



## Original article

# Muscle impairment in MRI affect variability in treatment response to nusinersen in patients with spinal muscular atrophy type 2 and 3: A retrospective cohort study

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## Abstract

**Background:** Real-world data have shown variability in treatment responses to nusinersen in spinal muscular atrophy (SMA). We investigated whether the magnitude of muscle impairment assessed by magnetic resonance imaging (MRI) at baseline can predict the treatment response.

**Methods:** We retrospectively assessed the clinical data in relevance to the thigh and pelvic MRI taken before the nusinersen treatment. A total of 16 patients with SMA types 2 and 3 (age = mean [SD]; 9.2 [4.6] year) receiving nusinersen treatment were enrolled. The T1-weighted MRI images of the pelvis and thigh were scored for muscle fatty infiltration and atrophy. The minimally clinically important difference (MCID) was considered as gaining at least 3 points of Hammersmith Functional Motor Scale-Expanded (HFMSE) from baseline.

**Results:** Of these 16 individuals, 14 had been treated for at least 15 months with baseline data. At 15 months, seven individuals obtained MCID in HFMSE. Baseline muscle MRI score could not differentiate the two groups; however, individuals who obtained MCID had significantly less severe scoliosis. In addition, there was a significant and negative relationship between baseline MRI score and the change of score in HFMSE after 15 months of treatment. Further, baseline Cobb angle along with MRI score also indicated the correlation to the degree of change in motor function.

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**Conclusion:** The degree of muscle damage may confer the variability in response to nusinersen in SMA types 2 and 3. Muscle MRI score along with the severity of scoliosis assessed at baseline may help to predict the motor function change.

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**Keywords:** 5q spinal muscular atrophy; Skeletal muscle; Magnetic resonance imaging; Hammersmith Functional Motor Scale-Expanded; Antisense oligonucleotide

## 1. Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive disorder that affects motor neurons in the anterior horn of the spinal cord. Patients manifest with slowly progressive, generalized muscle weakness [1]. The major cause of SMA is deletion or mutation of the survival motor neuron 1 (*SMN1*) gene located on chromosome 5q, which decreases the amount of SMN protein. There are five types of 5q SMA (type 0,1,2,3,4) depending on the onset age and the severity of symptoms. The most severe is the infantile-onset type 0 with severe muscle weakness and respiratory insufficiency, and the mildest is the adult-onset type 4 with milder weakness who do not normally cause respiratory problems. Patients with SMA type 2 develop their symptoms between 6 and 18 months. They may sit independently, but they cannot walk. Patients with SMA type 3 manifest their symptoms after age 18 months, and their ability to walk is preserved.

Supportive care such as rehabilitation, respiratory, and feeding management had been the only available therapy for patients with SMA until the approval of nusinersen. This drug is an antisense oligonucleotide that can positively modify the disease course [2–5]. It binds to the SMN2 pre-mRNA and promotes exon 7 inclusion, which leads to increased production of SMN protein [6]. Hammersmith Functional Motor Scale-Expanded (HFMSE) is a motor function scale which has been used in several clinical trials to assess the efficacy of nusinersen in SMA. [3,4]. The minimally clinically important difference (MCID) is the smallest change that is considered to be beneficial for the patient, and the previous studies have shown that the changes beyond  $\pm 2$  points on HFMSE can be considered as clinically meaningful change in SMA types 2 and 3 [7–9].

From the results and experiences of both clinical trials and real-world data, it is known that the response to nusinersen varies among the patients [2,3,5,10–12]. The reason for this variability of treatment responses has been of critical interest for both patients and clinicians; however, some studies reported that the treatment initiation at an earlier stage of the disease may provide better effect [11,13], which is supported by the promising results from clinical trials of nusinersen in presymptomatic infants with SMA [14]. For patients with SMA types 2 and 3, there were reports suggesting age

and baseline motor function scores were predictive of the changes in motor function after the initiation of nusinersen [15,16].

On the other hand, muscle magnetic resonance imaging (MRI) is a useful tool to noninvasively evaluate the degree and the involvement pattern of muscles for neuromuscular disorders. A recent report defined the patterns of muscle MRI image changes in the lower limb of SMA types 2 and 3 by scoring the degree of fatty infiltration and atrophy in T1-weighted sequences acquired axial planes [17]. The report also showed the correlation between the muscle MRI score and the simultaneously assessed HFMSE score. This report did not clarify whether the patients had been treated with any of the disease modifying drug, and have not compared the longitudinal change of HFMSE score with the muscle MRI score. Nonetheless, the results from this report suggested a potential but reasonable hypothesis for the variability in treatment responses; that is, individuals having a more severe change in muscles are potentially at higher risk of showing less functional improvements with innovative treatments such as nusinersen [17].

Recent development in the 5q SMA treatment has brought the approval of not only nusinersen but also onasemnogene abeparvovec and risdiplam. Under the presence of multiple treatment choices, many efforts have been made to understand the possible patterns of drug efficacy [18]. Further, although data are becoming available for the effect of nusinersen, there still remain questions over the relevant factors that discriminate responders and the non-responders of the treatment, which needs to be addressed [19]. Here we assessed the baseline muscle MRI imaging scores and motor functional score changes in patients with SMA types 2 and 3 who had been treated with nusinersen. The aim was to investigate whether the severity of fatty and atrophic change in muscles may predict the extent of motor function change after nusinersen treatment.

## 2. Subjects/ methods

This was a single-center, retrospective analysis of medical information obtained in Japan aiming to investigate the correlation between the baseline magnitude of muscle impairment and the change in gross motor function after initiation of nusinersen treatment.

## 2.1. Subjects

The medical records of the patients with SMA who visited the department of Child Neurology, National Center of Neurology and Psychiatry (NCNP), Tokyo, Japan between 2000 to November 2021 were reviewed. This identified a total of 56 patients, of which two had deceased. Of the 54 patients, 16 individuals received nusinersen treatment in the department of Child Neurology, NCNP and all of them were included in the study. The clinical data were retrospectively obtained from the medical records. Following the approved nusinersen regimen for non-infantile SMA in Japan, patients were intrathecally injected with the drug at weeks 0, 4, 12 for loading, and every 6 months thereafter. All the patients received 12 mg/time of nusinersen as indicated in the manufacturer's instruction (<https://pins.japic.or.jp/pdf/newPINS/00066991.pdf> [in Japanese]). The individuals underwent pelvic and thigh MRI studies, motor function, and X-ray exams for scoliosis at baseline. X-rays to examine scoliosis were taken either in a supine or sitting position or in a position where it was safe for the patient. Cobb angles were manually measured as shown elsewhere. These tests were followed up every time or occasionally as they received nusinersen.

## 2.2. Muscle MRI scoring

MRI exams were done in 3.0 T MRI (either in Achieva; Philips Healthcare, Best, the Netherlands; or MAGNETOM Verio; Siemens, Erlangen, Germany). For the analysis, we used turbo-spin echo T1-weighted sequences on the axial plane of the bilateral pelvis and thighs in contiguous slices (5 mm thickness, gap 3–11 mm, Repetition Time [TR] 550–728 ms, and Echo Time [TE] 8.4–8.4 ms). Most of the patients except for those who could not remain still during the MRI procedure did not receive any sedation.

The examined parts through their whole extension were as follows: muscles of the pelvis (gluteus maximus, gluteus medius, gluteus minimus) and the thighs (vastus lateralis, vastus intermedius, vastus medialis, rectus femoris, gracilis, sartorius, adductor longus, adductor brevis, adductor magnus, semitendinosus, biceps femoris, semimembranosus). A semi-quantitative evaluation of fatty infiltration of the muscle was done according to Mercuri grading. Specifically, fatty infiltration of muscles was graded as follows [17,20]:

Grade 0: normal appearance.

Grade 1: scattered small areas of or increased density by MRI.

Grade 2a (2.0): numerous discrete areas of increased density < 30 % of the volume of the muscle.

Grade 2b (2.5): numerous discrete areas of increased density with beginning confluence, 30 %–60 % of the volume of the muscle.

Grade 3: washed-out appearance due to confluent areas of increased density with muscle still present at the periphery.

Grade 4: end-stage appearance, muscle replaced by increased density connective tissue and fat.

To assess the atrophy in a method that has been previously reported, thigh muscles were grouped to anterior (rectus femoris, vastus lateralis, vastus intermedius, vastus medialis), medial (adductor magnus, adductor brevis, adductor longus, sartorius, gracilis), and posterior (semimembranosus, semitendinosus, biceps femoris) compartments [17]. Specifically, muscle atrophy was graded as following the grading system as previously described [17], as well as referring to the pre-existing images in our institute who apparently had normal muscle volume:

Grade 0: normal muscle.

Grade 1: peripheral muscle volume loss.

Grade 2: volume loss is more than peripheral but is < 50 %.

Grade 3: >50 % of muscle volume loss.

Muscle MRI images were assessed independently by two evaluators (one pediatric neurologist and one radiologist), who had experiences of examining muscle images of neuromuscular disorders. The MRI images were scored by using the numbers obtained from the grading procedure. These MRI scores were calculated by summing the numbers of Mercuri grades and atrophy grades, which were considered as the scores for the muscle damages (0 being normal and 69 being the most severe). The average of the scores from two evaluators was adopted for further assessment.

The representative muscle MRI images and the scores are shown in Fig. 1.

## 2.3. Motor function assessment

The HFMSE, a widely used scale for patients with SMA, was used to assess gross motor function [21]. The HFMSE includes 33 items, with a maximum motor function score equal to 66, whereas the lowest motor function score is 0. The HFMSE was evaluated by physical therapists that were trained to assess patients with neuromuscular disorders. As previous studies have shown that the changes beyond  $\pm 2$  points on HFMSE are a clinically meaningful change in SMA types 2 and 3 [7–9], individuals who had HFMSE score change of three or more were considered to have obtained MCID by nusinersen in this study.

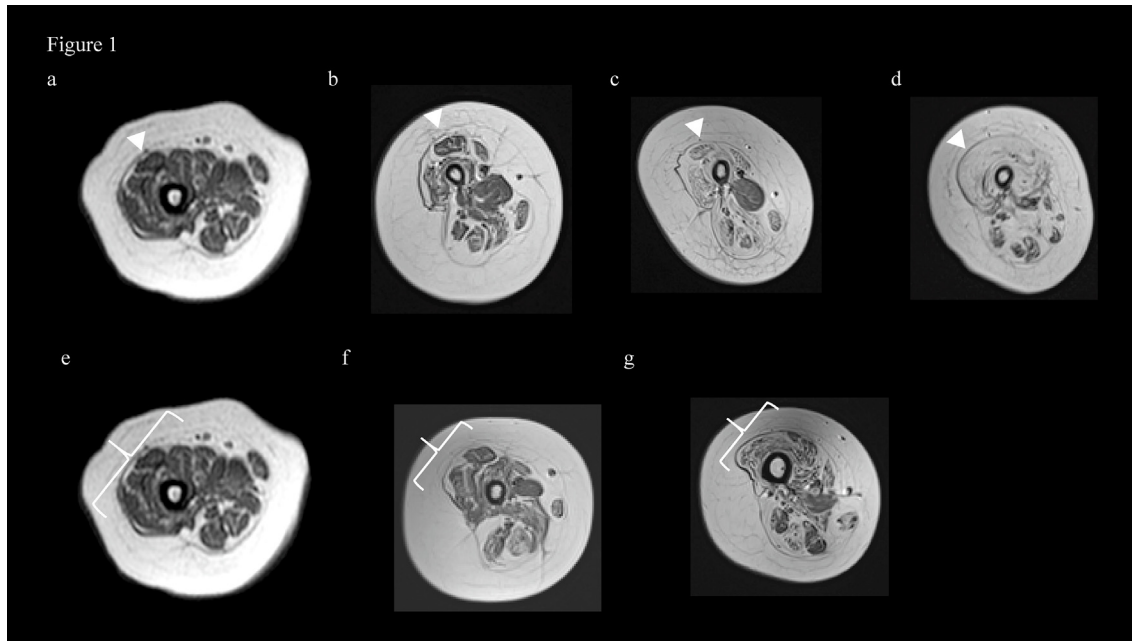


Fig. 1. Representative images of muscle MRI for Mercuri and atrophy grading. a. Patient 5. Arrow head shows Mercuri grade 1, rectus femoris. b. Patient 6. Arrow head shows Mercuri grade 2, rectus femoris. c. Patient 10. Arrow head shows Mercuri grade 3, rectus femoris. d. Patient 1. Arrow head shows Mercuri grade 4, vastus lateralis. e. Patient 5. Bracket shows atrophy grade 1, anterior compartment. f. Patient 7. Bracket shows atrophy grade 2, anterior compartment. g. Patient 12. Bracket shows atrophy grade 3, anterior compartment.

#### 2.4. Statistical analysis

Analyses were conducted using SPSS Statistics 23 (Armonk, NY: IBM Corp). Testing for normality was done with the Shapiro–Wilk test. The comparison of variables between the individuals with apparent improvement and those who did not after nusinersen treatment was assessed with two tailed Student’s *t*-test for continuous variables and Fisher’s exact test for categorical variables. The correlation between age–Cobb angle, age–MRI score, MRI score–Cobb angle was evaluated either by Spearman’s rank or Pearson correlation coefficient depending on the distribution of the values.

The MRI score (fatty infiltration and atrophy scores) was non-parametric data; therefore, interrater correlation of the scores from two evaluators was assessed by Spearman’s rank correlation coefficient. The comparison of MRI scores of each muscle between the individuals with apparent improvement with nusinersen and those with unclear response was assessed by Mann–Whitney *U* test. The relationship between baseline muscle MRI score, Cobb angle, age, and HFMSE score change (15 months of treatment–baseline) was modeled using either simple or multiple linear regression analysis.

#### 2.5. Ethics

The ethics approval for this study was obtained from the National Center of Neurology and Psychiatry in Tokyo, Japan (A2021-058).

### 3. Results

There were 16 individuals who received nusinersen (type 2, *N* = 8; type 3, *N* = 8) and underwent pelvic and thigh muscle MRI testing. The clinical characteristics of these 16 individuals are summarized in Table 1. All of the patients were either SMA type 2 or 3, and the baseline age at the beginning of treatment ranged from 2 to 18 years old. Six patients could walk independently at the initiation of nusinersen treatment (Patients 7, 9, 11, 12, 13, 14) and one could walk with aid (Patient 5). Two patients were receiving nocturnal noninvasive positive pressure ventilation (Patients 1, 3), and three had experiences of scoliosis surgery (Patients 6, 10, 15;

Table 1  
Summary of the enrolled patients.

<i>N</i>	16
Sex F:M	6:10
Baseline age mean (SD) year	9.2 (4.6)
SMA type 2 ( <i>N</i> )	8
SMA type 3 ( <i>N</i> )	8
SMN2 copy number 2 ( <i>N</i> )	1
SMN2 copy number 3 ( <i>N</i> )	12
SMN2 copy number 4 ( <i>N</i> )	3
Baseline HFMSE mean (SD)	27.1 (22.9)
Ventilator ( <i>N</i> )	2, NPPV
Gavage feeding ( <i>N</i> )	0

*N*, number; F, female; M, male; SD, standard deviation.

HFMSE, Hammersmith Functional Motor Scale-Expanded.

NPPV, noninvasive positive pressure ventilation.

patients 6 and 15 underwent surgery during the study period). Since the treatment initiation varied among the patients, the duration of nusinersen treatment ranged from 3 months (3 doses) to 45 months (10 doses).

To investigate the reliability of muscle evaluation using MRI, we assessed the correlation between the two evaluators' scores. Bilateral pelvis and thigh muscle MRI (fatty infiltration and atrophy scores) of 16 patients were assessed by two independent evaluators. The two values showed a strong positive correlation ( $r_s = 0.827$ ,  $p < 0.001$ ) (Suppl. Fig. 1). For further study, we used the mean values of the two evaluators. The muscle MRI scores ranged from 18.75 to 51.75 (0 being normal and 69 being the most severe) (Table 2).

Of the 16 individuals, 15 were treated for at least 15 months (mean [SD] = 33.8 [7.8]). Of the 15 patients, one had neither baseline HFMSE nor MRI data; therefore, it was not possible to judge the treatment effect for this patient. For the 14 patients who had baseline data and serial testing results during the treatment period, there were variabilities in their gross motor functional change from baseline assessed by HFMSE (HFMSE score last treated-baseline, mean [SD] = 2.9 [5.9]) (Fig. 2). The overall mean change in HFMSE at 15 months of treatment from baseline was 4.4 (SD = 4.9). Referring to the previous reports [7–9], the individuals were subgrouped according to their changes in HFMSE from baseline to 15 months after treatment. The results revealed seven individuals with a MCID in gross motor function (HFMSE score change  $\geq 3$  from baseline) and seven individuals without (HFMSE score change  $< 3$  from baseline). The comparison of the baseline characteristics between the individuals with and without MCID is shown in Table 3. The individuals with MCID had less severe scoliosis at baseline ( $p = 0.02$ ). On the other hand, the same group had younger age and lower muscle MRI scores although the differences were statistically nonsignificant (Table 3). Although precise age of onset for each patient could not be collected due to the nature of the retrospective study (Table 2), all individuals without MCID (Patient 1, 4, 6, 8, 10, 11,12) had a major time lag from onset until receiving nusinersen ( $> 5$  years); whereas only three out of seven individuals with a MCID had a major time lag (Patient 9, 13, 16).

We further examined the MRI scores of each muscle, to compare between the individuals who obtained MCID and those who did not. This revealed relatively preserved adductor longus and adductor brevis in both groups to a similar degree (Fig. 3). There was less fat infiltration in the individuals obtaining MCID; however, the differences were nonsignificant.

The change in HFMSE after 15 months of nusinersen treatment was correlated with baseline MRI score, and the estimated regression coefficient (B) was  $-0.261$  [95 % (CI)  $-0.488$ ,  $-0.034$ ],  $p = 0.028$ ) (Fig. 4). Baseline

Cobb angle or baseline age also showed significant relationship with HFMSE change (Cobb angle: B =  $-0.093$ , [ $-0.171$ ,  $-0.014$ ],  $p = 0.024$ ) and (age: B =  $-0.534$ , [ $-1.065$ ,  $-0.03$ ],  $p = 0.049$ ). We further performed a multiple regression analysis using baseline MRI score and Cobb angle as explanatory variables and HFMSE changes at 15 months of treatment as the outcome variable. As age was correlated with the baseline muscle MRI score ( $r = 0.583$ , Pearson correlation coefficient) and the Cobb angle ( $r_s = 0.590$ , Spearman's rank correlation coefficient), we did not include age in the regression model. The baseline MRI score and Cobb angle indicated the negative correlation with HFMSE change (baseline MRI score B =  $-0.192$ , [ $-0.41$ ,  $0.026$ ],  $p = 0.079$ ) and (Cobb angle B =  $-0.069$ , [ $-0.145$ ,  $0.006$ ],  $p = 0.069$ ).

#### 4. Discussion

According to our study, the intensity of fatty infiltration and atrophy of the muscle evaluated at baseline correlated to the degree of motor functional change after nusinersen treatment in patients with SMA types 2 and 3. This underscores the importance of early diagnosis and treatment to obtain the maximum effect of nusinersen. In addition, the assessment with both baseline muscle MRI score and Cobb angle correlated with the nusinersen efficacy. Technical difficulty of nusinersen administration in patients with severe scoliosis have been addressed [22,23], and it has been shown that deformation of spine correlates to the motor function in patients with SMA [24]. However, there were scarce information on how the severity of scoliosis affects the effect of nusinersen. Findings from our study suggested that the degree of muscle involvement and scoliosis at baseline may affect gross motor function improvement after therapeutic intervention.

Recent reports have shown that the age along with baseline HFMSE [15] or a higher SMN2 copy number [25] correlates to the magnitude of motor functional change in patients with SMA treated with nusinersen. Others have reported that there were no statistically significant differences between patients presenting with 2 and those presenting with 3 copies of SMN2 gene [26]. In our study, there was no difference in SMN2 gene copy number between the individuals who obtained MCID and who did not. On the other hand, although the significance was not robust due to the small sample size in our study, age-related treatment response (B =  $-0.534$ , 95 % CI [ $-1.065$ ,  $-0.03$ ],  $p = 0.049$ ) was observed.

In our population, the overall mean change in HFMSE score from baseline was 4.4 (SD = 4.9). A previous study showed that HFMSE has changed in 80 % of untreated patients with SMA types 2 and 3 within the range of  $\pm 2$  points [7]. Over approximately 1 year observation period, one recent study demonstrated that

Table 2  
Pelvic and thigh muscle MRI assessed by Mercuri grading and atrophy grading.

Pt	SMA type	Onset age (M)	Age at MRI (Y)	Ambulation	Ventilator	Baseline HFMSE	HFMSE change (15M-baseline)	Mercuri grading													Atrophy grading			Total score		
								Pelvis			Thigh										Anterior	Medial	Posterior			
								GM	GME	GMI	LV	IM	MV	RF	GR	SA	AL	AB	AM	ST					BF	SM
1	2	6–8	10.3	N-A	NPPV	0	2	3.5	3.5	3.5	4	4	4	3.5	1.75	2.75	0.5	1	3	2.75	2.75	2.75	2	2	3	50.25
2	2	9–10	2.1	N-A	N	6	14	1.5	1.75	1.75	1.5	1.5	1.5	1.5	1	0.5	0.5	1.5	1.5	1	0.5	0.5	2	1.5	2	23.5
3	2	8	4.2	N-A	NPPV	4	4	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2	2	0.5	1.5	2.25	1.75	1.75	1.75	1	1	1.5	34.5
4	2	6–12	11.6	N-A	N	3	-1	3	3.5	3.5	4	4	4	3	3	2.5	1	1	2	2.75	2.75	2.75	3	1.5	3	50.25
5	2	15	3.1	A	N	30	11	1	1	1.75	1	1	1	1	1	0.5	1	1	1	1	1	1	1	1	1.5	18.75
6	2	16	12.2	N-A	N	15	-1	1.75	2.25	2.25	2.25	2.5	2.25	2	1.5	2	1	1.5	2.25	2	2	2	2.5	1.5	2.5	36
7	3	18–24	4.2	A	N	42	10	2.25	2.25	2.25	2.25	2	2	2.25	2.25	2.25	1	1	2.25	2.25	2	2	2	2	2	36.25
8	3	18–24	6.9	N-A	N	29	1	2	2	2	2	2	2	1	1	0.5	1	1	1.5	1.5	1.5	2.5	2	2.5	30	
9	3	24	13.7	A	N	48	5	3	3.5	3.5	2.25	2.25	2.25	2.25	2.25	2.25	1.75	2.25	2.25	2.25	2.25	2.25	1	1	1	39.5
10	2	15	13.1	N-A	N	5	1	4	3.5	3.25	3	3	3	3	2.5	3	1	2	3.5	3	3	3	3	2	3	51.75
11	3	24	7.4	A	N	52	1	1.5	1.5	2.5	1	1.5	1	1	1	1	1	1	1	1	1	1	1	1	1	21
12	3	29	18.2	A	N	54	1	2.25	2.75	3	3	3	2.5	2.5	2.5	1.5	2.5	2.5	2.25	2	3	2	2.5	2.5	44.75	
13	3	36	14.0	A	N	51	10	1	1	1	2.25	2.25	2.25	1.75	1.5	1.5	0.5	1	1.5	1.5	1	1.5	2	1	1.5	26
14	3	36	8.6	A	N	63	NA**	1.5	1	1	1.75	1.75	2.25	1	0.5	2	1.5	1	0.5	1.75	1.75	1.75	0.5	0	1	22.5
15	3	18	12.3*	N-A	N	NA	NA	2.5	2.25	2.25	1.75	1.75	1.5	1.5	0.5	1	0.5	0.5	1.5	1.5	1.75	1.75	1.5	0.5	1	25.5
16	2	11	6.3	N-A	N	4	4	1.75	2.5	2.5	1.5	1.5	1.5	1.75	1	1.5	1	1.5	1.75	2.25	2	2	2.5	2.5	2.5	33.5
Mean (SD)								2.19 (0.86)	2.30 (0.90)	2.41 (0.81)	2.25 (0.90)	2.28 (0.87)	2.22 (0.88)	2.03 (0.75)	1.58 (0.77)	1.80 (0.74)	0.89 (0.42)	1.33 (0.54)	1.86 (0.80)	1.92 (0.65)	1.83 (0.71)	1.84 (0.67)	1.91 (0.82)	1.41 (0.66)	1.97 (0.76)	34.00 (10.94)

Pt, patient; SMA, spinal muscular atrophy; M, months; Y, years; HFMSE, Hammersmith Functional Motor Scale-Expanded

NA, not available; A, ambulatory; N-A, non-ambulatory; NPPV, noninvasive positive pressure ventilation; N, none.

GM, Gluteus maximus; GME, Gluteus medius; GMI, Gluteus minimus; LV, Lateral vastus; IM, Intermedius vastus; MV, Medial vastus; RF, Rectus femoris; GR, Gracilis; SA, Sartorius; AL, Adductor longus; AB, Adductor brevis; AM, Adductor magnus; ST, Semitendinosus; BF, Biceps femoris; SM, Semimembranosus

\* No baseline MRI available, and the image was taken 22months after treatment initiation. Otherwise, age of MRI indicates age of nusinersen initiation.

\*\* Had treatment for < 15 months.

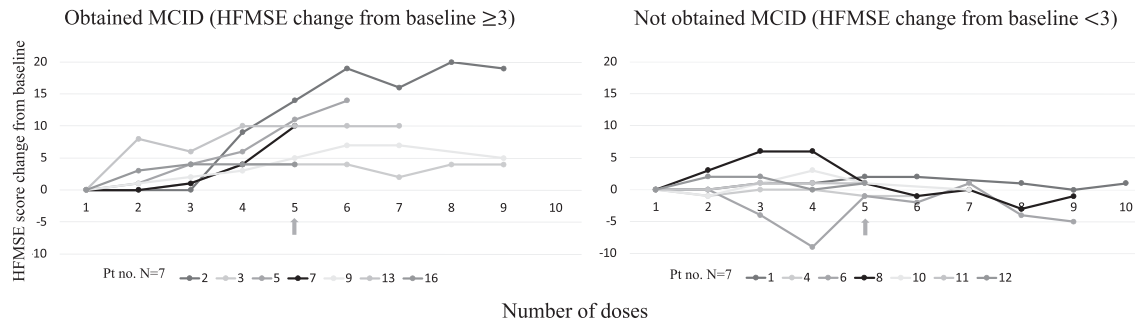


Fig. 2. HFMSE score change from baseline at the 15th month of treatment, Legend: There was variability in treatment response. The HFMSE score of seven individuals changed  $\geq 3$  from baseline (considered as those who obtained MCID), and the other seven had score change  $< 3$  from baseline. Two out of 16 patients were not included (One patient had  $< 15$  months of treatment, and one had no baseline data). HFMSE, Hammersmith Functional Motor Scale-Expanded MCID, minimally clinically important difference Arrow indicates 15 months from baseline.

Table 3

Comparison of the clinical features (baseline) depending on the drug response.

	MCID -	MCID +	p-value
N	7	7	
Sex F:M	4:3	1:6	0.266
Age mean (SD)y	11.4 (3.8)	6.8 (5.0)	0.077
SMA type 2 (N)	4	4	1.000
SMA type 3 (N)	3	3	–
SMN2 copy no 3(N)	7	4	0.192
SMN2 copy no 4(N)	0	3	–
Baseline HFMSE mean (SD)	22.6 (22.9)	26.4 (21.4)	0.750
Cobb angle mean (SD)	47.6 (35.7)	10.4 (8.5)	0.020
NPPV (N)	1	1	1.000
Muscle MRI score mean (SD)	40.6 (11.9)	30.3 (7.6)	0.077

N, number; F, female; M, male; SD, standard deviation.

SMA, spinal muscular atrophy; HFMSE, Hammersmith Functional Motor Scale-Expanded.

NPPV, Noninvasive positive pressure ventilation.

MCID, minimally clinically important difference (HFMSE change from baseline  $\geq 3$ ).

SMA type 2 and 3 patients treated with nusinersen have a mean change of 1.53 to 3.7 in HFMSE score [16,18,27], whereas the natural history data showed a mean change of  $-1.9$  to  $-0.56$  in the non-treated population [7,18,27–29]. In addition, a study focused on caregivers' point of view suggested that score gain of at least two in each HFMSE ability may justify the treatment [9]. Taken together, considering HFMSE score change of  $\geq 3$  as MCID is reasonable, and the motor function change observed in our cohort suggests a positive effect of nusinersen.

When treated individuals with HFMSE change  $\geq 3$  were considered to have MCID based on the previous report [7–9], half the patients gained MCID in our study. The baseline muscle MRI score could not clearly differentiate those who obtained MCID from those who did not (Table 3). Stabilization of the HFMSE score may also indicate a beneficial effect for the patients who manifest progressive course, and longer period assessment is necessary to understand the true benefits

of nusinersen. Therefore, future study with larger number of patients in longer term is necessary to adequately answer the utility of muscle MRI score to predict the efficacy prior to the treatment.

It is known that in the untreated population, patients with SMA types 2 and 3 who are younger than age 5 and 7 can have a spontaneous positive change in HFMSE score [18]. Five out of 14 patients were in this age range in our study, showing HFMSE change between 1 and 14, with only one patient showing a score change of  $< 3$  (Table 3). The inclusion of younger patients could be the reason for the relatively better outcomes in our cohort. Another point to be issued was that there was one case (case 11) who had relatively preserved muscle score but did not respond well to nusinersen (MRI score 21, 15 months HFMSE change from baseline 1). We sought for the factor that could have affected the outcome, such as scoliosis severity or any health related event, however, we could not identify a reasonable explanation. There could be factors outside the current assessment that affect the motor function improvements in patients with SMA treated with nusinersen, which should be addressed in future.

What is noteworthy is that the Japanese regimen for patients with SMA types 2 and 3 follows the CHERISH regimen [3], which was the regimen used in a clinical trial for SMA types 2 and 3 before the approval of nusinersen. This means that our cohort had received nusinersen less frequently compared with the patients in most other countries who follow ENDEAR regimen [2], where the drug is administered in three loading doses with 14-day intervals, followed by a fourth dose after a month, and then every 4 months thereafter. Due to the small number assessed, it is not conclusive, but our data may add some insight to the understanding of the optimal dosage of this drug.

Muscle MRI has been used to diagnose and speculate the disease progression in various neuromuscular diseases. Not much had been reported for patients with SMA, but a report from Brogna et al. systematically

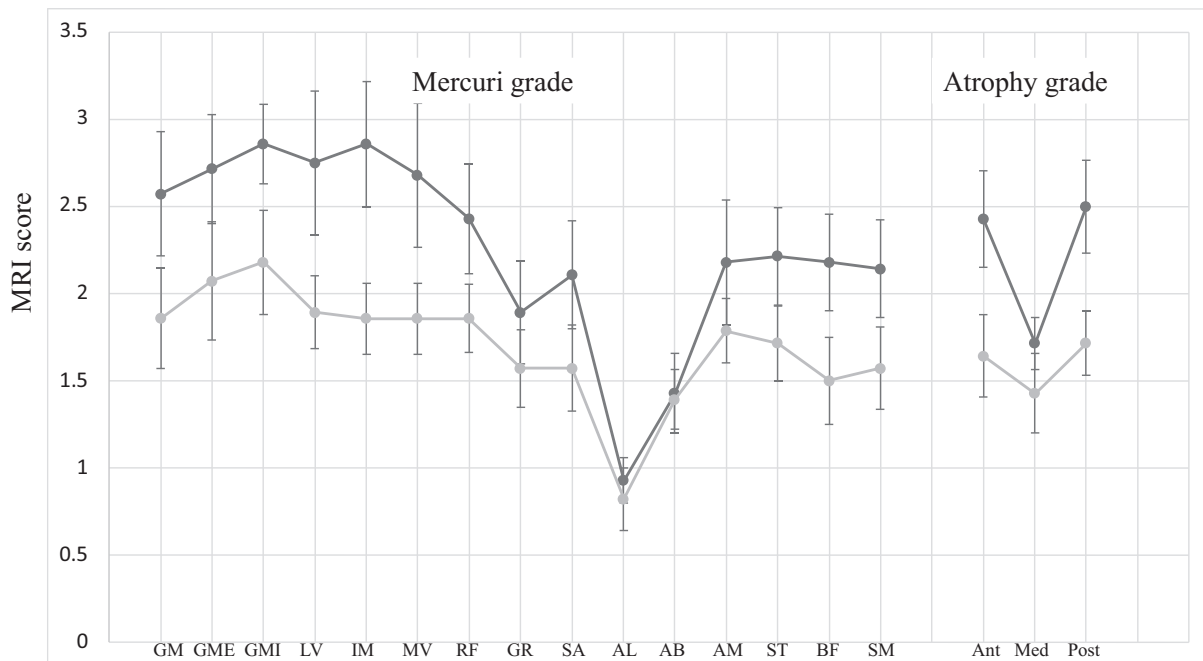


Fig. 3. Comparison of individual muscle between the individuals obtaining MCID and those who did not. Legend: Adductor longus and adductor brevis were relatively preserved in both groups. The mean and the standard error of the mean of MRI scores are shown. GM, Gluteus maximus; GME, Gluteus medius; GMI, Gluteus minimus; LV, Lateral vastus; IM, Intermedius vastus; MV, Medial vastus; RF, Rectus femoris; GR, Gracilis; SA, Sartorius; AL, Adductor longus; AB, Adductor brevis; AM, Adductor magnus; ST, Semitendinosus; BF, Biceps femoris; SM, Semimembranosus; Ant, Anterior compartment; Med, Medial compartment; Post, Posterior compartment; Gray line: Group who obtained minimally clinically important difference (MCID), HFMSE, Black line: Group who did not obtain MCID.

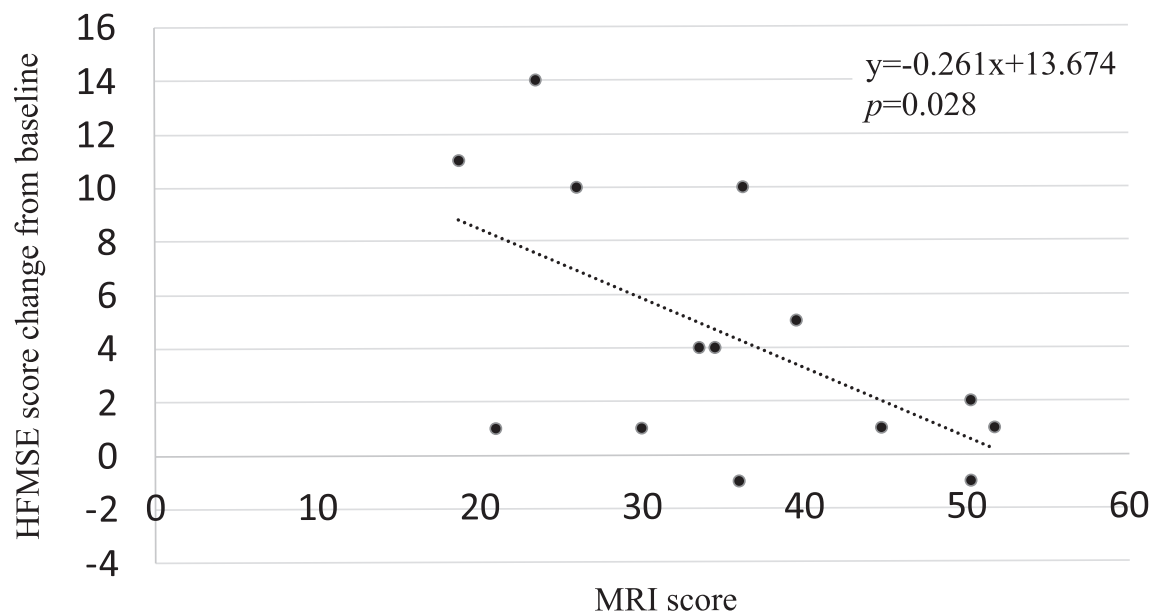


Fig. 4. The relationship between MRI score and HFMSE change at 15 months HFMSE, Hammersmith Functional Motor Scale-Expanded.

investigated the muscle involvement pattern in SMA types 2 and 3 using T1-weighted images [17]. The study demonstrated a versatile method to visually analyze the muscle in a semiquantitative manner using a well-known Mercuri grade [20] along with atrophy assessment. In terms of interrater reliability of the muscle MRI evalua-

tion, Brogna et al. showed concordance of over 90 % among the different examiners [17]. In our study, there was a strong and significant correlation between the scores measured by two evaluators; however, when the absolute scores were compared, there were differences among the evaluators (Suppl. Fig. 2). This suggests that



when used in a clinical setting, it is better to assess muscle MRI by a single evaluator or a single team to avoid the discrepancy in evaluation. Further, a more objective way to analyze muscle MRI has been reported, which the automatic calculation of muscle impairment ration based on T1-weighted MR images were developed [30]. This method may enable a study to have less margin of errors, which should be addressed in future.

The main limitation of this study is that the MRI evaluation was confined to the thigh and pelvic muscles, and the motor function testing was done only by HFMSE. Several outcome measures other than HFMSE including the 6-minute walk test, Revised Upper Limb Module (RULM), and amyotrophic lateral sclerosis (ALS) Functional Rating Scale (ALS-FRS) have been demonstrated, especially for adult patients with SMA [31]. Assessments using multiple modules may reflect the patients' condition more accurately and expect to provide a further understanding of the disease and the drug; therefore, such research should be planned in the future. A more accurate method to quantify the magnitude of muscle impairment may be achieved by using specific sequences such as T2, Dixon, and diffusion tensor imaging [17,32]. In addition, whole-body MRI is drawing attention in the field of neuromuscular disorders, which also provides a wider view of the disease [17].

Additional limitation is the small sample size and the retrospective nature of the study. Nonetheless, this is the first report that studied the direct relationship between muscle impairment and the change in motor function under innovative therapeutic intervention.

This study provides the information to further understand the factors that affect variability in treatment response to nusinersen in SMA types 2 and 3. The magnitude of muscle impairment potentially affects the outcome in gross motor function, and T1-weighted muscle MRI images can be useful to assess muscle involvement. In addition, scoliosis may also affect the outcome. These insights may help both patients and clinicians to understand what to expect from the drug in each case. A larger study may confirm the more accurate prediction model for outcome after nusinersen treatment, and in that case, muscle MRI score, Cobb angle, and age are the candidate variables to be explored.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Authors' contributions

YSM and HK conceived the study. YSM, HY, AI, and ET collected the data. YSM and EC evaluated the MRI images. MO supported statistical analysis. KM and HY interpreted the motor function assessments. YSM, MO, KM, NS, MS, SI, and HK analyzed and interpreted the data. All authors read and approved the final version of the manuscript.

### Conflict of interest disclosures

YSM has received speaking fees from Biogen. YSM has received a fee for attending the advisory board held by Novartis. YSM was the sub-investigator of the clinical trial sponsored by Chugai Pharmaceutical (risdiplam). KM has received fees for speaking and supervising information material for Biogen. KM is receiving a fee for being a member of the research executive committee for Chugai Pharmaceutical. KM has received fees from Biogen for speaking and supervising information materials. HY has received fees from Biogen for speaking and supervising information materials. AI and ET were the subinvestigators of clinical trials sponsored by Chugai Pharmaceutical (risdiplam). SI has received scholarship grant, speaking fees, and fee for attending the advisory board from Chugai Pharmaceutical. SI has received speaking fees from Novartis. HK was the principal investigator of clinical trials sponsored by Chugai Pharmaceutical (risdiplam). HK has received fees from Biogen for speaking and supervising information materials. HK has received fees for attending the advisory board held by Biogen and Novartis. The other authors declare no competing interests.

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### Availability of data and materials

The datasets used and analyzed during the present study are available from the corresponding author on reasonable request.

## Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.braindev.2022.11.002>.

## References

- [1] Kolb SJ, Kissel JT. Spinal muscular atrophy: a timely review. *Arch Neurol* 2011;68:979–84.
- [2] 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;377:1723–32.
- [3] Mercuri E, Darras BT, Chiriboga CA, Day JW, Campbell C, Connolly AM, et al. Nusinersen versus Sham Control in Later-Onset Spinal Muscular Atrophy. *N Engl J Med* 2018;378:625–35.
- [4] Darras BT, Chiriboga CA, Iannaccone ST, Swoboda KJ, Montes J, Mignon L, et al. Nusinersen in later-onset spinal muscular atrophy: Long-term results from the phase 1/2 studies. *Neurology* 2019;92:e2492–506.
- [5] Finkel RS, Chiriboga CA, Vajsar J, Day JW, Montes J, De Vivo DC, et al. Treatment of infantile-onset spinal muscular atrophy with nusinersen: final report of a phase 2, open-label, multicentre, dose-escalation study. *Lancet Child Adolesc Health* 2021;5:491–500.
- [6] Hua Y, Sahashi K, Hung G, Rigo F, Passini MA, Bennett CF, et al. Antisense correction of SMN2 splicing in the CNS rescues necrosis in a type III SMA mouse model. *Genes Dev* 2010;24:1634–44.
- [7] Mercuri E, Finkel R, Montes J, Mazzone ES, Sormani MP, Main M, et al. Patterns of disease progression in type 2 and 3 SMA: Implications for clinical trials. *Neuromuscul Disord* 2016;26:126–31.
- [8] Coratti G, Pera MC, Montes J, Pasternak A, Scoto M, Baranello G, et al. Different trajectories in upper limb and gross motor function in spinal muscular atrophy. *Muscle Nerve* 2021;64:552–9.
- [9] Pera MC, Coratti G, Forcina N, Mazzone ES, Scoto M, Montes J, et al. Content validity and clinical meaningfulness of the HFMSE in spinal muscular atrophy. *BMC Neurol* 2017;17:39.
- [10] Pane M, Coratti G, Sansone VA, Messina S, Bruno C, Catteruccia M, et al. Nusinersen in type 1 spinal muscular atrophy: Twelve-month real-world data. *Ann Neurol* 2019;86:443–51.
- [11] Pane M, Coratti G, Sansone VA, Messina S, Catteruccia M, Bruno C, et al. Type I SMA “new natural history”: long-term data in nusinersen-treated patients. *Ann Clin Transl Neurol* 2021;8:548–57.
- [12] Audic F, de la Banda MGG, Bernoux D, Ramirez-Garcia P, Durigneux J, Barnerias C, et al. Effects of nusinersen after one year of treatment in 123 children with SMA type 1 or 2: a French real-life observational study. *Orphanet J Rare Dis* 2020;15:148.
- [13] Hoy SM. Nusinersen: A Review in 5q Spinal Muscular Atrophy. *CNS Drugs* 2021;35:1317–28.
- [14] De Vivo DC, Bertini E, Swoboda KJ, Hwu WL, Crawford TO, Finkel RS, et al. Nusinersen initiated in infants during the presymptomatic stage of spinal muscular atrophy: Interim efficacy and safety results from the Phase 2 NURTURE study. *Neuromuscul Disord* 2019;29:842–56.
- [15] Coratti G, Pane M, Lucibello S, Pera MC, Pasternak A, Montes J, et al. Age related treatment effect in type II Spinal Muscular Atrophy pediatric patients treated with nusinersen. *Neuromuscul Disord* 2021;31:596–602.
- [16] Pera MC, Coratti G, Bovis F, Pane M, Pasternak A, Montes J, et al. Nusinersen in pediatric and adult patients with type III spinal muscular atrophy. *Ann Clin Transl Neurol* 2021;8:1622–34.
- [17] Brogna C, Cristiano L, Verdolotti T, Pichiecchio A, Cinnante C, Sansone V, et al. MRI patterns of muscle involvement in type 2 and 3 spinal muscular atrophy patients. *J Neurol* 2020;267:898–912.
- [18] Coratti G, Cutrona C, Pera MC, Bovis F, Ponzano M, Chieppa F, et al. Motor function in type 2 and 3 SMA patients treated with Nusinersen: a critical review and meta-analysis. *Orphanet J Rare Dis* 2021;16:430.
- [19] Gidaro T, Servais L. Nusinersen treatment of spinal muscular atrophy: current knowledge and existing gaps. *Dev Med Child Neurol* 2019;61:19–24.
- [20] Mercuri E, Lampe A, Allsop J, Knight R, Pane M, Kinali M, et al. Muscle MRI in Ullrich congenital muscular dystrophy and Bethlem myopathy. *Neuromuscul Disord* 2005;15:303–10.
- [21] Finkel R, Bertini E, Muntoni F, Mercuri E. 209th ENMC International Workshop: Outcome Measures and Clinical Trial Readiness in Spinal Muscular Atrophy 7–9 November 2014, Heemskerk. The Netherlands *Neuromuscul Disord* 2015;25:593–602.
- [22] Carrera-García L, Muchart J, Lazaro JJ, Expósito-Escudero J, Cuadras D, Medina J, et al. Pediatric SMA patients with complex spinal anatomy: Implementation and evaluation of a decision-tree algorithm for administration of nusinersen. *Eur J Paediatr Neurol* 2021;31:92–101.
- [23] Zhang J, Cui X, Chen S, Dai Y, Huang Y, Zhang S. Ultrasound-guided nusinersen administration for spinal muscular atrophy patients with severe scoliosis: an observational study. *Orphanet J Rare Dis* 2021;16:274.
- [24] Stepiń A, Gajewska E, Rekowski W. Motor Function of Children with SMA1 and SMA2 Depends on the Neck and Trunk Muscle Strength, Deformation of the Spine, and the Range of Motion in the Limb Joints. *Int J Environ Res Public Health* 2021;18.
- [25] Osredkar D, Jílková M, Butenko T, Loboda T, Golli T, Fuchsová P, et al. Children and young adults with spinal muscular atrophy treated with nusinersen. *Eur J Paediatr Neurol* 2021;30:1–8.
- [26] Aragon-Gawinska K, Seferian AM, Daron A, Gargaun E, Vuillerot C, Cancas C, et al. Nusinersen in patients older than 7 months with spinal muscular atrophy type 1: A cohort study. *Neurology* 2018;91:e1312–8.
- [27] Wadman RI, van der Pol WL, Bosboom WM, Asselman FL, van den Berg LH, Iannaccone ST, et al. Drug treatment for spinal muscular atrophy types II and III. *Cochrane Database Syst Rev* 2020;1:Cd006282.
- [28] Coratti G, Messina S, Lucibello S, Pera MC, Montes J, Pasternak A, et al. Clinical Variability in Spinal Muscular Atrophy Type III. *Ann Neurol* 2020;88:1109–17.
- [29] Coratti G, Pera MC, Lucibello S, Montes J, Pasternak A, Mayhew A, et al. Age and baseline values predict 12 and 24-month functional changes in type 2 SMA. *Neuromuscul Disord* 2020;30:756–64.
- [30] Nakayama T, Ishiyama A, Murakami T, Kimura E, Kuru S. Automatic calculation of Mercuri grades from CT and MR muscle images. *Brain and Development* 2019;41:870–7.
- [31] Walter MC, Wenninger S, Thiele S, Stauber J, Hiebele M, Greckl E, et al. Safety and Treatment Effects of Nusinersen in Longstanding Adult 5q-SMA Type 3 - A Prospective Observational Study. *J Neuromuscul Dis* 2019;6:453–65.
- [32] Otto LAM, Froeling M, van Eijk RPA, Asselman FL, Wadman R, Cuppen I, et al. Quantification of disease progression in spinal muscular atrophy with muscle MRI—a pilot study. *NMR Biomed* 2021;34:e4473.