Nusinersen induces detectable changes in compound motor action potential response in spinal muscular atrophy type 1 patients with severe impairment of motor function

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20 text pages, 1 Figure and 2 Tables

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Abstract

Background: Most long-term affected spinal muscular atrophy (SMA) type 1 patients have severe impairment of motor function and are dependent on mechanical ventilation with tracheostomy. The efficacy and safety of nusinersen in these patients have not been established.

Methods: We retrospectively evaluated the efficacy of intrathecal nusinersen treatment in patients with SMA type 1 who continued treatment for at least 12 months. There were three patients enrolled in our study (3, 4 and 16 years of age) who had severe impairment of gross motor function without head control or the ability to roll over. All three needed mechanical ventilation with tracheostomy and tube feeding. Motor function was assessed using the Children`s Hospital of Philadelphia infant test of neuromuscular disorders (CHOP-INTEND) and the caregivers' evaluations. Concurrently, we examined nerve conduction longitudinally and compared compound motor action potential (CMAP) amplitudes.

Results: All patients continued nusinersen administration without significant adverse events for more than three years. While CHOP-INTEND scores did not remarkably increase, according to the caregivers, all three patients had improved finger or facial muscle movements that enabled them to make their intentions understood. Some

CMAPs before treatment were not identified but became traces after nusinersen administration.

Conclusions: The improvement in motor function that leads to smoother communication could be a basis for continuing nusinersen treatment. Currently available motor function scorings are not efficient for assessing therapeutic interventions in SMA patients with medical care complexity. Longitudinal nerve conduction studies could be an objective indicator.

Keywords:

Spinal muscular atrophy, nusinersen, severe impairment of motor function, nerve conduction study, compound motor action potential.

1. Introduction

Spinal muscular atrophy (SMA) is a neuromuscular disease caused by degeneration of motor neurons and the major etiology is a deficiency of survival motor neuron (SMN) protein due to a bi-allelic loss of function mutation or deletion in the SMN1 gene located in 5q (1). Nusinersen, an antisense oligonucleotide drug, has been approved for SMA patients of all ages in Japan since 2017. The therapeutic target of nusinersen is *SMN2*, a paralogue gene of *SMN1*. Intrathecal nusinersen modifies splicing in exon 7 and increases the production of full-length SMN protein (2), leading to rescue of motor neuron functions. SMA type 1, a severe phenotype with infantile onset, has fewer *SMN2* copies, usually two (3, 4). Most individuals with SMA type 1 either die without any medication, or are dependent on medical care, including mechanical ventilation with tracheostomy by two years of age (5, 6). Although a phase III trial of nusinersen in SMA type 1 patients showed clear improvement in motor milestones, the patients were all under seven months of age (7). A subsequent double-blind randomized controlled study including a broader age of patients also showed the efficacy and safety of nusinersen but did not include patients with a tracheostomy (8). Even referring to the latest systematic review of real-world studies, the efficacy of nusinersen in long-term affected patients, especially with medical complexity, has not

been adequately assessed (9). Therefore, we investigated the impact of nusinersen in long-term survivors of SMA type 1 with severe impairment of motor function and performed longitudinal nerve conduction studies in these patients.

2. Patients/Methods

This is a retrospective study conducted in the Department of Pediatrics at Hokkaido University Hospital. This study was approved by the ethical board of Hokkaido University Hospital (approval no. 023-0166).

2.1. Patients

Inclusion criteria were as follows: i) infantile-onset type 1 SMA; ii) treatment with nusinersen after the age of two years; and iii) nusinersen treatment continued for more than 12 months. Three individuals met the inclusion criteria and were included in our study. The profiles of these patients are summarized in Table 1. Genetic testing demonstrated no copy in *SMN1* and two copies in *SMN2* in all three patients. Nusinersen was started in Patients 1-3 at three, four and 16 years of age, respectively. Gross motor function was severely impaired in all individuals where they could only move their fingers or toes, and face slightly. They also had joint contracture in upper

and lower extremities. Only Patient 3 had scoliosis. Patient 3 could use a communication tool customized to her finger movements. The other two patients expressed their intention only by slight facial changes or simple gestures using fingers, for example, the thumbs-up movement. All patients depended on mechanical ventilation with tracheostomy and tube feeding. Written informed consent concerning this study was obtained from the patients' guardians.

2.2. Methods

The first evaluation time point was before nusinersen administration (before treatment), the second was before the fourth loading dose (two months), and the third was at the latest visit (latest). Motor function was assessed by the Children's Hospital of Philadelphia infant test of neuromuscular disorders (CHOP-INTEND). CHOP-INTEND is used to assess motor function in infant and toddlers with severe neuromuscular diseases such as SMA type 1. The test consists of sixteen items (spontaneous and reflex movements) measuring muscle strength of the neck, trunk, and proximal and distal extremities, and is scored from zero (no response) to four (complete response) for each item. The total core ranges from 0 to 64, with higher scores indicating better motor function. Subtle changes in motor function and general condition, including respiratory_

condition, were assessed mainly by the caregiver's evaluation at a medical interview, supported by video recordings at hospital visits. For nerve conduction studies, we recorded M waves using an electromyogram (Nihon Kohden, Tokyo, Japan). Several laboratory technicians performed the studies according to the same examination protocol used in our institution. No sedatives were used. We compared the maximum amplitude of the compound motor action potentials (CMAPs) of the median and tibial nerves before and after treatment. The CMAP amplitude was measured between the negative and positive peak (peak to peak). When a CMAP was detected but the wave shape was unclear with a significantly low amplitude unable to be measured, it was labelled as a 'trace', as described in a previous study (10)**.**

3. Results

3.1. Changes in motor function

All patients underwent intrathecal nusinersen administration without significant adverse events for more than three years. Patient 2 could move her lower extremities just a little bit after nusinersen administration. The item of spontaneous movement in lower extremity changed from zero to one point, and her total CHOP-INTEND score increased from one to two points. In Patients 1 and 3, the CHOP-INTEND scores

remained at two points, in other words, the items of spontaneous movement in upper and lower extremity were each one point. On the other hand, their caregivers reported subjective improvement in their motor function. All patients could move their fingers widely and quickly. In Patient 1, his thumbs-up sign became more visible resulting in smoother communication (Video content). Patient 3 was able to operate her communication tool more accurately using her fingers more smoothly. In the evaluative item of spontaneous movement in upper extremity of CHOP-INTEND, the score will increase one to two when wrist movement is observed, so improvements of their fingers were not reflected in the CHOP-INTEND score. Patient 1 and 3 could also move facial muscles more vividly, making it easier to make their intentions understood. Because facial muscle movement is not included in the evaluative items of CHOP-INTEND, these improvements were also not reflected in CHOP-INTEND. General condition including respiratory condition was stable in all patients with no change in respirator settings. One patient's caregiver (Patient 3) reported the response that her respiratory condition had improved because the frequency of oxygen desaturation had decreased.

3.2. Changes in nerve conductions studies (CMAP amplitude)

Before treatment most nerve stimulation responses were not detected or very subtle

and unable to be measured. At the two-month time point, some waveforms invisible at baseline became traces. At the latest time point, all waveforms became traces and some waveform amplitudes became measurable, aligning with some of the subjective improvements (Table 2). The longitudinal evolution of nerve conduction throughout nusinersen treatment is shown in Figure 1. The CMAP changes in Patient 1 were the most apparent of the three patients. Patient 2 could slightly move her lower extremities, which may be related to the CMAP of the tibial nerve being traceable. The CMAP changes in Patient 3 were barely apparent throughout treatment despite her subjective improvement.

4. Discussion

Recent studies in SMA type 1 patients of all ages treated with nusinersen have indicated that earlier treatment produces a higher improvement of motor function (11, 12). On the other hand, some patients treated after the first two years show clinically meaningful improvement even if the increase in scores is small (12). In all these previous studies (11)-(13), only small percentages of patients were dependent on mechanical ventilation with tracheostomy at the start of nusinersen treatment (0%, 9.4 % and 19.7%, respectively). In the latest systematic review (9), more than half of

the studies were designed with respiratory support as an endpoint. Therefore, the efficacy and safety of nusinersen in SMA patients with severe impairment of motor function who depend on permanent mechanical ventilation have not been established. When nusinersen is administered to these patients, their condition should be carefully monitored, and the efficacy should be periodically evaluated to determine whether to continue administration. In long-term affected SMA type 1 patients, existing motor function scoring scales, such as CHOP-INTEND, are not sensitive enough to evaluate treatment efficacy. In our patients, even a slight improvement in facial muscles and finger movements led to clear intention. All the caregivers were highly satisfied with the improvement, even if it was slight, which could not have happened in the natural course of the disease. While the significant costs of treatment cannot be ignored, the improvement that can lead to smoother communication would be meaningful and could be the basis for continuing treatment.

CMAP amplitude is a well-established method for tracking neurophysiological progression in SMA (14, 15). Without therapeutic intervention, CMAP amplitude decreases in an age-dependent manner (6). In a clinical trial of nusinersen, 36% of infants who were treated with nusinersen showed an increase in peroneal CMAP amplitude to at least 1 mV (7). Infants who commenced nusinersen treatment during

199 their presymptomatic stage achieved motor milestones consistent with normal
200 development, and concomitantly their CMAP amplitude remained stable (16). A recent
201 CMAP study in Taiwan of new-born SMA screening showed a rapid increment in post-
202 treatment CMAP could predict better treatment outcomes (17). The longitudinal CMAP
203 data in our patients demonstrated initially undetectable waveforms that became traces
204 throughout treatment, aligning with some of their subjective improvement. The patients
205 with shorter disease duration tended to have greater CMAP changes. Although their
206 amplitudes were lower than those treated at early onset or when presymptomatic, and
207 were not enough to recover gross motor function, nusinersen could make an impact on
208 motor nerve system, even in the later phase of the disease.
209 SMN protein is an essential molecular chaperone for the biogenesis of spliceosomal
210 small nuclear ribonucleoproteins, which carry out splicing of pre-mRNA transcripts.
211 SMN protein has also been shown to play important roles in neuronal repair and
212 development in axons and dendrites (18). It is thought that the increase in SMN protein
213 level raises residual function in some way.
214 Followed by nusinersen, risdiplam and *SMN1* gene replacement therapy called
215 onasemnogene abeparvovec have become important treatment options (19, 20). For
216 SMA type 1 patients with a long time from onset, undergoing a lumbar puncture is no

small burden. Although adverse events attributed to lumbar puncture were not frequent and most were temporary (21, 22), the repetitive nature of the procedure might increase incidental adverse events. Risdiplam may replace nusinersen for some patients whose burdens need to be taken into consideration. As with nusinersen, risdiplam should also be evaluated closely to determine whether to continue administration. A longitudinal nerve conduction study, including measuring CMAP amplitude, could be an objective indicator that would help in treatment decisions, including whether to continue the oligonucleotide drug or not.

This study has several limitations. Firstly, SMA is a rare disease and candidates are generally admitted to a single center, leading to our small sample size. Secondly, there is the possibility of variations in the procedure and health conditions for the nerve conduction studies.

In Japan, SMA type 1 patients can receive medical care at home through the universal health insurance system, and some live through adolescence despite severe impairment of motor function (23) and have continued to receive nusinersen treatment (24, 25). A study focusing on eye movements using a pair-matching task was recently reported as one of the objective evaluation methods for treatment efficacy in SMA patients with loss of gross motor function (26). Collaboration with other facilities is

desired and further investigations, including CMAP and other non-invasive and less burdensome methods, are needed to objectively evaluate the efficacy of therapeutic interventions.

Authors' contributions

YU and KK conceived and designed the study. YU, KK, KE, NO, SK, TG, MN, SN, MN, and HS collected the clinical data and performed the treatments. NO collected the laboratory data. YU, NO, KE, and HS analyzed and interpreted the data. YU, KE, HS, and AM prepared the draft manuscript. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

References

1. Lefebvre S, Bürglen L, Reboullet S, Clermont O, Burlet P, Viollet L, et al. Identification and characterization of a spinal muscular atrophy-determining gene. Cell. 1995;80(1):155-65.

2. Corey DR. Nusinersen, an antisense oligonucleotide drug for spinal muscular atrophy. Nat Neurosci. 2017;20(4):497-9.

3. Kaneko K, Arakawa R, Urano M, Aoki R, Saito K. Relationships between long-term observations of motor milestones and genotype analysis results in childhood-onset Japanese spinal muscular atrophy patients. Brain Dev. 2017;39(9):763-73.

4. Calucho M, Bernal S, Alías L, March F, Venceslá A, Rodríguez-Álvarez FJ, et al. Correlation between SMA type and SMN2 copy number revisited: An analysis of 625 unrelated Spanish patients and a compilation of 2834 reported cases. Neuromuscul Disord. 2018;28(3):208-15.

5. Finkel RS, McDermott MP, Kaufmann P, Darras BT, Chung WK, Sproule DM, et al. Observational study of spinal muscular atrophy type I and implications for clinical trials. Neurology. 2014;83(9):810-7.

6. Kolb SJ, Coffey CS, Yankey JW, Krosschell K, Arnold WD, Rutkove SB, et al. Natural history of infantile-onset spinal muscular atrophy. Ann Neurol. 2017;82(6):883-91.

7. Finkel RS, Mercuri E, Darras BT, Connolly AM, Kuntz NL, Kirschner J, et al. Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy. N Engl J Med. 2017;377(18):1723-32.

8. Acsadi G, Crawford TO, Müller-Felber W, Shieh PB, Richardson R, Natarajan N, et al. Safety and efficacy of nusinersen in spinal muscular atrophy: The EMBRACE study. Muscle Nerve. 2021;63(5):668-77.

9. Erdos J, Wild C. Mid- and long-term (at least 12 months) follow-up of patients with

278 spinal muscular atrophy (SMA) treated with nusinersen, onasemnogene abeparvovec, risdiplam
279 or combination therapies: A systematic review of real-world study data. Eur J Paediatr Neurol.
280 2022;39:1-10.
281 10. Imai T, Minami R, Kameda K, Ishikawa Y, Okabe M, Nagaoka M, et al. Attenuated SEPs
282 with no latency shifts in a family with hereditary spastic paraplegia. Pediatr Neurol. 1990;6(1):13-
283 6.
284 11. Aragon-Gawinska K, Seferian AM, Daron A, Gargaun E, Vuillerot C, Cances C, et al.
285 Nusinersen in patients older than 7 months with spinal muscular atrophy type 1: A cohort study.
286 Neurology. 2018;91(14):e1312-e8.
287 12. Pane M, Coratti G, Sansone VA, Messina S, Bruno C, Catteruccia M, et al. Nusinersen
288 in type 1 spinal muscular atrophy: Twelve-month real-world data. Ann Neurol. 2019;86(3):443-
289 51.
290 13. Pechmann A, Langer T, Schorling D, Stein S, Vogt S, Schara U, et al. Evaluation of
291 Children with SMA Type 1 Under Treatment with Nusinersen within the Expanded Access
292 Program in Germany. J Neuromuscul Dis. 2018;5(2):135-43.
293 14. Swoboda KJ, Prior TW, Scott CB, McNaught TP, Wride MC, Reyna SP, et al. Natural
294 history of denervation in SMA: relation to age, SMN2 copy number, and function. Ann Neurol.
295 2005;57(5):704-12.
296 15. Lewelt A, Krosschell KJ, Scott C, Sakonju A, Kissel JT, Crawford TO, et al. Compound
297 muscle action potential and motor function in children with spinal muscular atrophy. Muscle
298 Nerve. 2010;42(5):703-8.
299 16. De Vivo DC, Bertini E, Swoboda KJ, Hwu WL, Crawford TO, Finkel RS, et al.
300 Nusinersen initiated in infants during the presymptomatic stage of spinal muscular atrophy:
301 Interim efficacy and safety results from the Phase 2 NURTURE study. Neuromuscul Disord.

2019;29(11):842-56.

17. Weng WC, Hsu YK, Chang FM, Lin CY, Hwu WL, Lee WT, et al. CMAP changes upon symptom onset and during treatment in spinal muscular atrophy patients: lessons learned from newborn screening. Genet Med. 2021;23(2):415-20.

18. Beattie CE, Kolb SJ. Spinal muscular atrophy: Selective motor neuron loss and global defect in the assembly of ribonucleoproteins. Brain Res. 2018;1693(Pt A):92-7.

19. Baranello G, Darras BT, Day JW, Deconinck N, Klein A, Masson R, et al. Risdiplam in Type 1 Spinal Muscular Atrophy. N Engl J Med. 2021;384(10):915-23.

20. Waldrop MA, Karingada C, Storey MA, Powers B, Iammarino MA, Miller NF, et al. Gene Therapy for Spinal Muscular Atrophy: Safety and Early Outcomes. Pediatrics. 2020;146(3).

21. Haché M, Swoboda KJ, Sethna N, Farrow-Gillespie A, Khandji A, Xia S, et al. Intrathecal Injections in Children With Spinal Muscular Atrophy: Nusinersen Clinical Trial Experience. J Child Neurol. 2016;31(7):899-906.

22. Darras BT, Farrar MA, Mercuri E, Finkel RS, Foster R, Hughes SG, et al. An Integrated Safety Analysis of Infants and Children with Symptomatic Spinal Muscular Atrophy (SMA) Treated with Nusinersen in Seven Clinical Trials. CNS Drugs. 2019;33(9):919-32.

23. Ito M, Yamauchi A, Urano M, Kato T, Matsuo M, Nakashima K, et al. Epidemiological investigation of spinal muscular atrophy in Japan. Brain Dev. 2022;44(1):2-16.

24. Wataya T, Takasaki S, Hoshino M, Makioka H, Nakamura G, Matsuda N. Real-world safety of nusinersen in Japan: results from an interim analysis of a post-marketing surveillance and safety database. Int J Neurosci. 2023;133(8):851-63.

25. Tachibana Y, Takasaki S, Hoshino M, Makioka H, Jin M. Real-world safety and effectiveness of nusinersen, a treatment for spinal muscular atrophy, in 401 Japanese patients:

results from an interim analysis of post-marketing surveillance. Int J Neurosci. 2022, in press. doi: 10.1080/00207454.2022.2095270.

26. Yae Y, Yuge K, Maeda T, Ichinose F, Matsuo M, Kobayashi O, et al. Exploratory evaluation of an eye-tracking system in patients with advanced spinal muscular atrophy type I receiving nusinersen. Front Neurol. 2022;13:918255.

Figure legends

Figure 1. Changes in compound motor action potential (CMAP)

(**A**) CMAPs of median and tibial nerve stimulation in Patient 1 (a three-year-old boy). At baseline, no response to nerve stimulation was recorded (left panels). At two months, the CMAPs became traces (middle panels). At the latest visit (after continuing nusinersen for three years and seven months), the CMAPs of both the median and tibial nerves became clear enough to measure the amplitudes (right panels), in spite of them being much lower than the normal standards. (**B**) In Patient 2 (a four-year-old girl), the CMAP of median nerve stimulation at two months became a trace more clearly than at baseline (left and middle panels) and at the latest visit (after continuing nusinersen for three years and seven months), the CMAP amplitude increased enough to measure (right panel). The responses to tibial nerve stimulation became traces at two months and the latest visit (middle and right panels). (**C**) In Patient 3 (a 16-year-old girl), the CMAP amplitude of median nerve stimulation did not increase with continuing treatment. In tibial nerve stimulation, at baseline and two months (left and middle panels), no response was recorded. At the latest visit (after continuing nusinersen for three years and four months), the CMAP became a trace (right panel).

Video content

Changes of finger movement in Patient 1 (4-year-old boy).

From his guardians we obtained permission to publish this video content. The first half of the video was taken before nusinersen treatment. He could move his fingers weakly. As treatment continued, although the area he could move was limited to his fingers, the motion became wider and quicker. The second half of the video was taken after three years of continuing treatment. He could communicate his intentions with a thumbs-up sign.

Figure1

A

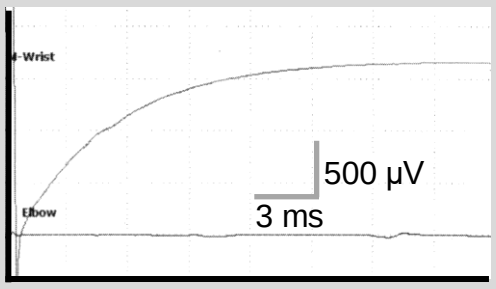
Before treatment

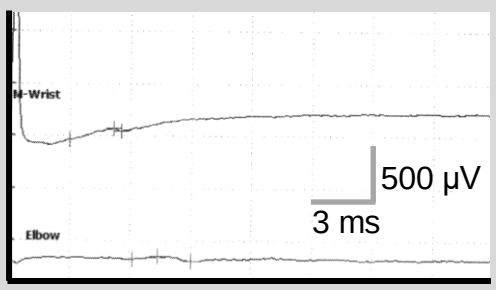
2 months

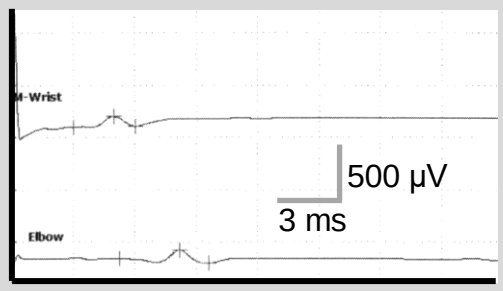
Latest

Case1

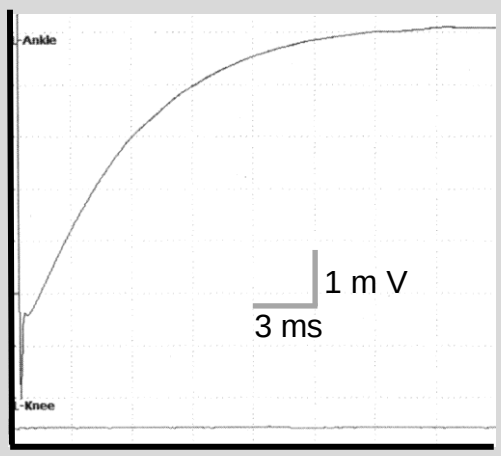
Median

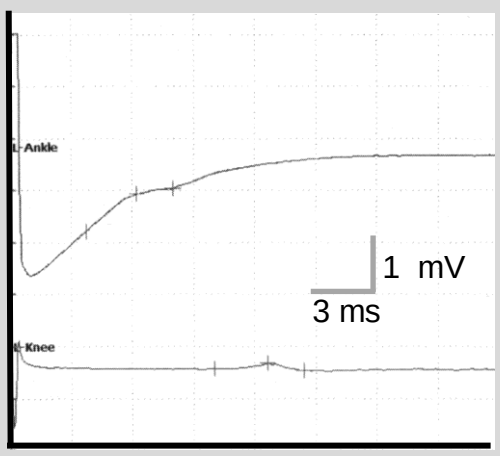


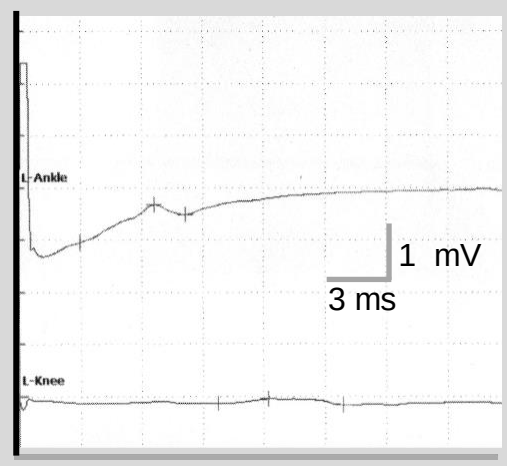




Tibial



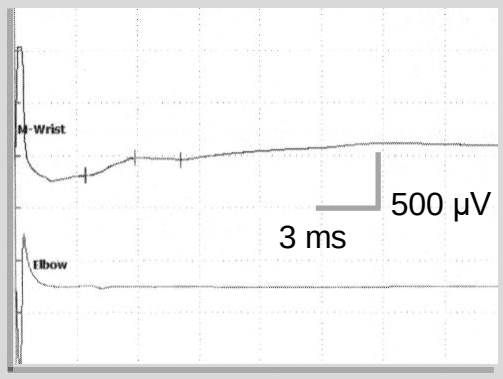


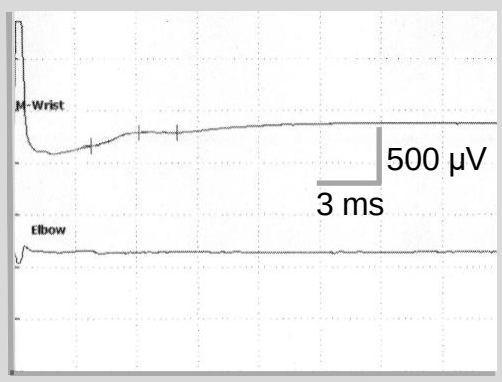


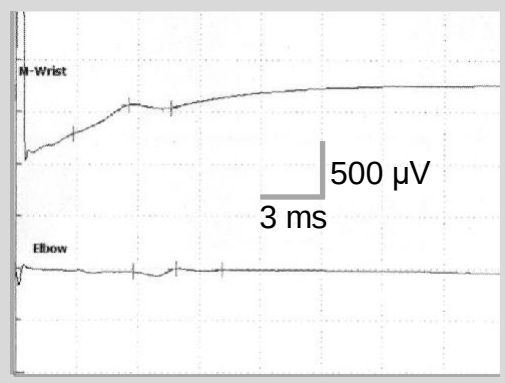
B

Case 2

Median







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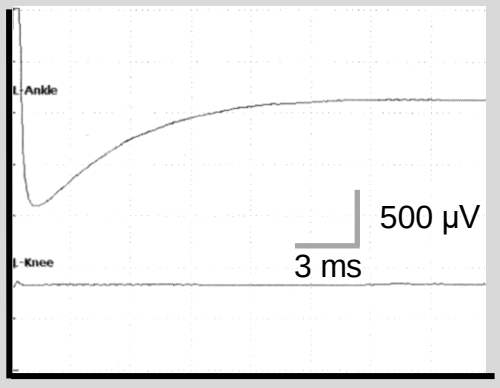
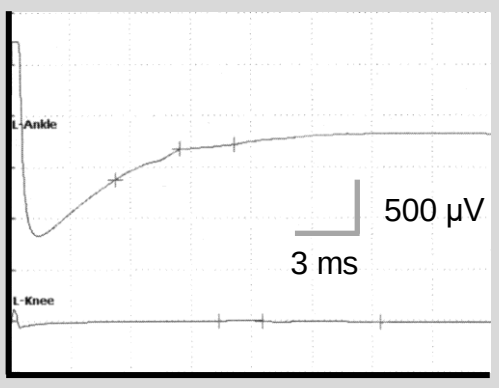
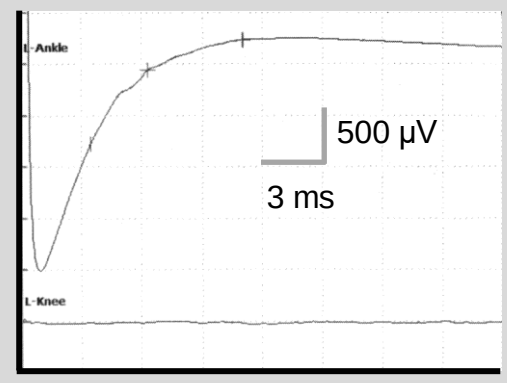


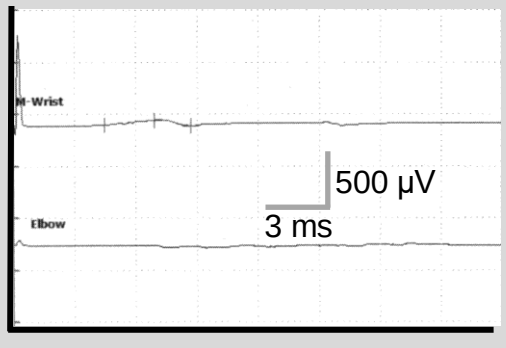


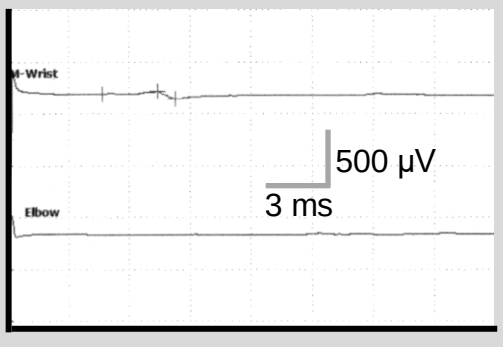


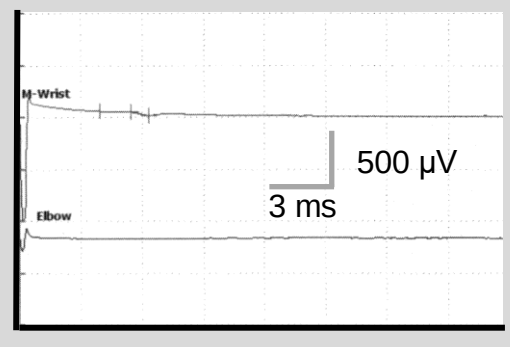
C

Case 3

Median







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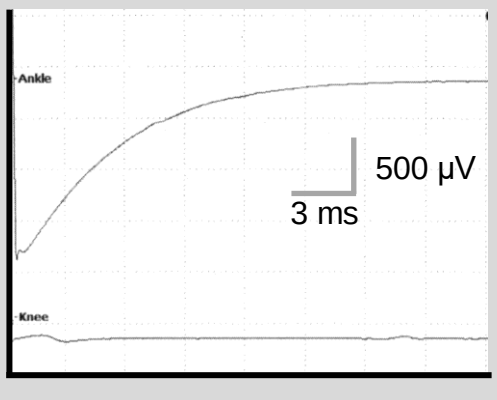

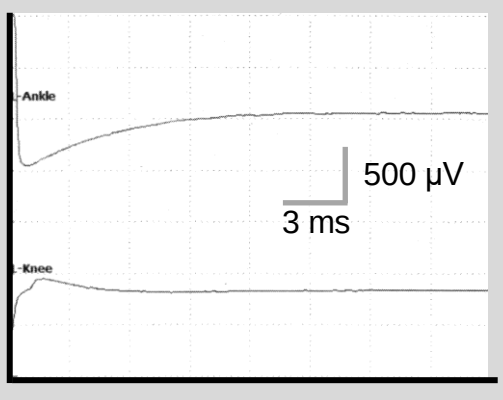

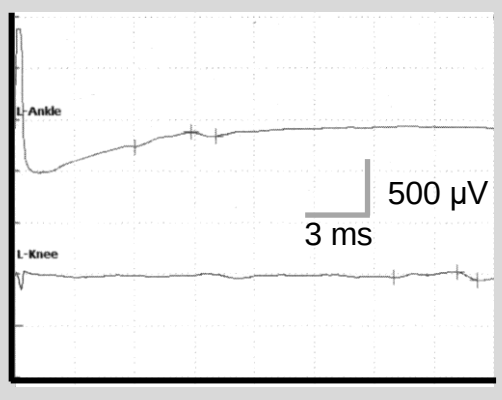

Table1

Table 1. Summary of patient clinical profiles

Patient	1	2	3
Age at the start of treatment	3 y, 4 mo	4 y, 3 mo	16 y, 1 mo
Duration of treatment	3 y and 7 mo	3 y and 7 mo	3 y and 4 mo
SMN2 copy number	2	2	2
Medical care			
Tracheostomy	+	+	+
Mechanical ventilation	+	+	+
Tube feeding	+	+	+
Motor function			
CHOP-INTEND (Before treatment)	2	1	2
CHOP-INTEND (Latest)	2	2	2
Physical findings			
Joint contracture in upper extremities	+	+	+
Joint contracture in lower extremities	+	+	+
Scoliosis	-	-	+

Abbreviations: CHOP-INTEND, Children's Hospital of Philadelphia infant test of neuromuscular disorders

Table2

Table 2. Longitudinal data of CMAP amplitude and subjective improvement

	Before treatment	2 months	Latest
Patient 1			
CMAP - Median nerve (μV)	ND	Trace	90.8
Tibial nerve (μV)	ND	Trace	181.5
Subjective improvement			
Finger		+	+
Foot		-	-
Facial muscle		+	+
Respiratory condition		-	-
Patient 2			
CMAP - Median nerve (μV)	Trace	Trace	26.7
Tibial nerve (μV)	ND	Trace	Trace
Subjective improvement			
Finger		+	+
Foot		+	+
Facial muscle		-	-
Respiratory condition		-	-
Patient 3			
CMAP - Median nerve (μV)	Trace	Trace	Trace
Tibial nerve (μV)	ND	ND	Trace
Subjective improvement			
Finger		+	+
Foot		-	-
Facial muscle		+	+
Respiratory condition		-	+

Abbreviations: CMAP, compound motor action potential; ND, not detected