand, the wavelet transform (WT) method, based on a time-frequency (TF) wavelet decomposition, can quantify a time-varying energy of the signal in each frequency band's oscillatory activity.[31] This method provides a better compromise between time and frequency resolution[32] than previously proposed ERD/ERS methods using FFT filtering.[33] Hence, we analyzed the data concerning ERD/ERS using FFT and the intertrial variance method at first and secondly reanalyzed using WT to confirm the results of FFT analysis. The time resolution of a WT is better at high frequencies whereas its frequencies resolution is better at lower frequencies. WT is well suited for the dynamic of EEG signal, in which low frequencies change more slowly than higher frequencies,[29] as general in cognitive tasks evoking Fm theta.

## METHODS AND MATERIALS

### Subjects

Eight healthy volunteers (24-33 years, six males), who had frequently showed Fm theta (Figure 1) in preliminary EEGs, were recruited. All subjects reported that they were in good physical health and had normal hearing. After a complete description of the study was presented to the subjects, all subjects gave informed consent for the protocol, which was approved by the Institutional Review Board of the Juntendo Institute of Mental Health.

### Experimental tasks and procedure

To elicit Fm theta, subjects played a videogame while simultaneously being presented with "passive" auditory oddball stimuli that required no response. Subjects were instructed to ignore the auditory stimuli and concentrate on the videogame.

### Videogame

Subjects played Tetris® on a video screen that subtended a visual angle of 5.4° x 5.4°. Tetris is a puzzle game where seven different types of blocks continuously fall from above and the player must arrange them to make horizontal rows of bricks. Completing any row causes those blocks to disappear and the rest above move downwards. The blocks above gradually fall faster, and the game is over when the screen fills up and blocks can no longer fall from

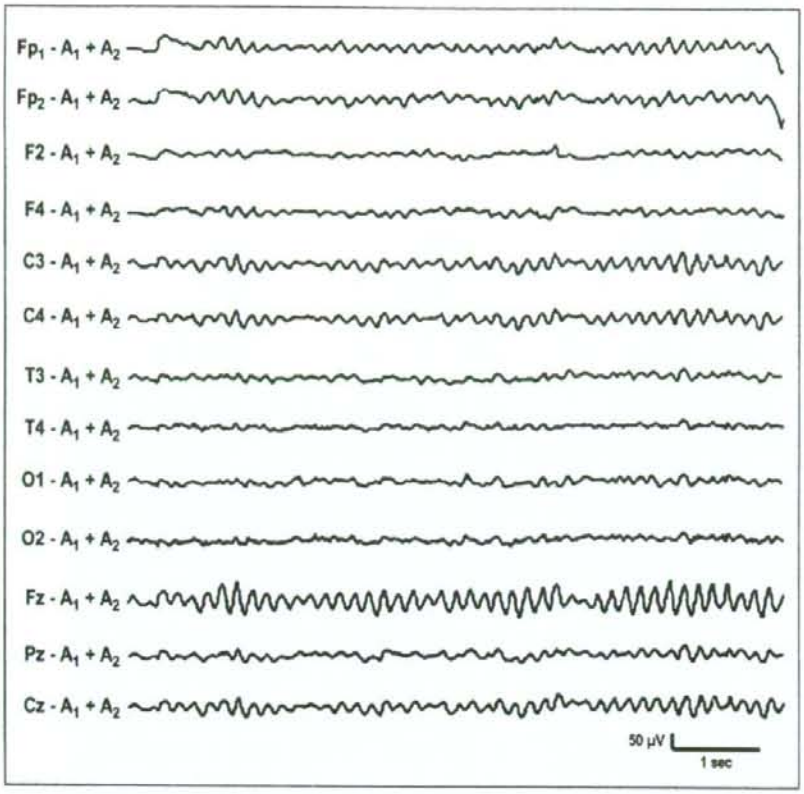


**Figure 1. Representative EEG tracings of Fm theta observed in a male subject.**
The subject showed rhythmic theta activities at midline Fz and Cz leads during the video game task in the preliminary EEG test. Note that the montage of EEG sites in the preliminary tests is different from that in digital recording for ERP and ERD/ERS analyses.

the top. Tetris requires the subjects to maintain a high level of sustained attention and concentration. Subjects practiced the Tetris game for 5 minutes prior to the start of the dual-task condition.

**Auditory oddball stimuli**

The auditory stimuli consisted of tones (sine waves) with a duration of 80 ms, including 10 ms rise and fall times. The frequency of the standard tones (**standards**) (probability = 0.85) and the oddball or deviant tones (**deviants**) (probability = 0.15) were 1,000 and 1,050 Hz, respectively, and the onset-to-onset interval was randomized between 1500-2000 ms. A computer with custom-designed software generated the auditory stimuli and controlled their timing and presentation. Tones were presented binaurally at a constant listening level (75 dB sound pressure level) through electrically shielded headphones. The subjects were instructed to avoid unnecessary eye movement and eye blinking during the session.

**EEG recording**

EEG was recorded using Ag/AgCl disk electrodes placed at 10 scalp sites ($Fp_1$, $Fp_2$, Fpz, $F_3$, $F_4$, Fz, $C_3$, $C_4$, Cz and Pz of the International 10/20 System). Recording electrodes were referenced to the nose. In addition, a ground electrode was placed at the forehead. A bipolar electrode pair was placed above and over the outer canthus of the right eye to record the electrooculogram (EOG). Impedances of all electrodes were maintained below 5 K . EEG data was recorded and analyzed using a Brain Atlas 2 and Ceegraph system (Bio-logic). During the task, the EEG and EOG were continuously digitized at 250 Hz per channel and stored on a computer disk using a 0.1-100 Hz on-line filter. The EEG was filtered off-line with a bandpass of 0.1-35 Hz (-3dB points; 6 dB/octave). The digital event markers for the deviants or standards were synchronized to the stimulus onsets and were stored. EEG epochs of 1024 ms duration (256 points) (100 ms prestimulus baseline, 924 ms poststimulus) associated with each stimulus type were excised from the continuous records. The root mean square voltage of the EOG channel was computed to identify and discard epochs associated with eye movements and blink artifacts. All single trial epochs were baseline corrected prior to subsequent processing. Epochs contaminated by EOGs, blinks, or muscle artifacts exceeding an artifact rejection threshold of ±80 μV at any electrode were omitted from the analysis. The experimental task was continued until 400 clean deviant epochs were obtained.

**Criteria of Fm theta**

The criteria for defining Fm theta were as follows: (1) 5.5-7.5 Hz rhythmical sinusoidal configuration with a

maximum at Fz or Cz, (2) markedly higher amplitudes as compared with background activity, and (3) duration exceeding 1 s.[1,2]

**EEG analysis**

Artifact-free epochs were segregated according to the two types of auditory stimuli. Each raw EEG epoch was visually screened, and epochs where Fm theta was observed throughout, or where no Fm theta appeared at all, were identified. For each of the resulting four types of epochs, (standards and deviants with and without Fm theta), ERD/ERS and ERP were constructed. Fifty artifact-free epochs were analyzed for each type of epoch.

**ERP**

After filtering off-line with a bandpass of 0.1-35 Hz, the EEG epochs of standards and deviants both with and without Fm theta were averaged for each subject individually, and then across all subjects to compute group-average ERPs.

**ERD/ERS (FFT)**

The EEG data were filtered with FFT bandpass filtering so that the theta (5.5-7.5 Hz) band activity was extracted.

The calculation of the variance by the intertrial variance method is as follows:

$$\text{Variance}_{(j)} = \frac{1}{N-1}\sum_{i=1}^{N}(X_{(i,j)} - \bar{X}_{(j)})^2$$

$$\bar{X}_{(j)} = \frac{1}{N}\sum_{i=1}^{N}X_{(i,j)}$$

Where N = total number of trials, $X_{(i,\,j)}$ = j-th sample of the i-th trial of the bandpass filtered data and $\bar{X}_{(j)}$ = mean of the data at the j-th sample (averaged over all bandpass filtered trials).[24] In power methods, phase-locked activity is not excluded, because $\bar{X}_{(j)}$ is not subtracted from $X_{(i,j)}$. The ERD was quantified as the percentage change of the intertrial variance at each sample point relative to the average intertrial variance in the 100 ms prestimulus baseline as a reference interval. Individual subject's ERD/ERS for each condition was calculated using intertrial methods, and then low-pass filtered at 4 Hz to obtain reliable power estimate. Individual subject's ERD/ERS was averaged across all subjects to compute a group-average ERD/ERS.

ERD/ERS of the slow alpha (8-10 Hz) and the fast alpha (10-12 Hz) band were also constructed using epochs without Fm theta.

**ERD/ERS (WT)**

We modified the procedures of Tallon-Baudry et al[31] and Mazaheri et al.[29] The time-varying energy in the single trial was calculated by convolving the recorded activity with complex Morlet's wavelets that have a Gaussian shape both in the time domain and in the frequency domain around its central, and then varied in width and frequency to obtain the energy at different frequencies in the spectrogram. Since the Morlet's wavelet is a modulated Gaussian curve, time points at the beginning and end of the wavelet window would show an attenuation of the frequencies.[23] We subjected the entire 1024 ms epochs to the WT, but examined only the time point interval from the stimulus onset to 800 ms.

The time-varying energy of the signal in a frequency band is the square norm of the result of the convolution of a complex wavelet with the signal. The time resolution of a WT is better at high frequencies whereas its frequencies resolution is better at lower frequencies. Analyses of the resultant spectrogram were obtained by calculating time-varying energy at 119 different frequencies from 2 to 35.71 Hz (Figure 2). After averaging the time-varying energy of each single trial in the individual, ERD/ERS was assessed in terms of percentage change scores from the mean energy during the reference period (0-100 ms from the stimulus onset). ERD/ERS of the theta band were obtained by averaging the time-varying energy change (%) at 5.00, 5.56, 5.95, 6.58, 6,94, 7.35 Hz.

According to the method of Mazaheri and Picton,[29] we did not subtract the spectrogram of averaged ERP (phase-locked activity) from the averaged spectrogram from the individual EEG trial. The usage of the subtraction method to remove phase-locked evoked activity does not fully remove the power of the ERP from spectrum, and the validity of the subtraction method has not been established.[29,34,35]

**Statistical analysis**

ERD/ERS of the theta band at Fz and Cz, where the Fm theta is usually the greatest, was analyzed by means of an analysis of variance (ANOVA) with repeated measures. For FFT data, ANOVA was conducted using five time-windows with time duration of 200 ms; 1000 ms (prestimulus 100 ms and poststimulus 900 ms) was separated down to 200 ms. For WT data, 800 ms (poststimulus 800 ms) was separated down to eight windows of 100 ms. ERD/ERS data were averaged over time-window for each electrode and each type of epoch within an individual subject.[13,18,19,23] There were four within-subject factors, Electrode (Fz, Cz), Stimulus (standard, deviant), Fm-theta (epochs with or without Fm theta), and Time (five time-windows). Reduced degrees of freedom (Greenhouse-Geisser) were used when appropriate to counter violations of the sphericity assumption underlying ANOVA with repeated measures (epsilon values are provided). Alpha values of 0.05 were considered significant. All statistics were performed using SPSS for Windows (SPSS, Chicago, IL).

## RESULTS

**ERP**

On the waveforms for the standards and deviants, during the periods with or without the appearance of Fm theta (Figure 3), ERP components, N100, P200, and N200, were clearly identified for both the standards and deviants. No prominent deflections of P3a or P3b were observed. It might be because the oddball task was completely passive and the auditory stimuli were not distractive or novel.

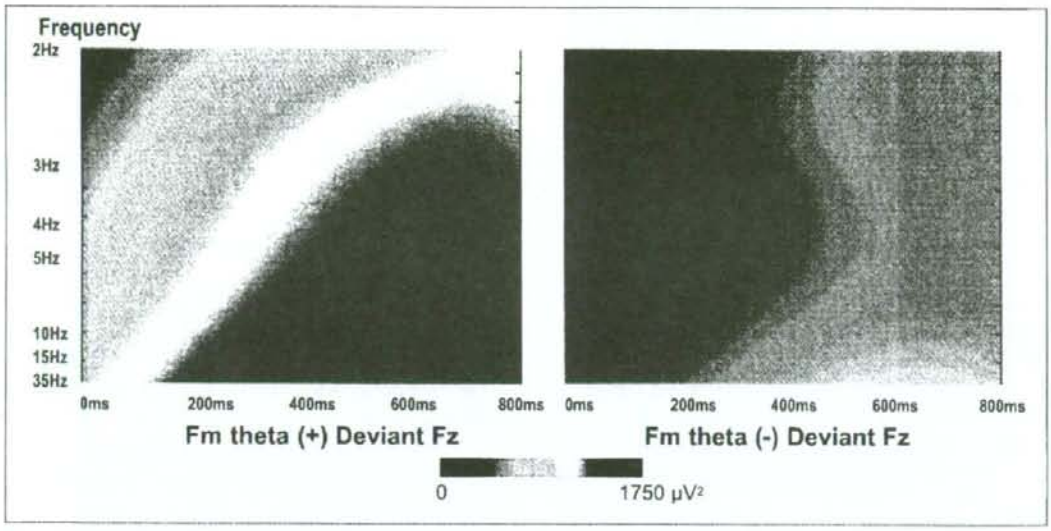


**Figure 2. Examples of WT spectrogram (deviants, Fz).**
Each of the wavelet estimations of the time changes in energy at a particular frequency, averaged across single trials, grand averaged across subjects, was presented with x-axis for time, y-axis for frequency (not yet baseline-subtracted or converted on the percentage of the average of baseline) and the stimulus onset indicated at 0ms. Energy values are coded on a color scale. Note that the time resolution of a WT is better at high frequencies whereas the frequencies resolution is better at lower frequencies.

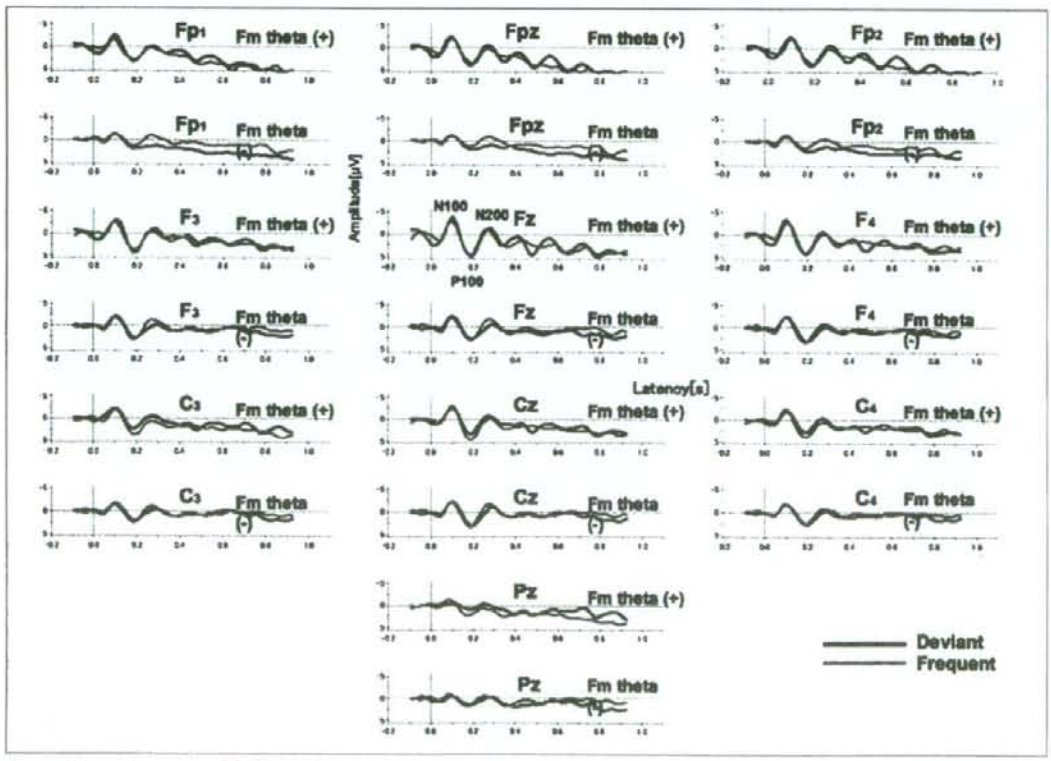


**Figure 3. Grand average of ERP waveforms.**
ERP components, N100, P200, and N200 were apparently recognized for both deviants and standards.

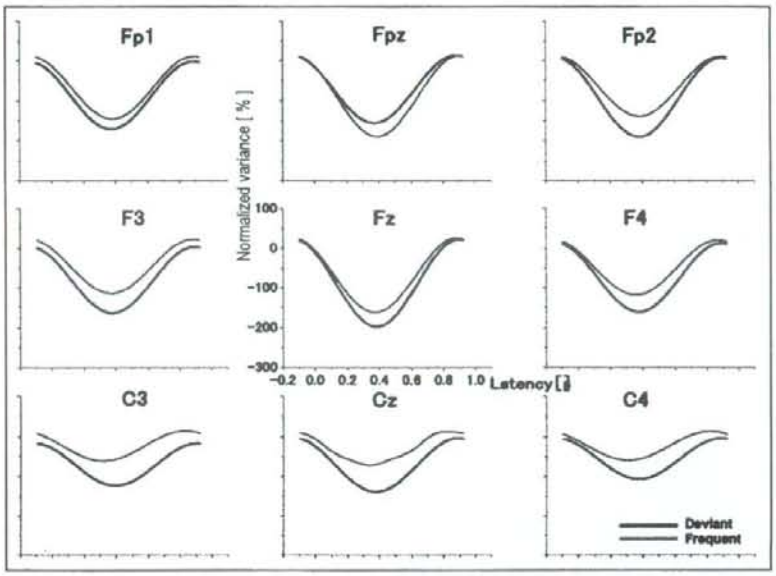


**Figure 4.**
**Grand averaged theta ERD during the periods with the appearance of Fm theta (FFT).**
During the periods when Fm theta appeared, apparent theta ERD was observed at Fz and Cz.

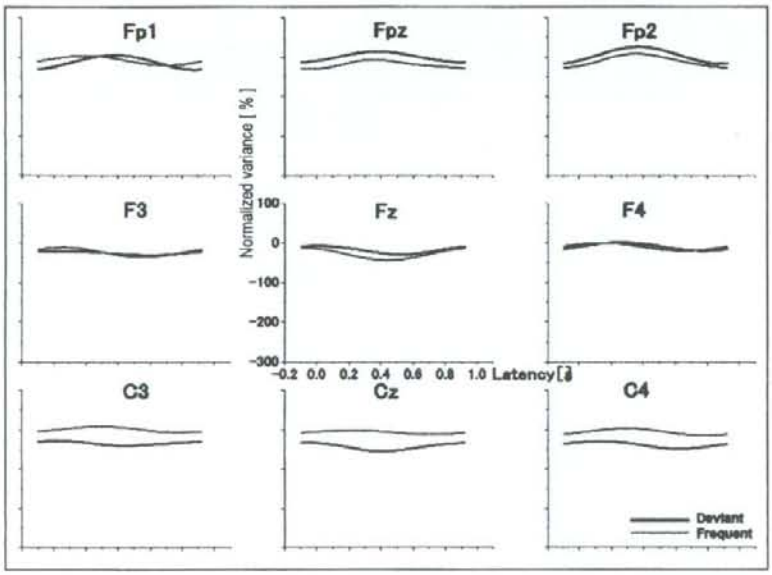


**Figure 5.**
**Grand averaged theta ERD during the periods without the appearance of Fm theta (FFT).**
During the periods without Fm theta, no apparent ERD/ERS was recognized for either the standards or deviants.

**ERD/ERS (FFT)**

During the periods with Fm theta, ERD of the theta band was clearly visible, peaking approximately 400 ms after the onset of stimulus with a deflection decrease of more than 100 % from the baseline for both the standards and deviants (Figure 4). During the periods without Fm theta, however, no apparent ERD/ERS was recognized for either the standards or deviants (Figure 5). ANOVA revealed significant effects of Stimulus [$F(1,7) = 10.220$, $p = 0.015$], FM-theta [$F(1,7) = 6.418$, $p = 0.039$] and Time [$F(4,28) = 6.914$, $p = 0.030$, $\varepsilon = 0.271$]. ANOVA also revealed significant interactions of Fm-theta x Time [$F(4,28) = 10.332$, $p = 0.008$, $\varepsilon = 0.319$], which indicated that the ERD with the appearance of Fm theta was more prominent than that during the periods without Fm theta. The interaction of Fm-theta x Time was confirmed by the post-hoc test results that during the period of 100-500 ms ERD with Fm theta were more prominent than that without Fm theta (100-300ms; $p = 0.012$, 300-500ms; $p = 0.022$). No clear ERS/ERD of the alpha band was observed (not shown).

**ERD/ERS (WT)**

ERD and ERS of theta band indicated biphasic or triphasic deflections during the wavelet window for both the standards and deviants. As in the FFT data, the ERD was

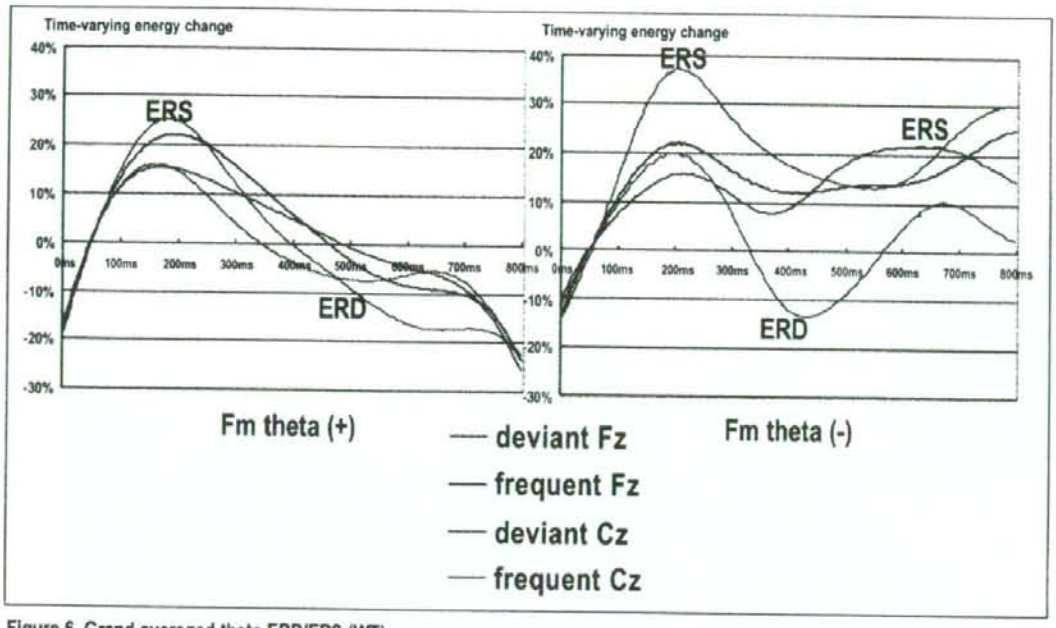


**Figure 6. Grand averaged theta ERD/ERS (WT).**
ERD and ERS of theta band indicated diphasic or triphasic deflections during the wavelet window for both the standards and deviants. ERD was seen 400-600 ms after the onset of stimulus, whereas ERD was preceded by ERS at 160-200 ms. ERD was followed by ERS at 600-800 ms during the periods without Fm theta. However, during the periods with Fm theta, no apparent ERS following ERD was seen.

seen 400-600 ms after the onset of stimulus, whereas ERD was preceded by ERS at 160-200 ms both during the periods with and without Fm theta. Furthermore, ERD was followed by ERS at 600-800 ms during the periods without Fm theta. During the periods with Fm theta, however, no apparent ERS following ERD was seen for either the standards or deviants (Figure 6). ANOVA revealed significant effects of Fm-theta [$F(1,7) = 31.649$, $p = 0.001$] and Time [$F(7,49) = 9.210$, $p = 0.001$, $\varepsilon = 0.345$]. ANOVA also revealed significant interactions of, Fm-theta x Time [$F(4,28) = 6.857$, $p = 0.013$, $\varepsilon = 0.243$]. Post-hoc test revealed that ERS with Fm theta was more prominent than that with Fm theta during the period of 600-800 ms (500-600 ms, $p = 0.001$; 600-700 ms, $p = 0.003$; 700-800 ms, $p = 0.002$).

## DISCUSSION

We found that during periods with clear Fm theta, ERD of the theta band was seen at the front midline region. These findings suggest that Fm theta is desynchronized in relation to additional external stimuli independent of the task evoking the Fm theta.

The midline subcortical structures, including the thalamus and hippocampus, may play important roles in controlling interhemispheric synchronization of Fm theta.[36] It was also hypothesized that the appearance of Fm theta during consecutive mental tasks reflects alternative activities of the prefrontal-medial superficial cortex and ACC in approximately a 40 to 120 degree phase shift during one Fm theta.[37] Alternative fire of the two regions may be operated by subcortical structures such as the thalamus, and may be closely related to the ongoing cortico-ACC feedback required for the recruitment of neuronal subpopulations into a coherent distribution network. The control of cortico-ACC activity may contribute to suppressing unnecessary regions and activating necessary ones in performing the engaging task.[37] Such a "positive suppression" control probably results in a blood flow increase in the prefrontal medial cortex including ACC[38,39] and in generating Fm theta.[37]

In the FFT data, the ERD with the appearance of Fm theta was more prominent than that during the periods without Fm theta. The ERD of Fm theta might be revealing the mechanism that processes external auditory stimuli automatically and prevents the attentional resources from being unnecessarily allocated to the auditory stimuli. More amplified ERD during the period with Fm theta compared to that without Fm theta might reflect more efficient "positive suppression" by cortico-ACC activity. However, although "Tetris" requires a certain amount of sustained attention, there was no direct control on the "amount" of attention allocated to the task. A task with established different levels of difficulty should be a better method to control for allocation of resources.

Electrophysiological studies of working memory indicate that persistent firing of cells underlies working memory.[40] The fact that theta is induced by working memory

tasks suggests that this firing may have an oscillatory character.[23] Jensen and Lisman[41,42] proposed an oscillatory model that similar phase coding may be important in multi-item working memory with different memory items active at different phases of the theta cycle. Bastiaansen and Hagoort[43] supported the hypothesis that theta activity plays a functional role in cell assembly formation, a process which may constitute the neural basis of memory formation and retrieval. Another possible function of theta is to rapidly encode information directly into long-term memory by synaptic modification[44,45] or to synchronize different regions of the cortex that participate in the task.[46] On the other hand, passive oddball stimuli used here are generally used to elicit a component of ERPs, Mismatch Negativity (MMN). Näätänen[47] has proposed a model for the role of automaticity and attention in the processing of acoustic stimuli in which all auditory sensory information produced by preconscious processing is stored for a temporary period in the form of precise neuronal representations of sensory memory (memory trace). Another possible function of Fm theta induced by Tetris, and desynchronized in relation to oddball stimuli, may reflect information processing modeled by the multi-item working memory task required for executing Tetris and the simultaneous auditory processing using memory trace described by Näätänen's model.

In the WT data, ERD was followed by ERS during the periods without Fm theta. However, during the periods with Fm theta, no apparent ERS following ERD was noted. ERS during the periods without Fm theta might indicate the further processing of auditory information free from the processing reflected by Fm theta.

To our knowledge, there have been a few reports regarding Fm theta in clinical populations accompanied by cognitive deficits such as schizophrenia, depression, or ADD/ADHD (attention deficit disorder/attention deficit hyperactivity disorder). Fm theta has been reported to be closely related to personality and anxiety level. Fm theta was likely to appear more markedly in those subjects who were more extraverted, less neurotic and less anxious. A subject with low MAO (monoamine oxidase) activity will show more Fm theta and is likely to be extraverted. Anxiety in subjects with Fm theta may be mainly correlated with 5-HT1A (5-hydoxytryptamine) receptor function. On the other hand, the PANSS (positive and negative syndrome scale) scores of Fm theta group of schizophrenic patients were higher than those of non-Fm theta group. Plasma levels of dopamine and homovanillic acid were lower and prolactin level was lower in Fm theta group compared with those in non-Fm theta group. Furthermore, patients with quantitative neurometric EEG features of the frontal theta excess responded to stimulants despite their classification of ADD or affective disorder. Summarizing there findings, Fm theta in healthy subjects may reflect expeditious processing, which is interfered by anxiety, however, Fm theta in clinical populations may stem from their pathological states or low vigilance. Further studies in such clinical populations will be required to elucidate mechanisms underlying Fm theta under concentration for a mental task and the frontal theta activity in a resting state.

Finally, we should address the limitations of the present study. Firstly, we could not recognize the difference of the ERD/ERS between the deviant and frequent stimuli. Even though the passive auditory oddball stimuli was preconscious, the deviants, which require the subjects to update their memory buffer according to the present context instated by the standards, might demand attentional resources to a greater extent than the standards. Modification of the experimental designs or further analyses for other frequency bands such as gamma or alpha might be needed. Secondly, the findings of the FFT data were not completely compatible with the WT data. Advantages and disadvantages of the both methods should be assessed minutely for the study of Fm theta. Thirdly, detailed analyses other than ERD/ERS, such as the peak range of Fm theta within and across individuals, the differences of cognitive processing reflected by the lower or upper range of theta, or correlation between ERD/ERS and the task performances or duration of Fm theta, would be needed in the future.

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# Genetic Association Between Notch4 Polymorphisms and Alzheimer's Disease in the Japanese Population

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It is shown that Notch 4 plays important roles in the pathogenesis of Alzheimer's disease (AD). To investigate whether three single nucleotide polymorphisms (SNPs) of the Notch4 gene are associated with AD, the three SNPs were genotyped by a polymerase chain reaction–restriction fragment length polymorphism method for 243 AD patients and 130 age-matched controls. We also confirmed the linkage disequilibrium among these three SNPs of the gene using the EH program. The three SNPs did not seem to alter risk for AD. Our study suggests that SNPs studied are not associated with AD. The linkage disequilibrium of this locus indicates that there is genetic heterogeneity in the Notch4 gene. We could not confirm the previous synergetic associations of the 5′ untranslated region (rs367398) C/C genotype in apolipoprotein E ε4 bearers in AD patients. Potential markers nearby the 5′ untranslated region polymorphism might affect risk for AD.

THE genes HLA-DR3, CREBLI, RAGE (the receptor for advanced glycosylation end products), and Notch4 are located within the major histocompatibility complex (MHC) 6p21.3 locus. This genetic region seems to provide good candidates for Alzheimer's disease (AD) (1–3). Missense mutations of the Notch3 gene cause CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy), a form of dementia with stroke (4). In addition, AD might have a pathogenesis similar to that of CADASIL (5). Symptoms of dementia observed in two diseases have suggested a relationship between the Notch family and AD. The presenilins are known to interact with several proteins including the Notch 1–4 proteins, and a dysfunction of these interactions may play a role in AD pathogenesis (6,7). Although Lambert and colleagues (8) showed negative results for two single nucleotide polymorphisms (SNPs) of the gene, genetic studies of the gene were not fully performed. In this study, we focused on three SNPs—rs367398, rs2071282, and rs422951 to confirm the genetic association. These three SNPs cover a 3.3-kb region from the 5′ untranslated region to exon 4 of the Notch4 gene, and were studied in a Japanese cohort of AD cases with age-matched controls.

## Materials and Methods

Sporadic AD cases ($n = 243$, ratio of men to women = 106:137) were obtained from the in-/outpatients of the hospitals where the authors work. All the AD cases were diagnosed according to National Institute of Neurological and Communication Disorders and Stroke–Alzheimer's Disease and Related Disorders Association (NINCDS–ADRDA) criteria. The controls ($n = 130$, ratio of men to women = 56:74) were obtained from healthy volunteers with no history of neuropsychiatric diseases or dementia. The mean age of the AD group (69.4 years, standard deviation 9.9, range: 38–90) was not significantly different from that of the control group (70.3 years, standard deviation 9.0, range: 51–93). Japanese samples are genetically homogenous, and the collected case–control samples are geographically matched. The purpose and significance of this study was explained in detail to each patient and family, and all participants provided their written informed consent. The study protocol was approved by the Ethics committee of the Juntendo University School of Medicine.

Genomic DNA was extracted from white blood cells of peripheral bloods by using the Nucleon II kit (Scott Lab Bioscience, U.K.). Information on the SNPs of each gene was derived from the SNP database (dbSNP) established by the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/). The polymorphisms of the Notch4 gene were studied by a polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) analysis method (Table 1). The distribution of genotypes between the AD cases and their controls were compared using chi-square tests (a $p$ value of $< .05$ was considered statistically significant). For SNP rs2071282, Fisher's exact test was performed. To assess linkage disequilibrium (LD) between each SNP, the estimate haplotype frequencies (EH) program (http://linkage.rockefeller.edu/ott/eh.htm) was used.

## Results

The distribution of the three polymorphisms is shown in Table 2. Logistic regression tests have shown that there were no associations among age, sex, and each

Table 1. Details of PCR–RFLP Method to Determine Each SNP of the Notch 4 Gene

| SNP | Primers | RFLP Method |
|---|---|---|
| rs367398 | 5′-tagtgttcctccactcttcctc-3′ | *MspI* |
| C/T | 5′-agtgaaggggggctgcattccac-3′ | C allele: 190, 40 bp |
| | | T allele: 230 bp |
| rs2071282 | 5′-cttcgggacttctgttcagcc-3′ | *HinfI* |
| C/T | 5′-aggcagaggtgaaaggtggag-3′ | C allele: 300, 20 bp |
| | | T allele: 156, 144, 20 bp |
| rs422951 | 5′-cgaagatgtggatgagtgtga-3′ | *HaeIII* |
| A/G | 5′-agcaatacagtcatccaggtt-3′ | A allele: 154 bp |
| | | G allele: 130, 24 bp |

*Note*: PCR–RFLP = polymerase chain reaction–restriction fragment length polymorphism; SNP = single nucleotide polymorphism.

polymorphism. There was no significant difference in genotypic distribution for any polymorphism (rs367398: $df = 2$, $\chi^2 = 0.24$, $p = .89$; rs2071282: $df = 2$, $\chi^2 = 0.0006$, $p = .99$; rs422951: $df = 2$, $\chi^2 = 0.14$, $p = .93$) between the AD participants and their controls. Analysis of the distribution of the allele of three SNPs showed negative results (rs367398: $df = 1$, $\chi^2 = 0.10$, $p = .81$; rs2071282: $df = 1$, $\chi^2 = 0.0001$, $p = .99$; rs422951: $df = 1$, $\chi^2 = 0.03$, $p = .93$). Strong LD was shown between rs367398 and rs422951 in our Japanese participants ($p < .001$). LD between rs367398 and rs2071282 ($p < .001$) was weaker than LD between rs367398 and rs422951. Two SNPs, rs2071282 and 422951, were also in weak LD in Japanese samples ($p < .02$).

## Discussion

To the best our knowledge, this is the first follow-up study evaluating the association between SNPs of the Notch4 gene and AD. Our results indicate that the three SNPs of the Notch4 gene did not alter risk for AD in our Japanese cohort. In a previous genetic study, Lambert and colleagues (8) analyzed two SNPs, rs387071 (in the promoter region) and rs367398. They reported an increased risk for AD associated with the C/C genotype of rs367398 in apolipoprotein E (Apo E) ε4 bearers. In the present study, logistic regression analysis by Apo E ε4 allele were performed to each SNP of the Notch4 gene, and suggested no associations between any of the Notch4 SNPs and the Apo E ε4 allele (data not shown). The discrepancy between their results and ours might be attributed to the differences between ethnic groups or a low penetrance effect. Lambert and colleagues observed no relationship between rs367398 and amyloid β loads. It is possible that a marker yet to be studied near this locus, and not rs367398 itself, might play a synergetic role with Apo E ε4. We also confirmed that rs367398 was in strong LD with rs422951 in the AD and the control groups. Rs2071282 showed weaker LD with rs367398 and rs422951. Because it was suggested that there might be more variants between rs2071282 and rs367398, other SNPs around this genetic locus should be searched in other populations.

Table 2. Comparison of the Notch4 Gene SNP Frequencies

| | C/C | C/T | T/T | C Allele | T Allele |
|---|---|---|---|---|---|
| rs367398 | | | | | |
| AD | 62 (0.27) | 118 (0.52) | 47 (0.21) | 232 (0.52) | 212 (0.48) |
| Control | 34 (0.26) | 71 (0.55) | 25 (0.19) | 139 (0.53) | 121 (0.47) |
| *p* Value | | | .89 | | .81 |
| rs2071282 | | | | | |
| AD | 201 (0.86) | 31 (0.13) | 2 (0.01) | 433 (0.93) | 35 (0.07) |
| Control | 109 (0.86) | 17 (0.13) | 1 (0.01) | 235 (0.93) | 19 (0.07) |
| *p* Value | | | .99 | | .99 |
| | A/A | A/G | G/G | A Allele | G Allele |
| rs422951 | | | | | |
| AD | 132 (0.58) | 82 (0.36) | 15 (0.06) | 346 (0.76) | 112 (0.24) |
| Control | 77 (0.59) | 44 (0.34) | 9 (0.07) | 198 (0.76) | 62 (0.24) |
| *p* Value | | | .93 | | .92 |

*Note*: SNP = single nucleotide polymorphism; AD = Alzheimer's disease.

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ORIGINAL ARTICLE

# Long-term effect of donepezil for Alzheimer's disease: Retrospective clinical evaluation of drug efficacy in Japanese patients

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## Abstract

**Background:** Alzheimer's disease (AD) is common in the Japanese population. In 1999, donepezil was authorized in Japan for the treatment of AD. However, because the time since donepezil was authorized is relatively short, there are few reports regarding the long-term effects of donepezil in Japanese AD patients.

**Methods:** In the present study, the clinical features of 72 AD patients treated at Juntendo University Urayasu Hospital were examined retrospectively in order to examine the long-term effects of donepezil. Sixty-two AD patients had been administrated donepezil. The effect of donepezil was evaluated using the Revised Hasegawa Dementia Scale (HDS-R). In patients with increased points on the HDS-R after 6 months, treatment was regarded as effective, whereas patients in whom there was no change in points on the HDS-R were classified as 'no change'; finally, in patients with decreased points on the HDS-R after 6 months, treatment was regarded as non-effective.

**Results:** The duration of medication was divided into four groups: (i) 6 months (16 cases; 26%); (ii) 1 year (16 cases; 26%); (iii) 2 years (13 cases; 21%); and (iv) more than 3 years (11 cases; 18%). Donepezil treatment was stopped in six patients (10%) because of adverse effects. Thus, the 56 patients who continued with donepezil treatment were categorized as follows: treatment was effective in 27 cases (48%), no change was found in five cases (9%) and, in 24 cases (43%), donepezil treatment was found to be non-effective. Differences in treatment efficacy were not related to sex, apolipoprotein E genotype or medical history. Comparative studies for the age of onset of AD and points of the HDS-R before administration of donepezil suggest that donepezil tended to be effective in patients in whom AD developed at 71–80 years of age and with 16–20 points of the HDS-R. In patients in whom donepezil was effective, cognition returned to the state before treatment 2 years later. However, the marked degradation of points on the HDS-R was not seen in these cases. Conversely, the long-term consequences for patients in whom donepezil was not effective after 6 months were similar to those for patients not treated with donepezil.

**Conclusion:** Donepezil improved the dementia symptoms in patients in whom it was effective over the long term. We suggest that the presence of drug efficacy after 6 months is an indicator that long-term treatment with donepezil is warranted. In particular, donepezil was effective in cases with mild to moderate AD.

***Key words:*** *Alzheimer's disease, dementia, donepezil, Japanese patient, long-term effect.*

## INTRODUCTION

In Japan, Alzheimer's disease (AD) was first recorded in 1952 and it is recognized as a common disease in the Japanese population.[1]

It has been reported that East Asian people, including the Japanese, had a low incidence of dementia until 1990.[2] However, AD became the most common disease in demented Japanese patients after 1990.[3]

Given this situation, in 1999 donepezil was authorized for the treatment of AD in Japan.[4] However, because the time since donepezil was authorized is relatively short, there are few reports regarding the long-term effects of donepezil in Japanese AD patients.[5,6]

To examine the long-term effect of donepezil, we studied the clinical features of AD patients treated at Juntendo University Urayasu Hospital.

## METHODS

The clinical features of 72 AD patients were examined. All patients were diagnosed by DSM-IV criteria as 'dementia of the Alzheimer's type'. Vascular dementia was excluded by means of brain magnetic resonance imaging findings. Sixty-two cases (25 men, 37 women; mean age 72.4 years) had been prescribed donepezil and a further 10 cases (four men, six women; mean age 77.1 years) had not been treated with donepezil.

We investigated the degree of dementia symptoms using of the Revised Hasegawa Dementia Scale (HDS-R).[7,8] The effect of donepezil was evaluated by HDS-R scores. Donepezil treatment in patients in whom there was an increase in points on the HDS-R after 6 months was regarded as effective, whereas patients in whom there was no change in points on the HDS-R were classified as 'no change'; finally, in patients with decreased points on the HDS-R after 6 months, treatment was regarded as non-effective.

In addition, clinical features, such as sex, age of onset, medical history, apolipoprotein E genotype and degree of dementia before administration, were investigated to explore their relationship with the clinical effect of donepezil.

Informed consent for the use of data was obtained verbally from the patients or from their caregivers according to procedures approved by the institutional review board of Juntendo University Urayasu Hospital.

## RESULTS

The duration of medication was divided into four groups: (i) 6 months (16 cases; 26%); (ii) 1 year (16 cases; 26%); (iii) 2 years (13 cases; 21%); and (iv) more than 3 years (11 cases; 18%). Donepezil treatment was stopped in six patients (10%) because of adverse effects, including three cases of digestive symptoms, one case of aggression, one case of delirium and one case of rhabdomyolysis. Those adverse effects disappeared following the discontinuation of donepezil and temporary medical treatment.

After 6 months treatment, the effects of donepezil were found to be effective in 27 cases (48%), no change was found in five cases (9%) and, in 24 cases (43%), donepezil treatment was found to be non-effective.

Figure 1 shows the mean differences in points on the HDS-R. In patients in whom donepezil was effective, cognition returned to the state before treatment 2 years later. However, the marked degradation of points on the HDS-R was not seen in these cases. Conversely, the long-term consequences for patients in whom donepezil was not effective after 6 months were similar to those for patients not treated with donepezil.

Comparative studies of the age of onset of AD and points on the HDS-R before administration suggest that donepezil tends to be effective in cases in whom AD developed at 71–80 years of age and with 16–20 points of the HDS-R (Figs 2,3).

Differences in the effectiveness of donepezil were not related to sex, medical history or apolipoprotein E genotype (Figs 4–6).

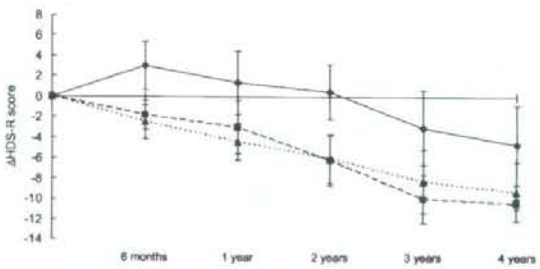


**Figure 1** Mean (±SD) differences in Revised Hasegawa Dementia Scale (HDS-R) scores in patients in whom donepezil treatment was deemed effective or producing no change (———) or non-effective (------) and patients who were not treated with donepezil ($n = 10$; ·····).

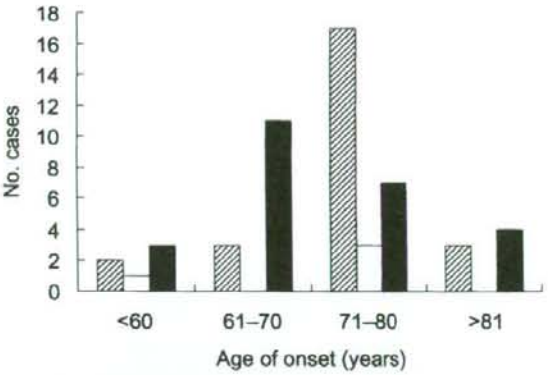


**Figure 2** Differences in the effects of donepezil according to age of onset of Alzheimer's disease in patients in whom donepezil treatment was deemed effective (▨), producing no change (□) or non-effective (■).

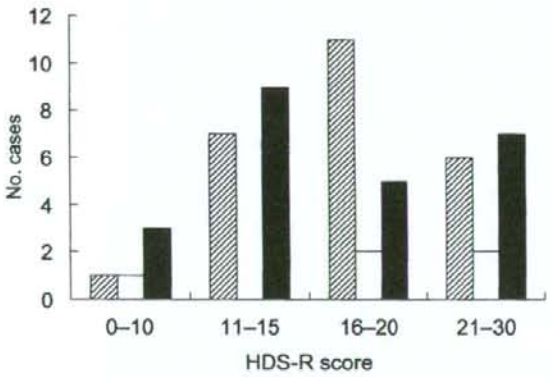


**Figure 3** Differences in the effects of donepezil according to Revised Hasegawa Dementia Scale (HDS-R) scores before administration in patients in whom donepezil treatment was deemed effective (▨), producing no change (□) or non-effective (■).

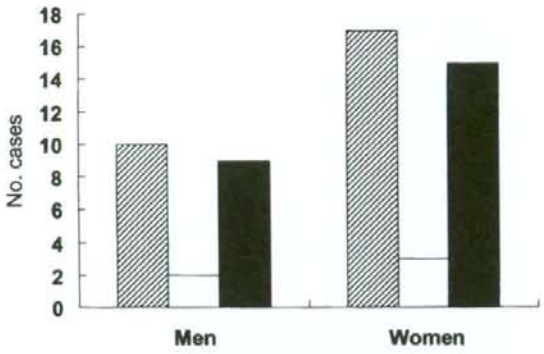


**Figure 4** Differences in the effects of donepezil according to gender in patients in whom donepezil treatment was deemed effective (▨), producing no change (□) or non-effective (■).

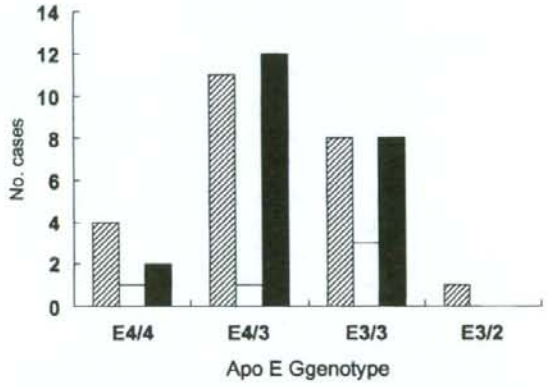


**Figure 5** Differences in the effects of donepezil according to apolipoprotein (Apo) E genotype in patients in whom donepezil treatment was deemed effective (▨), producing no change (□) or non-effective (■).

## DISCUSSION

In the present study, 56 of 62 cases were able to take donepezil. Of the 56 cases treated with donepezil, treatment was found to be effective (an improvement on the HDS-R following donepezil) or resulted in no change in 32 patients. Because both inhibition of the progression of dementia and an improvement of dementia symptoms are recognized as effects of donepezil,[9,10] the results of the present study suggest that donepezil was effective in 57% of patients. This is almost as effective as conventional treatment of AD in Japan.[5]

Figure 3 shows that the effect of donepezil was maintained for approximately 2 years and that sudden degradation of points on the HDS-R was not seen in patients in whom donepezil treatment was effective after their symptom progressed.

However, the long-term consequences of AD in cases in which donepezil treatment had been non-effective after 6 months were similar to patients who had not been administered donepezil. Because there has been a report published stating that the long-term treatment with donepezil is not cost effective,[11] the administration of donepezil without specific aims must be avoided. On the basis of the results of the

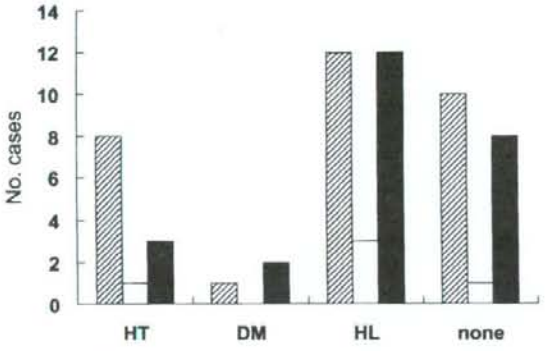


**Figure 6** Differences in the effects of donepezil according to medical history in patients in whom donepezil treatment was deemed effective (▨), producing no change (□) or non-effective (■). HT, hypertension; DM, diabetes mellitus; HL, hyperlipidemia.

present study, we suggest that the presence of drug efficacy after 6 months is an indicator that long-term treatment with donepezil is warranted.

A medical history of hypertension, diabetes mellitus and hyperlipidemia is assumed to indicate risk factors for the development of AD.[12,13] However, there was no difference in the effect of donepezil between the cases with hypertension, diabetes mellitus and hyperlipidemia. This suggests that the effectiveness of donepezil treatment is not affected by patient age or the medical history of AD patients.

According to some reports,[6,14,15] the effect of donepezil in patients with the E4 gene is inferior to that in patients without this genotyped. However, in the present study, there was no difference in the effect of donepezil between patients with and without the E4 gene. This supports previous reports that the effect of donepezil is not affected by apolipoprotein E genotype.[16,17]

Specifically, we found that donepezil was effective in patients who developed AD between 71 and 80 years of age or who had a point on the HDS-R of 16–20 before administration of the drug. This suggests that donepezil is effective in patients with mild to moderate AD, which is in accord with published reports.[18,19]

Until now, the long-term effects of donepezil have been investigated in the US,[7,8] but the number of reports remains insufficient, especially for Asian populations. The results of the present study suggest that donepezil is effective for Japanese AD patients in the long term.

In the present study, we evaluated the degree of dementia using the HDS-R, which was used originally as a screening test. In the future, the relationship between the points obtained on the screening test and dementia symptoms must be investigated in detail.

## ACKNOWLEDGMENTS

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