level shown by high HbA_{1c} might impair endogenous insulin secretion after meal load, but has little effect on endogenous insulin secretion after glucagon load. The lack of influence of HbA_{1c} on CPR-6min might be helpful to evaluate reserve capacity of endogenous insulin secretion, even when glycemic control is poor enough to deteriorate postprandial insulin secretion. In contrast, PPCPR is affected by HbA_{1c} and might reflect the state of deteriorated insulin secretion by glucose toxicity that may be recovered by improved glycemic control.

In stepwise regression analysis, HbA_{1c} was not important to predict FCPR, but was important to predict PPCPR. In simple correlation, HbA_{1c} was significantly negatively correlated not only with PPCPR, but also with FCPR, whereas the *P*-value and *r*-value for FCPR were larger and smaller, respectively, compared with those for PPCPR (Table 2). Taken together, these findings suggest that glucose toxicity might deteriorate not only postprandial insulin secretion, but also fasting insulin secretion, whereas postprandial insulin secretion might be more vulnerable to glucose toxicity than to fasting insulin secretion.

The suppressive effect of glucose toxicity on insulin secretion *in vivo* might be attributable to impairment of β-cell responsiveness to glucose[22] and to impairment of incretin effect[23,24]. However, it is important to understand why glucagon-stimulated CPR is preserved despite severe impairment of glucose-stimulated CPR before treatment to improve hyperglycemia[21]. This remains largely unknown, but our hypothesis based on an *in vitro* study is that deteriorated intracellular glucose metabolism plays an important role in impaired glucose-induced insulin secretion[25] and that increased intracellular cyclic adenosine 3′,5′-monophosphate concentration derived from glucagon stimulation ameliorates impaired intracellular glucose metabolism to improve suppressed insulin secretion[26].

A recent study showed that indices using CPR correlate well with β-cell mass by analysis of β-cell areas of samples obtained during pancreatectomy and serum levels of CPR before operation[27]. Thus, PPCPR might reflect not only β-cell mass, but also reversible impairment of endogenous secretion as a result of chronic glucose elevation.

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The authors declare no conflict of interest.

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Long-term safety and efficacy of exenatide twice daily in Japanese patients with suboptimally controlled type 2 diabetes

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ABSTRACT

Aims/Introduction: We assessed the long-term (52 weeks) safety and efficacy of exenatide b.i.d. in Japanese patients with type 2 diabetes and suboptimal glycemic control.

Materials and Methods: Participants completing a 24-week randomized controlled trial of exenatide (5 μg, 10 μg or placebo b.i.d.) were invited to continue in a 28-week open-label extension study (5 μg, $n = 64$; 10 μg, $n = 53$). Safety measures included treatment-emergent adverse events (TEAE). Efficacy measures included change from baseline in glycosylated hemoglobin A1c (HbA_{1c}) levels, proportion of participants achieving HbA_{1c} target levels, fasting and seven-point, self-monitored blood glucose concentrations (SMBG), 1,5-anhydroglucitol concentrations, and bodyweight.

Results: A total of 60 and 49 participants in the exenatide 5 and 10 μg groups completed the study. The 52-week incidence of TEAE considered by investigators as related to the study drug was 80.6% (58/72) and 88.9% (64/72) in the exenatide 5 and 10 μg groups, respectively. Mild hypoglycemia and nausea were the most common TEAE. Most TEAE occurred during the first 24 weeks. Eight participants experienced serious adverse events. Exenatide treatment was associated with sustained decreases in HbA_{1c} values, with 33.3–47.9% of participants achieving <6.9% HbA_{1c}, sustained decreases in fasting plasma glucose concentrations and SMBG, and sustained increases in 1,5-anhydroglucitol concentrations. Exenatide 10 μg was associated with sustained weight loss.

Conclusions: Long-term exenatide treatment had a similar safety profile to that observed previously and was efficacious in improving glycemic control in Japanese patients with suboptimally controlled type 2 diabetes. This trial was registered with ClinicalTrials.gov (no. NCT00577824). (J Diabetes Invest, doi: 10.1111/j.2040-1124.2011.00137.x, 2011)

KEY WORDS: Exenatide, Japan, Type 2 diabetes mellitus

INTRODUCTION

The prevalence of type 2 diabetes mellitus in Japan is increasing[1–3]. Indeed, findings from a recent survey carried out by the Japanese Health Service Bureau suggested that approximately 8.9 million Japanese have glycosylated hemoglobin A1c (HbA_{1c}) values ≥6.5% or are taking glucose-lowering medication and are therefore highly likely to have diabetes[4]. The same survey also suggested that approximately 21.1 million Japanese have HbA_{1c} values between 6.0% and 6.5%, and therefore may have diabetes[4]. Unfortunately, currently available treatments for type 2 diabetes in Japan, including insulin, sulfonylurea (SU), biguanide (BG) and thiazolidinedione (TZD), do not always provide adequate glycemic control[5–7], and can have adverse side-effects, such as hypoglycemia and weight gain[8,9]. Given the increasing prevalence of type 2 diabetes in Japan and the risks associated with current treatment, there is a need for new therapies that provide adequate glycemic control.

Exenatide is a glucagon-like peptide-1 receptor agonist that has been shown to improve glycemic control, decrease bodyweight and improve β-cell function in patients with type 2 diabetes from Western countries[10–15]. Consequently, exenatide b.i.d. has been approved in the USA and Europe for use as adjunct therapy, with diet and exercise, for patients with type 2 diabetes who have not achieved adequate glycemic control with metformin (Met), SU or a combination of Met and SU. Exenatide has also been approved in the USA for use as monotherapy adjunct to diet and exercise, and as adjunct therapy with TZD or combined Met and TZD. We have recently reported the findings of the first phase III, double-blind, randomized controlled trial of exenatide b.i.d. in Japan[16]. After 24 weeks of adjunct treatment with exenatide, we found that participants with type 2 diabetes and suboptimal glycemic control had improved glycemic control and, at a 10-μg b.i.d. dose, decreased bodyweight. Exenatide also had a favorable safety profile and was generally well tolerated. In October 2010, exenatide b.i.d. was approved in Japan as an adjunct therapy for patients with type 2 diabetes

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who had not achieved adequate glycemic control with SU, alone or in combination with BG or TZD.

The purpose of the present extension study was to determine the long-term (52 weeks) safety and efficacy of adjunct exenatide treatment in Japanese patients with type 2 diabetes and suboptimal glycemic control.

MATERIALS AND METHODS

Study Design

The present study was a 28-week, open-label extension study carried out at 23 centers in Japan. Participants were enrolled immediately after completing a 24-week, double-blind, randomized controlled trial (ClinicalTrials.gov registration number NCT00577824)[16]. In the 24-week trial, a total of 181 participants were randomized (1:2:2) to receive placebo, exenatide 5 μg or exenatide 10 μg b.i.d. Participants in the placebo group were further randomized (before starting the 24-week trial) to receive exenatide 5 μg or exenatide 10 μg during the extension study. Throughout the 52-week study, participants were instructed to inject exenatide (Eli Lilly and Company, Indianapolis, IN, USA) or placebo 15 min before both the morning and evening meals; however, injections made between 0 and 60 min before meals were allowed.

The study was approved by the Institutional Review Board of each participating center and was carried out in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. All study participants provided written informed consent.

Participants

The main study inclusion criteria at the time of enrolment in the initial study[16] were as follows: a diagnosis of type 2 diabetes mellitus according to the Japan Diabetes Society (JDS)[17] and the World Health Organization[18] definitions; currently on SU monotherapy, SU and BG combination therapy, or SU and TZD combination therapy without any change in dose within 90 days of enrolment; suboptimal glycemic control as defined by the JDS[17] (i.e. HbA_{1c} value ≥ 7.4% and ≤10.4%); age between 20 and 75 years; and bodyweight ≥ 50 kg. Patients taking α-glucosidase inhibitors or short-acting insulin secretion inducers were eligible for inclusion, but stopped taking these drugs before beginning the study.

The main study exclusion criteria at the time of enrolment in the initial study[16] were as follows: treatment with exogenous insulin or any drug affecting gastrointestinal motility within 90 days of enrolment; currently on triple therapy (i.e. SU, BG and TZD); any clinically significant gastrointestinal or hepatic disorder; suspected malignant tumor or a history of malignant tumor within 5 years of enrolment; serum creatinine ≥ 1.5 mg/dL in men or ≥1.4 mg/dL in women; systolic blood pressure (sitting) ≥ 160 mmHg or diastolic blood pressure (sitting) ≥ 100 mmHg; fasting plasma glucose ≥ 250 mg/dL or casual blood glucose ≥ 350 mg/dL or at least one episode of severe hypoglycemia within 90 days of enrolment; and women who were breastfeeding, pregnant, intending to become pregnant during the study, not practicing a reliable method of contraception within 90 days of enrolment or unable to practice a reliable method of contraception during the study.

Interventions

From week 24 to week 28 (i.e. the first 4 weeks of the extension study), all participants received exenatide 5 μg, b.i.d. Participants who received exenatide (5 μg or 10 μg) in the 24-week trial received the same dose of exenatide from week 28 to week 52 (exenatide 5 μg and exenatide 10 μg groups, respectively). Participants who received placebo in the 24-week trial received exenatide 5 μg (placebo/exenatide 5 μg group) or exenatide 10 μg (placebo/exenatide 10 μg group) from week 28 to week 52.

Baseline clinical characteristics of participants in the exenatide groups were determined at week 0 and included all participants who received at least one dose of exenatide in the 24-week trial. Baseline clinical characteristics of participants in the placebo/exenatide groups were determined at week 24. Follow-up assessments were made every 4 weeks.

Efficacy Outcomes

The following efficacy outcomes were recorded: change from baseline in HbA_{1c} values; the proportion of participants who achieved an HbA_{1c} value < 7.4% and <6.9% at week 52; fasting plasma glucose concentrations; seven-point, self-monitored blood glucose concentrations (SMBG; measured before breakfast, before lunch, before dinner, 2 h after starting each meal and before bedtime); bodyweight; serum lipid concentrations (total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglyceride); and glycemic control, as indicated by 1,5-anhydroglucitol concentrations. Please note that all HbA_{1c} values in the present study are expressed as National Glycohemoglobin Standardization Program equivalent values (i.e. JDS HbA_{1c} value + 0.4%)[17]. We used the JDS definition of good glycemic control (i.e. HbA_{1c} value < 6.9%)[17].

All laboratory tests were carried out using standard methods at a central laboratory (Bio Medical Laboratories Inc., Tokyo, Japan).

Safety Outcomes

Treatment-Emergent Adverse Events

Treatment-emergent adverse events (TEAE) were coded and summarized using the Medical Dictionary for Regulatory Activities (version 12.0) and were defined as events that occurred during the treatment period or worsened from baseline. Only common TEAE, defined as TEAE with an incidence >10% in the exenatide 10 μg group during the 52-week study, and serious adverse events (SAE) are described.

Hypoglycemia

Hypoglycemia was defined as the presence of signs or symptoms of hypoglycemia, regardless of blood glucose concentration. Decreased blood glucose was defined as a blood glucose

concentration < 70 mg/mL in the absence of signs or symptoms of hypoglycemia.

Exenatide Antibody Status

Participants were considered to have treatment-emergent exenatide antibodies if antibodies were undetectable at baseline and detectable (a titer ≥ 25) at any subsequent visit. Participants were also considered to have treatment-emergent exenatide antibodies if antibodies were detectable at baseline and the titer increased by three dilution factors at any subsequent visit. Exenatide antibody concentrations were measured by Millipore Corporation (St. Charles, MO, USA) using a solid-phase enzyme-linked immunosorbent assay[10].

Statistical Analysis

Continuous variables are presented as mean ± standard deviation (SD). Categorical variables are presented as frequency and percentage.

RESULTS

Participant Disposition

Of the 152 participants who completed the 24-week trial, 150 were enrolled in the 28-week extension study, and 137 completed the extension study (Figure 1). A total of 13 participants discontinued the extension study. The most common reason for discontinuation was adverse events (8/13 participants).

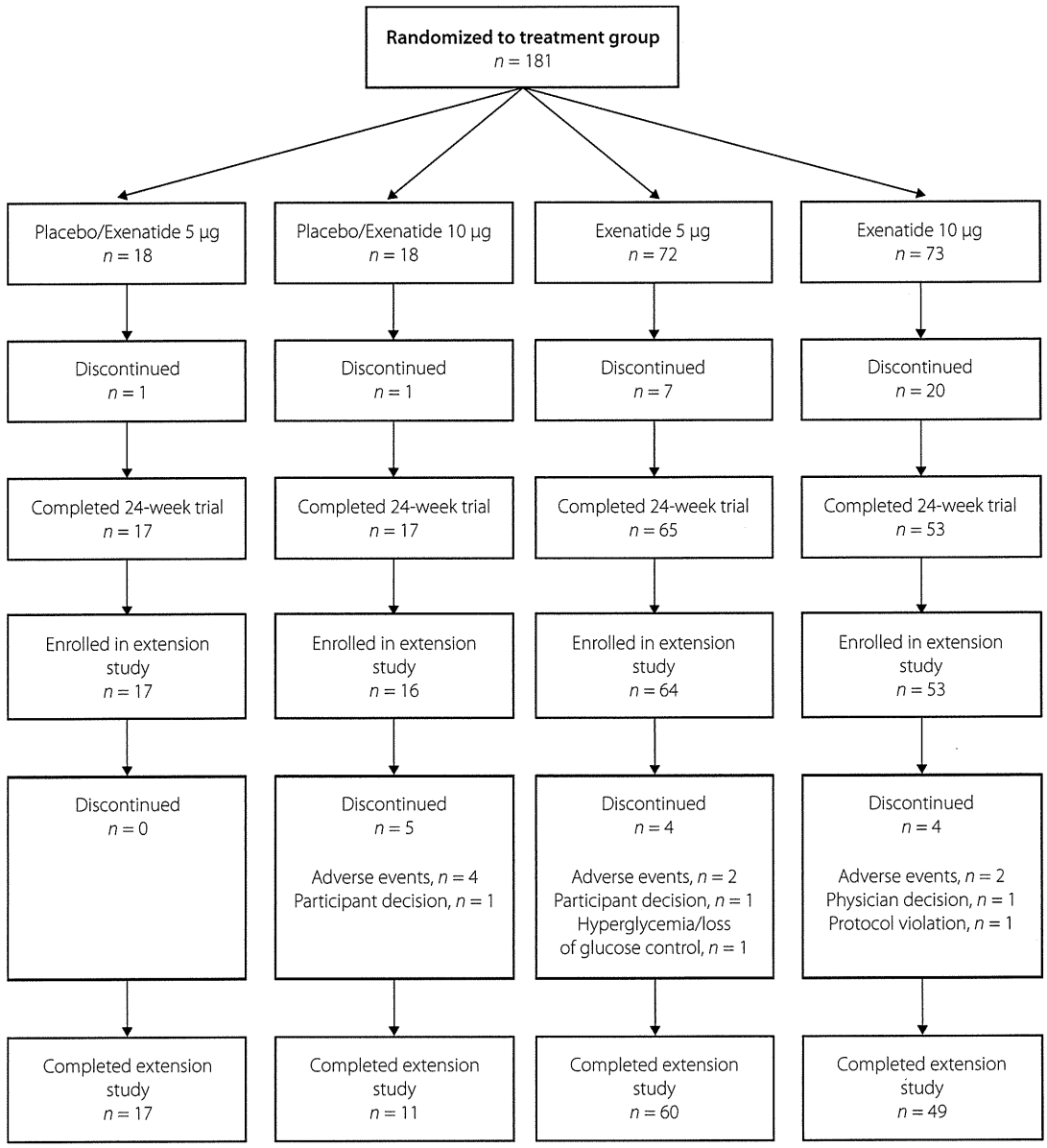

Figure 1 | Participant flow diagram.

Table 1 | Baseline characteristics of Japanese participants with type 2 diabetes mellitus and suboptimal glycemic control

Characteristic	Placebo/exenatide 5 μg (*n* = 17)*	Placebo/exenatide 10 μg (*n* = 16)*	Exenatide 5 μg (*n* = 72)†	Exenatide 10 μg (*n* = 72)†
Men, *n* (%)	12 (71)	10 (63)	49 (68)	49 (68)
Age (years)	54 ± 12	59 ± 11	59 ± 9	59 ± 10
Weight (kg)	67 ± 10‡	72 ± 17‡	67 ± 11	69 ± 11
BMI (kg/m^2)	24.6 ± 3.2‡	26.4 ± 5.2‡	25.0 ± 4.1	25.8 ± 3.9
Duration of type 2 diabetes (years)	11.7 ± 5.5	12.7 ± 7.8	12.2 ± 6.3	11.6 ± 7.0
HbA_{1c} (%)	8.6 ± 0.9‡	8.1 ± 1.2‡	8.7 ± 0.8	8.6 ± 1.0
Fasting plasma glucose (mg/dL)	161 ± 35‡	147 ± 29‡	164 ± 42	164 ± 39
Total cholesterol (mg/dL)	195 ± 27‡	192 ± 19‡	204 ± 36	202 ± 31
HDL cholesterol (mg/dL)	52 ± 10‡	57 ± 13‡	57 ± 15	55 ± 11
LDL cholesterol (mg/dL)	120 ± 27‡	115 ± 19‡	124 ± 28	125 ± 27
Triglycerides (mg/dL)	140 ± 73‡	106 ± 49‡	133 ± 95	131 ± 70
Oral anti-diabetic agents§				
SU alone, *n* (%)	1 (5.9)	1 (6.3)	4 (5.6)	8 (11.1)
SU + α-GI, *n* (%)	1 (5.9)	2 (12.5)	1 (1.4)	4 (5.6)
SU + BG, *n* (%)	7 (41.2)	7 (43.8)	33 (45.8)	27 (37.5)
SU + BG + α-GI, *n* (%)	4 (23.5)	5 (31.3)	22 (30.6)	13 (18.1)
SU + BG + meglitinide derivative, *n* (%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.4)
SU + TZD, *n* (%)	3 (17.6)	0 (0.0)	6 (8.3)	12 (16.7)
SU + TZD + α-GI, *n* (%)	1 (5.9)	1 (6.3)	6 (8.3)	7 (9.7)

Values are mean ± standard deviation, except where indicated.
*Includes participants who were enrolled in the 28-week extension study.
†Includes participants who were enrolled in the initial 24-week trial and received at least one dose of exenatide.
‡These values were determined at the beginning of the 28-week extension study.
§Taken at the time of providing informed consent.
α-GI, α-glucosidase inhibitors; BMI, body mass index; BG, biguanide; HbA_{1c}, glycosylated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SU, sulfonylurea; TZD, thiazolidinedione.

Baseline Clinical Characteristics

Participants in the different groups had similar baseline clinical characteristics (Table 1). The proportions of participants taking different combinations of oral anti-diabetic drugs were similar between the groups.

Efficacy Outcomes

Exenatide treatment was associated with sustained decreases in HbA_{1c} values (Figure 2a). In the exenatide 5 μg and exenatide 10 μg groups, the decreases in HbA_{1c} values that occurred during the first 24 weeks of treatment were generally maintained from week 24 to week 52. In the placebo/exenatide 5 μg and placebo/exenatide 10 μg groups, HbA_{1c} values were decreased from baseline (week 24) to week 52. Decreases from baseline in HbA_{1c} values were greater in the exenatide 10 μg and placebo/exenatide 10 μg groups than in the exenatide 5 μg and placebo/exenatide 5 μg groups.

A greater proportion of participants in the exenatide 10 μg and placebo/exenatide 10 μg groups achieved HbA_{1c} target levels than participants in the exenatide 5 μg and placebo/exenatide 5 μg groups. Among participants who received exenatide for 52 weeks, 32 of 45 (71.1%) and 30 of 59 (50.8%) achieved an HbA_{1c} level of <7.4% in the exenatide 10 μg and exenatide 5 μg groups, respectively. Similarly, 8 of 11 (72.7%) participants in the placebo/exenatide 10 μg group achieved an HbA_{1c} level of <7.4% compared with 8 of 17 (47.1%) participants in the placebo/exenatide 5 μg group. Among participants who received exenatide for 52 weeks, 23 of 48 (47.9%) and 20 of 60 (33.3%) participants achieved an HbA_{1c} level of <6.9% in the exenatide 10 μg and exenatide 5 μg groups, respectively. Similarly, 7 of 11 (63.6%) participants in the placebo/exenatide 10 μg group achieved an HbA_{1c} level of <6.9% compared with 3 of 17 (17.6%) participants in the placebo/exenatide 5 μg group.

Fasting plasma glucose concentrations decreased from baseline in all groups after the start of exenatide treatment. The decrease in fasting plasma glucose concentration from baseline in participants in the exenatide 5 μg group and the exenatide 10 μg group after 24 weeks of treatment was maintained during the extension study. The mean (±SD) change from baseline in fasting plasma glucose concentration in the exenatide 5 μg group was −29.6 ± 37.1 mg/dL at week 24 and −16.4 ± 41.4 mg/dL at week 52. The mean (±SD) change from baseline in fasting plasma glucose concentration in the exenatide 10 μg group was −36.0 ± 36.3 mg/dL at week 24 and −29.8 ± 39.0 mg/dL at week 52. The mean (±SD) change from week 0

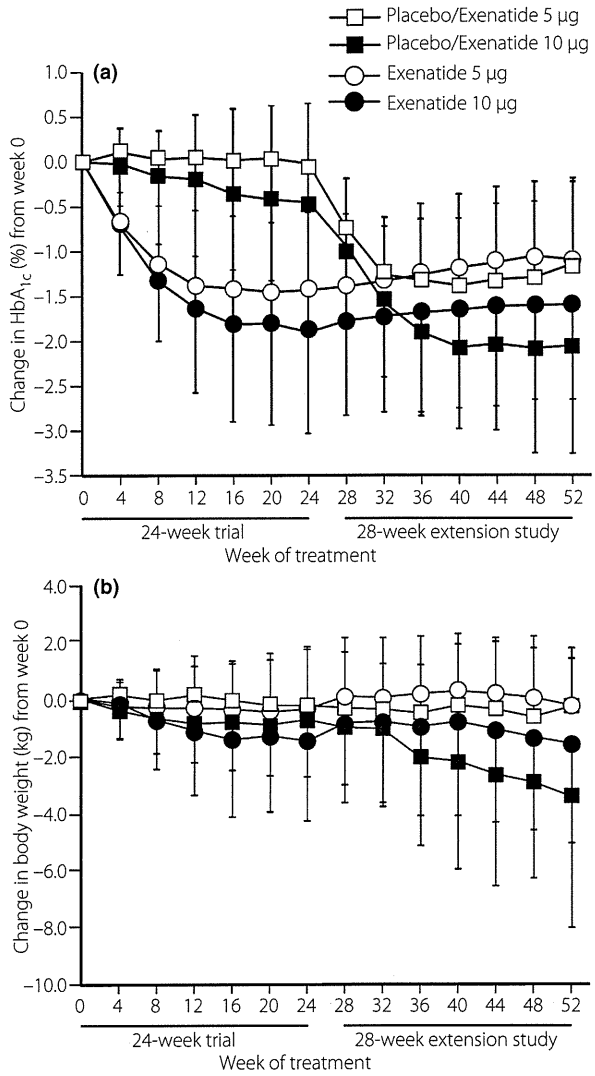

Figure 2 | Change from week 0 in (a) glycosylated hemoglobin A1c (HbA_{1c}) values and (b) bodyweight after treatment with exenatide (5 or 10 μg) b.i.d. Participants in the placebo/exenatide groups received placebo for the first 24 weeks and exenatide for the following 28 weeks. Participants in the exenatide groups received exenatide for the entire 52 weeks. Values are means, with standard deviations shown as upward (placebo/exenatide 5 μg [*n* = 16–18] and exenatide 5 μg [*n* = 60–72] groups) or downward (placebo/exenatide 10 μg [*n* = 11–17] and exenatide 10 μg [*n* = 48–72] groups) error bars. Note: data were not available for all participants.

in fasting plasma glucose concentration in the placebo/exenatide 5 μg group was −8.2 ± 23.3 mg/dL at week 24 (baseline for this group) and −19.1 ± 36.9 mg/dL at week 52. The mean (±SD) change from week 0 in fasting plasma glucose concentration in the placebo/exenatide 10 μg group was −3.2 ± 27.1 mg/dL at week 24 (baseline for this group) and −31.6 ± 27.0 mg/dL at week 52.

Seven-point, SMBG were decreased in all treatment groups at week 52 compared with baseline (Figure 3). Decreases from baseline were most pronounced at the after breakfast and after dinner time-points.

Bodyweight decreased from baseline in the exenatide 10 μg group and in the placebo/exenatide 10 μg group, but did not change in either the exenatide 5 μg group or the placebo/exenatide 5 μg group (Figure 2b). The bodyweight decrease from baseline in participants in the exenatide 10 μg group after 24 weeks of treatment was maintained during the extension study.

Plasma total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglyceride concentrations were generally decreased from baseline or similar to baseline in the exenatide 5 μg and exenatide 10 μg groups at weeks 24 and 52, and in the placebo/exenatide 5 μg and placebo/exenatide 10 μg groups at week 52 (data not shown). Plasma concentrations of all lipids were within the normal range in all groups during the entire study.

Exenatide treatment was associated with sustained increases from baseline in 1,5-anhydroglucitol concentrations. The mean (±SD) increases from baseline (week 0) in 1,5-anhydroglucitol concentrations were 5.7 ± 4.4 μg/mL and 5.5 ± 4.4 μg/mL at week 24, and 3.3 ± 3.7 μg/mL and 4.5 ± 4.1 μg/mL at week 52 in the exenatide 5 μg group and the exenatide 10 μg group, respectively. The mean (±SD) increases from baseline (week 24) in 1,5-anhydroglucitol concentrations were 2.7 ± 3.5 μg/mL and 6.7 ± 4.9 μg/mL at week 52 in the placebo/exenatide 5 μg group and the placebo/exenatide 10 μg group, respectively.

Safety Outcomes

The majority of common TEAE in the exenatide 5 μg and exenatide 10 μg groups occurred during the first 24 weeks, as shown by similar incidence rates between the first 24 weeks and the entire 52 weeks (Table 2). Mild hypoglycemia and nausea were the most common TEAE. With the exception of six episodes of moderate hypoglycemia in two patients in the exenatide 10 μg group, all episodes of hypoglycemia were mild in severity. In all groups, the incidences of hypoglycemia and nausea were highest within the first 4–8 weeks after starting exenatide treatment. Few participants who were randomized to receive exenatide for 52 weeks experienced hypoglycemia (5/144 participants) or nausea (1/144 participants) for the first time during the extension study. No TEAE specifically occurred during the extension study only (data not shown).

For the entire 52 weeks, the incidence of TEAE considered by the investigators to be related to the study drug was 80.6% (58/72 participants) in the exenatide 5 μg group and 88.9% (64/72 participants) in the exenatide 10 μg group.

A total of eight participants experienced SAE during the extension study.

One participant in the placebo/exenatide 10 μg group and one participant in the exenatide 5 μg group experienced

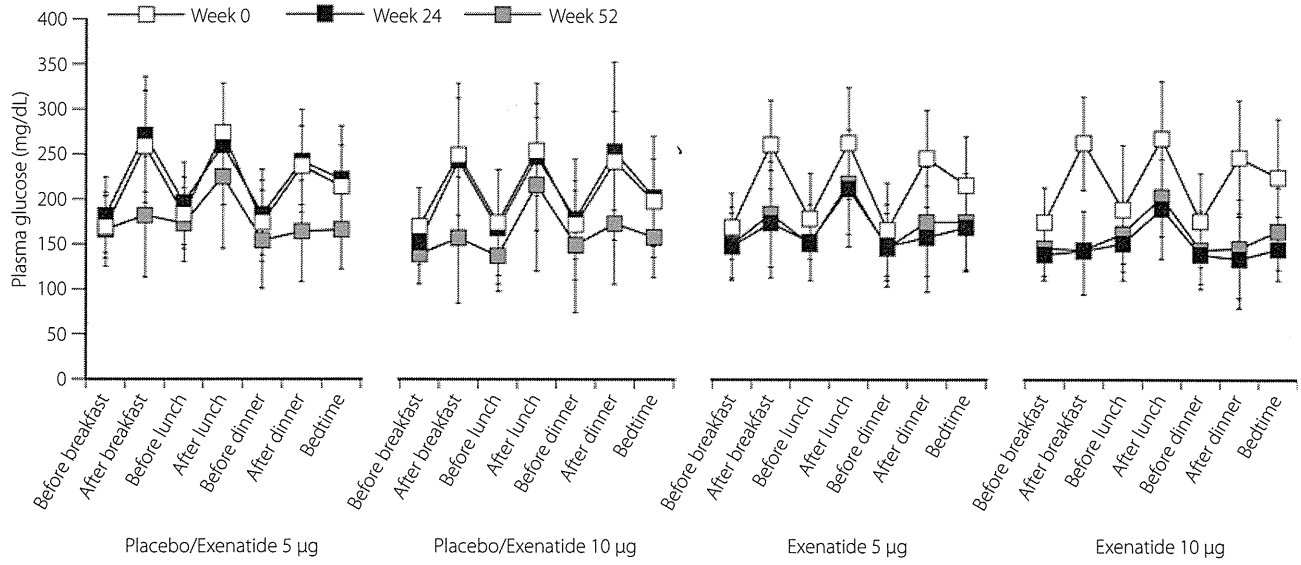

Figure 3 | Changes in seven-point, self-monitored blood glucose concentrations after treatment with exenatide (5 or 10 µg) b.i.d. Plasma glucose concentrations were measured before breakfast, before lunch, before dinner, 2 h after starting each meal and before bedtime. Participants in the placebo/exenatide groups received placebo for the first 24 weeks and exenatide for the following 28 weeks. Participants in the exenatide groups received exenatide for the entire 52 weeks. Values are mean ± standard deviation (n = 16–18 in the placebo/exenatide 5 µg group; n = 11–17 in the placebo/exenatide 10 µg group; n = 59–72 in the exenatide 5 µg group; n = 46–72 in the exenatide 10 µg group). Note: data were not available for all participants.

Table 2 | Common treatment-emergent adverse events during the 24-week, double-blind study (24 weeks: placebo and exenatide groups), the extension study (28 weeks: placebo/exenatide groups only) and the entire study (52 weeks cumulative: exenatide groups only)

Treatment-emergent adverse event*	24 Weeks	28 Weeks		24 Weeks		52 Weeks	
	Placebo (n = 35)	Placebo/exenatide 5 µg† (n = 17)	Placebo/exenatide 10 µg† (n = 16)	Exenatide 5 µg (n = 72)	Exenatide 10 µg (n = 72)	Exenatide 5 µg (n = 72)	Exenatide 10 µg (n = 72)
Hypoglycemia, n (%)	8 (22.9)	5 (29.4)	12 (75.0)	37 (51.4)	42 (58.3)	40 (55.6)	44 (61.1)
Nausea, n (%)	3 (8.6)	6 (35.3)	8 (50.0)	18 (25.0)	26 (36.1)	18 (25.0)	27 (37.5)
Blood glucose decreased, n (%)	4 (11.4)	4 (23.5)	4 (25.0)	10 (13.9)	18 (25.0)	14 (19.4)	24 (33.3)
Nasopharyngitis, n (%)	8 (22.9)	4 (23.5)	3 (18.8)	8 (11.1)	9 (12.5)	24 (33.3)	22 (30.6)
Vomiting, n (%)	1 (2.9)	1 (5.9)	4 (25.0)	3 (4.2)	12 (16.7)	4 (5.6)	15 (20.8)
Constipation, n (%)	1 (2.9)	1 (5.9)	1 (6.3)	10 (13.9)	11 (15.3)	12 (16.7)	13 (18.1)
Abdominal discomfort, n (%)	1 (2.9)	1 (5.9)	2 (12.5)	7 (9.7)	9 (12.5)	10 (13.9)	12 (16.7)
Decreased appetite, n (%)	1 (2.9)	0 (0)	1 (6.3)	7 (9.7)	9 (12.5)	7 (9.7)	9 (12.5)
Anorexia, n (%)	1 (2.9)	0 (0)	3 (18.0)	2 (2.8)	8 (11.1)	2 (2.8)	8 (11.1)

*Treatment-emergent adverse events with an incidence >10% in the exenatide 10 µg group during the first 24 weeks are shown.
†These participants received placebo for the first 24 weeks and exenatide for the following 28 weeks.

pancreatitis. Both participants discontinued the study and recovered uneventfully. Both participants had gallstones, a known cause of pancreatitis; however, the investigators could not rule out a relationship between exenatide treatment and pancreatitis.

One participant in the exenatide 5 µg group experienced pneumonia, which was considered to be unrelated to the study procedures. However, the investigators could not rule out a relationship between pneumonia and exenatide treatment.

Three participants experienced two different SAE, including cerebral infarction and hemiplegia (placebo/exenatide 5 µg group, n = 1), cerebral circulatory failure and calculus ureteric (exenatide 5 µg group, n = 1), and diabetic retinopathy and retinal vein occlusion (exenatide 10 µg group, n = 1). Other SAE during the extension study included brain stem infarction (exenatide 10 µg group, n = 1) and large intestine carcinoma (exenatide 10 µg group, n = 1). All of these SAE were considered by the study investigators to be unrelated to exenatide treatment and the study procedures.

There were no increases between week 24 and week 52 in the proportion of participants in the exenatide 5 µg and exenatide

10 μg groups who tested positive for the exenatide antibody. The proportion of participants who tested positive for the exenatide antibody at week 24 was 63.1% (41/65) and 60.4% (32/53) in the exenatide 5 μg and exenatide 10 μg groups, respectively. Corresponding proportions at week 52 were 63.3% (38/60) and 61.2% (30/49).

Exenatide antibody status did not appear to influence changes in HbA_{1c} values or the incidence of TEAE, including hypoglycemia and nausea (data not shown).

DISCUSSION

This is the first study to examine the long-term (52 weeks) safety and efficacy of exenatide b.i.d. in Japanese participants with type 2 diabetes and suboptimal glycemic control. Importantly, we observed a very low incidence of common TEAE during the second 28 weeks of treatment. We also found that the efficacy of exenatide (as shown by improved glycemic control) previously observed after 24 weeks of treatment[16] was maintained after 52 weeks of treatment. Our findings suggest that long-term exenatide has a similar safety profile to that observed in previous studies and is efficacious for improving glycemic control in Japanese patients with suboptimally controlled type 2 diabetes.

We found that long-term treatment with exenatide was associated with generally mild severity TEAE, a low incidence of TEAE in the extension study and a low proportion of participants who discontinued in the extension study because of TEAE. In keeping with the findings of previous studies of Caucasian participants with type 2 diabetes[11–14,19,20], we found that hypoglycemia and nausea were the most common TEAE associated with exenatide treatment and that the severity of these TEAE was generally mild to moderate. Also consistent with previous findings was our finding that TEAE were generally more common during the earlier stages of treatment than during the later stages of treatment[11–13,19]. Further, more than 75% of participants who received exenatide completed the 52-week study. Similar completion rates have been reported in long-term studies of exenatide involving Caucasian participants with type 2 diabetes[14,21,22].

Two participants in the present study experienced pancreatitis during the treatment period. Although a relationship between exenatide treatment and pancreatitis could not be ruled out by the study investigators in either case, both participants had gallstones, a known cause of pancreatitis. Furthermore, type 2 diabetes is also a known risk factor for acute pancreatitis[23–25]. Several recent studies found that exenatide treatment was not associated with an increased risk of pancreatitis compared with other anti-diabetic treatments[24,26,27]. Nevertheless, the prescribing information for exenatide acknowledges that postmarketing cases of acute pancreatitis have been reported[28].

We found that long-term exenatide treatment was associated with sustained improvement in glycemic control, as shown by decreased HbA_{1c} values from baseline, an increased proportion of participants achieving target HbA_{1c} values, decreased fasting plasma glucose concentrations and SMBG, and increased 1,5-anhydroglucitol concentrations. Other researchers have also consistently reported that long-term (1–3 years) exenatide treatment was associated with sustained improvement in glycemic control in Caucasian participants with type 2 diabetes[14,19–22]. The present findings suggest that exenatide treatment is effective for long-term glycemic control in Japanese patients with type 2 diabetes.

Similar to previous long-term studies of Caucasian patients with type 2 diabetes and suboptimal glycemic control[14,19–22,29], we found that exenatide 10 μg treatment was associated with sustained weight loss in Japanese participants with type 2 diabetes and suboptimal glycemic control. Our finding that exenatide 10 μg was associated with sustained weight loss is important given that bodyweight is known to influence glycemic control[30]. Also worth noting is our finding that participants receiving exenatide 10 μg lost weight despite continuing to take oral anti-diabetics, which are known to cause weight gain[8,9]. Interestingly, we found that exenatide 5 μg treatment was not associated with weight loss. Variable effects of exenatide 5 μg on weight loss have been reported in previous, shorter duration studies involving Caucasian participants with type 2 diabetes[11,13]. These different findings may reflect differences in the mean baseline body mass index of the study participants. Indeed, DeFronzo *et al.*[12] reported that only participants with an initial body mass index ≥ 30 kg/m^2 had significant weight loss after 30 weeks of exenatide 5 μg treatment, whereas all participant groups in the present study had a mean baseline body mass index < 30 kg/m^2.

The strengths of the present study include the multicenter design, the duration of exenatide treatment, the assessment of multiple indicators of glycemic control and the high participant retention rate during the extension study. The present study, however, does have several limitations that must be acknowledged, including the absence of a group who received placebo for 52 weeks and the lack of control of participant dietary and exercise habits. We did, however, include a placebo group for the initial 24-week trial in which pronounced differences were observed between the placebo and exenatide treatment groups[16]. Although different dietary and exercise habits might have confounded our results, we have no reason to believe that these habits would have been markedly different between groups. Furthermore, dietary and exercise habits are rarely strictly controlled in 'real world' clinical settings; hence, our demonstration of efficacy and weight loss (in the exenatide 10 μg group) despite this lack of control is important. Finally, we acknowledge that monitoring beyond 52 weeks is necessary to evaluate the continued efficacy of exenatide in Japanese patients with type 2 diabetes.

In conclusion, our findings from this open-label extension study show that long-term (52 weeks) treatment with exenatide b.i.d. has a safety profile similar to that observed previously and is associated with sustained improvement in glycemic control in Japanese patients with type 2 diabetes. Taken together, our

findings suggest that exenatide may be a viable treatment option for Japanese patients with type 2 diabetes and suboptimal glycemic control.

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Cognitive and affective impairments of a novel SCA/MND crossroad mutation Asidan

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Background: A variety of hereditary spinocerebellar ataxia (SCA) develops a broad spectrum of both ataxia and non-ataxia symptoms. Cognitive and affective changes are one such non-ataxia symptoms, but have been described only in hereditary SCAs with exonic CAG gene expansion.

Methods: We newly found intronic hexanucleotide GGCCTG gene expansion in NOP56 gene as the causative mutation (=SCA36) in nine unrelated Japanese familial SCA originating from Asida river area in the western part of Japan, thus nicknamed Asidan for this mutation. These patients show unique clinical balance of cerebellar ataxia and motor neuron disease (MND), locating on the crossroad of these two diseases. In the nine families, 14 patients were clinically examined and genetically confirmed to Asidan. In the present study, we examined cognitive and affective analyses on 12 patients (seven men and five women) who agreed to join the examination with average age at onset of 53.1 ± 3.2 years, average duration of 12.1 ± 5.2 years, and current average age at 65.1 ± 6.2 years.

Results: The 12 Asidan patients demonstrated a significant decrease in their frontal executive functions measured by frontal assessment battery (FAB) and Montreal cognitive assessment (MoCA) compared with age- and gender-matched controls, whilst mini-mental state examination (MMSE) and Hasegawa dementia score-revised (HDS-R) were within normal range. The decline of frontal executive function was related to their disease duration and scale for the assessment and rating of ataxias (SARA). They also demonstrated mild depression and apathy. Single-photon emission tomography (SPECT) analysis showed that these Asidan patients showed decline of regional cerebral blood flow (rCBF) in a particular areas of cerebral cortices such as Brodmann areas 24 and 44–46.

Conclusion: These data suggest that the patients with Asidan mutation show unique cognitive and affective characteristics different from other hereditary SCAs with exonal CAG expansion or MND.

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Introduction

Spinocerebellar ataxia (SCA) is a neurodegenerative disorder consisted of heterogeneous subgroups of hereditary cases with late onset, progressive dysarthria, and gait/limb ataxias. We have reported that some hereditary SCAs such as SCA1, SCA2, SCA3, and SCA6 slightly affect motor neuron system with skeletal muscle atrophy [1–5], which was recently confirmed by Schmitz-Hübsch *et al.* [6]. Cognitive and affective characteristics have also been described in SCA1 [7], SCA2 [8,9], SCA3 [10–12], and SCA6 [13–15]. Motor neuron disease (MND) and amyotrophic lateral sclerosis (ALS) are another neurodegenerative disorder again consisted of heterogeneous subgroups of hereditary and sporadic cases with adult onset, progressive motor palsy, and skeletal/tongue muscle atrophy. More than 10 subtypes of hereditary ALS have been reported

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[16], which do not usually develop cerebellar ataxia, but sometimes accompany cognitive and affective symptoms [17,18].

We have reported unique cases with the combination of SCA and MND/ALS symptoms [19,20] and have newly found GGCCTG hexanucleotide repeat expansions within NOP56 gene as the causative mutation in the previous and additional several families [21]. The patients usually develop walking unsteadiness at around age 50 years, soon followed by dysarthria, tongue fasciculation, and atrophy, moderate to severe skeletal muscle atrophy with fasciculation and hyperreflexia, and limb ataxia in subsequent 10–20 years and usually survive until age 80 years or more. Speech disturbance is characterized initially by cerebellar slurred type, which is eventually modified by motor system involvement with tongue atrophy and fasciculation [19–21]. Because most of these cases are originally found along 'Asida' river located in the west part of Japan, we have been calling this mutation as 'Asidan'. Physico-clinical and pathological details will be described elsewhere.

NOP56 is involved in RNA processing with forming RNA foci [21] similar to some hereditary SCAs with pentanucleotide ATTCT repeat expansion (SCA10, ref. [22–23]) and TGGAA repeat expansion (SCA31, ref. [24]) as well as ALS with TAR DNA-binding protein 43 (TDP43, ref. [25]) and fused-in-sarcoma (FUS, ref. [26,27]) gene mutations. However, unlike those hereditary SCAs and ALS, the present Asidan cases show a unique clinical balance to develop a slowly progressive and a relatively pure cerebellar ataxia with moderate to severe motor involvement (both upper and lower motor signs). Based on this unique clinical characteristics having both SCA and MND phenotypes together, Asidan may locate on the crossroad of clinical spectrum between SCA and MND (Ikeda Y, Ohta Y, Kobayashi H, Okamoto M, Takamatsu K, Oota T, Yasuhiro M, Okamoto K, Koizumi A, Abe K, unpublished observation).

We hypothesized that cognitive and affective functions of these Asidan patients may also reside of the crossroad on those functions in SCA and MND/ALS. Therefore, in this report, we focused on their cognitive and affective characteristics, which may also reside on the crossroad of SCA and MND/ALS.

Patients and methods

We found NOP56 gene mutation (=SCA36) in nine unrelated Japanese familial SCA originating from Asida river area in the western part of Japan, thus nicknamed Asidan for this mutation [21]. Because the numbers of patients were small, we took inclusion criteria of as many Asidan patients as possible who were genetically proven, but excluded if they disagree to join the present study. So far 14 patients were clinically examined and genetically confirmed in the nine families. In the present study on cognitive and affective analyses, 12 patients (seven men and five women) agreed to join the examination with average age at onset of 53.1 ± 3.2 years, average duration of 12.1 ± 5.2 years, and current average age at 65.1 ± 6.2 years. Their educations were all 12 years, representing graduation of high school at age 18 (Table 1). Their GGCCTG hexanucleotide repeat expansions were measured and estimated with Southern blot analysis [21]. Clinical severities for SCA and dementia were evaluated with the scale for the assessment and rating of ataxias (SARA, higher worse, [28,29]) and clinical dementia rating (CDR, higher worse) scale, respectively. CDR relies on informant interview, and therefore, CDR scores were obtained from spouses or children for the Asidan patients and from any family members for the control subjects. Standard cognitive function was evaluated with mini-mental state examination (MMSE, lower worse, [30]) and Hasegawa dementia score-revised (HDS-R, lower worse, [31]). Frontal cerebral function was evaluated with frontal assessment battery (FAB, lower worse, [32,33]) and Montreal cognitive assessment (MoCA, lower worse, [34]). The tests of verbal fluency in FAB and five-word recall in MoCA were evaluated at the end of 1 min. Depressive state was evaluated with geriatric depression scale (GDS, higher worse, [35]). Vitality and apathy were evaluated with vitality index (VI, lower worse, [36]) and apathy score (AS, higher worse, [37,38]), respectively. For cognitive and affective assessment, age-, gender-, and education period-matched subjects (n = 94, 65.2 ± 10.0 years old, 56 men/38 women, education period 11.7 ± 1.6 years) were also examined as normal controls.

Magnetic resonance imagings (MRIs) were examined for their brain with T1- and T2-weighted images by axial, coronal, and sagittal slices. Frontal cerebral atrophy was calculated with the ratio of sagittal lengths of intracranial space minus the brain/sagittal length of intracranial space at the slice level of maximum anterior horn in T2-weighted slice. Regional cerebral blood flow (rCBF) of the patients was measured with ^{99m}Tc-ECD-SPECT (single-photon emission tomography), and the data were analyzed with easy Z-score imaging system (eZIS) for obtaining standardized deviation of their rCBF [39]. eZIS score is expressed as SD (standard deviation) from standardized normal control value, and the eZIS score more than two represents an evident decline of rCBF. All the data in the present study are expressed with mean ± SD, and statistical analyses

Table 1 Clinical summary of 12 patients of Asidan (sorted by duration > onset age of the disease) and 94 control subjects

Case No.	1	2	3	4	5	6	7	8	9	10	11	12	Asidan total mean ± SD	Control subjects (n = 94, age-, gender-, and education period-matched)
Onset age (years old)	57	51	52	56	55	51	48	49	52	52	58	55	53.1 ± 3.2	–
Disease duration (years)	6	7	7	7	8	11	13	13	16	17	19	21	12.1 ± 5.2	–
Current age (years old)	63	58	59	63	63	62	61	62	68	69	77	76	65.1 ± 6.2	65.2 ± 10.0
Gender	F	F	M	M	M	M	F	F	F	M	M	M	M7/F5	M56/F38
Education (years)	12	12	12	12	12	12	12	12	12	12	12	12	12.0 ± 0.0	11.7 ± 1.6
SARA (0–40)	13.0	14.0	9.0	14.0	20.5	24.0	28.0	22.0	25.5	34.0	25.0	26.0	21.3 ± 7.4	–
CDR (0–3)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MMSE (0–30)	30	30	28	29	26	29	30	30	27	25	22	28	27.9 ± 2.5	28.2 ± 2.7
HDS-R (0–30)	30	30	29	29	28	29	28	30	30	25	22	28	28.2 ± 2.4	28.1 ± 3.0
FAB (0–18)	15	17	17	17	14	12	15	16	10	11	6	11	13.4 ± 3.4*	15.8 ± 2.7
MoCA (0–30)	29	28	28	23	18	19	26	26	22	16	18	15	22.3 ± 5.0*	25.5 ± 2.4
GDS (0–15)	11	6	6	0	13	10	3	8	2	13	10	5	7.3 ± 4.3**	3.2 ± 3.3
VI (0–10)	9	10	9	9	8	8	9	8	10	10	9	9	9.0 ± 0.7	9.7 ± 0.6
Apathy score (0–42)	21	27	14	4	26	17	14	7	3	17	38	39	18.9 ± 11.9*	8.4 ± 5.2
Frontal lobe atrophy (%)	1.2	1.8	1.6	1.6	2.3	4.5	4.8	4.6	4.4	5.5	4.8	4.6	3.5 ± 1.6	
eZIS (SD decrease)														
Area 24	<2	2.2	<2	< 2	2.4	2.5	2.6	c.n.o.	3.2	2.9	2.8	3.8	2.8 ± 0.5**	
Area 44–46	<2	<2	<2	2.4	3.1	3.4	3.1	c.n.o.	4.7	3.2	3.5	4.3	3.5 ± 0.7**	

CDR, clinical dementia rating; FAB, frontal assessment battery; GDS, geriatric depression scale; MMSE, mini-mental state examination; SARA, scale for the assessment and rating of ataxias; c.n.o. = consent not obtained.
(Bracket in each score represents the range of respective score.)
*P < 0.05 and **P < 0.01 compared with the control.

were performed by nonparametric Dunnet method with $P < 0.05$ as significant for comparison between two groups of the Asidan patients and the control subjects.

The present study was approved by the Ethical Committee of Graduate School of Medicine, Dentistry and Pharmaceutical Science, Okayama University.

Results

As shown in Table 1, the SARA scale ranged from 9.0 to 34.0 with average of 21.3 ± 7.4 (53.3 ± 18.5% of full scale 40). Eight items of SARA scale were analyzed with dividing into four symptom categories relating to gait disturbance (walking, standing, and heel-knee test), speech disturbance (speech), pure truncal ataxia (sitting stability), and upper limb ataxia (finger chasing test, finger-nose test, and diadochokinesis). The four symptom categories of SARA ataxia scale showed decreases in gait disturbance ranging 6–18 (sum full score 18) with 12.3 ± 4.0 (mean ± SD, 68.3% of full score), speech disturbance ranging 1–6 (full score 6) with 3.1 ± 1.2 (51.7% of full score), pure truncal ataxia ranging 0–4 (full score 4) with 1.7 ± 1.2 (41.5% of full score), and upper extremity (UE) ataxia ranging 2–7 (sum full score 12) with 4.2 ± 1.6 (35.0% of full score), respectively (Fig. 1).

Typical physical pictures of Asidan patients are shown in Fig. 2a–d. The patients usually developed gait unsteadiness first, followed by wide-based standing (Fig. 2a) and slurred speech with tongue fasciculation, then developed a swallowing difficulty with a marked tongue atrophy (Fig. 2b), and finally developed leg and hands/forearm atrophies with fasciculations and neurogenic electromyography findings (Fig. 2c and d). Most of the patients showed hyperreflexia in four limbs. On MRI examination, cerebellar vermis atrophy was found in all 12 patients, but was kept mild from the early (Fig. 2e, arrow) to the advanced (Fig. 2g, arrow) stages. Cerebellar hemisphere and brain stem (Fig. 2e and 1g, arrowheads) were usually spared. Frontal lobe atrophy ranged from 1.2 to 5.5%, which was related to the disease duration (Table 1). In cases with advanced stage, frontal lobe atrophies were evident (Fig. 2h, arrows) with corresponding anterior horn dilatation (Fig. 2h, arrowheads).

Analysis of eZIS with ^{99m}Tc-ECD-SPECT showed a decrease in cerebellar vermis and hemisphere from early (Fig. 2i and 1k, arrows) to advanced (Fig. 2l and 1n, arrows) stages. Brodmann cerebral cortical areas 44–46 (area 44, pars opercularis of inferior frontal gyrus; area 45, pars triangularis of inferior frontal gyrus; area 46, dorsolateral prefrontal cortex) showed a slight decrease

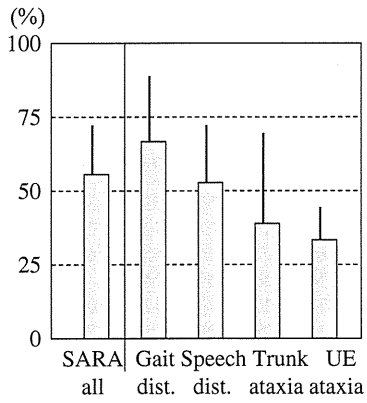

Figure 1 (Left) Average scale for the assessment and rating of ataxias (SARA) ataxia scale in all 12 Asidan patients, and (Right) those with dividing into four symptom categories relating to gait disturbance (walking, standing, and heel-knee test), speech disturbance (speech), pure truncal ataxia (sitting stability), and upper extremity (UE) ataxia (finger chasing test, finger-nose test, and diadochokinesis). Data are expressed as % of respective full scale. Note gait disturbance is the highest symptoms, and UE ataxia the lowest.

in rCBF at the early stage (Fig. 2j, arrowhead) with a progressive decrease to the advance stage (Fig. 2m, arrowhead). Although rCBF was not reduced in Brodmann area 24 (ventral frontal cingulate gyrus) at the early stage (Fig. 2k, arrowhead), it showed an evident decrease at the advanced stage (Fig. 2n, arrowhead) with eZIS score of 2.6-3.8 SD of the control after 13 years of onset (Table 1, bottom). Asidan patients showed a statistically significant decrease in rCBF in eZIS score in Brodmann areas 24 (2.8 ± 0.5, $^{**}P < 0.01$) and 44–46 (3.5 ± 0.7, $^{**}P < 0.01$) (Table 1).

Cognitive and affective functions in the Asidan patients and 94 controls are summarized in the Table 1. Amongst these functions, FAB ($^{*}P < 0.05$), MoCA ($^{*}P < 0.05$), GDS ($^{**}P < 0.01$), and apathy ($^{*}P < 0.05$) scores were significantly decreased in the Asidan patients compared with the controls. The number of hexonucleotide GGCCTG repeat expansion of these patients ranged from 1700 to 2300 (2120 ± 215, mean ± SD). As shown in Fig. 3, there was no correlation between the number of GGCCTG expansion and MMSE (dark circles and solid line, $r = 0.20265$, $P > 0.05$) or FAB (dark squares and dotted line, $r = 0.10909$, $P > 0.05$) cognitive scores. Correlation between the number of GGCCTG expansion and age at onset was not also found ($r = 0.28682$, $P > 005$, data not shown). In contrast, there was slight correlations between MMSE and the disease duration (years, Fig. 4, left panel, dark circles and solid line, $r = -0.43287$, $^{*}P < 0.05$) or SARA scale (Fig. 4, right panel, dark circles and solid line, $r = -0.39144$, $^{*}P < 0.05$) and stronger correlations between FAB and the disease course (years, Fig. 4, left panel, dark squares and dotted line, $r = -0.76475$, $^{**}P < 0.01$) and between MoCA and SARA scale (Fig. 4, right panel, dark diamonds and dotted line, $r = -0.69269$, $^{**}P < 0.01$).

In six FAB subcategories, motor programming was the most impaired to 1.58 ± 1.31 (mean ± SD, 52.7% of full score 3, $^{**}P < 0.01$ vs. control = 2.83 ± 0.45), then verbal fluency to 1.83 ± 0.94 (61.0% of full score 3, $^{*}P < 0.05$ vs. control = 2.37 ± 0.56), similarities/conceptualization to 1.83 ± 1.03 (61.0% of full score 3, $^{*}P < 0.05$ vs. control = 2.78 ± 0.54), Go/no Go selection to 2.42 ± 0.79 (80.7% of full score 3, P = ns vs. control = 2.31 ± 0.97), conflicting instructions to 2.67 ± 0.65 (89.0% of full score 3, P = ns vs control = 2.90 ± 0.41), and prehension behavior to 2.92 ± 0.29 (97.3% of full score 3, P = ns vs. control = 2.99 ± 0.31), respectively (Fig. 5, left panel). Verbal fluency test in FAB took 40–50 s in the control subjects and < 35 s in the Asidan patients.

In seven MoCA subcategories, five-word recall was the most impaired to 1.92 ± 1.93 (mean ± SD, 38.4% of full score 5, $^{**}P < 0.01$ vs. control = 3.19 ± 1.48), then language to 1.50 ± 1.00 (50.0% of full score 3, $^{*}P < 0.05$ vs. control = 1.84 ± 0.66), visuospatial executive function to 3.08 ± 1.51 (61.6% of full score 5, $^{*}P < 0.05$ vs. control = 4.33 ± 0.81), attention to 4.58 ± 1.51 (76.3% of full score 6, P = ns vs. control = 5.11 ± 0.96), abstraction to 1.58 ± 0.67 (79.0% of full score 2, P = ns vs. control = 1.85 ± 0.39), naming to 2.67 ± 0.65 (89.0% of full score 3, P = ns vs. control = 2.86 ± 0.38), and orientation to 6.00 ± 0.00 (100.0% of full score 6, P = ns vs. control = 5.97 ± 0.18), respectively (Fig. 5, right panel). The five-word recall test in MoCA took 30–40 s in the control subjects and < 30 s in the Asidan patients.

Discussion

The present study first showed that the patients with intronic hexanucleotide GGCCTG expansion with NOP56 mutation (Asidan) demonstrated normal cognitive scores measured by standard screening cognitive tests (MMSE and HDS-R) and CDR, but that their frontal lobe function detected with FAB and MoCA demonstrated a significant decline compared with age-, gender-, and education period-matched controls (Table 1, Fig. 3–5). CDR was insensitive to this type of cognitive impairment (Table 1). Important contributions of cerebellar activity for normal cognitive function have been pointed out by many reports [40–45], and in fact, cognitive reductions have also been reported in

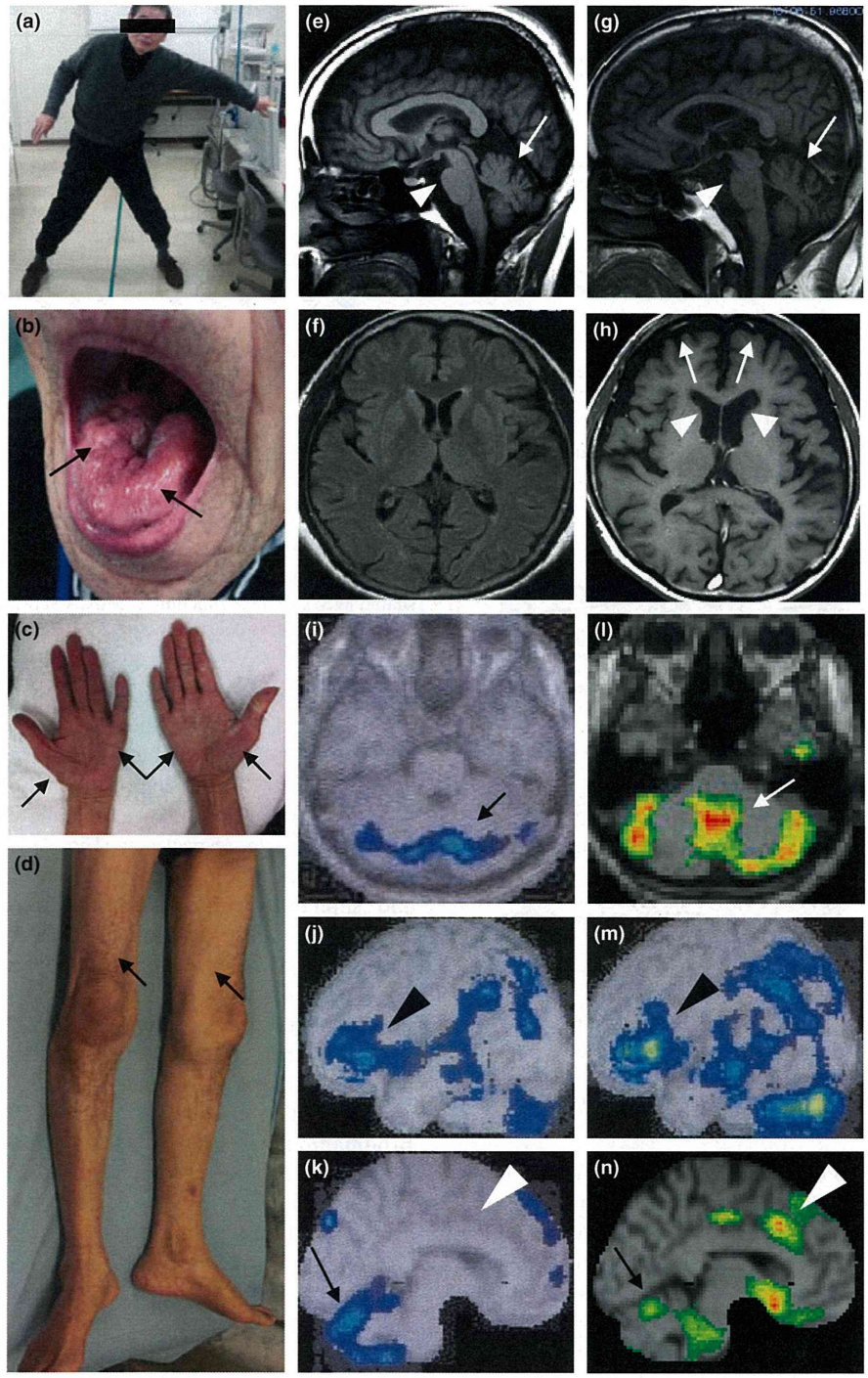

Figure 2 (a–d) Typical physical pictures of Asidan patients with a strong standing/walking disability (a), tongue atrophy with fasciculation (b, arrows), thenar and hypothenar atrophies (c, arrows), and leg atrophy with fasciculation (arrows). Magnetic resonance imaging MRI images with an early stage patient (e, f) and an advanced stage patient (g, h). Note cerebellar atrophies (e and g, arrows) without brain stem atrophy (e and g, arrowheads), and frontal lobe atrophy in the advanced stage (h, arrows) with corresponding anterior horn dilatation (h, arrowheads). Examples of eZIS analysis with ^{99m}Tc-ECD-SPECT in an early stage patient (i–k) and an advanced stage patient (l-m) show rCBF reduction in cerebellar vermis and hemisphere from the early (i and k, arrows) to advanced stage (l and n, arrows) and also show the progressive rCBF decline in Brodmann areas 44–46 (j and m, arrowheads) with decline in Brodmann area 24 only at the advanced stage (k and n, arrowheads).

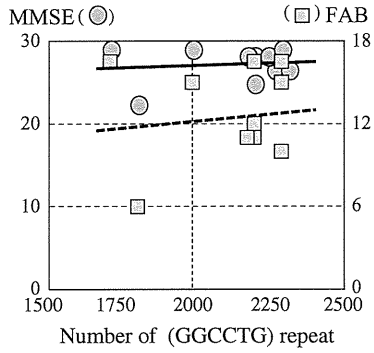

Figure 3 Plots of the number of GGCCTG repeat expansion and the scores of mini-mental state examination (MMSE) (dark circles and solid line) and FAB (dark squares and dotted line). No correlation between number of GGCCTG repeat expansion and MMSE or FAB cognitive score.

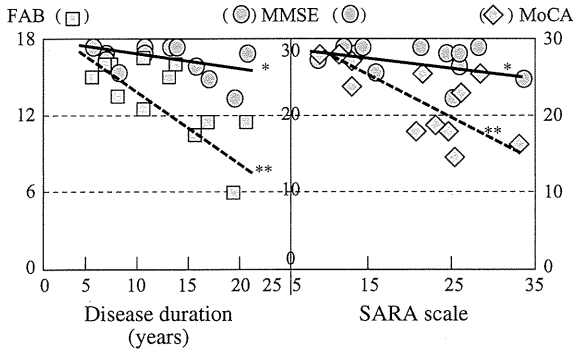

Figure 4 Slight correlations between MMSE and the disease course (left panel, dark circles and solid line, $r = -0.43287$, $^{*}P < 0.05$)) and scale for the assessment and rating of ataxias (SARA) score (right, dark circles and solid line, $r = -0.39144$, $^{*}P < 0.05$), and stronger correlations between FAB and the disease course (left, dark squares and dotted line, $r = -0.76475$, $^{**}P < 0.01$) and between MoCA and SARA score (right, dark diamonds and dotted line, $r = -0.69269$, $^{**}P < 0.01$).

SCA1, 2, 3, and 6 especially in executive frontal function [6,46,47]. A recent report found a decline of frontal attentional and executive functions with mild depressive mood in 4 types of hereditary SCA with a severer reduction in SCA1 > SCA2 > SCA3 > SCA6 in this order [48]. Recent studies suggested a more impact of brain stem (SCA 1–3) on attentional/executive declines and depressive mood than simple cerebellar dysfunction (SCA6) [47,48]. Those studies were mainly performed with hereditary SCA with exonic CAG repeat expansion, but not intronic hexanucleotide expansion such as Asidan.

Although MMSE and FAB scores of the present Asidan patients were not correlated with the numbers of GGCCTG hexanucleotide repeat expansion (Fig. 3),

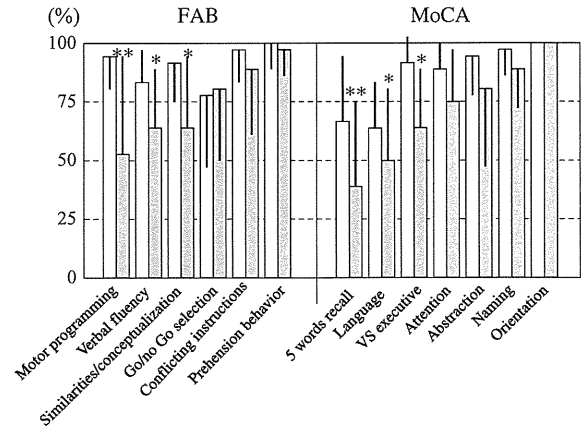

Figure 5 Each six subcategories in FAB (left panel) and seven in MoCA (right panel) of the control (white bars) and Asidan patients (dark bars). Data are expressed as % mean ± SD of the respective full score. Note strong impairments in motor programming, verbal fluency, and similarities/conceptualization as FAB subcategory and in five-word recall, language, and visuospatial (VS) executive function as MoCA subcategory. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs control.

MMSE were slightly correlated with the disease duration and SARA scale (Fig. 4) with age of an alternative covariate (Table 1, case #11, MMSE 22 at age 77). Their frontal lobe dysfunction in FAB and MoCA were well correlated with the disease duration and SARA scale, respectively (Fig. 4). Amongst 6 FAB subcategories, motor programming, verbal fluency, and similarities/conceptualization were decreased to 52.7–61.0% of the respective full score (Fig. 5, left), and five-word recall, language, and visuospatial executive function were decreased to 38.4–61.6% of the respective full score amongst 7 MoCA subcategories (Fig. 5, right). These declines in the frontal lobe function were correlated with the frontal lobe atrophy and respective decline of their rCBF especially in Brodmann areas 24 and 44–46 (Table 1, Fig. 2f–n). These results suggest that the reduction of frontal executive function may be a common cognitive characteristic between exonic CAG repeat expensions (SCA 1, 2, 3, and 6) and intronic hexanucleotide expansion such as Asidan. Because Abrahams *et al.* ([49,50]) indicated an importance of motor control for evaluating verbal fluency in PET activation study, a motor control is preferable when there is any evidence of motor impairment. In the present experiment, the Asidan patients finished verbal fluency test in FAB and five-word recall test in MoCA within 30–35 s with no more words coming out until 1 min of examination, which suggests an actual disturbance of verbal fluency and may partly excuse the lack of motor control in this non-activation study.

On the other hand, affective characteristics of these hereditary SCA have not been fully described. Compared with age-, gender-, and education period-matched controls, GDS score in Asidan patients was worse to 7.3 ± 4.3 (Table 1). We have previously reported a depressive state of SCA1 and 3 patients and their family members detected by self-rating depression scale (SDS, [51,52]) in an occasion of genetic testing [53]. A recent report confirmed such a mild depressive mood in exonic CAG expansion of hereditary SCAs (mainly SCA1, 2, and 3) [48]. Unlike another type of pure cerebellar hereditary ataxia SCA6, the moderate increase in GDS to 48.7% of full score suggests a subclinical involvement of the brain stem (such as Raphe nucleus) in these Asidan patients (Table 1). Our present study also first showed that a slight decrease of VI and a significant increase of AS than the control (Table 1), suggesting a slight unwillingness (apathy) in these Asidan patients. Because such VI and AS were not examined in the past for hereditary SCA, a possible presence of this apathy in other types of hereditary SCAs may be a future subject to be studied.

In addition to pure cerebellar ataxia, Asidan patients also develop typical lower/upper motor neuron involvement clinically identical with ALS. Thus, a comparison of the cognitive and affective functions of Asidan with MND/ALS is also important. Subtle executive deficits are found in a large proportion of ALS, whilst only a small proportion shows fronto-temporal dementia (FTD). Although ALS patients developed affective and behavioral changes [17,18,25,54], they showed only a slight intelligent decline detected by Raven's Coloured Matrices test [55]. Emotional lability in ALS may be related to glutamatergic and serotoninergic neurotransmitter systems [56], and Taylor *et al.* [57] recently established that the prevalence of depression in ALS is not lower than that of patients with other motor disorders. Hereditary ALS revealed reductions of verbal fluency and executive function and high AS [58]. Previous reports showed reductions of rCBF in the fronto-temporal, fronto-parietal, and prefrontal cerebral cortex of ALS [59,60] or no such reductions [49]. Of interest was a subtle rCBF decrease in the anterior cingulate gyrus of ALS without dementia with 3D-SSP [60] similar to our result (Fig. 2n, arrowhead).

Previous studies suggested an active participation of cerebellum both in cognitive and affective functions independent from its dedicated motor control [50,61,62]. PET activation study showed rCBF reduction in areas 4, 8, 9, 10, and 46 [49]. Our Asidan cases did not show general decreases in the fronto-parieto-temporal cerebral cortices, but showed an evident decrease in the particular cerebral cortical areas of Brodmann areas 24 (ventral frontal cingulate gyrus) and 44–46 (pars opercularis of inferior frontal gyrus, pars triangularis of inferior frontal gyrus, dorsolateral prefrontal cortex) (Fig. 2j–n, arrowheads), which suggest that these decreases of rCBF are not simply related to ALS pathology but a unique finding in this Asidan mutation. There are number of limitations in the present study. Several of the FAB and MoCA subscores rely on motor coordination, which may be impaired by the patients' cerebellar deterioration. Therefore, we must be cautious in attributing such changes to purely frontal lobe function, even if there are some subtle frontal neuroimaging changes. We would also conduct future studies either to use tests that control all forms of motor impairment or to perform functional neuroimaging tests such as fMRI simultaneously with neuropsychological testing.

In summary, the present study described cognitive and affective impairments of a novel hereditary cerebellar ataxia with motor neuron involvement (Asidan) in relation to brain imagings. The decline of frontal executive function was related to their disease duration and SARA scale. They also demonstrated mild depression and apathy. These data were not reported before in other hereditary SCAs with exonal CAG expansions or ALS.

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