

Three-Year Follow-Up of High-Dose Ubiquinol Supplementation in a Case of Familial Multiple System Atrophy with Compound Heterozygous *COQ2* Mutations

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Published online: 1 February 2017

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Abstract We report a 3-year follow-up of high-dose ubiquinol supplementation in a case of familial multiple system atrophy (MSA) with compound heterozygous nonsense (R387X) and missense (V393A) mutations in *COQ2*. A high-dose ubiquinol supplementation substantially increased total coenzyme Q₁₀ levels in cerebrospinal fluid as well as in plasma. The patient was at the advanced stage of MSA, and the various scores of clinical rating scales remained stable without changes during the 3 years. The cerebral metabolic ratio of oxygen measured by ¹⁵O₂ PET, however, increased by approximately 30% after administration of ubiquinol, suggesting that ubiquinol can improve mitochondrial oxidative metabolism in the brain. It also suggests the therapeutic potential of ubiquinol for patients with MSA with *COQ2* mutations. Further clinical trials of administration of high-dose ubiquinol to MSA patients are warranted.

Keywords Multiple system atrophy · *COQ2* · Coenzyme Q₁₀ · Ubiquinol

Introduction

Multiple system atrophy (MSA) is a progressive neurodegenerative disease clinically characterized by autonomic failure in addition to various combinations of parkinsonism, cerebellar ataxia, and pyramidal dysfunction [1]. Whole-genome sequence analysis in combination with linkage analysis has revealed homozygous or compound heterozygous mutations in *COQ2* in two of the six multiplex Japanese families with MSA [2]. *COQ2* encodes an enzyme in the biosynthetic pathway of coenzyme Q₁₀ (CoQ₁₀) [3]. Indeed, the total CoQ₁₀ levels in frozen brain tissues and lymphoblastoid cell lines from patients with MSA carrying homozygous (M128V-V393A/M128V-V393A) and compound heterozygous mutations (R387X/V393A) were substantially lower than those from control subjects [2]. These observations suggest the efficacy of CoQ₁₀ supplementation for patients with MSA, in particular, for those with *COQ2* mutations.

CoQ₁₀ is a lipophilic molecule that functions as an essential carrier for electron transport in the mitochondrial respiratory chain and as an endogenous antioxidant [4]. Patients with primary CoQ₁₀ deficiency, a severer phenotype than MSA, caused by genetic defects in genes involved in the CoQ₁₀ biosynthetic pathway, have been reported to respond well to CoQ₁₀ supplementation [5–11]. Herein, we present the detailed clinical features of a patient with familial MSA carrying compound heterozygous *COQ2* mutations (R387X/V393A) [2] and report the outcome of a high-dose ubiquinol (reduced form of CoQ₁₀) supplementation to this patient.

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Materials and Methods

Patient

The patient, a 60-year-old male, is an affected member of a previously described Japanese multiplex family with MSA (II-4 in Family 12) [2]. He carried compound heterozygous nonsense (R387X, c.1159C>T) and missense (V393A, c.1178T>C) mutations in *COQ2* (NM_015697.7) [2, 12].

The patient gradually noticed slurring of speech, unsteadiness of gait, increased urinary frequency, and erectile dysfunction at the age of 44. Examination at the age of 45 revealed jerky pursuit of eye movements, gaze-evoked nystagmus, and scanning speech. Lower limb movements were uncoordinated when performing the heel-knee-shin test. His gait was ataxic, and he was unable to perform tandem gait. He had urinary urgency, frequent urination, and erectile dysfunction. He also had frequent orthostatic symptoms attributable to hypotension. His cognition and visual acuity were normal. Brain magnetic resonance imaging (MRI) showed the hot cross bun sign in the pontine base and mild atrophy of the pons and cerebellum. He was then diagnosed as having “familial MSA” because his elder sister had a similar presentation with dysarthria and unsteady gait with the onset at the age of 50. She died at the age of 61. He started intermittent catheterization at the age of 47 and had frequent orthostatic symptoms once a week at the age of 48. He became wheelchair-bound at the age of 50 and subsequently became bed-ridden owing to his severe orthostatic symptoms. At the age of 56, his speech was unintelligible most of the time due to the severe ataxic dysarthria. At the age of 58, endoscopic gastrostomy and laryngotracheal separation were performed owing to his recurrent aspiration pneumonia. At the age of 59, his renal function gradually worsened (serum creatinine level, 1.0–1.5 mg/dl).

Study Design

An open-label dose escalation trial for this patient with familial MSA with compound heterozygous *COQ2* mutations (R387X/V393A) was designed to evaluate the safety and tolerability of high-dose ubiquinol, to assess pharmacokinetics, and to obtain the clinical data including the scores of clinical rating scales [Barthel Index [13], Scale for the Assessment and Rating of Ataxia (SARA) [14], International Cooperative Ataxia Rating Scale (ICARS) [15], and Unified Multiple System Atrophy

Rating Scale (UMSARS) [16]]. Positron emission tomography (PET) was carried out to measure the cerebral blood flow (CBF) and the cerebral metabolic rate of oxygen (CMRO₂). The protocol of this study (UMIN000010712) was reviewed and approved by the institutional review board of the participating institutions. Informed consent was obtained from the patient and his legal representative prior to the initiation of this study, in accordance with the Declaration of Helsinki. Ubiquinol in the powder form (Kaneka QH ubiquinol) was provided by Kaneka Corporation (Tokyo, Japan) and was administered via a gastrostomy tube.

After baseline assessment, supplementation was started at 600 mg of ubiquinol/day (given once a day), with the dosage increased to 840 mg/day at week 2 and to 1200 mg/day at week 6. The 1200-mg/day dosage was maintained until week 8. When no adverse events were observed during this period, the patient resumed taking 1200 mg of ubiquinol/day after an interval of 8 weeks and remained taking ubiquinol at this same dose for over 3 years to date.

Biochemical Analysis

Peripheral blood was collected into heparinized tubes to obtain plasma samples. BD Vacutainer CPT Cell Preparation Tubes (BD, Franklin Lakes, NJ) with sodium heparin were used for separation of peripheral blood mononuclear cells (PBMCs). The total CoQ₁₀ levels (sum of ubiquinol and ubiquinone levels) in plasma, PBMCs, and cerebrospinal fluid (CSF) were measured by high-performance liquid chromatography (HPLC) with electrochemical detection (ECD) [17] or with tandem-mass spectrometry (LC-MS/MS). For measurement of the total CoQ₁₀ level in CSF by HPLC with ECD, 400 μl of CSF was mixed with 1600 μl of isopropanol. An aliquot of 1500 μl was evaporated to dryness and resolved in 120 μl of isopropanol and 30 μl of distilled water. An aliquot of 80 μl of the supernatant was directly injected onto the HPLC equipped with the separation column (SHISEIDO CQ-S: 150 mm × 2.0 mm I.D.) with the mobile phase of 50 mM sodium perchlorate in methanol/distilled water (95/5, v/v). Measurement of the total CoQ₁₀ level in additional CSF samples obtained after 12, 24, and 36 months of ubiquinol supplementation was conducted as follows. Twenty microliters of CSF was diluted to 5 ml with phosphate-buffered saline and mixed with 8 ml of diethyl ether and 10 μl of internal standard solution (200 ng/ml of CoQ₈ in isopropanol). After the solution was vigorously mixed, the supernatant separated

by centrifugation was evaporated to dryness and resolved in 20 μ l of isopropanol. Quantitation of CoQ₁₀ was accomplished by LC-MS/MS using Nexera X2 and LCMS-8060 (Shimadzu, Japan).

Measurement of the CBF and the CMRO₂ by PET

PET studies were carried out with ¹⁵O-labeled tracers (¹⁵O₂, C¹⁵O₂, and C¹⁵O). The C¹⁵O₂ and ¹⁵O₂ steady-state methods were used to measure the CBF and CMRO₂, respectively [18, 19]. PET scanning was performed during C¹⁵O₂ (370 MBq/min) or ¹⁵O₂ (740 MBq/min) gas inhalation after equilibrium had been reached. C¹⁵O PET (1110 MBq/min) was performed to measure cerebral blood volume (CBV) that was used for correction of CMRO₂ [19–21]. PET imaging was performed using a PET scanner (Headtome/SET2400 W, Shimadzu).

For quantitative analysis, brain PET images before and after ubiquinol supplementation were intrasubjectively coregistered and morphologically normalized to the brain PET template using statistical parametric mapping 8 and MATLAB version R2014a (MathWorks Inc., Natick, MA, USA). The regions of interest (ROIs) were manually placed on the cortical ribbon of the upper frontal, lower frontal, Rolandic, lateral and medial parietal, temporal, and occipital areas and on the striatum, thalamus,

cerebellar hemisphere, and cerebellar vermis on the morphologically normalized CBF PET images obtained before supplementation. These ROIs were automatically applied to other PET images, and each ROI value was then computationally calculated.

Results

Ubiquinol Supplementation and Clinical Outcome

The analysis of the total CoQ₁₀ levels in plasma and PBMCs revealed a significant increase after 2 weeks of ubiquinol supplementation at 600 mg/day (Table 1). The total CoQ₁₀ levels in plasma and PBMCs remained similar for another 4 weeks at 840 mg/day, and a subsequent 2-week administration of ubiquinol at 1200 mg/day led to substantial increases in the total CoQ₁₀ levels in the plasma and the PBMCs. The CoQ₁₀ level in CSF increased from 0.22 to 3.79 ng/ml after 2 weeks of 840 mg/day, and a similar level of 3.64 ng/ml was observed after 2 weeks of 1200 mg/day. Eight weeks after the last supplementation of ubiquinol, the total CoQ₁₀ levels in plasma, PBMCs, and CSF returned to baseline levels.

Since we did not observe any adverse events at 1200 mg/day dosage and ubiquinol supplementation at 1200 mg/day led to higher total CoQ₁₀ levels in the plasma and the PBMCs compared with those observed with 840 mg/day, we

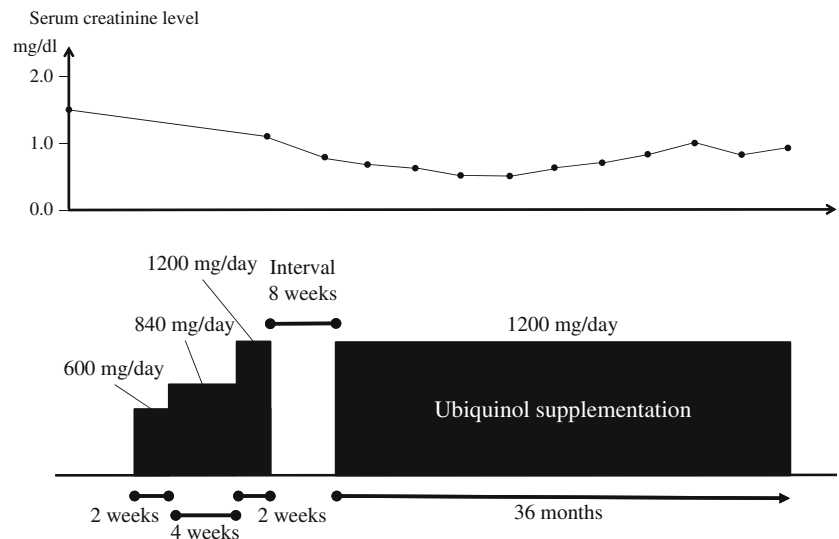
Table 1 Biochemical analyses

	Total CoQ ₁₀ (μ g/ml) in plasma	Total CoQ ₁₀ /free cholesterol in PBMCs (nM/mM)	Total CoQ ₁₀ in CSF (μ g/ml)
Reference (mean, standard deviation, number of controls)	0.72, 0.42, $n = 39$ (healthy controls) [31]	Not available	0.35×10^{-3} , 0.20×10^{-3} , $n = 23$ (disease controls)
Before supplementation	0.33	281	0.22×10^{-3}
After 2 weeks of 600 mg/day	5.04	1493	Not tested
After 2 weeks of 840 mg/day	4.02	1344	3.79×10^{-3}
After 4 weeks of 840 mg/day	4.43	1636	Not tested
After 2 weeks of 1200 mg/day	7.86	2047	3.64×10^{-3}
After 8 weeks of discontinuation	0.48	467	0.25×10^{-3}
After 6 months of 1200 mg/day	4.15	1894	Not tested
After 12 months of 1200 mg/day	7.62	1891	7.36×10^{-3a}
After 24 months of 1200 mg/day	4.92	Not tested	9.14×10^{-3a}
After 36 months of 1200 mg/day	4.78	Not tested	14.06×10^{-3a}

Total CoQ₁₀, ubiquinol + ubiquinone

^a Measured by LC-MS/MS

Fig. 1 Summary of clinical course with ubiquinol supplementation. After the baseline assessment, the patient was started on ubiquinol at 600 mg/day (given once a day), with the dose increased to 840 mg/day at week 2 and to 1200 mg/day at week 6. The 1200-mg/day dose was maintained until week 8. After an interval of 8 weeks, the patient resumed taking ubiquinol at 1200 mg/day. To date, he remains taking ubiquinol at this same dose for over 3 years. *BW* body weight, *sCre* serum creatinine level



decided to maintain the 1200-mg/day dosage. After supplementation of 1200 mg/day was resumed, the CoQ₁₀ levels in plasma and PBMCs returned to levels similar to those observed after the initial 2 weeks of 1200 mg/day and maintained at similar levels throughout the following period. The CoQ₁₀ levels in CSF were 7.36, 9.14, and 14.06 ng/ml after 12, 24, and 36 months of 1200 mg/day, respectively. Thus, the total CoQ₁₀ levels in CSF gradually increased after resuming ubiquinol supplementation at 1200 mg/day for the following period (Table 1).

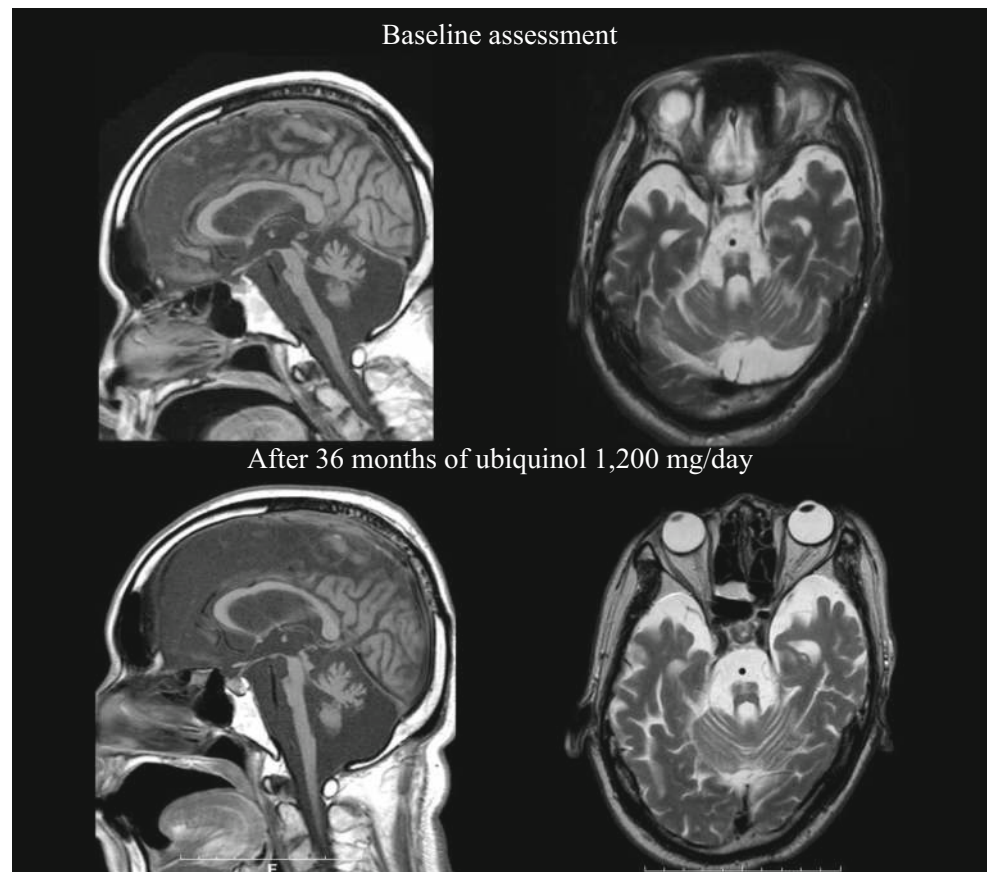
The patient continued to take 1200 mg of ubiquinol/day for over 3 years (Fig. 1). During the entire course, we did not observe any adverse events that were considered to be

associated with the ubiquinol supplementation throughout the entire study period. After 36 months of supplementation, evaluation of scores of clinical rating scales (Barthel index, SARA, ICARS, and UMSARS) showed no remarkable changes (Table 2). The brain MRI findings also remained unchanged for the 3 years (Fig. 2). It was notable that his serum creatinine level gradually declined over 36 months (from 1.45 to 0.95 mg/dl) (Fig. 1). His body weight decreased in the first 16 weeks (48.5 to 41.0 kg), but gradually increased over 36 months (41.0 to 46.0 kg) after increasing his daily calorie intake (from 900 to 1500 kcal/day).

Table 2 Scores of clinical rating scales

	Barthel index	SARA	ICARS (dynamic)	ICARS (static)	UMSARS Part I	UMSARS Part II	UMSARS Part IV
Before supplementation	0	40	50	34	47	49	5
After a 2-week supplementation at 600 mg/day	0	40	50	34	47	47	5
After a 4-week supplementation at 840 mg/day	0	40	50	34	47	51	5
After a 2-week supplementation at 1200 mg/day	0	39	50	34	47	49	5
After a 6-month supplementation at 1200 mg/day	0	39	51	34	47	50	5
After a 12-month supplementation at 1200 mg/day	0	39	51	34	47	51	5
After an 18-month supplementation at 1200 mg/day	0	40	52	34	47	51	5
After a 24-month supplementation at 1200 mg/day	0	40	51	34	47	50	5
After a 30-month supplementation at 1200 mg/day	0	40	50	34	47	49	5
After a 36-month supplementation at 1200 mg/day	0	40	50	34	47	49	5

Fig. 2 Brain MR images of patient. Upper images were obtained at 60 years of age (before supplementation of ubiquinol), and lower images were obtained at 63 years of age (after 36 months of ubiquinol 1200 mg/day). T1-weighted sagittal images (*left*) and T2-weighted axial images at the middle regions of the pontine base (*right*). They show gross atrophy of the infratentorial structures especially in the pons, middle cerebellar peduncle, and cerebellum



Measurement of the CBF and the CMRO₂ by PET

Figures 3 and 4 show CBF and CMRO₂ images, respectively, and Table 3 summarizes the quantitative measurements before and after 2 weeks of ubiquinol supplementation at 1200 mg/day. At the baseline, CBF decreased and CMRO₂ markedly decreased in the entire brain. For example, the CBF and CMRO₂ in the Rolandic area were 30.6 ml/100 ml/min [reference level [19], 44.6, 5.6 (mean, standard deviation)] and 1.81 ml/100 ml/min [reference level [19], 3.3, 0.5 (mean, standard deviation)], respectively. After 2 weeks of ubiquinol supplementation at 1200 mg/day, CBF remained unchanged as compared with the baseline level (Fig. 3 and Table 3). In contrast, the CMRO₂ markedly increased by approximately 30% in the entire brain (Fig. 4 and Table 3), although it did not reach normal levels. For example, the CMRO₂ in the Rolandic area increased from 1.81 to 2.15 ml/100 ml/min [reference level, 3.3, 0.5 (mean, standard deviation) [19]].

Discussion

In the present single case study of a patient with familial MSA carrying compound heterozygous mutations in *COQ2*, administration of high-dose ubiquinol led to a substantial increase in the total CoQ₁₀ levels not only in the plasma and PBMC but also in the CSF. Although previous reports have failed to show the increase in total CSF CoQ₁₀ level by ubiquinone or ubiquinol supplementation, which was caused possibly due to the insufficient dose (300 mg/day) [25], this is the first study showing that ubiquinol supplementation at 840 and 1200 mg/day clearly elevated the total CSF CoQ₁₀ level. CoQ₁₀ has been reported to be poorly absorbed, and its bioavailability varies among formulations [26]. Previous dose escalation studies (up to 3000 mg/day) using chewable tablets of ubiquinone in patients with Parkinson disease, amyotrophic lateral sclerosis, and Huntington disease concordantly showed that the total plasma CoQ₁₀ levels reached the plateau levels of approximately 7.0–7.5 μg/ml after multiple doses of

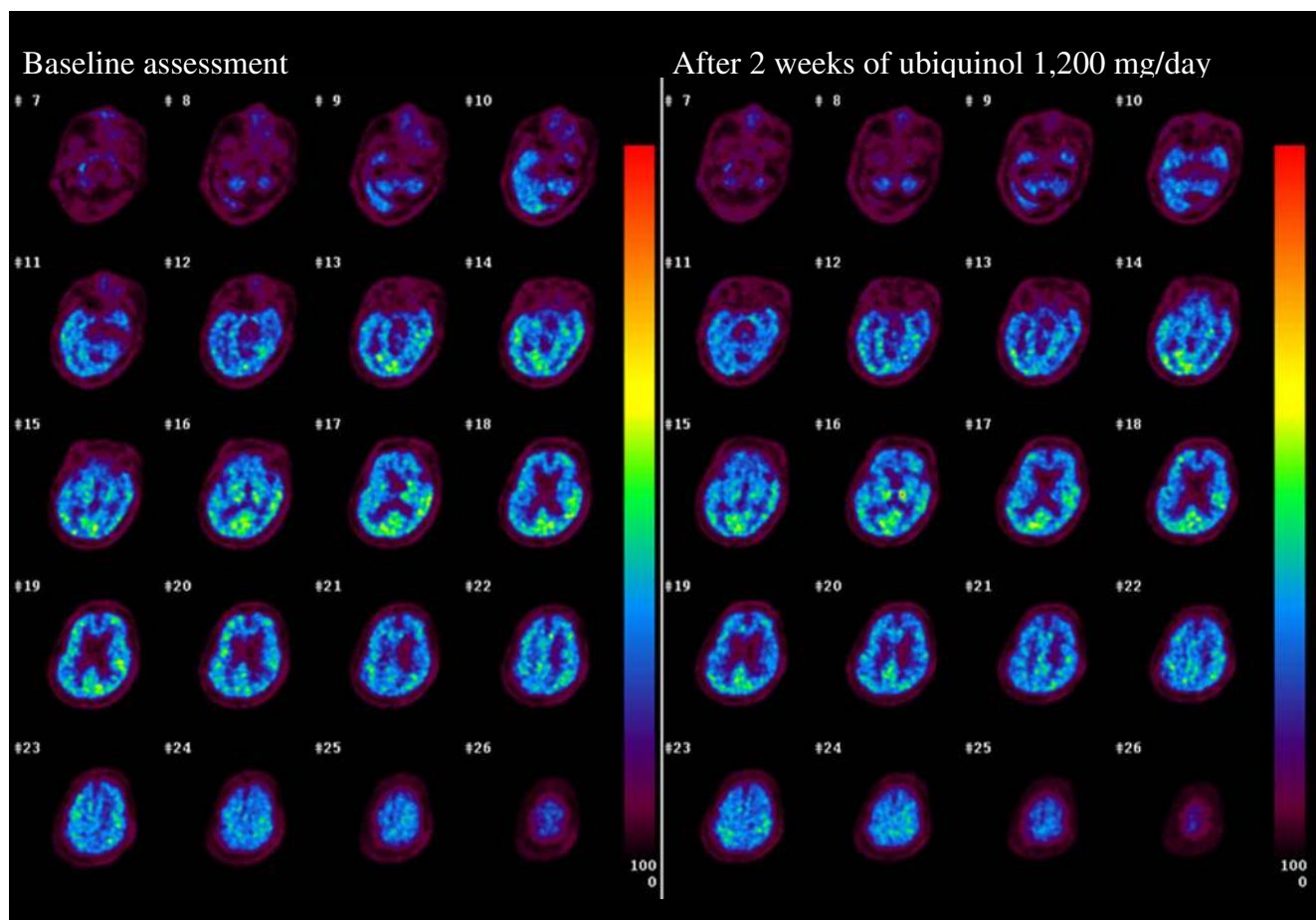


Fig. 3 CBF images before and after ubiquinol supplementation at 1200 mg/day

2400 mg/day [22–24]. When assessing the bioavailability of ubiquinol in this study, the trough concentrations of total CoQ₁₀ in plasma were 5.04 µg/ml for 600 mg/day, 4.02 µg/ml for 840 mg/day, and 7.86 µg/ml for 1200 mg/day 2 weeks after the daily intake of ubiquinol. Furthermore, another previous study using ubiquinol showed that mean total plasma CoQ₁₀ levels were 2.61 µg/ml for 90 mg/day, 3.66 µg/ml for 150 mg/day, and 6.53 µg/ml for 300 mg/day 2 weeks after a daily intake of ubiquinol [27]. These observations indicate that ubiquinol is better absorbed in the gastrointestinal tract than ubiquinone, and we conclude that the ubiquinol dose of 1200 mg/day is sufficient for achieving a plateau of total CoQ₁₀ level in plasma.

Remarkably, CMRO₂ increased without an increase in CBF after administration of 1200 mg of ubiquinol, which suggests that ubiquinol improved cerebral mitochondrial oxidative metabolism. Despite the increase in the CMRO₂, however, we did not detect any obvious neurological improvements as determined by the rating scales, presumably owing to the advanced stage of neurodegeneration. Notably, his serum creatinine level gradually declined during the ubiquinol

supplementation over 36 months (from 1.45 to 0.95 mg/dl). Because renal involvement has been frequently observed in patients with primary CoQ₁₀ deficiency caused by genetic defects in CoQ₁₀ biosynthesis [28–30], the renal dysfunction in the patient was likely caused by CoQ₁₀ deficiency and was ameliorated by ubiquinol supplementation. He also showed weight loss in the first 16 weeks of supplementation (48.5 to 41.0 kg). We extensively investigated the cause of his weight loss. However, we did not find chronic infectious diseases, malignancies, extremity edema, pleural effusion, or ascites in this patient during the entire study period. He gradually regained his body weight over 36 months (41.0 to 46.0 kg) after increasing his daily calorie intake. Despite the body weight changes, his general health condition remained stable.

Conclusions

The current study suggests that high-dose ubiquinol supplementation (up to 1200 mg/day) is tolerable and improves cerebral mitochondrial oxidative metabolism, which may alter

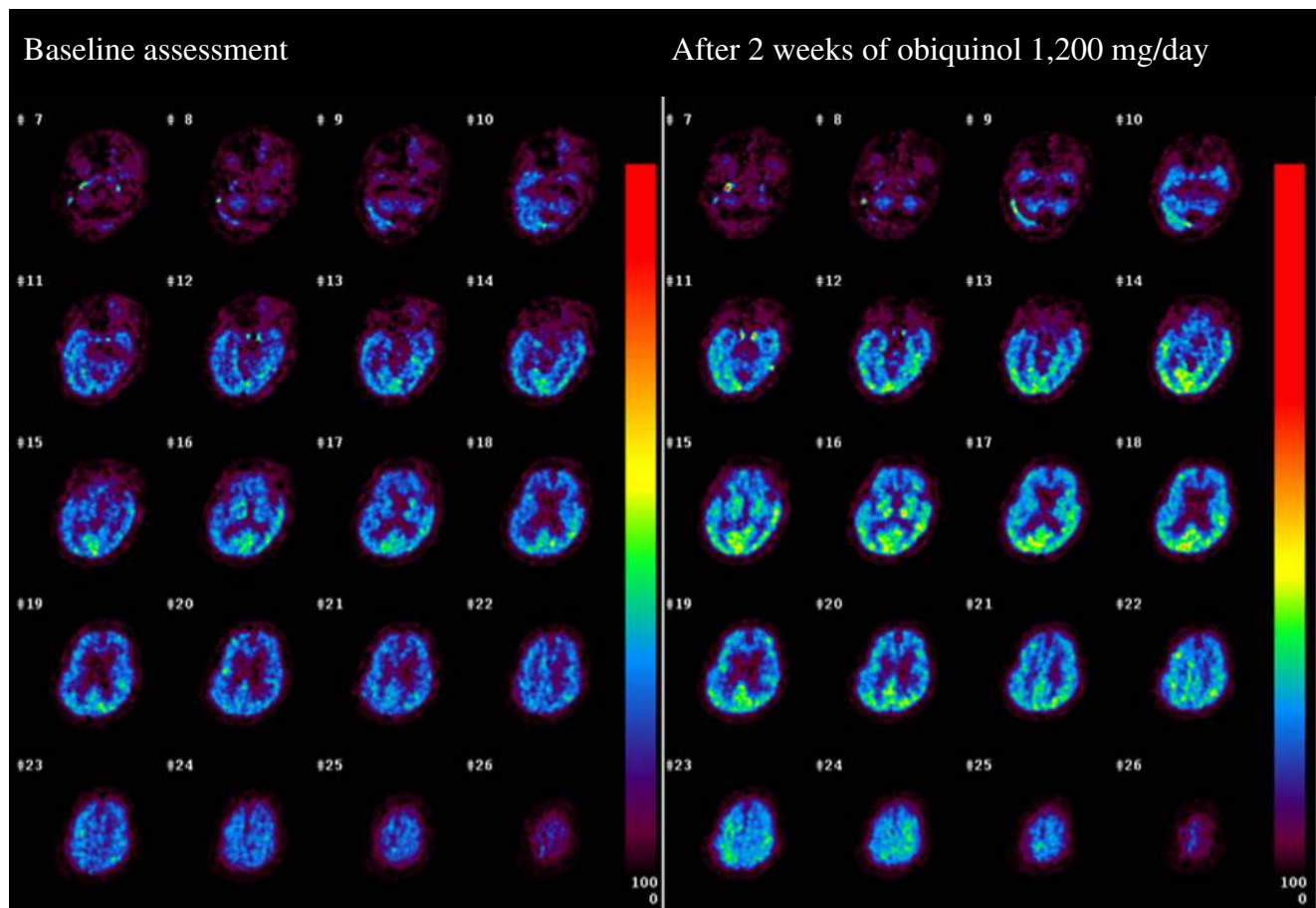


Fig. 4 CMRO₂ images before and after ubiquinol supplementation at 1200 mg/day

the natural history of MSA progression especially when applied in the early phase of MSA in patients with genetic defects in the CoQ₁₀ biosynthetic pathway. Further clinical trials including administration of ubiquinol to MSA patients

carrying heterozygous *COQ2* mutations as well as to patients without mutations in *COQ2* are warranted. Prospective randomized controlled trials will be undertaken to further extend these initial promising observations.

Table 3 CBF and CMRO₂ in major brain areas before and after ubiquinol supplementation

Region	CBF (ml/100 ml/min)		CMRO ₂ (ml/100 ml/min)	
	Before	After	Before	After
Upper frontal	28.8	27.7	1.78	2.17
Lower frontal	29.7	29.8	1.72	2.23
Rolandic	30.6	29.7	1.81	2.15
Lateral parietal	30.8	29.9	2.03	2.52
Medial parietal	35.3	42.1	2.05	3.12
Temporal	31.5	30.3	2.11	2.65
Occipital	38.2	37.5	2.63	3.17
Striatum	30.4	29.7	1.92	2.56
Thalamus	35.3	44.5	2.08	3.20
Cerebellar hemisphere	24.3	24.3	1.44	1.89
Cerebellar vermis	23.7	22.4	1.33	1.58

Acknowledgements We thank Midori Nagase for her technical assistance. This study was supported in part by KAKENHI [Grant-in-Aid for Scientific Research on Innovative Areas (22129001 and 22129002)] to S.T. from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and a Grant-in-Aid [H23-Jitsuyoka (Nanbyo)-Ippan-004 and “the Research Committee for Ataxic Diseases” of the Research on Measures for Intractable Diseases] from the Ministry of Health, Welfare and Labour, Japan, to S.T. Ubiquinol in the powder form (Kaneka QH ubiquinol) was provided by Kaneka Corporation (Tokyo, Japan).

Compliance with Ethical Standards The protocol of this study (UMIN000010712) was reviewed and approved by the institutional review board of the participating institutions. Informed consent was obtained from the patient and his legal representative prior to the initiation of this study, in accordance with the Declaration of Helsinki.

Conflict of Interest The authors declare that they have no conflict of interest.

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