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Early Onset of Diabetes Mellitus Accelerates Cognitive Decline in Japanese Patients with Mitochondrial Myopathy, Encephalopathy, Lactic Acidosis, and Stroke-Like Episodes

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Approximately 80% of patients with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) carry the A3243G mutation in the mitochondrial tRNALeu (UUR) gene. Conversely, this mutation has also been identified as one of the most prevalent genetic abnormalities in patients with diabetes mellitus. Mitochondrial diabetes mellitus complicated with MELAS is relatively common, and 12.5% of patients with the A3243G mutation develop MELAS after being diagnosed with diabetes mellitus. However, the clinical impact of diabetes mellitus in MELAS patients remains unclear. Therefore, we retrospectively studied 14 Japanese MELAS patients with the A3243G mutation: three men and eleven women, with the mean age of 48.0 (± 15.4) years. Eight patients had been diagnosed with diabetes mellitus prior to the diagnosis of mitochondrial disease, and all of them were treated with insulin. The other six included four patients with concurrent diagnosis of diabetes and mitochondrial disease, one patient diagnosed with diabetes after the diagnosis of mitochondrial disease, and one patient without developing diabetes currently. We thus compared the patients' characteristics between those with and without early onset of diabetes mellitus. Cognitive decline (75.0% vs. 0%; $p = 0.03$) and poor glycemic control with severe hypoglycemic events (75.0% vs. 16.7%; $p = 0.05$) were more common in MELAS patients with a prior diagnosis of diabetes than in those without the prior diagnosis of diabetes. Our data suggest that the latent progress of cognitive decline is accelerated because of early onset of diabetes mellitus in MELAS patients.

Keywords: cognitive decline; hypoglycemia; MELAS; mitochondrial diabetes mellitus; A3243G mutation
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Introduction

Mitochondrial dysfunction induces various organ dysfunctions in humans (Tuppen et al. 2010). The classical and most well-known clinical disorder caused by mitochondrial DNA mutations is mitochondrial myopathy. It includes mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) (Yatsuga et al. 2012). Approximately 80% of MELAS patients show the A3243G mutation in the mitochondrial tRNALeu (UUR) gene (Goto et al. 1992).

The A3243G mutation has been identified as one of the most prevalent genetic abnormalities in patients with diabetes mellitus (van den Ouweland et al. 1995), with a prevalence rate of 0.5%-2.8% of the general diabetic population in Japan (Suzuki et al. 2003). Moreover, mitochondrial diabetes mellitus complicated with MELAS is relatively common in those with the A3243G mutation, and 12.5% of diabetic patients with the A3243G mutation develop MELAS after being diagnosed with diabetes mellitus (Suzuki et al. 2003).

However, because both MELAS and mitochondrial diabetes mellitus are rare in common clinical settings, only few studies detail the clinical relationship and natural course of these conditions (Suzuki et al. 2003). Although the diagnosis of mitochondrial disease may be delayed and potentially increase the severity of MELAS, it remains challenging in clinical settings. Given the chronic, deleteri-

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ous effects of diabetes mellitus on MELAS progression, MELAS patients with diabetes are more likely to demonstrate a gradual disease progression (Yatsuga et al. 2012). Here we report a retrospective single-center study of 14 Japanese MELAS patients with the A3243G mutation. This study aimed to evaluate the clinical presentation of MELAS, including the interval between the diagnoses of diabetes and mitochondrial disease as well as the consequential clinical impact of diabetes at a Japanese community hospital.

Patients and Methods

Patients and study design

We conducted a retrospective single-center study at the Osaka Red Cross Hospital between April 2005 and March 2015. This study was approved by the Institutional Review Board of the Osaka Red Cross Hospital. Through a chart review, we identified 14 patients with MELAS, associated with the A3243G mutation. The patients' medical records were evaluated by neurologists certified by the Japanese Society of Neurology and diabetologists certified by the Japan Diabetes Society (JDS). Eligible patients included patients who were at least 18-year old and referred to a neurologist for evaluation by their primary care physician to obtain a definitive diagnosis of MELAS. Such confirmation would be based on the diagnostic criteria of the MELAS Study Committee in Japan, as previously reported (Yatsuga et al. 2012). Diagnosis of mitochondrial disease was defined as a proof of mitochondrial dysfunction according to at least one Category B criterion of the MELAS study committee in Japan. The Category B criteria comprise three items: 1) high lactate levels in the plasma and/or cerebrospinal fluid (CSF) or deficiency of mitochondria-related enzyme activities, 2) mitochondrial abnormalities based on a muscle biopsy, and 3) definitive mitochondrial gene mutations (Yatsuga et al. 2012). The A3243G mutation was confirmed in peripheral leukocytes, skeletal muscles, or urinary epithelial cells, as previously reported (Suzuki et al. 2003; McDonnell et al. 2004). Diagnosis of diabetes mellitus was based on the diagnostic criteria of JDS, and the values for glycated hemoglobin (HbA1c) were described with 0.25% added to the 1.02-fold JDS values (Committee of the Japan Diabetes Society on the Diagnostic Criteria of Diabetes Mellitus et al. 2010). We herein emphasize that we distinguished the diagnosis of diabetes mellitus from that of mitochondrial diabetes as one of the mitochondrial diseases. Thus, in this study, the diagnosis of diabetes mellitus does not indicate the diagnosis of mitochondrial diabetes, since many cases were recognized as diabetes mellitus but were not classified into mitochondrial diabetes mellitus in clinical settings. Severe hypoglycemia was defined as low blood glucose levels, requiring assistance from another person to treat that as previously reported (Seaquist et al. 2013). Since MELAS could lead to the development of deafness, aphasia, and/or cortical blindness, the presence of cognitive decline was diagnosed by neurologists via at least two clinical examinations: an interview with the caregiver and the routine neuropsychiatric assessment, including the Mini-Mental State Examination, revised Hasegawa's dementia scale, or clock-drawing test (El-Hattab et al. 2015). We simultaneously analyzed the clinical progression of MELAS using the total scores of section 1 and 2 of the Japanese Mitochondrial Disease Rating Scale (JMDRS) (Yatsuga et al. 2012) and the total scores of section I and II of the Newcastle Mitochondrial Disease Adult Scale (NMDAS) (Schaefer et al. 2006).

Statistical analysis

Statistical analyses were performed using Statcel3 (OMS, Tokyo, Japan) and StatView 5.0 software (SAS Institute, Inc., Cary, NC, USA). All data are expressed as mean ± standard deviation (SD). Student's or Welch's t-test, Fisher's exact test, and Yates' correction were used. The statistical level of significance level was $p < 0.05$.

Results

Patient characteristics

The clinical characteristics of the 14 MELAS patients with the A3243G mutation are summarized in Table 1. Patients were aged 26-72 (mean, 48.0 ± 15.4) years, and three of the 14 patients (21.4%) were male. Patients were of a short stature (height, 150.3 ± 8.0 cm) and lean [body mass index (BMI), 16.5 ± 2.0 kg/m^2]. In addition, a high prevalence of diabetes mellitus (92.9%) and deafness (92.9%) was observed among the patients (Table 1). All patients were seronegative for anti-glutamic acid decarboxylase antibodies. Only one patient who had no family history of diabetes presented without diabetes during the follow-up period. However, all patients with diabetes had a family history of diabetes mellitus. Ten patients were probands with a mitochondrial disease. Mitochondrial disease was diagnosed at an average age of 39.0 ± 15.5 years, whereas diabetes mellitus was diagnosed at a mean age of 30.6 ± 12.1 years. The interval between the diagnosis of diabetes mellitus and mitochondrial disease was 8.9 ± 11.4 years. There were eight patients who had been diagnosed with diabetes mellitus prior to the diagnosis of mitochondrial disease, four patients with concurrent diagnoses, and one patient who was diagnosed with diabetes after the diagnosis of mitochondrial disease. The symptoms or reasons given, which led patients to seek medical advice and consequent diagnosis of mitochondrial disease were as follows; genealogical research (four patients, 28.6%), stroke-like episode (three patients, 21.4%), seizure (two patients, 14.3%), hearing loss (two patients, 14.3%), gait disturbance (two patients, 14.3%), headache (one patient, 7.1%), unconsciousness (one patient, 7.1%), and diabetes mellitus (one patient, 7.1%).

Comparison of patients with and without a prior diagnosis of diabetes mellitus

The clinical characteristics of patients with and those without a diagnosis of diabetes mellitus prior to that of mitochondrial disease are presented in Table 2. Patients with a diagnosis of diabetes prior to that of mitochondrial disease tended to be older than those without, although this difference was not statistically significant (53.6 ± 13.8 years vs. 40.5 ± 13.0 years; $p = 0.08$). The current cognitive decline was significantly more common in patients with a prior diagnosis of diabetes than those without (75.0% vs. 0%; $p = 0.03$). Those with a prior diagnosis of diabetes suffered with the condition for a significantly longer duration than those without (24.0 ± 11.7 years vs. 8.8 ± 5.8 years; $p = 0.03$). These patients displayed relatively poor glycemic

Table 1. Patients' characteristics.

Age (year)	48.0 ± 15.4
Sex: male / female	3 / 11
Height (cm)	150.3 ± 8.0
Body weight (kg)	38.0 ± 6.7
Body mass index (kg/m^2)	16.5 ± 2.0
Diabetes mellitus [n (%)]	13 (92.9)
Without diabetes mellitus [n (%)]	1 (7.1)
Deafness [n (%)]	13 (92.9)
Muscle biopsy [n (%)]	8 (57.1)
Ragged-red fiber [n (%)]	7 (87.5)
Proband / family [n]	10 / 4
Age of diagnosis as mitochondrial disease (year)	39.0 ± 15.0
Age of diagnosis as diabetes mellitus (year)	30.6 ± 12.1
Duration of mitochondrial disease (year)	9.0 ± 5.3
Diabetic duration (year)	18.2 ± 12.1
Interval between the diagnosis of diabetes mellitus and mitochondrial disease (year)	8.9 ± 11.4
Prior diagnosis of diabetes mellitus [n (%)]	8 (57.1)
Concurrent diagnosis of diabetes mellitus and mitochondrial disease	4 (28.6)
Posterior diagnosis of diabetes mellitus [n (%)]	1 (0.1)

control (HbA1c, 9.8% ± 1.7% vs. 7.4% ± 3.4%; $p = 0.07$) with a reduced insulin secretory capacity (fasting C-peptide index 3 years after the diagnosis of mitochondrial disease, 0.29 ± 0.19 vs. 1.33 ± 0.89; $p = 0.06$) and a higher prevalence of severe hypoglycemia (75.0% vs. 16.7%; $p = 0.05$). In a comparison between patients with and without severe hypoglycemia regardless of the timing of diabetic diagnosis, a higher prevalence of cognitive decline was observed among the patients with severe hypoglycemia, although it was not statistically significant ($p = 0.05$).

Although both groups revealed an early requirement for insulin therapy after the diagnosis of diabetes, only one diabetic patient was treated without insulin therapy in the group without a prior diagnosis of diabetes. CSF lactate and pyruvate concentrations were significantly lower in patients with a prior diagnosis of diabetes than those without (CSF lactate, 31.0 ± 4.8 mg/dL vs. 48.8 ± 8.1 mg/dL, $p < 0.01$; CSF pyruvate, 1.3 ± 0.1 mg/dL vs. 1.9 ± 0.2 mg/dL, $p < 0.01$). Although the severity of mitochondrial disease at the time of the diagnosis of diabetes was relatively lower (JMDRS, 1.5 ± 1.5 vs. 11.0 ± 12.6, $p = 0.21$; NMDAS, 6.8 ± 3.3 vs. 22.0 ± 14.2, $p = 0.10$), it was not statistically significant. In contrast, the severity of mitochondrial disease at the time of diagnosis in patients with a prior diagnosis of diabetes was relatively higher (JMDRS, 19.1 ± 10.5 vs. 9.8 ± 11.9, $p = 0.19$; NMDAS, 29.3 ± 14.7 vs. 20.7 ± 14.1, $p = 0.35$), and the current severity scores also revealed the same tendency (JMDRS, 30.7 ± 8.9 vs. 19.0 ± 11.7, $p = 0.12$; NMDAS, 42.8 ± 15.4 vs. 30.0 ± 13.4, $p = 0.12$). However, these differences were not statistically significant. Moreover, the clinical progression of MELAS was almost equivalent between the two patient groups (changes in JMDRS after the diagnosis of mitochondrial disease, 9.0 ± 10.1 vs. 9.2 ± 6.0, $p = 0.98$; changes in NMDAS after diagnosis of mitochondrial disease, 9.7 ± 16.1 vs. 9.3 ± 10.8, $p = 0.98$).

The clinical status of patients with a diagnosis of diabetes mellitus prior to that of mitochondrial disease

A summary of the indexed patients with a diagnosis of diabetes mellitus prior to that of mitochondrial disease is presented in Table 3. The interval between the diagnosis of diabetes mellitus and mitochondrial disease was 15.6 ± 10.4 years. In addition, during their diabetic follow-up, the patients demonstrated −8.1 ± 2.7 kg and −3.7 ± 1.4 kg/m^2 changes in body weight and BMI, respectively. With regard to the clinical progression of MELAS, JMDRS and NMDAS revealed a 17.6 ± 10.9 and 22.5 ± 15.0 increase during the diabetic follow-up prior to the clinical diagnosis of mitochondrial dysfunction.

Discussion

Mitochondrial diabetes mellitus complicated with MELAS is relatively common in patients with the A3243G mutation. The clinical impact of diabetes on MELAS remains unclear and has been rarely studied. As previously reported, some patients with the A3243G mutation develop MELAS after the diagnosis of diabetes mellitus, whereas others develop MELAS simultaneously or before the diagnosis. Furthermore, others develop either MELAS or diabetes (Suzuki et al. 2003). Our study also showed a variable clinical course of MELAS and diabetes mellitus in adult MELAS patients with the A3243G mutation. Our patients showed clinical characteristics comparable to previous studies, including age at the time of the diagnosis of mitochondrial disease (Table 1) (Suzuki et al. 2003; Yatsuga

et al. 2012).

In this study, we revealed the interval and clinical progress between the diagnosis of diabetes and mitochondrial disease in MELAS patients who had been referred by primary physicians to neurologists at a Japanese community hospital (Table 3). The mean period for proof of mitochondrial dysfunction was over 10 years. During this interval, significant increases in the clinical rating scales for mitochondrial disease were observed. However, JMDRS and NMDAS at the time of the diagnosis of mitochondrial disease were not significantly different between patients with and without a prior diagnosis of diabetes (Tables 2, 3). Moreover, in our study, the decrease in body weight during diabetic follow-up was also considerable, despite the early introduction of insulin therapy (Tables 2, 3). The weight loss may be attributed to muscle atrophy and an increase in energy consumption combined with hypercatabolism because of insulin deficiency. However, a body composition assessment was not conducted in this study. Thus, body weight changes could be informative for an early diagnosis of mitochondrial disease during diabetic follow-up. In addition, our data suggest that a lack of suspicion of

Table 2. Comparison between the patients with and without prior diagnosis of diabetes mellitus.

	Patients with prior diagnosis of DM	Patients without prior diagnosis of DM	*p*
Number	8	6	
Age (year)	53.6 ± 13.8	40.5 ± 13.0	0.08
Sex: male / female	2 / 6	1 / 5	0.62
Body weight (kg)	35.3 ± 6.8	40.2 ± 4.9	0.33
BMI (kg/m^2)	15.9 ± 1.1	17.1 ± 2.4	0.45
DM (n)	8	5	0.43
Current cognitive decline [n (%)]	6 (75.0)	0 (0)	0.03
Proband / family (n)	5 / 3	5 / 1	0.41
Age of diagnosis as DM (year)	29.6 ± 10.8	32.2 ± 12.8	0.98
Age of diagnosis as MD (year)	45.3 ± 14.0	30.7 ± 12.2	0.06
Diabetic duration (year)	24.0 ± 11.7	8.8 ± 5.8	0.03
Duration of MD (year)	8.4 ± 4.9	9.8 ± 5.4	0.65
Body weight at diagnosis of DM (kg)	44.3 ± 6.9	37.8 ± 5.4	0.16
Body weight at diagnosis of MD (kg)	36.5 ± 6.6	41.1 ± 11.3	0.43
BMI at diagnosis of DM (kg/m^2)	19.7 ± 1.7	16.7 ± 1.9	0.03
BMI at diagnosis of MD (kg/m^2)	16.2 ± 1.5	17.9 ± 4.1	0.47
HbA1c at diagnosis of DM (%)	10.1 ± 1.7	10.9 ± 3.4	0.76
Current HbA1c (%)	9.8 ± 2.2	7.4 ± 1.5	0.07
Patients with history of severe hypoglycemia [n (%)]	6 (75.0)	1 (16.7)	0.05
Insulin therapy [n (%)]	8 (100.0)	4 (66.7)	0.38
Years from diagnosis of DM to insulin therapy (year)	4.0 ± 4.8	2.2 ± 3.1	0.53
Total insulin per body weight (units/day/kg)	0.55 ± 0.28	0.39 ± 0.42	0.60
Fasting C-peptide index 3 year after diagnosis of MD	0.29 ± 0.19	1.33 ± 0.89	0.06
Blood lactate at diagnosis of MD (mg/dL)	26.6 ± 13.2	25.9 ± 5.0	0.94
Blood pyruvate at diagnosis of MD (mg/dL)	1.1 ± 0.3	1.7 ± 0.5	0.08
CSF lactate at diagnosis of MD (mg/dL)	31.0 ± 4.8	48.8 ± 8.1	< 0.01
CSF pyruvate at diagnosis of MD (mg/dL)	1.3 ± 0.1	1.9 ± 0.2	< 0.01
JMDRS at diagnosis of DM	1.5 ± 1.5	11.0 ± 12.6	0.21
JMDRS at diagnosis of MD	19.1 ± 10.5	9.8 ± 11.9	0.19
Current JMDRS	30.7 ± 8.9	19.0 ± 11.7	0.12
Changes of JMDRS after diagnosis of MD	9.0 ± 10.1	9.2 ± 6.0	0.98
NMDAS at diagnosis of DM	6.8 ± 3.3	22.0 ± 14.2	0.10
NMDAS at diagnosis of MD	29.3 ± 14.7	20.7 ± 14.1	0.35
Current NMDAS	42.8 ± 15.4	30.0 ± 13.4	0.12
Changes of NMDAS after diagnosis of MD	9.7 ± 16.1	9.3 ± 10.8	0.98

BMI, body mass index; DM, diabetes mellitus; n, patients' number; MD, mitochondrial disease; HbA1c, glycated hemoglobin; CSF, cerebrospinal fluid; JMDRS, total scores of Section 1 and 2 of Japanese mitochondrial disease rating scale; NMDAS, total scores of Section I and II of Newcastle Mitochondrial Disease Adult Scale.

Table 3. The differences in clinical status between the diagnosis of diabetes mellitus and of mitochondrial disease in the patients with prior diagnosis of diabetes mellitus.

Interval between the diagnosis (years)	15.6 ± 10.4
Changes of body weight (kg)	−8.1 ± 2.7
Body weight changes per year (kg/year)	−0.95 ± 0.98
Changes of body mass index (kg/m^2)	−3.7 ± 1.4
Body mass index changes per year (kg/m^2·year)	−0.40 ± 0.35
Changes in JMDRS	17.6 ± 10.9
Yearly changes in JMDRS	1.3 ± 0.5
Changes in NMDAS	22.5 ± 15.0
Yearly changes in NMDAS	1.7 ± 0.6

JMDRS, total scores of Sections 1 and 2 of Japanese Mitochondrial Disease Rating Scale; NMDAS, total scores of Sections I and II of Newcastle Mitochondrial Disease Adult Scale.

mitochondrial disease may be an obstacle for an early diagnosis of this condition in Japanese clinical settings. This is supported by our results, which show that all primary physicians and diabetologists diagnosed the patients as having insulin-dependent diabetes mellitus at an early stage after diagnosis of diabetes in this study, although a previous study has already reported the clinical features of Japanese mitochondrial diabetes mellitus (Suzuki et al. 2003).

Moreover, the effects of diabetes on the latent progress of cognitive decline in MELAS patients were particularly notable. Our study revealed that there was a high prevalence of cognitive decline among relatively young patients with a prior diagnosis of diabetes (Table 2). The prevalence was significantly higher than that observed among patients without a prior diagnosis of diabetes. This is partially attributable to the observation that patients with a prior diagnosis of diabetes were older than those without it. However, the patients with a prior diagnosis of diabetes were not as old as the patients who experienced cognitive decline in common clinical settings. According to previous studies in Japan, 3.6% of patients with mitochondrial diabetes mellitus exhibited cognitive decline (Suzuki et al. 2003) and 21.1% of patients with adult-form MELAS exhibited memory loss (Yatsuga et al. 2012). However, the former study only included 12.5% of MELAS patients and excluded patients who had MELAS before the diagnosis of diabetes. The latter study also demonstrated a high prevalence of cognitive decline in MELAS patients and our data corroborate this finding. However, in the previous study, MELAS diagnosed with a mutation other than A3243G were also included, but did not focus on the diabetic effects. Therefore, our study provides novel and useful data, confirming that early onset of diabetes can accelerate cognitive decline in MELAS.

It is well known that MELAS patients develop cognitive decline mainly because of stroke-like episodes. However, CSF lactate and pyruvate concentrations were significantly lower in MELAS patients with a prior diagnosis of diabetes than those without it (Table 2). This suggests that the cognitive decline in MELAS patients may be caused not only by a failure in energy metabolism but also the possibility of relatively low CSF lactate and pyruvate levels at the time of the diagnosis of MELAS in diabetic patients.

To date, mitochondrial respiratory failure and oxidative stress can contribute to cognitive decline (Simoncini et al. 2015). It has been reported that MELAS patients without stroke-like episodes can also develop cognitive decline (Finsterer 2009). The accumulation of somatic mitochondrial DNA mutations accelerates oxidative damage, increases the production of reactive oxygen species, and promotes amyloid accumulation (Simoncini et al. 2015). Moreover, recent studies have shown that diabetes itself could be a risk factor for cognitive decline, and a history of severe hypoglycemia is associated with a greater risk of cognitive decline (Whitmer et al. 2009; Gudala et al. 2013). Therefore, we may infer that diabetes mellitus can influence cognitive function in MELAS patients. Our results support the evidence of a clinical relationship between the two diseases because patients with a prior diagnosis of diabetes tended to have poor glycemic control, relatively low insulin secretion, and experience more severe hypoglycemic events than those without (Table 2). In a comparison between patients with and without severe hypoglycemia regardless of the time of the diagnosis of diabetes, a higher prevalence of cognitive decline was observed among the patients with severe hypoglycemia. Moreover, patients with a prior diagnosis of diabetes had a significantly longer diabetic duration than those without (Table 2). Thus, diabetic duration and severe hypoglycemia may contribute to cognitive decline among patients with early onset of diabetes. However, it is difficult to determine which diabetic factor among diabetic duration and hypoglycemia has the highest effect because the number of patients in our study was very small. Our study may indicate that primary physicians and diabetologists should recognize the early diagnosis of mitochondrial diabetes mellitus and distinguish this from other types of insulin-dependent diabetes mellitus and aim to avoid severe hypoglycemia. Our study suggests that neurologists should recognize the risk of cognitive decline in MELAS patients with early onset of diabetes. These are the patients that are most likely to be treated with insulin because cognitive decline itself could significantly impact self-administered insulin therapy.

In our study, MELAS patients with early onset of diabetes tended to show delayed diagnosis of mitochondrial disease (Table 2). This suggests that the presence of diabetes results in a delayed development of significant neuromuscular symptoms. In particular, our study demonstrated that the two groups of patients had a comparable age at the time of the diagnosis of diabetes and severity of MELAS at the time of the diagnosis of mitochondrial disease (Table 2). These data indicate that patients with a diagnosis of diabe-

tes prior to that of mitochondrial disease potentially have lower levels of abnormal mitochondrial accumulation in their neuromuscular systems (Yatsuga et al. 2012). However, in our study, once significant symptoms had developed, an exacerbatory effect of diabetes mellitus on MELAS progression was not identified. This was evidenced by changes in both JMDRS and NMDAS, which were almost equivalent during comparable follow-up periods between the two groups (Table 2).

There are several limitations to this study. This is a preliminary small-scale, single-center retrospective study. Moreover, to evaluate the actual clinical conditions related to mitochondrial diseases in Japan, we focused on MELAS patients who were initially referred from primary physicians and local diabetologists to neurologists at a community hospital. Therefore, further studies are warranted to examine the natural history of MELAS and the clinical impact of diabetes mellitus in MELAS patients. In particular, the diabetes mellitus factor that could potentially have an effect on cognitive decline in MELAS patients should be examined.

In conclusion, this study demonstrated that the interval and clinical progression between the diagnosis of diabetes and mitochondrial disease in MELAS patients were not negligible. Although it is possible that there is a lack of suspected cases of mitochondrial disease in Japanese clinical settings, our findings indicate that weight loss could be informative for the early diagnosis of mitochondrial disease. Moreover, this study also revealed the possibility of an effect of diabetes mellitus in the acceleration of cognitive decline in MELAS patients with early onset of diabetes.

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Conflict of Interest

The authors declare no conflict of interest.

References

Committee of the Japan Diabetes Society on the Diagnostic Criteria of Diabetes Mellitus; Seino, Y., Nanjo, K., Tajima, N., Kadowaki, T., Kashiwagi, A., Araki, E., Ito, C., Inagaki, N., Iwamoto, Y., Kasuga, M., Hanafusa, T., Haneda, M. & Ueki, K. (2010) Report of the committee on the classification and diagnostic criteria of diabetes mellitus. *J. Diabetes Investig.*, **1**, 212-228.

El-Hattab, A.W., Adesina, A.M., Jones, J. & Scaglia, F. (2015) MELAS syndrome: clinical manifestations, pathogenesis, and treatment options. *Mol. Genet. Metab.*, **116**, 4-12.

Finsterer, J. (2009) Mitochondrial disorders, cognitive impairment and dementia. *J. Neurol. Sci.*, **283**, 143-148.

Goto, Y., Horai, S., Matsuoka, T., Koga, Y., Nihei, K., Kobayashi, M. & Nonaka, I. (1992) Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS): a correlative study of the clinical features and mitochondrial DNA mutation. *Neurology*, **42**, 545-550.

Gudala, K., Bansal, D., Schifano, F. & Bhansali, A. (2013) Diabetes mellitus and risk of dementia: a meta-analysis of prospective observational studies. *J. Diabetes Investig.*, **4**, 640-650.

McDonnell, M.T., Schaefer, A.M., Blakely, E.L., McFarland, R., Chinnery, P.F., Turnbull, D.M. & Taylor, R.W. (2004) Noninvasive diagnosis of the 3243A > G mitochondrial DNA mutation using urinary epithelial cells. *Eur. J. Hum. Genet.*, **12**, 778-781.

Schaefer, A.M., Phoenix, C., Elson, J.L., McFarland, R., Chinnery, P.F. & Turnbull, D.M. (2006) Mitochondrial disease in adults: a scale to monitor progression and treatment. *Neurology*, **66**, 1932-1934.

Seaquist, E.R., Anderson, J., Childs, B., Cryer, P., Dagogo-Jack, S., Fish, L., Heller, S.R., Rodriguez, H., Rosenzweig, J. & Vigersky, R. (2013) Hypoglycemia and diabetes: a report of a workgroup of the American Diabetes Association and the Endocrine Society. *Diabetes Care*, **36**, 1384-1395.

Simoncini, C., Orsucci, D., Caldarazzo Ienco, E., Siciliano, G., Bonuccelli, U. & Mancuso, M. (2015) Alzheimer's pathogenesis and its link to the mitochondrion. *Oxid. Med. Cell. Longev.*, **2015**, 803942.

Suzuki, S., Oka, Y., Kadowaki, T., Kanatsuka, A., Kuzuya, T., Kobayashi, M., Sanke, T., Seino, Y. & Nanjo, K.; Research Committee or Specific Types of Diabetes Mellitus with Gene Mutations of the Japan Diabetes Society (2003) Clinical features of diabetes mellitus with the mitochondrial DNA 3243 (A-G) mutation in Japanese: maternal inheritance and mitochondria-related complications. *Diabetes Res. Clin. Pract.*, **59**, 207-217.

Tuppen, H.A., Blakely, E.L., Turnbull, D.M. & Taylor, R.W. (2010) Mitochondrial DNA mutations and human disease. *Biochim. Biophys. Acta*, **1797**, 113-128.

van den Ouweland, J.M., Lemkes, H.H., Gerbitz, K.D. & Maassen, J.A. (1995) Maternally inherited diabetes and deafness (MIDD): a distinct subtype of diabetes associated with a mitochondrial tRNA(Leu)(UUR) gene point mutation. *Muscle Nerve Suppl.*, **3**, S124-S130.

Whitmer, R.A., Karter, A.J., Yaffe, K., Quesenberry, C.P. Jr. & Selby, J.V. (2009) Hypoglycemic episodes and risk of dementia in older patients with type 2 diabetes mellitus. *JAMA*, **301**, 1565-1572.

Yatsuga, S., Povalko, N., Nishioka, J., Katayama, K., Kakimoto, N., Matsuishi, T., Kakuma, T., Koga, Y.; Taro Matsuoka for MELAS Study Group in Japan (2012) MELAS: a nationwide prospective cohort study of 96 patients in Japan. *Biochim. Biophys. Acta*, **1820**, 619-624.