C-Peptide Replacement Therapy and Sensory Nerve Function in Type 1 Diabetic Neuropathy

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OBJECTIVE — C-peptide replacement in animals results in amelioration of diabetes-induced functional and structural abnormalities in peripheral nerves. The present study was undertaken to examine whether C-peptide administration to patients with type 1 diabetes and peripheral neuropathy improves sensory nerve function.

RESEARCH DESIGN AND METHODS — This was an exploratory, double-blinded, randomized, and placebo-controlled study with three study groups that was carried out at five centers in Sweden. C-peptide was given as a replacement dose (1.5 mg/day, divided into four subcutaneous doses) or a dose three times higher (4.5 mg/day) during 6 months. Neurological examination and neurophysiological measurements were performed before and after 6 months of treatment with C-peptide or placebo.

RESULTS — The age of the 139 patients who completed the protocol was 44.2 ± 0.6 (mean ± SE) years and their duration of diabetes was 30.6 ± 0.8 years. Clinical neurological impairment (NIA) (score >7 points) of the lower extremities was present in 86% of the patients at baseline. Sensory nerve conduction velocity (SCV) was 2.6 ± 0.08 SD below body height–corrected normal values at baseline and improved similarly within the two C-peptide groups ($P < 0.007$). The number of patients responding with a SCV peak potential improvement >1.0 m/s was greater in C-peptide–treated patients than in those receiving placebo ($P < 0.03$). In the least severely affected patients (SCV < 2.5 SD below normal at baseline, $n = 70$) SCV improved by 1.0 m/s ($P < 0.014$ vs. placebo). NIA score and vibration perception both improved within the C-peptide–treated groups ($P < 0.011$ and $P < 0.002$). A1C levels (7.6 ± 0.1% at baseline) decreased slightly but similarly in C-peptide– and placebo-treated patients during the study.

CONCLUSIONS — C-peptide treatment for 6 months improves sensory nerve function in early-stage type 1 diabetic neuropathy.

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Chronic hyperglycemia is a common feature of both type 1 and type 2 diabetes and an important factor for the development of microvascular complications. However, the functional and structural features of the complications for the two disorders show characteristic differences. Specifically, neuropathy in type 1 diabetes progresses more rapidly and shows a more marked decline of nerve conduction velocity than neuropathy in type 2 diabetes (1–4). The basis for the fall in conduction velocity are reduced endoneurial blood flow and diminished Na^+,K^+-ATPase activity in the nerve (5–8), causing sodium ion accumulation, axonal swelling, and, subsequently, a disruption of the paranodal-axoglial junctions and the paranodal ion-channel barrier (9,10). This phenomenon, termed axoglial dysjunction, is a characteristic finding in type 1 diabetes but occurs rarely or not at all in type 2 diabetes (1). Moreover, the functional and morphometric abnormalities of nociceptive C-fibers are more severe in type 1 diabetes (11). These considerations suggest that other factors in addition to hyperglycemia contribute to the pathogenesis of neuropathy in type 1 diabetes. In this context, it is noted that proinsulin C-peptide is lacking in type 1 but not in type 2 diabetes and that C-peptide is now reported to be a bioactive peptide with physiological effects of potential importance for cellular functions related to the development of diabetes complications.

C-peptide replacement in animal models of type 1 diabetes is accompanied by improved nerve function and amelioration of diabetes-induced morphological changes (12,13). Specifically, C-peptide treatment results in decreased paranodal swelling and demyelination, decreased axonal degeneration, reduced frequency of axoglial dysjunction, and augmented regenerative activity (13). Accompanying these beneficial effects of C-peptide is a partial restoration of the diabetes-induced reduction in Na^+,K^+-ATPase activity of the nerves (13,14). Moreover, C-peptide is known to have a stimulatory effect on endothelial nitric oxide synthase (15,16), thereby augmenting endoneurial blood flow (7,17). C-peptide also exerts neurotrophic effects and has an inhibitory effect on cellular apoptosis (for review, see ref. 18). Little information is available regarding the effect of C-peptide on nerve function in patients with type 1 diabetes, but after 3 months of C-peptide replacement in patients with subclinical neurop-

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Abbreviations: CMAP, compound muscle action potential amplitude; MCV, motor nerve conduction velocity; NIA, neuropathy impairment assessment; QST, quantitative sensory testing; SCV, sensory nerve conduction velocity; SCVi, sensory nerve conduction velocity measured at initial potential deflection; SCVp, sensory nerve conduction velocity measured at peak potential; VPT, vibration perception threshold.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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athy, sensory nerve conduction velocity was substantially improved by 2.7 m/s (19). C-peptide administration is also reported to result in improvement of autonomic nerve function in type 1 diabetes (20).

The aim of the present study was to examine whether C-peptide exerts a beneficial effect on peripheral nerve functional abnormalities in patients with type 1 diabetes and established peripheral neuropathy. Specifically, the effect of 6 months of C-peptide treatment on sensory nerve conduction velocity and other early signs of diabetic neuropathy in the lower extremities was investigated.

RESEARCH DESIGN AND METHODS

— In total, 526 patients with type 1 diabetes were screened for the following inclusion criteria: age between 18 and 55 years, diabetes duration >5 years, BMI <30 kg/m^2, A1C <12%, serum creatinine <120 μmol/l, and plasma C-peptide <0.15 nmol/l. Furthermore, the patients should have signs and/or symptoms of diabetic peripheral polyneuropathy, with reduced sensory nerve conduction velocity (SCV) in the sural nerves (<−1.5 SD from a body height–corrected reference value; see below) but with detectable action potentials in both sural nerves. The presence of diabetic neuropathy was established according to the San Antonio Conference criteria (21); i.e., a patient had at least two of the following four findings: *1*) clinical signs of polyneuropathy, *2*) symptoms of nerve dysfunction, *3*) nerve conduction deficits in at least two nerves, or *4*) quantitative sensory deficits. As for exclusion criteria, the patients were not receiving any treatment that might influence nerve function, e.g., cytotoxins or tricyclic antidepressive or antiepileptic agents, nor were they treated with Ca^{2+} channel blockers. Moreover, the patients did not have neuropathy for any reason other than diabetes and did not have a history of drug or alcohol abuse nor had they received a transplant (e.g., islets, kidney, or pancreas).

All patients were informed of the nature, purpose, and possible risks of the study before consenting to participate. The protocol was approved by the institutional human ethics committee of the Karolinska Institute and of the investigational sites and by the Swedish Medical Product Agency. The study was conducted in accordance with good clinical practice guidelines and the principles of the Declaration of Helsinki.

The study was carried out in a double-blinded, placebo-controlled, randomized fashion with three study arms: treatment with C-peptide low dose (1.5 mg/day corresponding to physiological replacement), C-peptide high dose (4.5 mg/day), or placebo (diluent) for 6 months. After giving their informed consent, the patients underwent physical examination, including an electrocardiogram, measurement of blood pressure, and clinical chemistry laboratory testing. In addition, samples for measurement of C-peptide plasma concentration were collected. Furthermore, neurological and neurophysiological examinations were carried out, as described below. In patients who fulfilled the inclusion criteria, neurophysiological evaluation and quantitative sensory testing (QST) were repeated on another day (2–14 days after the first assessment), and the mean of the two assessments was used as baseline value. Thereafter, the patients were randomly assigned to one of the three study groups and instructed to take the trial medication four times daily as subcutaneous injections of 20% of the daily study medication dose in conjunction with their regular insulin administration in the morning, at lunch, and at dinner and 40% of the daily dose at bedtime for a total of 6 months. Human C-peptide was produced recombinantly by Creative Peptides (Stockholm, Sweden). Every 6 weeks, the patients met with the study nurse for review of drug compliance and for safety assessments. After 6 months of treatment, assessments of neurophysiological variables and QST were repeated and performed in duplicate (on separate days 2–14 days apart), and a neurological evaluation was performed. Thereafter, the study was ended, and the trial medication was discontinued. Patients' compliance was checked by review of the patients' diaries and visual control of the returned medication vials. As an additional check, C-peptide concentrations in plasma samples taken after 3 months of treatment and at the end of the study were reviewed (evaluation performed after database closure).

Randomization and blinding of the trial medication were performed by the Karolinska Hospital Pharmacy, and source data verification was monitored by an external monitor (PharmAid, Stockholm, Sweden). The study was carried out at five centers in Sweden: Karolinska University Hospital at Huddinge and Solna, Lundby Hospital (neurophysiological assessments at the Sahlgrenska Hospital) in Gothenburg, Linköping University Hospital in Linköping, and Uppsala University Hospital in Uppsala.

Sensory function and neurophysiological assessments

Sensory nerve conduction properties were measured in the sural nerves bilaterally with regard to SCV and action potential amplitude. SCV was calculated both from the peak of the potential (SCVp), representing an average conduction velocity in the myelinated axons and from the initial potential (SCVi), representing the fastest conducting axons in the nerve. Motor nerve conduction velocity (MCV) and compound muscle action potential amplitude were measured bilaterally in the peroneal nerve. Surface electrodes and digital equipment were used for stimulation and recording (Keypoint; Dantec Medical, Skovlunde, Denmark). The assessments were performed under strictly standardized conditions in a warm room, with the legs warmed with heat pads for at least 10 min before the nerve conduction measurements to obtain skin temperatures >32°C. The reproducibility, measured as coefficient of variation for the SCV and MCV measurements, was 3 and 2%, respectively. QST was carried out bilaterally according to standardized procedures. A vibrating probe (Vibrameter; Somedic, Stockholm, Sweden, or Medoc Advanced Medical Systems, Ramat-Yishai, Israel) was applied over the first metatarsal and over the tibia (~10 cm below the knee) for evaluations of the vibration perception thresholds (VPTs). Heat and cold temperature thresholds were determined using the Marstock technique with a temperature-regulated probe (Thermotest; Somedic or Medoc Advanced Medical Systems) (22) starting at 32°C and automatically changed by a rate of 1°C/s. The probe was applied over the dorsum of the feet and over the tibial area. All measurements of sensation were estimated three times, and the mean was calculated. The interday reproducibility for VPT was 22% and the corresponding values for the heat and cold temperature thresholds were 15 and 21%, respectively.

Neurological examination and symptom assessment

The examination followed a fixed protocol and included sensory screening for touch, pinprick, vibration, and temperature, assessed on the big toes, and on the dorsum of the feet and the tibial regions.

The examination also included reflex testing at two levels and joint proprioception for the big toes. The different responses were graded as normal, decreased, or absent (0, 1, or 2 points, respectively) and a sum >7 points was considered a pathological finding. The presence of symptoms (numbness, allodynia, paresthesia, and pain) in the lower and upper extremities was recorded.

Analyses

Clinical chemistry variables, including A1C (Swedish Mono S method; upper reference value <5.3%), were determined according to standard procedures. C-peptide plasma levels were measured centrally by the Department of Clinical Chemistry, Karolinska University Hospital, Solna, Sweden, using a time-resolved fluoroimmunoassay (AutoDelfia; Wallac Oy, Turku, Finland).

Statistical methods

All data are presented as means ± SE. Nerve conduction data are presented both in absolute terms and as *z*-scores corrected for body height, to allow comparison of individual data from different patients. The *z*-scores were calculated as the observed value minus the mean of the reference value divided by its SD. QST data are presented as *z*-scores corrected for age. The reference values were estimated from linear regression analysis of data in a cohort of 63 healthy subjects (27 men and 36 women, 22–55 years of age, body height 150–196 cm). The Wilcoxon signed-rank test was used to compare baseline data and changes between and within groups (unless otherwise stated). As defined in the statistical analytical plan, the data were analyzed on a per protocol basis, i.e., including only those subjects who completed the protocol and did not show major protocol violations. The decision whether a protocol deviation was to be considered a minor or major deviation was made by a panel including the trial manager and the investigators before unblinding of the randomization code. The safety analysis dataset included all subjects who received at least one dose of C-peptide.

The predetermined primary analysis was to evaluate the change in SCV from baseline to 6 months in the per protocol patient population and to compare the effect of placebo with that of C-peptide, i.e., the low- and the high-dose groups combined. Power analysis was performed on the basis of previous published results (19), reporting a significant improvement (+2.7 m/s) in sensory nerve conduction velocity after 3 months of C-peptide replacement in type 1 diabetes patients with subclinical neuropathy and a common SD for the two groups (active and placebo) of 4.07 m/s. It was estimated that ~30 patients were required to discriminate (80% power and $P < 0.05$) between the active and the placebo patients.

RESULTS — One hundred sixty-one type 1 diabetic patients met the criteria for participation and were enrolled into the study. Of these, 17 ended their participation prematurely (2 in the low-dose group, 10 in the high-dose group, and 5 in the placebo group), 3 additional patients were considered as major protocol violators (not receiving an adequate dose), and 2 were screening failures. The results from these 22 patients were excluded from further analysis, except for safety evaluation, leaving 139 patients in the final dataset. The premature terminations were considered unrelated to the study medication. The following reasons were given: family reasons, heavy professional workload, objection to the extra injections, unstable blood glucose, increased incidence of hypoglycemic events, and pregnancy; one patient had a relapse of rheumatoid arthritis and declined to continue.

The characteristics of the 139 patients (61 women and 78 men) in the different study groups who completed the study showed no statistically significant differences for any of the baseline variables. Patients were, on average, 44.2 ± 0.6 years of age and had a diabetes duration of 30.6 ± 0.8 years. Their body height was 174.1 ± 0.9 cm, and BMI was 25.0 ± 0.2 kg/m^2. Regarding known microvascular complications, 54% of the patients reported having signs or symptoms of peripheral neuropathy, 45% had simplex retinopathy, and 41% had proliferative retinopathy, whereas only 13% reported microalbuminuria and 2% reported proteinuria. The patients' average insulin dose was 0.64 ± 0.01 IU · kg^{-1} · 24 h^{-1} and the level of glycemic control, as reflected by the A1C, was similar in the three groups, with an average A1C of 7.6 ± 0.1%. During and after the 6 months of treatment, there were no significant differences in A1C between the groups. However, A1C decreased 0.21 ± 0.09 ($P < 0.01$) and 0.03 ± 0.12% (NS) within the C-peptide low- and high-dose groups, respectively, and 0.42 ± 0.10% ($P < 0.001$) in the placebo group. The C-peptide plasma concentration at baseline was 0.02 ± 0.00 nmol/l, and the levels were measured again on two occasions, after 3 and 6 months of treatment. These results confirmed exposure to C-peptide in the patients in the C-peptide low- and high-dose groups. During the study there were no adverse drug reactions or adverse events that could be related to the trial medication nor were there any significant changes in safety variables (blood chemistry and vital signs).

The baseline SCV in the sural nerve in the diabetic patients was significantly reduced compared with normal (Table 1); SCVp was, on average, 2.6 ± 0.08 SD below normal, SCVi was 3.2 ± 0.08 SD below normal, and action potential was 4.6 ± 0.29 μV. Similarly, the MCV in the peroneal nerve was also significantly reduced in the patients (−2.9 ± 0.10 SD) (Table 1). There was no significant difference between the three treatment groups at baseline. QST revealed more markedly elevated thresholds in the feet than in the lower legs, especially to vibration (Table 1) and cold stimulation (cold temperature threshold in the feet 3.2 ± 0.19 SD above normal and in the lower legs 2.2 ± 0.15 SD). The corresponding values for heat stimulation were 1.2 ± 0.08 and 0.8 ± 0.09 SD. Pathological neurological findings assessed in the neurological examination (NIA >7 points) were present in 86% of the patients at baseline; the average NIA score was 18.0 ± 0.86 points. Of the randomly assigned patients, 35% reported subjective symptoms from the lower limbs at baseline, and six of these patients reported having symptoms including sensation of pain.

There was no statistically significant difference between the responses in the C-peptide low- and high-dose groups after 6 months (Table 1). Accordingly, the low- and high-dose C-peptide results were combined in the continued analyses. There was significant improvement from baseline for SCVp ($P < 0.007$) and SCVi ($P < 0.001$) in the C-peptide–treated patients, but these changes were not significantly different from those of the placebo group. The number of responders, defined as patients with an improvement in SCVp of >1 m/s (23), was significantly greater in the group receiving active treatment compared with those receiving placebo (37 and 19%; $P < 0.032$; Pearson χ^2 test). With a study duration of no more than 6 months it was anticipated that the

Table 1—*Neurophysiological and neurological results at baseline and change from baseline to 6 months of treatment*

		Change from baseline			
			Active combined	C-peptide	
	Baseline	Placebo		Low dose	High dose
n	139	47	92	53	30
Neurographic variables					
SCVp (m/s)	35.4 ± 0.31	0.24 ± 0.27	0.48 ± 0.19†	0.38 ± 0.27	0.63 ± 0.26*
SCVi (m/s)	44.2 ± 0.41	0.75 ± 0.38	0.93 ± 0.29†	0.77 ± 0.43*	1.14 ± 0.36†
MCV (m/s)	40.0 ± 0.41*	−0.53 ± 0.22*	−0.38 ± 0.15*	−0.25 ± 0.19	−0.57 ± 0.24*
VPT					
z-VPT foot	1.9 ± 0.12	0.03 ± 0.13	−0.09 ± 0.07	−0.07 ± 0.09	−0.12 ± 0.11
z-VPT lower leg	0.6 ± 0.10	−0.09 ± 0.08	−0.19 ± 0.06†	−0.18 ± 0.06*	−0.22 ± 0.13‡
Neurological examination					
NIA score (points)	18.0 ± 0.86	−0.9 ± 0.94	−2.2 ± 0.78†	−1.7 ± 0.97	−2.7 ± 1.31*

Data are means ± SE. Improvements for VPTs and the neurological assessment are indicated as negative differences. *z* indicates that the variable is expressed as a *z*-score, i.e., deviation from the corresponding reference value expressed in SD. *$P < 0.05$, †$P < 0.01$, and ‡$P = 0.057$ for differences within groups compared with baseline.

patients who were least affected at baseline may have a greater potential for improvement. Thus, a subgroup analysis was performed in the half of the patients who showed the least affected nerve conduction velocity at baseline (cutoff equal to the median SCVp, i.e., >−2.5 SD, $n = 70$; this analysis included 21, 31, and 18 patients in the placebo, low-dose, and high-dose groups, respectively, and with demographics similar to those of the entire group). In this group C-peptide administration for 6 months induced an improvement in SCVp of 1.03 m/s greater than that of the placebo group (C-peptide 0.61 ± 0.25 m/s and placebo −0.42 ± 0.29 m/s, $P < 0.014$) (Fig. 1). The corresponding improvement in SCVi in the C-peptide–treated patients was even greater (1.27 ± 0.36, $P < 0.001$) than that in SCVp. Analysis of the number of responders per treatment group in this patient population, i.e., patients with an improvement in SCVp >1 m/s, showed that there were 39% responders in the active group and 5% in the placebo group ($P < 0.004$).

The MCV decreased during the study period (Table 1). After 6 months of C-peptide administration, there were statistically significant improvements for VPT ($P < 0.01$) and NIA score ($P > 0.01$) within the C-peptide–treated group. The placebo group showed no significant changes in VPT or NIA score. No significant changes were observed for temperature perception or symptoms in any of the groups during the study (data not presented).

CONCLUSIONS — The present study shows for the first time in type 1 diabetic patients with established clinical neuropathy that C-peptide administration results in improvement of early neurological abnormalities that accompany type 1 diabetes. After 6 months of administration of C-peptide, there was a significant improvement in SCV, especially in patients whose nerve function was less affected at baseline (1.03 m/s improvement for the C-peptide patients vs. placebo, $P < 0.014$). A1C decreased slightly during the study, but this was most marked in the placebo group. Consequently, the improvements in nerve function after C-peptide were not related to changes in glycemic control. We have previously demonstrated a positive effect of C-peptide on SCV in type 1 diabetic patients with subclinical neuropathy (19). In the present study, the patients' mean duration of diabetes was three times longer, and the patients had more marked nerve dysfunction, probably including not only circulatory and metabolic changes but also varying degrees of structural changes. The difference in magnitude of SCV improvement emphasizes the importance of early intervention (23–25).

The beneficial effect of C-peptide on SCV was accompanied by significant improvements in VPT and in the NIA score within the C-peptide groups, although the latter changes were not statistically significantly different from those in the placebo group. The effect on VPT is well in agreement with the finding in SCV, because vibration perception is mediated primarily by large myelinated fibers such as the sural nerve. The NIA score represents the sum of several nerve qualities, in which large fiber functions represent the greater proportion of the score. Cold and heat perceptions, on the other hand, are

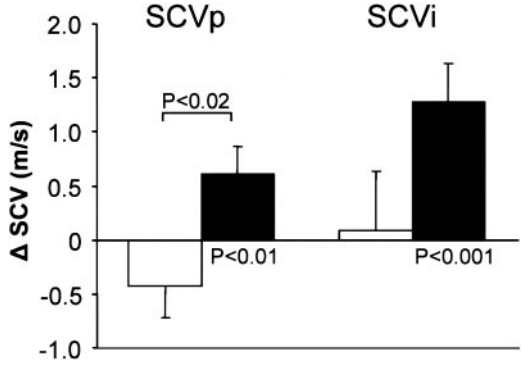

Figure 1— *Change in SCVp and SCVi after 6 months of C-peptide therapy* (n = *49,* ■*) and placebo administration* (n = *21,* □*) in the least affected half of the patients, i.e., those with SCVp >−2.5 SD at baseline. SCVi represents the fastest population of the axons in the nerve, whereas SCVp provides an estimate of the average conduction velocity.* P *values below the columns refers to within-group changes.*

mediated by the small A-δ fibers and C-fibers, respectively. In the present study, there were no detectable effects by C-peptide on small nerve fiber function, and it is conceivable that these fibers respond differently to intervention. This is in contrast to the finding of a beneficial preventive effect on nociceptive function after C-peptide administration in animals (11). Considering the results from QST and neurological evaluation, it should be noted that these variables cannot be measured with the same degree of precision and reproducibility as nerve conduction velocities, which are independent of the patient's participation.

In the present study C-peptide was administered four times daily in two different total doses. The low dose (1.5 mg/day) was calculated to represent a physiological replacement of C-peptide. Measurements of plasma concentrations confirmed expected exposures to C-peptide, although in a slightly lower range than anticipated. This may be related to less than full compliance with the treatment regimen, even though the predetermined criteria for compliance were fulfilled by the patients. The basis for the multiple C-peptide dose regimen used in the present study was to achieve a minimal effective plasma concentration for as many hours of the day as possible. This goal may have been achieved to a greater extent in the high-dose group, which may help to explain the tendency toward slightly, but not significantly, greater improvement in the higher dose group. In fact, the higher dose was not expected to result in a greater effect; the basis for this theory rests in studies of C-peptide binding to cell membranes (26) showing half saturation already at 0.3 nmol/l and full saturation at 0.9 nmol/l in several cellular systems. Thus, raising the concentration above this level would not be expected to elicit any further physiological effects. In fact, dose dependency for C-peptide in the concentration range of 0–1.0 nmol/l has been demonstrated in vitro as well as in vivo in rats and humans (27–29), but with concentrations above the physiological level the responses have not been greater. The findings for the higher dose group thus provide support for the view that C-peptide should be given as a physiological replacement.

As in our previous study (19), there was no improvement in MCV after the 6 months of C-peptide treatment. This lack of improvement may be related to the short study duration in relation to the initial conduction velocity deficit. In fact, lack of improvement in MCV was reported also for another intervention attempt involving 3 months' treatment duration (30), but with more prolonged treatment a tendency for improved MCV was noted (31). The deterioration rate of nerve functional measures is not linear and differs among types of nerves (32). It is conceivable that the different responses in motor and sensory nerves may be related to various sensitivity to factors such as hypoxia, reduced Na^+,K^+-ATPase activity, sorbitol accumulation, and response to growth factors (33).

In summary, C-peptide treatment in replacement doses for 6 months improves sensory nerve function in patients with diabetic neuropathy and mild to moderate nerve conduction abnormalities. The effect was most marked in the patients who had the least amount of disease at the onset of the study, as can be expected for a study that is of short duration at least in the context of diabetic neuropathy. Finally, the study results emphasize the need for early intervention in this disorder.

APPENDIX

In addition to the authors, the C-peptide study group includes the following participating investigators: Sten Andersson, MD, Karolinska University Hospital, Solna, Stockholm, Sweden; Mikael Elam, MD, PhD, Department of Neurophysiology, Sahlgrenska Hospital, Gothenburg, Sweden; Eva Svanborg, MD, PhD, and Nicola Reiser, MD, Department of Neurophysiology, Linköping University Hospital, Linköping, Sweden; Anna Sjölin, MD, PhD, Anna Stenborg, MD, and Roland Flink, MD, PhD, Departments of Medicine, Neurology, and Neurophysiology, respectively, Uppsala University Hospital, Uppsala, Sweden; Erik Moberg, MD, PhD, Per Oskarsson, MD, PhD, Tomas Andersson, MD, PhD, Benjamin Ribalta Stanford, MD, Martin Engvall, MD, and Cecilia Bungerfeldt, MD, Departments of Medicine, Neurophysiology, and Neurology, Karolinska University Hospital, Huddinge, Stockholm. In addition, two biostatisticians are included in the group: Björn Jonsson, PhD, and Anders Lindeberg, MSc, Stockholm, Sweden.

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