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ORIGINAL RESEARCH

Neurophysiological Characteristics in Type II and Type III 5q Spinal Muscular Atrophy Patients: Impact of Nusinersen Treatment

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Objective: This study aimed to observe the neurophysiological characteristics of type II and type III 5q spinal muscular atrophy (SMA) patients and the changes in peripheral motor nerve electrophysiology after Nusinersen treatment, as well as the influencing factors.

Methods: This single-center retrospective case–control study collected clinical data and peripheral motor nerve CMAP parameters from 42 5qSMA patients and 42 healthy controls at the Second Affiliated Hospital of Xi'an Jiaotong University (January 2021 to December 2022). It evaluated changes in motor function and CMAP amplitude before and after Nusinersen treatment.

Results: Our investigation encompassed all symptomatic and genetically confirmed SMA patients, consisting of 32 type II and 10 type III cases, with a median age of 57 months (29.5 to 96 months). Comparative analysis with healthy controls revealed substantial reductions in CMAP amplitudes across various nerves in both type II and type III patients. Despite the administration of Nusinersen treatment for 6 or 14 months to the entire cohort, discernible alterations in motor nerve amplitudes were not observed, except for a significant improvement in younger patients (≤36 months) at the 14-month mark. Further scrutiny within the type II subgroup unveiled that individuals with a disease duration ≤12 months experienced a noteworthy upswing in femoral nerve amplitude, a statistically significant difference when compared to those with >12 months of disease duration.

Conclusion: Motor nerve amplitudes were significantly decreased in type II and type III 5q SMA patients compared to healthy controls. Nusinersen treatment showed better improvement in motor nerve amplitudes in younger age groups and those with shorter disease duration, indicating a treatment-time dependence.

Keywords: 5q spinal muscular atrophy, neurophysiology, peripheral motor nerve, Nusinersen

Introduction

Spinal muscular atrophy (SMA) is a rare autosomal recessive genetic disorder. Deletion or mutation of the SMN1 gene on chromosome 5q13.2 has been identified as the most common cause of reduced full-length functional SMN protein, and it is the predominant subtype of SMA, accounting for the majority of cases.[1] SMA is characterized by progressive muscle atrophy and weakness due to the loss of motor neurons in the spinal anterior horn. A large-scale study found that carriers of SMN1 gene deletion have a frequency of 1/54 in the population, with an incidence rate of 1/11,000.[2–4] Based on the age of onset and the timing of milestone motor events, five types (0 to IV) have been established. Prenatal onset type 0 and infantile onset type I are the most severe and associated with increased mortality. Historically, SMA has been a major cause of monogenic infant mortality.[5,6] However, with the widespread use of gene-modifying therapies, this is expected to change significantly.

Previous meta-analyses have found significant differences in compound muscle action potential (CMAP) and motor unit number index (MUNIX) as neurophysiological biomarkers between SMA patients and healthy controls, specifically

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in the distal muscles of the upper limbs.[7] However, the placement of muscle electrodes in pediatric patients may cause pain and psychological fear, and the inability to cooperate in muscle contractions makes MUNIX examination difficult to perform. On the other hand, CMAP measurements are more feasible due to their good repeatability and non-invasive nature, making them more readily accepted by both patients and parents. CMAP has been shown to be strongly correlated with clinical classification, age, and SMN2 gene copy number in SMA.[8] In type I patients, CMAP amplitudes were found to be significantly low (mean 0.340 mV), well below the lower limit of normal (1.80 mV).[9] Additionally, another study reported significantly reduced CMAP amplitudes in 10 type II SMA patients compared to the normal values.[10] Previous studies on SMA patients have primarily focused on single limbs, particularly investigating the median nerve or ulnar nerve. However, there is a lack of research on the CMAP amplitudes of other peripheral motor nerves, such as the sciatic nerve or tibial nerve, despite the clinical characteristic of SMA patients showing a greater involvement of the lower limbs compared to the upper limbs, and a greater severity in the proximal muscles compared to the distal muscles.

Nusinersen is the first approved drug for the treatment of SMA. It is an antisense oligonucleotide that blocks the intronic silencer site from binding to SMN2 pre-mRNA, thereby promoting exon 7 transcription and increasing the quantity of full-length functional SMN protein.[11] The SMArtCARE Registry study found that Nusinersen has a positive effect on the majority of ambulant pediatric and adult SMA patients.[12] Currently, over 12,000 patients worldwide have received treatment with Nusinersen.[13] Previous studies have indicated significant improvements in motor function in SMA children following nusinersen therapy,[14,15] also with therapeutic efficacy observed in patients older than 12.[16] The increase in CMAP is considered to potentially reflect the treatment response.[17] Axente et al revealed a notable increase in CMAP amplitudes after 2 years of nusinersen treatment, showing a significant correlation with the motor function evolution in SMA type 1 patients.[18] Kariyawasam et al also found that nusinersen treatment significantly increased CMAP in patients with SMA.[19] However, there is still limited literature reporting on whether nusinersen can alter peripheral motor nerve electrophysiology, which peripheral motor nerves may be affected, and the factors associated with such changes. The objectives of this study are as follows: 1. To further understand the electrophysiological characteristics of the median nerve, ulnar nerve, axillary nerve, tibial nerve, peroneal nerve, and sciatic nerve in Type II and Type III SMA children compared to healthy controls. 2. To analyze the changes in CMAP amplitudes of the aforementioned motor nerves after Nusinersen treatment and identify influencing factors. This study aims to provide further data support for exploring new biomarkers in SMA patients, assisting physicians in determining the optimal timing for intervention, especially in patients with four or more copies of SMN2. It will also help patients, parents, and physicians in predicting the efficacy of Nusinersen treatment and establishing realistic treatment expectations.

Methods

Patients and Healthy Control Group

A total of 42 patients diagnosed with 5q spinal muscular atrophy (5qSMA) who received treatment at the Pediatric Department of the Second Affiliated Hospital of Xi'an Jiaotong University between January 2021 and December 2022 were enrolled, along with 42 healthy children who visited the Pediatric Outpatient Department for regular check-ups, matched for age and gender. This study was conducted with the approval of the Ethics Committee of the Second Affiliated Hospital of Xi'an Jiaotong University and was performed in accordance with the Helsinki II declaration. Informed consent forms were obtained from all participating researchers and guardians. The 42 patients exhibited varying degrees of clinical symptoms of muscle atrophy and weakness, and their genetic reports confirmed the diagnosis of 5qSMA. Clinical data, including baseline characteristics (age, gender, disease onset, medical history), genetic results, Hammersmith Functional Motor Scale scores, peripheral motor nerve electrophysiology, as well as Hammersmith scores and CMAP data just before treatment (T0) and at 6 months (T6) and 14 months (T14) of treatment, were collected for the patients.

Genetic Testing

Following the genetic testing procedure for 5qSMA patients, the copy numbers of SMN1 and SMN2 genes were initially examined using the MLPA method. If the SMN1 gene showed homozygous deletion, a diagnosis of 5qSMA was made. In cases where the SMN1 gene exhibited heterozygous deletion, nested PCR was subsequently performed to conduct first-generation

sequencing of the SMN1 gene, along with parental verification. If the patient met the criteria for compound heterozygous mutations, a diagnosis of 5qSMA was confirmed."

Patient Selection Criteria

The inclusion criteria for the patient group were as follows: 1. Age ≥24 months; 2. All patients presented with clinical symptoms consistent with 5qSMA and confirmed by genetic reports; 3. All patients received Nusinersen intrathecal treatment as scheduled and in the prescribed dosage; 4. Complete clinical and peripheral nerve electrophysiology data were available; 5. All 5qSMA patients underwent monotherapy with Nusinersen and had not previously received any other gene correction therapy. 6. All patients were treated for at least 6 months; 7. All patients signed informed consent. The exclusion criteria were as follows: 1. Occurrence of severe infections, hepatic or renal failure, fractures, or other significant events during the study period; 2. Patients below 24 months of age; 3. Incomplete clinical data, failure to follow up or receive intrathecal treatment as scheduled; 4. Genetic results not reported or not supportive of a 5qSMA diagnosis; 5. Patients without measurable CMAP amplitude were excluded."

The patients were divided into three subgroups based on the baseline age of 20 type II SMA patients who received 14 months of treatment: ≤36 months, >36 months and ≤60 months, and >60 months. Previous studies on the effect of Nusinersen on motor function scoring have shown different treatment responses between children below 60 months and those above 60 months, thus using 60 months as the cutoff.[20] In order to further observe if younger children have a better response to Nusinersen, we used 36 months as an additional cutoff point. Grouping information are shown in Figure 1.

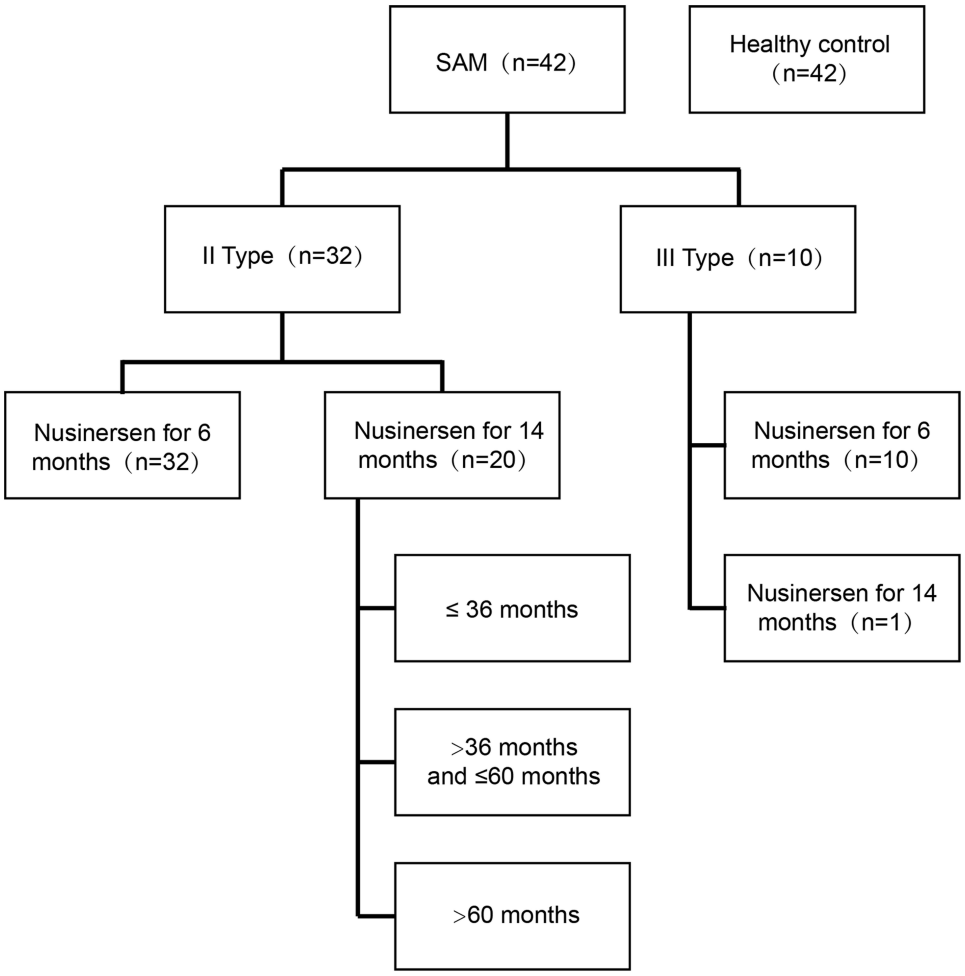

Figure 1 Grouping information. Nusinersen requires lifelong treatment. This study involved a total of 42 patients, all of whom received treatment for at least 6 months, with 21 patients receiving treatment for 14 months.

The healthy control group was recruited from children attending regular health check-ups at our Pediatric Outpatient Department. After matching for gender and age with the observation group, they signed informed consent forms to participate as healthy controls. The inclusion criteria for the healthy control group were as follows: 1. Age 2 years to 18 years or older; 2. Good physical health with no history of illness in the past month; 3. No underlying medical conditions or chronic diseases; 4. Informed consent obtained from the guardian and children aged 8 years or older. Children with chronic illnesses, premature birth, severe acute illnesses, any genetic neurologic disorders, or a family history of such conditions were excluded from the study.

Peripheral Motor Nerve Conduction Testing

Under ambient temperature of 25°C and a skin surface temperature of 31°C to 33°C, the motor conduction function of the ulnar nerve, median nerve, axillary nerve, peroneal nerve, tibial nerve, and femoral nerve was assessed using the HaiShen NDI-096 electromyography apparatus[9,21]. Surface electrodes were used for recording. For the ulnar nerve, median nerve, peroneal nerve, and tibial nerve, two stimulation points were selected, while for the axillary nerve and femoral nerve, one stimulation point was selected. Stimulation was applied at a threshold level of +5 to 10 mA current with a duration of 3 ms to elicit maximum CMAPs. The latency, amplitude, duration, and conduction velocity of the CMAPs were observed. The distal latency was measured from the stimulation point to the onset of the CMAP. Motor nerve conduction velocity (MNCV) was calculated by measuring the distance between the two stimulation points with a ruler and dividing it by the difference in latencies between the two points.

Nusinersen Treatment Protocol

All patients underwent intrathecal treatment with Nusinersen following the internationally accepted method. The loading phase was completed over the first two months, consisting of a total of four intrathecal injections, each with a dose of 12 mg. Subsequently, the maintenance phase followed, with a dose of 12 mg administered every four months. This treatment cycle was continued accordingly.

Hammersmith Functional Motor Scale-Expanded (HFMSE) Assessment

Prior to each intrathecal treatment, all patients underwent the Hammersmith Functional Motor Scale-Expanded assessment.[22] The measurement time points were baseline T0, 6 months of treatment (T6), and 14 months of treatment (T14). The scale ranges from 0 to 66, with higher scores indicating better motor function. These data were collected for statistical analysis.

Statistical Analysis

Descriptive statistics were used to analyze the clinical characteristics and outcomes. Categorical data were presented as numbers (percentages), while continuous variables were expressed as median (P25%, P75%). The non-parametric Kruskal–Wallis test was employed to compare data among the three groups, with the test statistic reported as H value. For comparisons between the two groups, the Mann–Whitney *U*-test was utilized, with the test statistic reported as a U value. All statistical analyses were performed using SPSS 24.0 software. A significance level of $p < 0.05$ was considered statistically significant for all analyses.

Results

Clinical Data of SMA Patients

A total of 42 cases of 5q SMA patients were included in this study. Among them, 24 were male and 18 were female. The median age was 57 months (range: 29.5 to 96 months), with a median onset age of 12.5 months (range: 8.0 to 18 months), and a median age of genetic diagnosis at 23.0 months (range: 14.8 to 36.5 months). In terms of clinical classification, there were 32 cases (76.2%) of Type II and 10 cases (23.8%) of Type III. Among the patients, 21 had spinal curvature. Homozygous mutations were observed in 37 cases (88.1%), and compound heterozygous mutations were observed in 5 cases (11.9%). The SMN2 copy number distribution was as follows: 5 copies in 5 cases (11.9%), 3

copies in 36 cases (85.7%), and 4 copies in 1 case (2.4%). At baseline, 17 cases (40.5%) had a Hammersmith score ≥20, 16 cases (38.1%) had a score ≥10 but <20, and 9 cases (21.4%) had a score <10. All 42 patients received 6 months of treatment, and among them, 20 cases (47.6%) showed an improvement in Hammersmith score ≥3 but <10 compared to baseline, while 6 cases (14.3%) showed an improvement of over 10 points. Among the 21 patients who received 14 months of treatment, 16 cases (80%) showed an improvement in Hammersmith score ≥3 compared to baseline. The above results are shown in Table 1.

Characteristics of CMAP Amplitude, Distal Latency, and Nerve Conduction Velocity in Type II and Type III SMA Patients

Peripheral motor nerve electrophysiology was compared between 10 cases of Type III SMA patients, 32 cases of Type II SMA patients, and age- and gender-matched healthy children. The results revealed that bilateral CMAP amplitudes at the median nerve, ulnar nerve, axillary nerve, peroneal nerve, tibial nerve, and femoral nerve in both Type II and Type III SMA patients were significantly lower than those in the healthy control group. Among them, the amplitudes of the femoral nerve and tibial nerve showed the most significant reduction, with lower amplitudes observed in the lower limbs compared to the upper limbs and in the proximal regions compared to the distal regions. Taking the distal amplitude of the right nerve as an example, the median amplitude of the femoral nerve in Type III patients was 1.08 mV, while in Type II patients it was 0.39 mV, both significantly lower than the healthy control group. The median amplitude of the tibial nerve in Type III patients was 6.57 mV, and in Type II patients it was 2.36 mV, both significantly lower than the healthy control group. There were no significant differences in distal latency and conduction velocity among the median nerve, ulnar nerve, peroneal nerve, and tibial nerve. The distal latency of the axillary nerve and the femoral nerve showed a slight prolongation in SMA patients compared to the control group, but remained within the normal range. As the primary abnormality affecting peripheral motor nerve electrophysiology in SMA is amplitude, subsequent analyses in this study focus mainly on this parameter. The above results are shown in Table 2.

Table 1 Clinical Data of 5q Spinal Muscular Atrophy (5qSMA) Patients

Variable		Results
Male: female		24:18
Age (months)		57.0(29.5,96)
Age of onset (months)		12.5(8.0,18.0)
Duration of disease (months)		39.5(15.75,74.25)
Age at genetic confirmation (months)		23.0(14.8,36.5)
Clinical typing	Type II (n, %)	32(76.2%)
	Type III (n, %)	10(23.8%)
Scoliosis	Yes (n, %)	21(50.0%)
	No (n, %)	21(50.0%)
Mutation type	Pure heterozygous deletion (n, %)	37(88.1%)
	Compound heterozygous mutation (n, %)	5(11.9%)
SMN2 copy number	2 (n, %)	5(11.9%)
	3 (n, %)	36(85.7%)
	4 (n, %)	1(2.4%)
Hammersmith score at baseline (n=42)	≥20 points (n, %)	17(40.5%)
	≥10 points and <20 points (n, %)	16(38.1%)
	<10 points (n, %)	9(21.4%)
Hammersmith score change From baseline to 6m (n=42)	Less than 3 points (n, %)	16(38.1%)
	≥3 points and <10 points (n, %)	20(47.6%)
	≥10 points (n, %)	6(14.3%)
Hammersmith score change From baseline to 14m (n=20)	Less than 3 points (n, %)	4(20%)
	≥3 points and <10 points (n, %)	9(45%)
	≥10 points (n, %)	7(35%)

Table 2 Comparison of Motor Nerve Conduction Velocity in Type II and Type III SMA Patients vs Healthy Control Group

Motor nerve				III Type (n=10)	II Type (n=32)	Control Group (n=42)	H Value	P value
Median nerve	Right	Distal latency (ms)		2.74(2.2,3.0)	2.30(1.9,2.5)	2.50(2.2,2.7)	4.406	0.104
		Distal wave amplitude (mV)		8.39(6.1,9.7)	5.63(3.4,7.8)	11.15(10.8,15.3)	39.827	0.000**
		Proximal wave amplitude (mV)		7.43(6.1,10.2)	4.60(2.8,7.1)	11.80(10.0,13.8)	45.362	0.000**
		Nerve conduction velocity (m/s)		59.00 (53.4,65.4)	56.30 (51.7,61.2)	58.35(55.6,62.5)	2.454	0.293
	Left	Distal latency (ms)		2.50(2.2,2.8)	2.40(2.1,2.8)	2.55(2.3,2.8)	1.847	0.397
		Distal wave amplitude (mV)		7.34(5.4,9.9)	3.83(2.7,7.4)	11.15(9.4,14.3)	41.643	0.000**
		Proximal wave amplitude (mV)		6.78(3.9,8.7)	3.74(1.8,6.3)	11.20(9.6,13.7)	48.582	0.000**
		Nerve conduction velocity (m/s)		59.65 (51.7,65.2)	56.55 (52.0,64.3)	59.70(53.8,64.9)	0.338	0.845
Ulnar nerve	Right	Distal latency (ms)		2.10(1.5,3.0)	2.13(1.8,2.6)	2.20(1.8,2.5)	1.885	0.386
		Distal wave amplitude (mV)		5.47(3.3,7.8)	1.63(1.0,3.7)	8.39(6.6,10.3)	53.772	0.000**
		Proximal wave amplitude (mV)		5.60(3.7,7.6) *	2.08(0.7,3.6)	7.95(6.4,11.2)	52.4	0.000**
		Nerve conduction velocity (m/s)		55.25 (53.4,62.5)	55.80 (53.0,59.7)	57.85(55.9,66.7)	5.575	0.067
	Left	Distal latency (ms)		2.25(1.9,2.8)	2.23(1.8,2.5)	2.20(1.9,2.3)	5.565	0.072
		Distal wave amplitude (mV)		5.37(1.8,6.5)	1.91(0.7,2.9)	7.36(6.8,11.6)	54.915	0.000**
		Proximal wave amplitude (mV)		4.85(2.1,6.2)	1.66(0.7,3.5)	7.55(6.6,10.9)	56.784	0.000**
		Nerve conduction velocity (m/s)		62.70 (53.0,66.5)	57.20 (50.6,61.9)	56.00(53.9,62.3)	2.129	0.345
Axillary nerve	Right	Erb	Latency (ms)	3.47(2.9,4.0)	2.93(2.5,3.2)	2.63(2.6,2.8)	17.595	0.000**
			Amplitude (mV)	7.95(4.6,10.2)	3.54(1.3,5.9)	11.15(10.4,13.9)	46.326	0.000**
	Left	Erb	Latency (ms)	3.15(2.9,4.0)	3.30(2.9,3.6)	2.70(2.4,2.8)	31.698	0.000**
			Amplitude (mV)	5.76(2.6,9.8)*	2.84(1.6,5.1)	13.70(11.5,14.8)	58.086	0.000**
Common peroneal nerve	Right	Distal latency (ms)		2.83(2.2,4.8)	2.35(2.0,3.4)	2.68(2.2,3.4)	1.536	0.464
		Distal wave amplitude (mV)		5.99(2.0,8.7)	2.25(0.7,3.3)	5.89(3.9,7.3)	30.351	0.000**
		Proximal wave amplitude (mV)		5.53(2.0,7.5)	2.22(0.8,3.8)	5.78(4.1,6.8)	31.273	0.000**
		Nerve conduction velocity (m/s)		48.00 (43.9,52.3)	46.75 (43.5,54.0)	50.30(48.0,55.2)	4.673	0.095
	Left	Distal latency (ms)		2.98(2.2,4.7)	2.40(1.9,2.7)	2.50(1.9,2.9)	4.301	0.116
		Distal wave amplitude (mV)		4.77(2.6,5.8)	2.29(1.3,3.8)	5.38(4.3,6.5)	29.265	0.000**
		Proximal wave amplitude (mV)		4.84(2.9,5.7)	2.21(0.8,3.8)	4.93(4.0,6.7)	25.109	0.000**
		Nerve conduction velocity (m/s)		48.50 (44.2,52.3)	47.70 (43.3,52.4)	51.35(47.5,55.4)	4.577	0.106

(*Continued*)

Table 2 (Continued).

Motor nerve			III Type (n=10)	II Type (n=32)	Control Group (n=42)	H Value	P value
Tibial nerve	Right	Distal latency (ms)	3.18(2.7,5.2)	2.83(2.0,3.5)	2.93(2.4,3.5)	1.523	0.478
		Distal wave amplitude (mV)	6.57(3.7,11.8)	2.36(1.1,3.9)	18.30(15.7,23.1)	63.44	0.000**
		Proximal wave amplitude (mV)	5.64(1.9,11.0)	1.51(0.6,3.4)	16.65(13.6,21.6)	60.569	0.000**
		Nerve conduction velocity (m/s)	50.85 (45.9,51.7)	50.30 (44.0,56.4)	50.30(45.6,52.5)	0.093	0.954
	Left	Distal latency (ms)	2.65(2.2,3.5)	2.80(2.2,3.4)	2.38(2.0,2.9)	3.106	0.212
		Distal wave amplitude (mV)	6.88(4.6,10.1)	1.70(0.6,2.9)	22.30(17.8,25.6)	62.787	0.000**
		Proximal wave amplitude (mV)	5.87(2.3,9.3)	1.15(0.4,2.8)	18.30(15.5,23.4)	62.36	0.000**
		Nerve conduction velocity (m/s)	47.60 (41.2,60.6)	46.00 (41.0,51.5)	48.30(44.7,51.8)	3.808	0.149
Femoral nerve	Right	Latency (ms)	3.44(2.8,4.1)	3.20(2.4,4.5)	2.15(1.7,2.5)	23.187	0.000**
		Wave amplitude (mV)	1.08(0.4,3.2)	0.39(0.1,0.8)	17.20(14.7,19.1)	63.069	0.000**
	Left	Latency (ms)	3.53(2.7,4.3)	3.23(2.5,4.0)	2.16(1.6,2.8)	21.331	0.000**
		Wave amplitude (mV)	1.34(0.3,4.2)	0.33(0.1,0.7)	15.85(13.7,18.2)	63.615	0.000**

Notes: *p<0.05, **p<0.01.

Changes in Peripheral Motor Nerve Amplitude in SMA Patients After Nusinersen Treatment

After Nusinersen treatment, there were no statistically significant differences in the amplitudes of the median nerve, ulnar nerve, axillary nerve, tibial nerve, peroneal nerve, and femoral nerve among 42 cases of 5q SMA patients, regardless of whether it was compared to baseline or after 6 months or 14 months of treatment. The above results are shown in Table 3.

Table 3 Comparison of Motor Nerve Amplitude Before and After Nusinersen Treatment in SMA Patients

Nerve	Location	Side	Amplitude(mV)Median (P25, P75)			Kruskal–Wallis Test Statistic H-value	p
			T0 (n=42)	T6 (n=42)	T14 (n=21)		
Median Nerve	Distal	Right	6.115(3.9,9.2)	6.075(4.6,8.3)	5.710(4.3,7.7)	0.148	0.928
		Left	4.570(3.1,8.4)	5.740(3.3,8.5)	6.420(3.7,10.1)	1.693	0.429
	Proximal	Right	5.080(3.0,7.8)	5.120(3.7,7.6)	4.800(3.4,6.1)	0.251	0.882
		Left	4.070(2.3,6.6)	4.950(2.2,6.9)	4.700(3.3,7.5)	1.505	0.471
Ulnar nerve	Distal	Right	2.065(1.2,4.9)	2.495(0.9,4.3)	2.040(1.5,4.3)	0.014	0.993
		Left	1.985(1.0,4.0)	2.160(1.1,4.9)	2.230(1.5,4.2)	0.532	0.767
	Proximal	Right	2.360(1.0,4.8)	2.355(1.3,4.5)	1.930(1.0,3.6)	0.526	0.769
		Left	1.955(0.9,4.7)	2.240(1.1,4.8)	2.180(1.2,3.6)	0.154	0.926
Axillary nerve		Right	4.480(1.6,7.0)	4.420(2.0,8.3)	3.590(2.5,4.5)	1.329	0.514
		Left	3.235(1.7,6.8)	3.170(1.9,8.4)	3.270(2.0,4.4)	0.535	0.765
Common peroneal nerve	Proximal	Right	2.450(0.9,4.4)	3.195(1.1,4.9)	2.200(0.7,3.4)	1.736	0.42
		Left	2.665(1.7,4.5)	2.620(1.3,4.3)	1.840(0.9,3.7)	2.558	0.278
	Distal	Right	2.375(1.0,4.5)	3.825(1.4,5.3)	1.770(0.8,3.6)	2.028	0.363
		Left	2.820(1.0,5.1)	2.695(1.1,4.4)	1.820(0.9,2.7)	2.417	0.299

(*Continued*)

Table 3 (Continued).

Nerve	Location	Side	Amplitude(mV)Median (P25, P75)			Kruskal–Wallis Test Statistic H-value	p
			T0 (n=42)	T6 (n=42)	T14 (n=21)		
Tibial nerve	Proximal	Right	1.630(0.9,4.2)	1.995(0.8,4.2)	1.790(0.5,4.2)	0.158	0.924
		Left	1.565(0.5,5.2)	1.770(0.7,4.4)	1.520(0.4,2.7)	0.865	0.649
	Distal	Right	2.705(1.3,5.6)	2.870(1.1,5.6)	2.030(1.1,4.4)	0.52	0.771
		Left	2.200(0.8,6.4)	2.285(0.9,5.6)	2.170(1.1,4.9)	0.045	0.978
Femoral nerve		Right	0.415(0.2,1.0)	0.685(0.2,1.7)	0.970(0.2,2.0)	1.724	0.422
		Left	0.495(0.1,0.9)	0.675(0.2,1.2)	0.590(0.3,1.5)	1.746	0.418

The Influence of Nusinersen on Peripheral Motor Nerve Amplitude in Different Age Groups of Type II SMA Children

Thirty-two cases of Type II SMA children were divided into three groups: The ≤36 months group (n=5), the >36 months and ≤60 months group (n=6), and the >60 months group (n=9). After 6 months of Nusinersen treatment, there were no significant improvements in the distal or proximal amplitudes of the bilateral median nerve, ulnar nerve, axillary nerve, peroneal nerve, tibial nerve, and femoral nerve compared to baseline in any of the groups. However, after 14 months of treatment, taking the right side as an example, the ≤36 months group showed a significant improvement compared to both the >36 months and ≤60 months group and the >60 months group in terms of the proximal amplitude of the median nerve, which increased by 2.12 (0.5, 2.9) mV from baseline. The proximal amplitude of the tibial nerve increased by 1.84 (1.2, 3.2) mV from baseline, and the amplitude of the femoral nerve increased by 1.06 (0.3, 2.8) mV from baseline, both showing statistically significant improvements. Similar results were reached for the left side as well. Results are shown in Figure 2.

Analysis of Factors Influencing Bilateral Femoral Nerve CMAP Amplitude in Type II SMA Patients After 14 Months of Nusinersen Treatment

A total of 20 Type II SMA patients were analyzed to identify other factors influencing the CMAP amplitude of the bilateral femoral nerves after 14 months of Nusinersen treatment. The results revealed that, apart from age, disease duration was an important factor affecting amplitude improvement. In patients with a disease duration of ≤12 months, the right femoral nerve amplitude increased by 1.060 (0.6, 2.8) mV, while the left side showed an increase of 2.090 (0.8, 3.2) mV. These improvements were significantly higher than those observed in patients with a disease duration >12 months, demonstrating a statistically significant difference. The above results are shown in Table 4.

Discussion

Peripheral Motor Nerve Electrophysiological Characteristics in Patients with SMA

In this study, significant differences were observed in the CMAP amplitudes of the median, ulnar, axillary, tibial, peroneal, and femoral nerves between Type II and Type III SMA patients and the healthy control group. Among these six pairs of motor nerves, the femoral nerve exhibited the lowest median amplitude, differing significantly from the control group. For instance, in the case of the distal amplitude of the right-side nerve, the median amplitude was 1.08 mV for Type III patients and 0.39 mV for Type II patients, both significantly lower than that of the healthy control group. The findings were generally consistent between the left and right sides. Although there was a slight prolongation of the distal latency for the axillary and femoral nerves, it remained within the normal range, possibly due to the lower amplitudes that affected the latency measurements. The electrophysiological characteristics observed in this study largely align with the clinical features of SMA, where the lower extremities are more affected than the upper extremities, and the proximal muscles are more affected than the distal muscles.[23]

The CMAP amplitude, obtained by recording the muscle depolarization following maximal stimulation of the motor nerves, indirectly reflects the number of intact and functioning motor neurons.[24] Previous electrophysiological studies

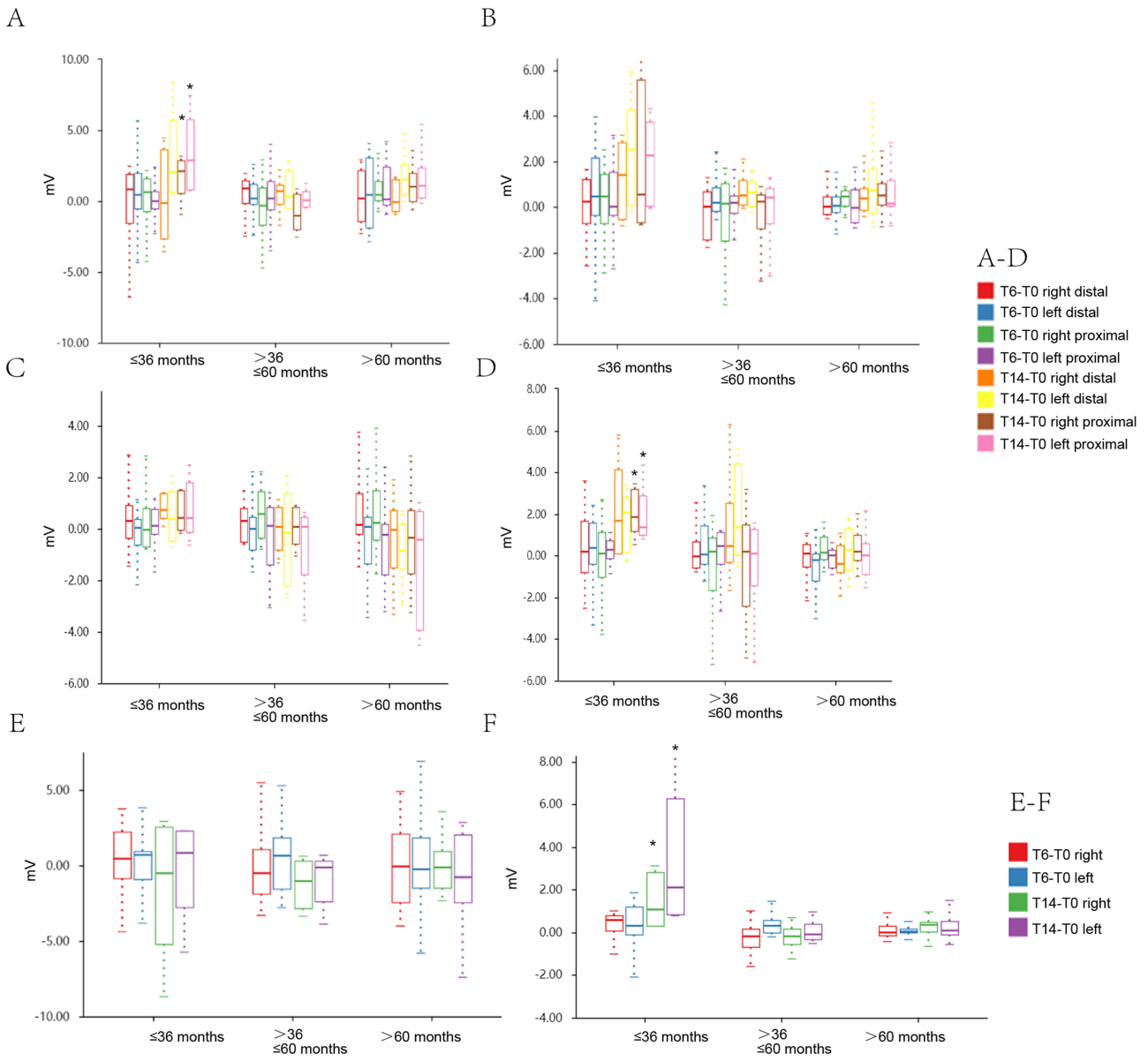

Figure 2 Changes in Peripheral Motor Nerve Amplitude in Different Age Groups of Type II SMA Children Treated with Nusinersen. *p<0.05. (**A**). Median nerve; (**B**). Ulnar nerve; (**C**). Common peroneal nerve; (**D**). Tibial nerve; (**E**) Axillary nerve; (**F**). Femoral nerve.

Note: After 14 months of treatment, the proximal amplitude of the median nerve in the ≤36 months group increased by 2.12 (0.5, 2.9) mV on the right side and by 2.9 (0.8, 5.8) mV on the left side, as shown in Figure (**A**). The proximal amplitude of the tibial nerve increased by 1.84 (1.2, 3.2) mV on the right side and by 1.36 (1.0, 2.9) mV on the left side, as shown in Figure (**D**). The amplitude of the femoral nerve increased by 1.06 (0.3, 2.8) mV on the right side and by 2.09 (0.8, 6.3) mV on the left side, as shown in Figure (**F**). These peripheral motor nerve amplitudes were significantly improved compared to both the >36 months and ≤60 months group and the >60 months group, with statistical significance. T0, just before treatment; T6, 6 months of treatment; T14, 14 months of treatment.

examining cross-sectional and longitudinal data in SMA have explored motor unit number estimation (MUNE) and CMAP and have demonstrated a strong correlation with SMA type, age, and SMN2 gene copy numbers.[25,26] In comparison to MUNE, CMAP is non-invasive, painless, requires less time, is more easily accepted by young patients, has good reproducibility, and is less influenced by changes in the expertise of the operator.[27,28]

Given the high costs of drug therapy, medical insurance expenses, and potential adverse reactions to medication, monitoring the time of symptom onset is crucial for patients seeking pre-symptomatic treatment. In this study, one Type III patient initially had a Hammersmith score of 62 (out of a maximum of 66), but the nerve conduction velocity of the lower extremities showed a significant decrease in femoral nerve amplitude: 0.75 mV on the right and 0.78 mV on the left, while the amplitudes of other peripheral nerves remained close to normal. Previous studies on peripheral nerve electrophysiology have mentioned the median,

Table 4 Analysis of Factors Influencing Bilateral Femoral Nerve Amplitude in Type II SMA Patients at 14 Months of Nusinersen Treatment

Variable		The Change Value of Femoral Nerve Amplitude Compared With Baseline	
		Right mV	Left mV
Gender	Male n=11	0.150(−0.2,0.5)	0.170(−0.2,0.9)
	Female n=9	0.460(−0.0,0.9)	0.470(−0.1,2.1)
	U-value	38	43.5
	P-value	0.382	0.648
Age group	≤36 months group n=5	1.060(0.3,2.8)	2.090(0.8,6.3)
	>36 months and ≤60 months group n=6	−0.185(−0.6,0.1)	−0.115(−0.3,0.4)
	>60 months group n=9	0.360(0.0,0.5)	0.090(−0.1,0.5)
	H-value	7.475	8.5
	P-value	0.024*	0.014*
Disease duration	Less than or equal to 12 months (n=5)	1.060(0.6,2.8)	2.090(0.8,3.2)
	Greater than 12 months and less than or equal to 36 months (n=4)	0.025(−0.3,0.2)	0.495(−0.1,7.1)
	Greater than 36 months (n=11)	0.050(−0.3,0.5)	0.010(−0.3,0.4)
	H-value	8.445	9.318
	P-value	0.015*	0.009**
Baseline Hammersmith value	≥20 points n=8	0.140(−0.6,0.9)	0.330(−0.5,1.0)
	≥10 points and <20 points n=8	0.375(0.0,0.9)	0.365(0.0,2.9)
	<10 points n=4	0.255(−0.2,0.4)	0.225(−0.1,0.5)
	H-value	0.938	1.129
	P-value	0.626	0.569
Hammersmith score change From baseline to 14m (n=20)	Less than 3 points n=11	0.150(−0.0,0.5)	0.090(−0.2,0.9)
	≥3 points n=9	0.460(−0.2,0.8)	0.440(0.0,2.1)
	U-value	40.5	40
	P-value	0.494	0.47

Notes: *p<0.05, **p<0.01.

ulnar, tibial, and peroneal nerves but have not addressed the femoral nerve.[26] In a study by Wen-Chin Weng,[26] it was noted that in Type I patients, CMAP amplitudes rapidly decline at the onset of clinical or subclinical symptoms and can be reversed after Nusinersen treatment, indicating that the rapid decline in CMAP amplitude can be used to detect clinical symptoms in children early for timely treatment. Among the six pairs of peripheral motor nerves in the upper and lower extremities, the femoral nerve exhibited the lowest amplitude compared to the control group, suggesting that it may be the nerve most susceptible to damage. Therefore, this study proposes, for the first time, that femoral nerve CMAP amplitude may serve as a potential biomarker for monitoring pre-symptomatic treatment and early detection of clinical symptoms in SMA patients, facilitating timely intervention.

The Impact of Nusinersen Treatment on Peripheral Motor Nerve Electrophysiology

In recent years, three therapeutic approaches have been approved for patients with SMA. Nusinersen, an SMN2 splicing modifier, received approval from the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) in 2017, becoming the first treatment for all types of SMA.[29]

This study revealed that, compared to baseline, there was no significant improvement in the CMAP amplitudes of the upper and lower limbs' peripheral motor nerves after 6 or 14 months of Nusinersen treatment, which differs from previous research. In an initial cohort study of a single-arm, open-label trial in infants with SMA type 1, a two-year follow-up of the ulnar nerve's CMAP amplitude showed improvement after gene therapy.[30] Didu Kariyawasam et al[31] demonstrated a significant increase in CMAP amplitudes after 18 months of Nusinersen treatment. Several factors may contribute to this discrepancy: 1) The follow-up duration in this study was up to 14 months, which might not have

allowed enough time for the peripheral motor nerves to respond to Nusinersen. Extending the observation period is necessary; 2) The patients in this group had a longer disease duration, with a median of 39.5 months, leading to irreversible loss of spinal anterior horn motor neurons, which could result in poor overall improvement in CMAP amplitudes. However, treatment helped maintain the CMAP amplitudes at least. Due to the lack of a control group with untreated cases and natural disease history, it cannot be concluded that the maintenance of these amplitudes is solely attributed to Nusinersen usage. Nevertheless, studies correlating the natural disease history and improvements in motor function scores suggest that for children older than 60 months, if the motor function score can be maintained after 12 months of treatment, Nusinersen is considered effective.[32] CMAP amplitude indirectly reflects the quantity of intact and functioning motor neurons, and it can be inferred that the stability of amplitudes in this group of patients indirectly indicates that their spinal anterior horn motor neurons did not experience further loss after treatment.

Nusinersen Treatment Significantly Improves Peripheral Motor Nerve Electrophysiology in Young Age and Short-Duration Type II SMA Patients

In this study, we further subdivided the type II SMA patients according to age to observe the impact of Nusinersen treatment on peripheral motor nerve electrophysiology in different age groups. The results showed no significant improvement in CMAP amplitudes across all groups after 6 months of treatment. However, after 14 months of treatment, there was a significant improvement in CMAP amplitudes of the median nerve, tibial nerve, and femoral nerve in the ≤36 months age group compared to the >36 months and ≤60 months age group, as well as the >60 months age group. This indicates that Nusinersen treatment can significantly increase CMAP amplitudes in younger age groups with a time-dependent effect. In contrast, there was no significant change in CMAP values before and after treatment in the older age group, suggesting that Nusinersen only stabilizes peripheral nerve electrophysiology in older SMA patients.

We further analyzed the changes in femoral nerve amplitudes in relation to gender, age, clinical classification, baseline Hammersmith score, Hammersmith score improvement, and disease duration. The results revealed no significant association between femoral nerve amplitude changes and gender, clinical classification, baseline score, and Hammersmith score improvement. However, there was a correlation with age and disease duration, where younger age and shorter disease duration were associated with a greater increase in femoral nerve amplitude.

Our study also observed that the improvement in CMAP amplitudes first occurred in the median nerve after 14 months of Nusinersen treatment in type II SMA patients. The lack of improvement in the ulnar and radial nerves can be attributed to previous studies focusing on a single peripheral nerve and lacking simultaneous comparison with other nerves. Similar findings were reported by R. Barrois[28] et al in their study of AVXS-101 treatment in type I SMA patients, where the improvement in CMAP amplitudes was most evident and occurred earlier in the median nerve. Additionally, our study found that the median nerve damage was less severe compared to the ulnar nerve, which is consistent with previous research on the natural history of SMA.[10] This phenomenon is known as the "split hand effect" where the median nerve is less affected than the ulnar nerve. Therefore, it is not surprising that the median nerve responds earlier to treatment. However, the reason why the femoral nerve, which is more severely affected in the lower limbs, responds earlier than the sciatic nerve remains unexplained.

Recent studies on Nusinersen treatment in patients have indicated the potential for motor neuron recovery in children but not in adults, suggesting an age-dependent response to treatment.[31,33] Similarly, Nusinersen did not significantly affect neuromuscular junction transmission in adult patients, but early intervention cannot be ruled out for favorable outcomes in pediatric patients.[34] A study on pre-symptomatic treatment with Nusinersen found a rapid reversal of CMAP amplitude after treatment initiation, further highlighting the greater improvement in CMAP amplitudes in younger age groups and shorter disease duration. Similar conclusions have been drawn regarding the improvement in motor function scores in pediatric SMA patients after Nusinersen treatment. Studies by Giorgia Coratti[35] et al have shown that the absolute increase in motor function scores in type II SMA patients older than 5 years is lower compared to younger age groups. Another retrospective observational study on Nusinersen treatment in pediatric and adult SMA patients also found that earlier initiation of treatment is associated with better treatment outcomes.[36] Didu Kariyawasam[31] et al reached similar conclusions, demonstrating a more significant increase in ulnar nerve amplitudes after Nusinersen treatment in children with shorter disease duration.

This study has several limitations. This study is a single-center retrospective case–control study, which may have selection bias. Additionally, although the healthy control group was matched for age and gender with the case observation group of type II and III SMA children, the influence of age and gender could not be completely eliminated in the statistical analysis, which may have had some impact on the conclusions. Furthermore, the absence of an untreated SMA control group in this study poses a limitation in evaluating the specific effects of the treatment. Finally, due to SMA being a rare disease, the sample size was small, which restricted our ability to conduct a reliable multivariate examination without the risk of overfitting or unreliable estimates, and the observation period was short. Future studies with larger sample sizes, conducted in multiple centers, and with longer follow-up periods are needed to obtain more reliable conclusions.

Conclusion

Children with type II and III SMA showed significantly lower CMAP amplitudes in the radial nerve, tibial nerve, peroneal nerve, median nerve, ulnar nerve, and axillary nerve compared to the healthy control group. However, the latency and conduction velocity were within the normal range. After Nusinersen treatment, there was no significant improvement in the overall CMAP amplitudes of the peripheral motor nerves. However, for younger children, the median nerve, femoral nerve, and tibial nerve showed a response to treatment after 14 months, with more pronounced improvement in femoral nerve amplitudes observed in children with shorter disease duration. In clinical practice, our findings stress the importance of tailored monitoring and treatment approaches, considering age, disease duration, and specific nerve involvement. Ongoing collaborative research efforts are crucial for advancing our understanding of SMA and developing more targeted therapies.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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