Table 2 Reasons patients in the US thought they were switched to levodopa-carbidopa, and why they were concerned about taking levodopa-carbidopa

Perceived reasons for being switched to LC[a]	Patients (%)[a]
My PD symptoms were getting progressively worse	55
My PD symptoms did not get worse, but I did not get good symptom control with previous treatments	16
I do not know/my doctor recommended it	15
I could not tolerate the side effects of previous treatments	10
Other reason	5
Concerns about taking LC	**Patients (%)**
Long-term side effects of LC, such as dyskinesias (uncontrolled movements, wiggles)	52
Benefits may begin to wear off sooner than desired	49
An indication that my PD might have advanced to a more severe stage	46
Immediate side effects of LC, such as nausea and vomiting	34
Fear that LC might make my PD worse	23
Being able to afford LC	21

Notes: [a]Respondents who were not initiated on levodopa (n = 110); patients who did not provide a specific response were excluded from this analysis.
Abbreviations: LC, levodopa-carbidopa; PD, Parkinson's disease.

Society of Japan conference held in Kyoto, the most commonly used daily dose of levodopa ranged from 300 mg to 400 mg.[8] For many Japanese providers, the highest daily dose of levodopa was 300 mg, even for patients with advanced PD. Consistent with this, about 17% of patients who were interviewed stated that they had been informed by their physician that their medication could no longer be increased, despite the suggestion of increasing motor disability.

Discussion

Despite being the most effective treatment for PD, the higher possibility of motor complications associated with levodopa may result in potential underdosing.[5] Although dyskinesias are often regarded as one of the most important complications of levodopa therapy,[9] this project suggests that dyskinesias were not a primary concern for patients surveyed in either the US or Japan. In the US, patients were more concerned about wearing-off, whereas other adverse effects, such as hallucinations, were of greater concern to Japanese patients. Interestingly, Japanese patients who had not yet experienced dyskinesia were more concerned about this adverse effect than those with a prior history of dyskinesia, possibly due to concern regarding the mental burden and hardship of the condition. Although primary care providers in the US recognized the importance of wearing-off, specialists considered dyskinesias to be of equal, if not greater, concern for patients. This suggests that patient concerns about dyskinesia may, in some cases, be overestimated by physicians, and may cause some hesitation when prescribing levodopa.

Patient perspectives on treatment options are, among other things, influenced by disease stage, symptom severity, and experience of adverse effects. Understanding patient attitudes towards PD therapies and the associated complications may help physicians devise individualized treatment strategies. There is currently a multitude of therapeutic options for patients with PD, and individual benefit varies significantly among patients. The benefit of efficient communication between the patient and the doctor in any culture cannot be overestimated, particularly when individualizing treatment. However, improved patient education and awareness is paramount for effective patient–physician communication. Patients need to understand the symptoms of PD, and be aware of the implications of certain therapies in order to be familiar with signs of disease progression or treatment complications.

The results of our US survey highlight a further discrepancy between physicians and patients as to reasons for initiation of levodopa-carbidopa therapy: while the majority of patients believed levodopa-carbidopa therapy was initiated because of progressive worsening of PD symptoms, 50% of family physicians and nearly a third of specialists initiated levodopa-carbidopa therapy at diagnosis. Furthermore, more than half of the US patients said they were at least somewhat concerned about taking levodopa-carbidopa, as a result of information obtained on the Internet or from physicians.

It is interesting to note that while US patients were most concerned about long-term side effects of their medication, such as dyskinesia and wearing-off, Japanese patients worried more about experiencing hallucinations. This is possibly due to the fact that the majority of Japanese patients in this study received dopamine agonist therapy. Hallucinations are more likely to occur with dopamine agonists than with levodopa,[6] and, in Japan, it is common clinical practice for patients with PD to be initiated on low-dose levodopa combined with dopamine agonists or amantadine. The higher use of dopamine agonists in Japan is also reflected in Japanese clinical trials compared with those conducted in the West.[10,11] Studies have reported a higher incidence of hallucinations in Japanese patients compared with Western patients, which is attributable to the higher doses of dopamine agonists used in the Japanese PD population.[12–14] Therefore, because hallucinations can impact on the quality of life of both patients and their caregivers,[15] it would seem pertinent for physicians in Japan to know how to avoid these adverse

effects and how to manage drug-induced psychotic symptoms should they arise.[15]

Regarding attitudes towards drug intake and dose increases, it is noteworthy that while almost all US physicians believe patients would rather reduce their pill burden, US patients themselves consider their biggest challenge to be wearing-off. In contrast, patients in Japan would rather increase their dose or dosing frequency in order to ameliorate their symptoms. Indeed, patients in Japan expressed a preference for obtaining symptomatic relief, even if that required an increase in medication dosing. This observation is strengthened further by the fact that this preference for symptomatic relief was similar between patients with or without wearing-off. In addition, one major discrepancy between patients and physicians, in both the US and Japan, related to dose increases. In the US, patients feared wearing-off, yet physicians were under the impression that patients wanted to restrict medication intake; However, in Japan, patients seek symptomatic relief, even if that results in an increase in medication. Despite this, the conference survey results indicated that physicians in Japan are reluctant to increase doses. This is supported by results of Japanese studies advocating the use of low doses of levodopa to avoid the development of motor complications.[12] Therefore, the findings demonstrate a need for improved communication between doctors and patients in both countries regarding dose increases, taking into account patient perspectives of adverse effects.

The difference between patient perspectives among Japanese and US patients is likely to stem from differences in medical practice for the management of PD. However, the underlying reasons for this difference are unclear. One possibility is that availability of certain antiparkinsonian therapeutic agents in the two countries may differ. For example, the triple combination therapy levodopa/carbidopa/entacapone is not yet available in Japan, whereas certain dopamine agonists, such as talipexole and droxidopa, are only marketed in Japan. Monotherapy with selegiline is not covered by Japanese health insurance, because it has not been approved by the local authorities.[16] Another possible difference that may influence decisions on therapy is the cost of the drug in the respective countries. In Japan, the cost of antiparkinsonian therapies is largely covered by government-funded Japanese health insurance. In the US, the cost of the drugs depends on the specific health care insurance scheme in which the patient in enrolled. However, given that the cost of levodopa is much lower than that of dopamine agonists, it is unlikely to play a significant role in determining whether to introduce levodopa or whether increases in its dosage or dosing frequency are required.[17] In fact, in Japan, the cost of levodopa and dopamine agonists will be covered by national insurance (at least for patients with Hoehn and Yahr stage III or higher), and is unlikely to be a driving factor for the choice of therapy used in this region. Therefore, the reason why the doses of levodopa used in Japan tend to be lower than in the West is unclear. Results from a retrospective study based at the Sapporo Azabu Neurosurgical Hospital in Japan suggested that lower doses of levodopa may be sufficient to achieve symptom control and may reduce or delay the appearance of motor complications compared with the higher doses of levodopa required to achieve symptom control in multinational, randomized, controlled trials.[12,18–20] The authors of the former study proposed that Japanese patients with PD may respond better to levodopa compared with their Caucasian counterparts, and speculated that variations in genetic background, pharmacokinetics, and lifestyle choices may contribute to this difference.[12] It is also likely that physicians in Japan are concerned about dyskinesias, which tend to be associated with levodopa, and try as much as possible to avoid the development of this complication.[12] Finally, a long-term anti-levodopa campaign, which focused on the potential neurotoxicity of levodopa, and interpretation of the 2002 Japanese practice guidelines for PD, may play a role in influencing attitudes in Japan.[16,21] Although, the seminal ELLDOPA (Earlier vs Later L-DOPA) study of levodopa in early PD patients has dispelled the notion that levodopa is neurotoxic,[22] concerns may still resonate with many Japanese physicians. However, underdosing with levodopa can be associated with a reduction in symptom control and, consequently, may impact patient quality of life.[5] In addition, the observations that dyskinesia is not a major concern for patients in this study and that patients in Japan prefer increasing the dose of medication to improve symptom control, suggests that physicians should not limit the dose of levodopa to avoid the development of dyskinesia.

This study set out to elucidate the perspectives of patients towards PD and antiparkinsonian therapy and to understand whether such views and concerns differ between patients in the US and those in Japan. Although in some cases (eg, those with cognitive or physical difficulties), the patient's caregiver may have completed the Japanese survey on behalf of the patient, this is unlikely to have affected the study results significantly. However, it should be noted that the way in which the two surveys were conducted varied slightly, and the results between the two countries may not be directly comparable. As such, some caution must be exercised when interpreting these results. Nevertheless, the study highlights some interesting similarities and differences between the

two populations, as well as differences between patient and physician perspectives in both countries.

Conclusion

In conclusion, this study suggests that patient perceptions about PD therapy may differ from the views of their physicians. Heightened understanding of patient concerns and attitudes towards PD treatments and their associated complications may help physicians to individualize optimal treatment strategies. Improving patient education and awareness about PD and medical therapy will be instrumental in enhancing patient–physician communication and, consequently, patient care and treatment outcomes.

Disclosure

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Clinical Study

The Efficacy of Pramipexole, a Dopamine Receptor Agonist, as an Adjunctive Treatment in Treatment-Resistant Depression: An Open-Label Trial

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Dopaminergic dysfunction is implicated in the pathophysiology of treatment-resistant depression. Although the efficacy of adjunctive pramipexole treatment has been demonstrated in treatment-resistant bipolar depression, such data are scarce for major depressive disorder (MDD). We recruited 17 patients with DSM-IV major depressive episode who have failed to respond to previous treatment with a selective serotonin reuptake inhibitor. Five patients were diagnosed as having bipolar II disorder and 12 as having unipolar MDD. Patients were monitored at an ambulatory care facility every two weeks until 12 weeks. Pramipexole was added to existing medication. Depression severity was assessed with the Hamilton Depression Rating Scale 21-item version (HDRS-21). The mean maximum dosage of pramipexole was 1.6 mg (SD 0.9). The HDRS-21 total score decreased from 19.4 (SD 3.8) at baseline to 7.2 (SD 5.4) at endpoint ($P < 0.000001$). Twelve patients (71%) were responders based on the definition of 50% or more reduction in the HDRS-21 score. Ten patients (59%) remitted (HDRS-21 total score at endpoint < 8). These results were almost unchanged when the sample was confined to patients with MDD. No serious adverse events were observed. Our findings indicate that pramipexole augmentation therapy may be effective and well tolerated in refractory depressed patients.

1. Introduction

It is well known that a significant proportion of patients with major depressive disorder fail to achieve remission with standard antidepressant therapies, even when optimally delivered. Such a condition is called treatment-resistant (or refractory) depression and represents a major challenge in everyday practice. Treatment-resistant depression can be classified into different stages based on the degree of treatment resistance; Thase and Rush [1] defined stage I treatment-resistant depression as the persistence of significant depressive symptoms, despite at least one adequate trial with one major class of antidepressant, stage II as stage I resistance plus failure of an adequate trial with an antidepressant in a different class from that used in stage I, and stage III as stage II resistance plus failure of an adequate trial with a tricyclic antidepressant.

As dopamine is involved in the regulation of motivation, volition, interest/pleasure, and attention/concentration, all of which are likely to be impaired in depressed patients, reduced dopamine neurotransmission is implicated in the pathophysiology of depression and thought to play an important role in the treatment-resistant depression [2]. Supporting this, preclinical studies have demonstrated the effectiveness of dopamine agonists in depression [3, 4], and we also reported antidepressant-like and anxiolytic-like effects of cabergoline in rats [5]. It would therefore be reasonable to assume that depressed patients who have not responded to multiple serotonergic and noradrenergic antidepressants may benefit from dopaminergic agents.

There are six dopamine agonists currently used in clinical practice mainly for Parkinson's disease: bromocriptine, cabergoline, pergolide, talipexole, ropinirole, and pramipexole. Ergot alkaloids (bromocriptine, cabergoline,

and pergolide) can cause serious, albeit rare, adverse events including valvular heart diseases whereas nonergot dopamine agonists (talipexole, ropinirole, and pramipexole) do not have such an effect on cardiac valves. Among the latter, pramipexole, a D_2/D_3 receptor agonist approved for the treatment of Parkinson's disease and restless legs syndrome, has been demonstrated to have antidepressant efficacy as an adjunctive treatment in treatment-resistant bipolar depression in two randomized placebo-controlled trials [6, 7].

On the other hand, evidence for efficacy of dopamine agonists in the treatment of refractory unipolar major depressive disorder (MDD) is scarce. To our knowledge, six studies have investigated the possible effect of adjunctive dopamine agonists in the treatment of refractory depression [8–13]. These studies have generally found marked improvement in depressive symptoms [8–11, 13]; however, most of these studies targeted stage I treatment-resistant depression, with only one study for stage II refractory depression [13]. The latter with an open-label design examined efficacy of adjunctive pramipexole in the treatment of 10 patients with stage II refractory depression during an 8-week follow-up period and showed substantial effect of pramipexole [13]. It is thus suggested that pramipexole augmentation, among various dopamine agonists, may be a worthwhile option for refractory depression. However, more studies are needed to clarify the efficacy of adjunctive pramipexole in the treatment of refractory depression.

In the present open-label trial, we aimed to examine the efficacy and safety of pramipexole as an adjunctive treatment in patients with treatment-resistant depression.

2. Methods

2.1. Study Design. From August 2009 to February 2011, we conducted a 12-week open trial of pramipexole augmentation in treatment-resistant depression at the National Center of Neurology and Psychiatry (NCNP) Hospital, Japan. Seventeen patients diagnosed as having DSM-IV major depressive episode were recruited from the outpatient clinic of the NCNP Hospital or from community through advertisements in free local magazines and our website announcement. All of the community patients had been regularly attending to their nearby hospital or clinic before the participation in the present trial. Diagnosis was made based on the DSM-IV criteria [14] by an experienced psychiatrist.

Eligible subjects were those who had persistence of significant depressive symptoms as defined by the total score on the Hamilton Depression Rating Scale 21-item version (HDRS-21) [15] of equal to or greater than 15, despite previous 6 weeks or more treatment with adequate dose of at least one selective serotonin reuptake inhibitor. All but one of the patients turned out to have failed to respond to multiple antidepressant trials, that is, stage II or III treatment-resistant depression according to the classification of Thase and Rush [16]. Exclusion criteria were being under 18 or over 64 years old, being pregnant, having a prior medical history of central nervous system disease or severe head injury, having a physical illness that could interfere with the present study, and exhibiting marked suicidality. Those who needed to drive a car were also not eligible because pramipexole is associated with a potential risk of sleep attack.

The protocol was approved by the Institutional Review Board of the NCNP, and the present trial was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines. After the nature of the study procedures had been fully explained, written informed consent was obtained from every subject.

2.2. Treatment. Patients visited the ambulatory care facility of the NCNP Hospital every two weeks up until 12 weeks. Pramipexole was added to each patient's current medication, with the initial dosage of 0.25 mg/day. Dosages were then titrated on case-by-case basis (up to 3 mg daily when needed). Other medication was essentially kept unchanged during the 12-week trial period except for a minor change of sleeping medication.

2.3. Assessments. Adherence to medication was ascertained by clinical interview. Depressive symptoms were assessed with the HDRS-21. Clinical status was assessed with the Clinical Global Impression of Severity (CGI-S) and Improvement (CGI-I) scales [17]. These assessments were made at each visit (i.e., every two weeks). Response to treatment was defined as a 50% or more reduction in the HDRS-21 total score from baseline to endpoint. Remission was defined as a score of 7 or less on the HDRS-21 at endpoint. Safety was determined by adverse event monitoring through clinical observation/interview (at each visit) as well as objective examinations including blood test, urinalysis, and electrocardiogram (at the first, 4-week, and 12-week visits).

2.4. Statistical Analysis. Averages are reported as means ± SD (standard deviation). All analyses were performed on the intent-to-treat basis, with the conservative last observation carried forward (LOCF), in patients with at least one available follow-up assessment. The paired t-test and the Wilcoxon signed-rank test were used to compare baseline and LOCF results of the HDRS and CGI-S, respectively. Statistical significance was set at two-tailed $P < 0.05$. Analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 18.0 (SPSS Japan, Tokyo).

3. Results

3.1. Baseline Demographics and Clinical Characteristics. Baseline demographics and clinical variables of the sample are summarized in Table 1. Seven males and 10 females, with mean age of 36.2 were enrolled. Of the total 17 patients with a current major depressive episode, 12 had MDD and 5 had bipolar II disorder (Table 2). Of the 12 MDD patients, 3 had comorbid dysthymic disorder. According to the guideline of Thase and Rush [16], one patient was classified as stage I treatment-resistant depression, 3 as stage II, and 13 as stage III.

Table 1: Demographic characteristics and clinical variables at baseline.

Variable	Value
Diagnosis: bipolar II disorder/major depressive disorder	
Bipolar II disorder, *n*	5
Major depressive disorder, *n*	12
Stage of treatment-resistant depression	
Stage II	4
Stage III	13
Age, years: mean (SD)	36.2 ± 9.2
Gender: female, *n* (%)	10 (58.8)
Experience of hospitalizations: Yes, *n* (%)	6 (35.3)
History of suicidal attempt: Yes, *n* (%)	4 (23.5)
Family history of psychiatric disorder within first-degree relatives: Yes, *n* (%)	6 (35.3)
Age at onset, years: mean (SD)	28.1 ± 7.6
Age at first contact to psychiatric service, years: mean (SD)	30.2 ± 8.1
HDRS-21 total score: mean (SD)	19.4 ± 3.8
CGI-S score: median (range)	5 (3–6)

HDRS-21: 21-item version of the Hamilton Depression Rating Scale.

The mean dosage of pramipexole at endpoint was 1.6 ± 0.9 mg/day. The maximum dosage was also 1.6 ± 0.9 mg/day. Seven patients were given relatively high dosages (i.e., equal to or greater than 2.0 mg/day), of whom four required the maximum dosage that we set at 3 mg/day.

3.2. Efficacy. As shown in Table 2, 12 patients (70.6%) were considered to be responders based on the definition of 50% or more reduction in the HDRS-21 score from baseline to endpoint. Ten patients (58.8%) remitted based on the definition of HDRS-21 score equal to or less than 7. The HDRS-21 total score decreased from 19.4 ± 3.8 at baseline to 7.2 ± 5.4 at endpoint ($t = 7.7$, $df = 16$, $P < 0.000001$). This significant reduction in HDRS-21 total score was replicated, when the sample was limited to 12 MDD patients (19.8 ± 4.0 to 7.6 ± 5.3; $t = 7.3$, $df = 11$, $P < 0.0001$). Figure 1 shows the mean scores over time on the HDRS-21 score of the sample ($n = 17$), based on the intent-to-treat analysis. This figure illustrates that the HDRS-21 score was reduced nearly by half within the first 4 weeks, followed by a further gradual reduction.

The CGI-S score decreased from 4.5 ± 0.7 (i.e., moderate to marked illness) at baseline to 2.5 ± 1.0 (i.e., borderline to mild illness) at endpoint (by Wilcoxon signed-rank test, $z = -3.4$, $P = 0.001$). This significant reduction in the CGI-S score was replicated when the sample was limited to 12 MDD patients (by Wilcoxon signed-rank test, $z = -3.0$, $P = 0.003$). The CGI-I score at endpoint indicated that 9 patients very much improved, 3 much improved, 3 minimally improved, and 2 showed no change.

3.3. Safety. Two patients dropped out from the study before the 12-week visit; one patient discontinued pramipexole at an early stage due to increased appetite and the other discontinued it after catching a common cold (Table 2). In total, adverse events likely to be caused by pramipexole were

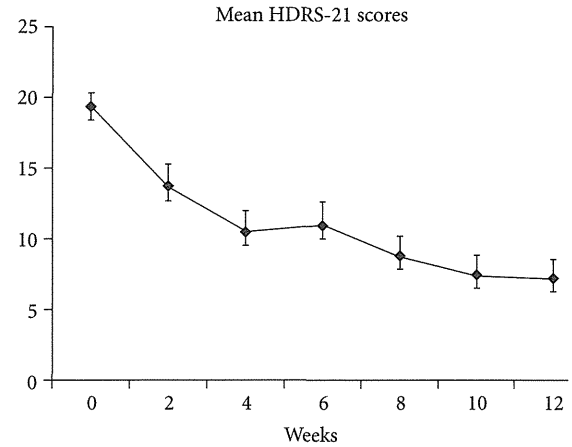

Figure 1: Mean scores over time on the Hamilton Depression Rating Scale (HDRS) 21-item version of the sample ($n = 17$), based on the intent-to-treat analysis. Error bars represent standard errors of the mean.

observed in 10 patients: nausea ($n = 3$), drowsiness ($n = 3$), orthostatic hypotension ($n = 2$), dry mouth ($n = 1$), insomnia ($n = 1$), agitation ($n = 1$), and increased appetite ($n = 1$). No serious events were seen except for persistent nausea in one patient which required an antiemetic drug.

3.4. Summary of the Studies Investigating Efficacy of Adjunctive Dopamine Agonist in Refractory MDD. Besides the present study, there have been six studies investigating the efficacy of adjunctive dopamine agonist in treatment-resistant unipolar MDD (Table 3). As for three studies that included depressed patients with bipolar disorder in addition to those with MDD [10–12], only the results for MDD patients are presented in this table. These studies overall showed substantial efficacy of dopamine agonist augmentation therapy in refractory depression, with the strongest evidence obtained

Table 2: Characteristics and outcome of the sample.

Patient number and DSM-IV diagnosis (Sex, Age)	Stage of treatment-resistant depression	Prior/concomitant medication (dosage: mg/day)	Follow-up visit (weeks)	Pramipexole dosage at endpoint (mg/day)	HDRS-21 decrease at endpoint (%)	Adverse events (reason for dropping out)
(1) BPII (M, 52)	Stage III	Amoxapine (125) Lithium (800)	12	1	100	None
(2) MDD, dysthymic disorder (F, 46)	Stage III	Fluvoxamine (50) Mirtazapine (15) Valproic acid (400)	4	0.25	27.3	Increased appetite (Increased appetite)
(3) BPII (M, 49)	Stage III	Amoxapine (75) Milnacipran (100) Sulpiride (150)	12	3	87.5	Orthostatic hypotension
(4) MDD (F, 41)	Stage III	Clomipramine (30) Lithium (600)	12	1	60.0	Nausea
(5) MDD (F, 38)	Stage III	Clomipramine (75) Sertraline (100)	12	1	58.8	Nausea
(6) MDD (M, 39)	Stage III		12	0.25	92.0	None
(7) MDD, dysthymic disorder (F, 28)	Stage III	Milnacipran (50) Aripiprazole (3)	12	1	83.3	None
(8) BPII (F, 30)	Stage III	Milnacipran (75) Aripiprazole (3) Valproic acid (300) Olanzapine (2.5)	12	3	6.3	Orthostatic hypotension
(9) MDD (M, 48)	Stage III	Duloxetine (20) Mirtazapine (30) Lithium (200) Mianserin (30)	12	2	41.2	Drowsiness
(10) MDD (M, 26)	Stage II	Paroxetine (40)	8	3	55.6	None (common cold)
(11) MDD (M, 32)	Stage III	Paroxetine (40) Sulpiride (600)	12	2	70.0	Nausea
(12) MDD, dysthymic disorder (M, 39)	Stage III	Paroxetine (40) Milnacipran (25)	12	3	17.4	Drowsiness
(13) MDD (F, 34)	Stage III	Clomipramine (150) Lithium (400) Sulpiride (150)	12	2	90.0	None

Table 2: Continued.

Patient number and DSM-IV diagnosis (Sex, Age)	Stage of treatment-resistant depression	Prior/concomitant medication (dosage: mg/day)	Follow-up visit (weeks)	Pramipexole dosage at endpoint (mg/day)	HDRS-21 decrease at endpoint (%)	Adverse events (reason for dropping out)
(14) MDD (F, 23)	Stage III	Amitriptyline (75) Sertraline (100) Valproic acid (400)	12	1.125	71.4	Agitation Insomnia Dry mouth
(15) BPII (F, 27)	Stage II	Mirtazapine (30) Carbamazepine (100)	12	1.5	31.3	Drowsiness
(16) MDD (F, 24)	Stage I	Sertraline (50)	12	1	73.3	None
(17) BPII (F, 40)	Stage III	Fluvoxamine (75) Paroxetine (20) Lithium (200)	12	1.5	81.3	None

MDD: major depressive disorder; BPII: bipolar II disorder; HDRS-21: 21-item version of the Hamilton Depression Rating Scale.

Table 3: Studies on the efficacy of adjunctive dopamine agonist in treatment-resistant unipolar major depressive disorder.

Study	*N* of subjects	Stage for treatment-resistant depression	Design	Dopamine agonist		Follow-up visit (weeks)	Efficacy (% responder)
				Drug	Dosage at endpoint (mg/day)		
The present study	12	mostly stage II and III	Open-label	pramipexole	1.47 ± 0.93	12	71[b]
Inoue et al. [8]	6	stage I	Open-label	bromocriptine	40.0[a]	6	67[b]
Izumi et al. [9]	20	stage I	Open-label	pergolide	0.59 ± 0.38[a]	4	40[c]
Sporn et al. [10]	20	stage I	Retrospective chart review	pramipexole	NA	NA	40[c]
Lattanzi et al. [11]	16	stage I	Open-label	pramipexole	0.95 ± 0.32[a]	16	64[d]
Cassano et al. [12]	7	stage I	Open-label	ropinirole	1.29	16	29[d]
Inoue et al. [13]	10	stage II	Open-label	pramipexole	1.3 ± 0.6[a]	8	60[d]

NA: not applicable.
[a]Maximum dosage.
[b]Defined as a reduction of 50% or more (from baseline to endpoint) in the Hamilton Depression Rating Scale total score.
[c]Defined as moderate to marked improvement (from baseline to endpoint) in the Clinical Global Impression-Improvement scale.
[d]Defined as a reduction of 50% or more (from baseline to endpoint) in the Montgomery-Asberg Depression Rating Scale total score.

for pramipexole. While most of the studies targeted stage I treatment-resistant depression, two recent studies including ours have observed marked efficacy of adjunctive dopamine agonist for stage II (or III) refractory depression.

4. Discussion

The main finding was that many of our treatment-resistant depressed patients responded to adjunctive pramipexole treatment. This finding is in line with those of previous studies [8–11, 13]. Moreover, the present study, using a larger sample, confirmed the finding of Inoue et al. [13], that this effect can also be observed in stage II refractory patients. In addition, the present study suggested that adjunctive pramipexole may be effective even for stage III treatment-resistant depression.

The majority of responders demonstrated the 50% or more reduction of HDRS-21 total score within the first 4 weeks of pramipexole treatment. Consistent with the present result, previous studies have shown that pramipexole augmentation in MDD brings a relatively rapid improvement in depressive symptoms [11, 13]. In treatment-resistant bipolar depression, two randomized controlled trials demonstrated that the addition of pramipexole to existing mood stabilizers resulted in a significant improvement in depressive symptoms [6, 7]. In MDD, there is one randomized controlled trial that investigated the effect of pramipexole, although that study did not examine "treatment-resistant" patients [18]. Thus, the evidence of pramipexole in refractory MDD has been scarce to date. Furthermore, efficacy of a dopamine agonist in refractory MDD patients whose degree of treatment resistance is explicitly defined as stage II or more has been examined only in one open trial [13], although the other studies investigating adjunctive pramipexole therapy in treatment-resistant depression may have included stage II MDD patients [8–12]. On the other hand, only one of the three dysthymic patients benefited from the pramipexole augmentation (Table 2). This suggests that pramipexole may be less effective in dysthymia than in pure MDD, although the small sample size does not allow any conclusions to be drawn regarding efficacy of pramipexole in dysthymia.

With respect to the dosage of pramipexole, a systematic review of the studies on pramipexole in mood disorders reported that the mean daily dose of pramipexole in the total 156 patients was 1.6 mg [19], which is almost identical to that in the present study. The final dosage of pramipexole varied widely between patients who showed response, from 0.25 to 3 mg/day. This indicates that pramipexole can exert its optimal therapeutic effect at a relatively low dose while higher doses may be needed in other cases. Since all of the four patients in whom pramipexole was increased up to 3 mg/day did not report serious adverse events, it may be suggested that the dosage be increased to 3.0 mg/day if the patient did not respond to lower dosage. Indeed, there is some evidence suggesting a dose-response relationship of pramipexole [19].

The mechanisms underlying the antidepressant effect of pramipexole are not elucidated. A recent neuroimaging study, however, showed that clinical improvement with pramipexole augmentation in bipolar depression was associated with a reduction in regional metabolism in orbitofrontal cortex, ventrolateral prefrontal cortex, and anteromedial prefrontal cortex [20]. This finding provides support for a role of the central dopaminergic system in the pathophysiology of depression since cerebral metabolic activity in these regions has been found to be elevated in depression [21, 22].

As for safety, no severe dopaminergic adverse events, such as delusions, hallucinations, and sleep attacks, were observed, although three patients experienced mild to moderate nausea. It is suggested that pramipexole has a lower risk of psychosis when used in depression than in Parkinson's disease [19], which may be attributable to relatively younger subjects in depression. In addition, no other serious side effects of pramipexole, including compulsive behavior and manic episodes, were seen in any of our patients. Our results therefore suggested good tolerability of pramipexole augmentation therapy for MDD. It should be noted, however, that the present 12-week trial was unable to examine any withdrawal effect of pramipexole.

There were several limitations to the current study. First and foremost, this is an open study that does not have placebo or active control groups, which may have led to some potential biases such as observer bias. Second, the small sample size prevented us from conducting post-hoc multivariate analyses to control for or stratify by demographic/clinical variables. Third, our sample was heterogeneous in terms of diagnosis and treatment-resistant stage, although this might rather be advantageous in terms of ecological validity. Finally, concomitant medication was not standardized.

In summary, pramipexole may be effective and relatively well tolerated in depressed patients who have failed to respond to previous medications. Future randomized controlled trials for treatment-resistant major depression are needed to prove the efficacy and safety of pramipexole.

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Heteroplasmic m.1624C>T mutation of the mitochondrial tRNA^{Val} gene in a proband and his mother with repeated consciousness disturbances

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ABSTRACT

Homoplasmic m.1624C>T mutation of the mitochondrial tRNA^{Val} gene was previously demonstrated to cause fatal neonatal Leigh syndrome. Here, we report the clinical phenotypes of a Japanese male and his mother with heteroplasmic m.1624C>T mutation. The 36-year-old male presented with repeated episodes of consciousness disturbance since the age of 25, cognitive decline, and personality change. Cerebrospinal fluid levels of lactate and pyruvate were elevated. His mother showed similar symptoms and course. The mutation m.1624C>T was identified heteroplasmically in the proband's muscle and leukocytes and in the mother's leukocytes. The heteroplasmy load decreased with age.

1. Introduction

Point mutations in mitochondrial DNA (mtDNA) are responsible for a group of mitochondrial encephalomyopathies, maternally inherited disorders characterized by impaired energy production. Several hundred mtDNA mutations are reported in the Human Mitochondrial Genome Database (Brandon et al., 2005) as being associated with mitochondrial diseases. Each cell has 2–100 mitochondria and each mitochondrion contains 4–10 copies of the mtDNA. Heteroplasmy is the presence of both normal and mutant mtDNA at different levels within the same cell or tissue and is reported to be associated with the phenotypic variation within mitochondrial disease (DiMauro and Moraes, 1993).

Here we describe the clinical phenotypes observed in a Japanese mother–child pair harboring heteroplasmic m.1624C>T mutation. This mutation, which maps within the MTTV gene that encodes an mt-tRNA (valine), affects the dihydrouridine loop that is highly conserved across species from yeast to human (McFarland et al., 2002). McFarland et al. reported severe sibling cases with homoplasmic m.1624C>T mutation, which caused infantile and fatal Leigh syndrome-like symptoms. Cytochrome c oxidase (COX)-deficient fibers were present in the skeletal muscle of these cases. The pathogenicity of the m.1624C>T mutation was confirmed by the extremely low steady-state levels of mt-tRNA^{Val} observed in m.1624C>T mutant cell lines and the cardiac and skeletal muscle of the patients. However, until now, there has been no report of a patient with heteroplasmic m.1624C>T mutation.

2. Patients and methods

2.1. Case reports

2.1.1. Proband

The proband is a 36-year-old male (Fig. 1A; II-3), who is 170.5 cm tall and weighs 61 kg (normal body mass index of 21). He is the third of four children of non-consanguineous Japanese parents. He was normal at

Abbreviations: ARMS, amplification refractory mutation system; COX, cytochrome c oxidase; CSF, cerebrospinal fluid; CT, computed tomography; Ct, threshold cycle; EEG, electroencephalogram; mtDNA, mitochondrial DNA; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; VARS2, valyl-tRNA synthetase 2; WAIS, Wechsler Adult Intelligence Scale.

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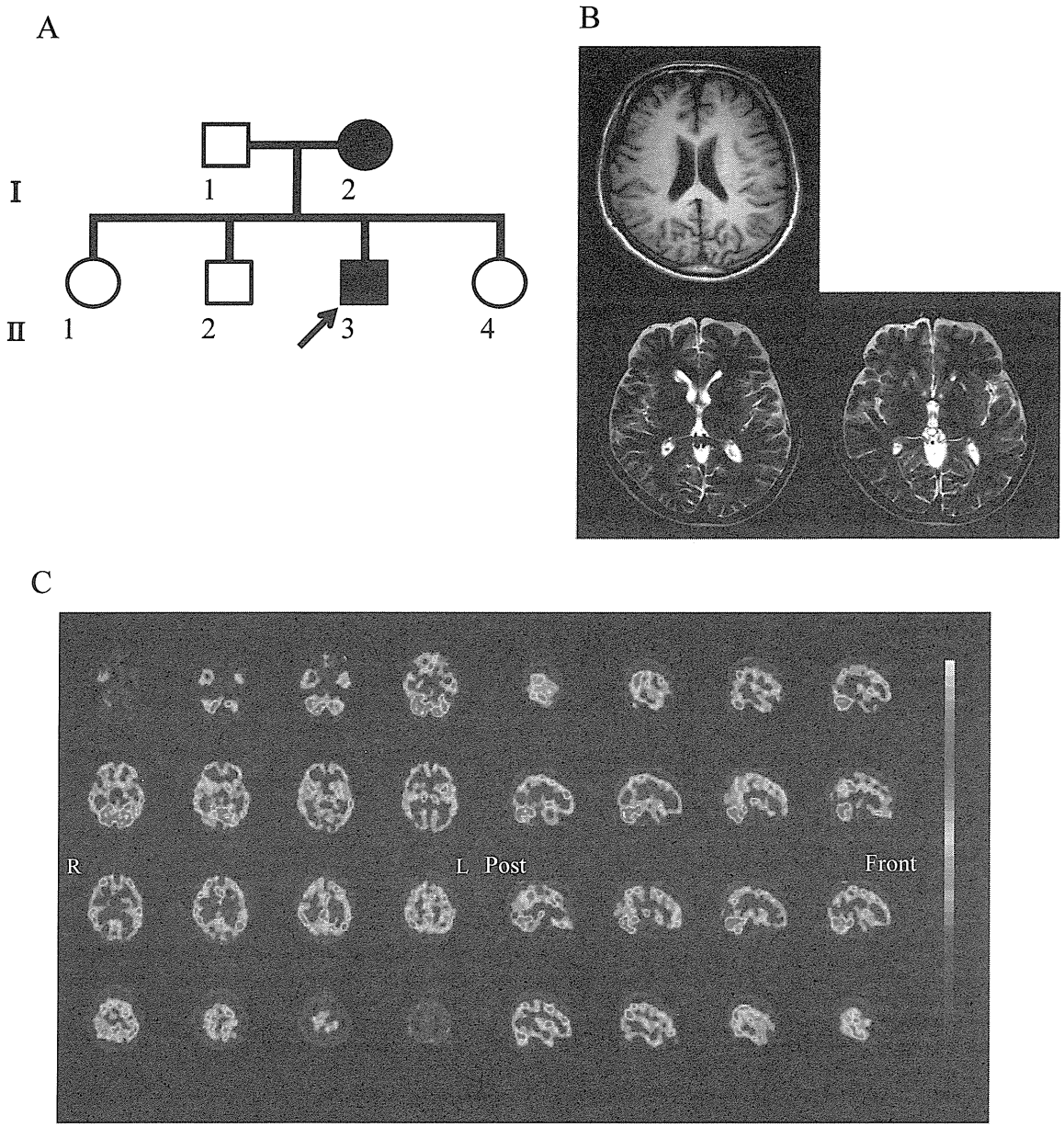

Fig. 1. Proband's family tree and brain imaging. (A) Pedigree of the family. The proband is indicated with an arrow. Affected and unaffected status is indicated by black and white symbols, respectively. (B) Brain magnetic resonance imaging of the proband revealed frontal lobe atrophy and high intensity on T2-weighted images and low intensity on T1-weighted images in the right caudate head and bilateral putamen. (C) Single photon emission computed tomography using technetium-99 m revealed reduction of cerebral blood flow in the proband's bilateral frontal lobe and the bilateral medial temporal lobe.

birth and grew well but dropped out of his high school. He then worked as a construction worker. His brother and sisters have neither neuromuscular nor neuropsychiatric symptoms. His personality gradually changed by age 36, developing irritability and emotional insecurity, which disrupted his relationships with others. He has been admitted to psychiatric hospital four times since he was 25 years old for repeated episodes of transient mild consciousness disturbance with delirious psychomotor agitation. These consciousness disturbances were not associated with lactic acidosis (data not shown). He presented with neuropsychiatric symptoms including disinhibition, cognitive decline, and personality change. He did not exhibit hallucination and delusion.

An electrocardiograph and echocardiogram at age 29 revealed left ventricular hypertrophy. Electromyograms of the right biceps and right rectus femoris showed normal findings. Deafness, diabetes, or ptosis were not observed. The concentrations of both lactate and pyruvate in the cerebrospinal fluid (CSF) at ages 25 and 29 were elevated, but those in the serum were not elevated at age 25 (Table 1). At age 29, the serum pyruvate level was elevated, but the lactate level was normal. Serum creatine kinase was transiently elevated at age 29. There were no abnormal findings on tests of liver and renal function and electrolyte levels. The levels of serum amino acids, urinary amino acids, and serum organic acids (except lactate and pyruvate) were within normal limits. He showed labile neurological signs (Table 2). Although his eye movements were normal at the age of 25, oculomotor disturbance and constriction of the visual fields appeared transiently at age 29. He showed a symmetrical tendon reflex and Babinski's signs were not observed at age 25. At age 29, increased left patellar tendon reflex and bilateral Babinski's signs

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Table 1
The concentration of lactate and pyruvate.

	Age	Lactate (10–20 mg/dl)	Pyruvate (0.62–1.2 mg/dl)
CSF[a]	25	28↑	1.3↑
	29	36↑	1.3↑
Peripheral blood	25	9	0.7
	29	16	1.1↑
Gas analysis	25	18 (pH 7.42)	N/A

[a] CSF = cerebrospinal fluid.

were observed, but these were not observed at age 36. Ataxic gait, slurred speech, and poor coordination were evident at ages 29 and 36. Consciousness disturbance and psychomotor agitation were significantly improved by carbamazepine treatment. Electroencephalograms (EEGs) showed a continuous 7-Hz slow wave rhythm at age 25. The slow wave abnormal EEG was improved after treatment with carbamazepine. Medication withdrawal-related relapses were observed several times. Disinhibition, cognitive decline, and personality change remained after treatment.

Table 3 shows the results of the neuropsychological examinations. His intelligence quotient score measured using the Wechsler Adult Intelligence Scale (WAIS)—Revised (Wechsler, 1981) and the WAIS-III (Wechsler, 1997) decreased slightly from 69 (WAIS-R) to 61 (WAIS-III) from ages 29 to 36. Assessment of frontal lobe function by the Trail-Making Test (Corrigan and Hinkeldey, 1987), the modified Stroop test (Ichiba et al., 2007), the Wisconsin card-sorting test (Nelson, 1976), and the word fluency test (Borkowski et al., 1967) revealed progressive impairment of frontal lobe functions from age 29 to 35. Brain magnetic resonance imaging at ages 25 (data not shown) and 35 (Fig. 1B) revealed mild frontal lobe atrophy and low and high signal intensity spots on T1- and T2-weighted images, respectively, in the right caudate head and bilateral putamen. Single photon emission computed tomography using technetium-99 m at the age of 25 revealed reduced cerebral blood flow in the frontal lobe (Fig. 1C). Muscle histopathology revealed moderate variability in fiber size but neither ragged-red fibers nor COX-deficient fibers.

2.1.2. Proband's mother

The proband's mother (Fig. 1A; II-2) is 65 years old. She was the first of four children of non-consanguineous Japanese parents. She was normal at birth and grew well. She worked as an office worker and got married at the age of 24. Since age 37, she has experienced repeated stroke-like episodes with a partial seizure with secondary generalization and status. Brain computed tomography (CT) revealed small low-density areas in the right occipital and parietal lobes, both of which had disappeared after 15 days. She was repeatedly admitted to general hospitals. She was admitted to a psychiatric hospital at age 38, because of psychomotor excitation with auditory and visual hallucinations and persecutory delusions. She heard noisy sounds as auditory hallucinations and saw flowers, clowns, and a yellow mattress as visual hallucinations. Deafness, diabetes, or ptosis were not observed. Serum pyruvate and lactate levels were normal. There were no abnormal findings on tests of liver and renal function and electrolyte levels.

Table 2
Neurological signs of the proband.

Neurological signs	Age		
	25	29	36
Oculomotor disturbance	(−)	(+)	(−)
Constriction of visual fields transiently	(−)	(+)	(−)
Increased left patellar tendon reflex	(−)	(+)	(−)
Babinski's signs	(−)	(+)	(−)
Ataxic gait	(−)	(+)	(+)
Slurred speech	(−)	(+)	(+)
Poor coordination	(−)	(+)	(+)

Table 3
Neuropsychological examinations.

Neuropsychological tests	Age	
	29	36
	WAIS-R[a]	WAIS-III
VIQ	73	63
PIQ	69	65
FIQ	69	61
Trail Making Test		
Part A	73 s	69
Part B	265 s	267 abort
Modified Stroop test		
Read around	48 s (error 1/50)	43 s (error 1/50)
Color naming	90 s (error 5/50)	88 s (error 16/50)
WCST[b]		
CAT1	3	2
CAT2	5	3
Word fluency test		
Category	8.0	8.0
Initial phoneme	3.3	1.7

[a] WAIS-R = Wechsler Adult Intelligence Scale—Revised.
[b] WCST = Wisconsin card sorting test.

CSF measurements of cell count, pressure, and total protein and sugar were also unremarkable although the CSF levels of pyruvate and lactate were not measured. She presented with transient consciousness disturbance, psychotic symptoms including auditory and visual hallucinations and persecutory delusions, and cognitive impairment including alexia, agraphia, and acalculia. On the fourth day of the admission, EEG revealed poor alpha rhythms, an excess of fast background, and asymmetrical occasional appearance of a slow wave. Neurological examination showed symmetrical tendon reflex, no paralysis, and no Babinski's signs. An echocardiogram revealed left ventricular hypertrophy. Brain CT revealed small low-density areas in the right occipital and parietal lobes, both of which had disappeared after a month. The EEG abnormality, consciousness disturbance, and psychiatric symptoms improved after treatment with carbamazepine and haloperidol, although the alexia, agraphia, and acalculia remained. She developed left-side paresis at the age of 48. Brain CT revealed a fresh infarction in the right parieto-occipital region. The alexia, agraphia, and acalculia were persistent.

2.2. Neuropsychological examination

The proband was examined using the WAIS-R (Wechsler, 1981), WAIS-III (Wechsler, 1997), the Trail-Making Test (Corrigan and Hinkeldey, 1987), the modified Stroop test (Ichiba et al., 2007), the Wisconsin card sorting test (Nelson, 1976), and the word fluency test (Borkowski et al., 1967).

2.3. Genetic analysis

Total DNA was extracted using a standard protocol from muscle biopsy samples and leukocytes. Written informed consent was obtained from all subjects. The research protocol and consent form were approved by the Institutional Review Boards of Kagoshima University and Ehime University.

2.4. Mutation analysis

The entire 16,569 bp of mtDNA was amplified in 27 overlapping polymerase chain reaction (PCR) fragments. All primers were designed using the 'Primer 3 plus' web interface (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). Annealing temperatures and times were determined according to the primer sequence and sequence abundance, respectively. Amplified products were purified using a QIAquick PCR purification kit (Qiagen, Hilden, Germany), labeled using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA), and

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directly sequenced on an ABI PRISM 3130 Avant Genetic Analyzer (Applied Biosystems) (Kato et al., 2011).

2.5. Quantification of mtDNA heteroplasmy

2.5.1. Primers for ARMS (Table 4)

To measure low proportions of mutant heteroplasmy, we designed mismatched primers for an amplification refractory mutation system (ARMS) assay (Newton et al., 1989). The 3′ end of the forward primer for the wild-type or mutated sequence was designed to amplify only the mutated or wild-type sequence, respectively (Bai and Wong, 2004; Genasetti et al., 2007). All primers were designed using the 'Primer 3 plus' web interface. Primer specificity was tested by gradient PCR to achieve the best yield and specificity at the most suitable annealing temperature, to design a real-time assay.

2.5.2. Quantitative real-time PCR

We measured the proportion of the m.1624C>T mtDNA mutation by real-time PCR using the THUNDERBIRD SYBR qPCR Mix (TOYOBO, Osaka, Japan). A range of 5 to 40 ng of total DNA in a volume of 20 μl was used. Nonspecific bands and primer dimers, which lead to inaccurate quantification by real-time PCR, were not observed.

Real-time PCR was performed with an ABI Prism 7300 (Applied Biosystems) and universal cycling conditions (2 min at 50 °C, 10 min at 95 °C, 40 cycles of 15 s at 95 °C, and 1 min at 60 °C). At least nine separate experiments were performed.

2.5.3. Preparation of DNA for assay calibration

We prepared calibration curves using known amounts of cloned plasmid DNA containing the wild-type or mutant sequence. A 712-bp PCR product generated using primers mtF1095 and mtR1787 (Table 4) was cloned into the pGEM-T vector (Promega, Madison, WI). One plasmid contained m.1624C (wild type) and the other contained m.1624T (mutant). Plasmid DNA was purified with a FastPlasmid Mini kit (Eppendorf, Hamburg, Germany) and a NucleoBond Xtra Midi Plus kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's protocols. To obtain accurate calibration curves, the empty pGEM-T vector was diluted into the reaction solution and the plasmid clone was digested with the restriction enzyme SpeI. The copy numbers of the wild-type and mutant DNA sequence were calculated based on the size and molecular weight of the plasmid DNA. The molecular weight of 1 kb of double-stranded DNA (6.6×10^5 g/mol) and Avogadro's number were needed for the calculation. The optimal concentrations of wild-type and mutant plasmid DNA were determined by PCR with the optimal ARMS primer for real-time PCR. In this way, we decided to use approximately 10^6 copies of the plasmid.

2.5.4. Measurement of mutant heteroplasmy

The calibration curves for both the wild-type and mutant mtDNA were included in each run. The copy number of the target sequence in the sample was calculated from the threshold cycle (Ct) number and the calibration curve. The proportion of m.1624C>T mutation was calculated from the copy number of the wild-type and mutant sequences.

Table 4
Primers.

Primers	Nucleotide positions	Primer sequences
For insert PCR		
F1095	1095–1114	5′TAGCCCTAAACCTCAACAGT3′
R1787	1787–1806	5′ATTTTTCATCTTTCCCTTGC3′
For ARMS		
1624C (Wild type)	1601–1624	5′CCAGAGTGTAGCTTAACACAAAGC3′
1624T (Mutation)	1601–1624	5′CCAGAGTGTAGCTTAACACAAAGT3′
Mismatch primers		
1624CC (Wild type)	1601–1624	5′CCAGAGTGTAGCTTAACACAAACC3′
1624CT (Mutation)	1601–1624	5′CCAGAGTGTAGCTTAACACAAACT3′

Underlined nucleotide corresponds to the mismatched nucleotide.

3. Results

3.1. Sequencing of mtDNA

Given that the proband's symptoms suggested a mtDNA defect, we sequenced the entire mtDNA from the proband's leukocytes and muscle and from the proband's mother's leukocytes. We identified a heteroplasmic m.1624C>T mutation (Fig. 2).

3.2. Quantification of the m.1624C>T heteroplasmy with ARMS

We drew calibration curves by plotting the logarithm of the plasmid copy number against the Ct value. For the calibration plots, we used step-wise dilutions of an m.1624T mutant plasmid as the template for real-time PCR with the ARMS method (Fig. 2). For each sample mtDNA, we plotted the Ct number on the calibration curves; we were then able to calculate the proportion of the m.1624C>T mutation in the sample.

The proportions of the m.1624C>T mutation in the proband's muscle at age 29, muscle at age 36, leukocytes at age 29, leukocytes at age 36, in his mother's leukocytes, and in a control individual's leukocytes were 88.8%, 59.7%, 47.8%, 34.0%, 17.2%, and 0%, respectively (Fig. 2).

4. Discussion

Several reports have demonstrated the existence of mtDNA mutations in patients with psychiatric disorders such as depression, bipolar disorder, schizophrenia, mental retardation, and Asperger syndrome (Fattal et al., 2006; Kato et al., 2011; Munakata et al., 2007; Rollins et al., 2009; Rossignol and Frye, 2012). In the present study, we identified heteroplasmic m.1624C>T mutation in the proband and his mother, who both suffered from neuropsychiatric symptoms including repeated episodes of consciousness disturbance and psychomotor agitation with elevation of lactate and pyruvate in the CSF. This suggests that the neuropsychiatric symptoms are strongly associated with mitochondrial dysfunction in these patients.

Despite the m.1624C>T mutation being present in the proband's muscle at 88.8% at age 25 and 59.7% at age 36, no muscle pathology or mitochondrial myopathy were observed except for variability in fiber size. This is in contrast to a previous study, in which COX-deficient fibers were present in the muscles of individuals with homoplasmic m.1624C>T mutation (McFarland et al., 2002). Jeppesen et al. (2006) demonstrated that there was a clear threshold m.3243A>G mutation level at which morphological abnormalities, including COX-deficient fibers and ragged-red fibers, occurred. Similarly, there may be a threshold m.1624C>T mutation level at which pathological abnormalities occur in skeletal muscle. In the present study, neuropsychological tests in the proband showed slowly progressive cognitive decline with frontal lobe dysfunction. The presence of neuropsychiatric symptoms, imaging findings, and elevation of lactate and pyruvate in the absence of muscle symptoms and pathology indicate that the condition is more similar to mitochondrial encephalopathy than it is to mitochondrial encephalomyopathy.

The proportions of mitochondrial heteroplasmy have been calculated by real-time PCR or restriction fragment length polymorphism (RFLP) analysis. However, RFLP analysis may not be sufficiently sensitive to detect a low proportion of mutated mtDNA. Bai et al. described that the validity of real-time quantitative PCR using allele-specific TaqMan probes was similar to that of RFLP analysis because nonspecific binding of the mutant TaqMan probe to wild-type target DNA, and vice versa, disrupts quantitative analysis if there is a low proportion of

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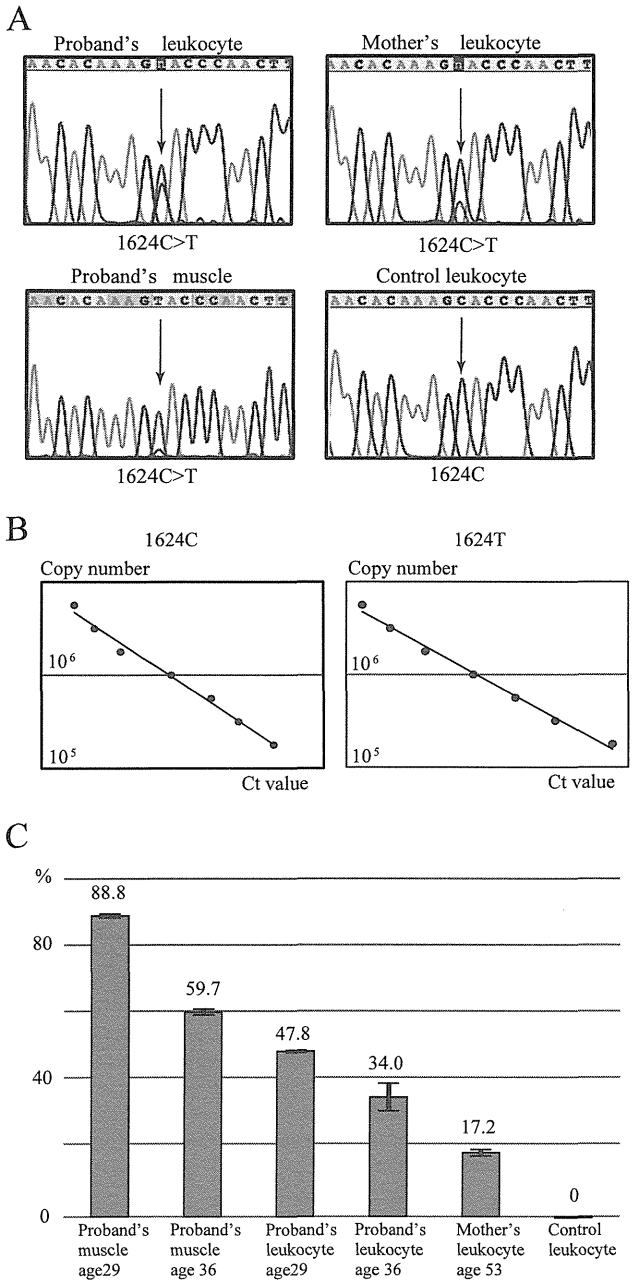

Fig. 2. Analysis of mtDNA. (A) Sequencing revealed m.1624C>T heteroplasmy in the proband's leukocytes and muscle and in the mother's leukocytes, but not in leukocytes from a control individual. The m.1624C>T mutation is indicated by arrows. In the mother's leukocytes, there is a low proportion of heteroplasmy, almost at the level of background noise. (B) Calibration curves of mutant mtDNA (m.1624T) and wild-type mtDNA (m.1624C). Step-wise dilutions of a plasmid containing the site of mutation were used as templates for real-time PCR with the ARMS method. Calibration curves were obtained by plotting the logarithm of the plasmid copy number against the Ct value. (C) Bar graph showing the proportions of the m.1624C>T mutation, calculated by plotting each Ct value on the calibration curves. The data are shown as mean ± SD ($n = 9$).

mutant mtDNA (Bai and Wong, 2004). These authors recommended the ARMS assay, which was developed to measure a low proportion of mutant heteroplasmy and is more accurate than using TaqMan probes in this situation. In the present study, we first detected heteroplasmy by mtDNA sequencing, although a low proportion of heteroplasmy can be masked in the background noise. As demonstrated in other studies (Bai and Wong, 2004; Genasetti et al., 2007; Sakiyama et al., 2011), using ARMS, we were able to detect variable proportions of the m.1624C>T mutation.

The proportion of heteroplasmic mtDNA is generally one determinant of phenotypic severity (Choi et al., 2010; Laloi-Michelin et al., 2009). Compared with the severely affected Leigh syndrome-like siblings with homoplasmic m.1624C>T mutation, our heteroplasmic cases showed milder phenotypes with respect to the age of onset and clinical features (Table 5); the lower proportion of mutant mtDNA might be responsible for this. However, the siblings' mother, who also harbored homoplasmic m.1624C>T mutation, suffered from comparatively milder symptoms, such as occasional migraine headaches, fatigue, and proximal muscle weakness (McFarland et al., 2002), implying that there may be additional modifying factors.

Rorbach et al. discussed how variations in the levels of VARS2 (called VARS2L in this paper) between tissue types and patients could underlie the difference in clinical presentation among individuals with homoplasmic m.1624C>T mutation (Rorbach et al., 2008). Our results agree that variable proportions of the m.1624C>T mutation, and consequently of VARS2, could cause differences in the clinical phenotype between individuals with m.1624C>T mutation.

We showed a reduction in the proportion of heteroplasmic m.1624C>T mutation in the proband's leukocytes and muscle over 7 years (Fig. 2). Similarly, previous reports (Olsson et al., 2001; 't Hart et al., 1996) have demonstrated that the proportion of heteroplasmic m.3243A>G mutation in leukocytes decreases with advancing age. This may be because of selection against high levels of pathogenic mtDNA in cells with rapid turnover (Olsson et al., 2001; 't Hart et al., 1996). Alternatively, another report revealed that, in diabetic patients, the somatic m.3243A>G mutation accumulated with age and the duration of diabetes (Nomiyama et al., 2004). In addition, the m.3243A>G mutation tends to accumulate and increase in abundance in post-mitotic cells with advancing age (Zhang et al., 1998). It is unknown why the load of m.1624C>T mutation in our proband's muscle reduced with age. A similar age-related decrease in m.13167A>G load with age (Zhang et al., 1998) was explained as follows: the mutation is generated during oocyte development or early embryogenesis, but the mutant molecules replicate more slowly than wild-type molecules do, leading to a decline in abundance with age. Post-mortem findings have indicated that the mutation load is similar among post-mitotic tissues (skeletal muscle, nervous system, and cochlea) (Macmillan et al., 1993; Shiraiwa et al., 1993). Thus, the proportion of m.1624C>T mutation, which is perhaps similar between muscle and brain, may be affected by slow

Table 5
Comparison of symptoms in the patients from this report and the patients studied by McFarland et al. (2002). qPCR analysis of heteroplasmy is provided for our family. The percentage of m.1624C>T heteroplasmy, onset of the ages and symptoms are given.

	Our patients		McFarland et al.'s (2002) patients	
	Proband	Mother	Mild case	Severe cases
Percentage of m.1624C>T heteroplasmy (%)				
Leukocyte	47.8 (age 29) 34.0 (age 36)	17.2 (age 53)	100 (age 35)	100 (infantile to <85 h after birth)
Muscle	88.8 (age 29) 59.7 (age 36)	NA[a]	100 (age 35)	100 (infantile to <85 h after birth)
Onset of age	25	38	<35	<0
Symptoms of onset	CD[b]	CD[b]	Unknown	Lethal or Leigh-symptoms

[a] NA = not available.
[b] CD = consciousness disturbance.

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replication. Further analyses, including brain biopsy or autopsy, will be required to test these predictions.

5. Conclusion

We identified heteroplasmic m.1624C>T mutation in a proband and his mother who suffered from repeated consciousness disturbance, cognitive decline, and personality change. In comparison with individuals with homoplasmic m.1624C>T mutation, our cases exhibited milder symptoms and course. Individual variability in the clinical features was apparent; this may depend upon the heteroplasmy load and unknown modifying factors.

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Case report

Novel *AGTR2* missense mutation in a Japanese boy with severe mental retardation, pervasive developmental disorder, and epilepsy

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Abstract

Angiotensin II type-2 receptor gene (*AGTR2*) mutations have been recently detected in patients with mental retardation. *AGTR2* plays a role in central nervous system development and cognitive functions. We identified a novel missense mutation of c.572G>A (p.G191E) in a 6-year-old boy showing severe mental retardation, pervasive developmental disorder, and epilepsy. This is the first report on *AGTR2* mutation in a Japanese boy with mental retardation.

Keywords: Angiotensin II type-2 receptor gene (*AGTR2*); Epilepsy; Mental retardation (MR); Pervasive developmental disorder (PDD)

1. Introduction

Mental retardation (MR) affects approximately 1–3% of the human population. Family studies demonstrated a relatively large number of X-linked MRs (XLMRs). This appears to explain the higher MR incidence in males than in females. Approximately 90 XLMR genes have been identified [1]. Angiotensin II type-2 receptor gene (*AGTR2*) is one of the genes causing XLMR.

Angiotensin II is largely known for its role in regulation of blood pressure and water electrolyte balance through one of its two receptors, AGTR1 and AGTR2. AGTR1 mediates major cardiovascular effects of angiotensin II, whereas the main function of AGTR2 remains unclear [2–4]. AGTR2-defective mice showed neurological involvement [5]. Mutant mice lacking AGTR2 had displayed an attenuated exploratory behavior and anxiety-like behavior [5] and significant impairment in learning performance on spatial memory tasks [6]. Recently, some *AGTR2* mutations have been detected in patients with MR, suggesting a possible AGTR2 function in the human central nervous system and cognitive development [2–4].

In March 2005, with an intention of organizing a repository for genetic analysis of MR at the National Center of Neurology and Psychiatry, our research group started collecting blood samples under informed consent and providing a diagnostic service for known genetic

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defects or chromosomal abnormalities. We also analyzed *AGTR2* in 221 patients with MR (203 males; 18 females) and identified a novel *AGTR2* missense mutation in a Japanese boy with MR, pervasive developmental disorder (PDD), and epilepsy. This is the first report describing the case with an evaluation of the clinical details.

2. Case report

The patient was a 6-year-old boy who was born uneventfully (body weight, 3100 g (+0.2 SD); head circumference, 35.0 cm (+1.2 SD)). His parents were non-consanguineous, paternal grandmother had depression, maternal grandmother had attention deficit hyperactivity disorder, also brothers of his maternal granduncle had epilepsy (Fig. 1). He acquired head control at 3 months, rolled over at 5 months, sat alone at 6 months, and walked unassisted at 1 year. At age 11 months, he developed Kawasaki disease but recovered without any sequelae. He learnt to speak some words after age 1 year, but presented echolalia. No further increase in vocabulary was observed. Moreover, he avoided eye contact, had communication problems, and showed persistency, hyperactivity, impulsivity, and poor concentration-ability. He was diagnosed with PDD-not otherwise specified.

The patient was first referred to our hospital at age 3 years to determine the cause of his MR. His height, body weight, and head circumference were 94.3 cm (+0.3 SD), 14 kg (+0.2SD), and 51.5 cm (+1.3 SD), respectively. His blood pressure at rest was normal. He had no dysmorphic or neurological abnormalities. Brain magnetic resonance imaging (MRI) showed no abnormality (Fig. 2A). Epileptic seizures first appeared clinically at age 3 years. They started with nausea, and he subsequently vocalized and collapsed; the seizures were suspected as being a localization-related epilepsy (frontal lobe epilepsy). Initially, seizure frequency was once a year, but gradually increased to once a month. An electroencephalogram (EEG) showed multifocal spikes or spike and waves, predominantly over the right frontal and bilateral occipital areas (Fig. 2B). Sodium valproate was started at age 6 years 2 months, and seizure frequency decreased to approximately once every 3–4 months. At age 6 years, his developmental quotient was 31 (1 year 10 months level) as determined by the Kyoto scale developmental quotient test. A flash-visual evoked potential (VEP) revealed a mild delayed latency of wave IV (P100), and his auditory brainstem response (ABR) showed a delayed latency of wave I. His cardiothoracic ratio was 53%, and electrocardiogram was normal. G-banded chromosome analysis showed a normal karyotype of 46, XY. Sequence analysis of *AGTR2* revealed a c.572G>A mutation leading to a p.G191E substitution (Fig. 3A and B). His mother was healthy and carried the mutation. Other relatives were unavailable for the study. This missense mutation had not been detected in previous studies and/or in normal controls (52 male; 51 females).

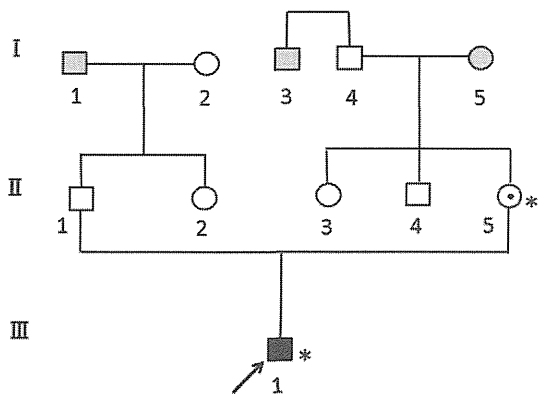

Fig. 1. Family tree of the patient. The arrow indicates the index patient and the dotted circle indicates the carrier female. Gray squares indicate some symptoms (I-1: depression, I-3: epilepsy, I-5: attention deficit hyperactivity disorder). Asterisk indicates family members tested for the mutation.

3. Discussion

Our patient showed symptoms such as severe MR, autistic features, and epilepsy, which are similar to those of previously studied patients with MR and *AGTR2* mutation [2–4]. His brain MRI showed no visible structural abnormality. However, flash-VEP suggested mild impairment of the optic pathway, and ABR indicated abnormal conductive function on the left side. These findings may suggest visual and auditory impairment. His EEG also showed significant paroxysmal abnormalities, which may reveal abnormal discharge of neurons. These abnormal findings may be related to cognitive impairment in this patient and are consistent with those in AGTR2-defective mice.

The association between *AGTR2* mutation and MR is controversial. Several studies indicated that *AGTR2* mutations published as causative have turned out to be possible polymorphisms because these substitutions were found in normal control, so *AGTR2* mutations do not directly cause MR [7,8]. However, the lines of evidence supporting the pathogenetic role of p.G191E are its low frequency, its absence in 154 normal X chromosomes from normal subjects, and its conserved position in the amino acid sequence of human, rat and mouse. This amino acid at position 191 located in the second extracellular loop of the *AGTR2*. This domain is important role in the binding of the ligand of AGTR2 [9].

This patient had developed with Kawasaki disease, a systemic vasculitis, without sequelae; its specific cause of unclear. It presumably results from the interaction