BRIEF COMMUNICATION | Genetics inMedicine

CMAP changes upon symptom onset and during treatment in spinal muscular atrophy patients: lessons learned from newborn screening

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Purpose: Early identification and treatment of spinal muscular atrophy (SMA) are crucial but difficult. In this study, we aimed to assess the significance of compound motor action potential (CMAP) amplitude in patients identified through a newborn screening program.

Methods: We initiated a large-scale population newborn screening program for SMA in Taiwan in 2014. Patients had access to treatment through clinical trials or expanded use programs. Symptomatic patients were evaluated regularly, including CMAP exams.

Results: Among 364,000 screened newborns, 21 were diagnosed with SMA. The incidence of SMA was around 1 in 17,000 live births, and 70% developed SMA type 1. All infants with two *SMN2* copies became symptomatic before the age of 1 month. CMAP amplitudes of 12 newborns were available, including 6 who were subsequently treated with nusinersen. We found that a rapid decrease of CMAP amplitude was an early predictor of symptom onset. Pretreatment CMAP and rapid increment of post-treatment CMAP could predict better treatment outcomes.

Conclusion: This study prospectively demonstrated the incidence of SMA and its types. Our results imply the importance of pretreatment CMAP amplitude and rapid reversal of post-treatment CMAP amplitude with regard to disease presentation and also treatment outcomes.

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Keywords: SMA type; incidence; nusinersen; compound motor action potential (CMAP); outcome

INTRODUCTION

Spinal muscular atrophy (SMA) is caused by the deletions or pathogenic variants of the survival motor neuron 1 gene (*SMN1*) (MIM 600354), leading to motor neuron degeneration and resulting in progressive muscle weakness and atrophy. Patients affected by the most common form of SMA, SMA type 1 (SMA1), usually experience symptom onset within the first few weeks of life. A few specific treatments are currently available for SMA, but early, or preferably presymptomatic, initiation of treatment seems to be critical.[1–5] We initiated a large-scale population newborn screening program (NBS) for SMA in Taiwan in 2014, and have successfully identified newborns with *SMN1* deletion.[6] In this report, we assess the impact of the early detection of SMA through this program, and the association between compound motor action potential (CMAP) amplitude and symptom onset and treatment outcomes.

MATERIALS AND METHODS

The SMA NBS has been described previously, with modification of the primers (sequence upon request) since 2018 to avoid the previously described false positives.[6] *SMN2* copies were confirmed both by a droplet digital polymerase chain reaction (ddPCR) assay using the original screening dried blood spot (DBS) sample and also a multiplex ligation-dependent probe amplification (MLPA) assay using DNA extracted from whole-blood samples of the same patients, as described in our previous publication.[6] *SMN2* c.859G>C modifier was also checked in genetically diagnosed SMA patients.[7] Patients had access to treatment through clinical trials or expanded use programs (NCT02386553, NCT02865109, NCT03837184, NCT03505099, NCT04042025). Infants with two copies of *SMN2* were treated immediately after the diagnosis, while those with three copies were followed monthly for symptom onset before starting treatment, and those with four copies were evaluated in outpatient clinics or followed by phone every 4 to 6 months. The evaluations included neurological examinations, developmental milestones, and CMAP, of which the maximal CMAP amplitude of the ulnar nerve was recorded by experienced pediatric neurologists. A minimum of four G1 placements over the hypothenar eminence were used to ensure

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the measurement of maximum CMAP, as described in previous studies.[8] The parents were taught to identify the symptoms of SMA. The phenotype of SMA was classified depending on the age of symptom onset.[9] Symptomatic patients also received multidisciplinary standard of care at the specific neuromuscular joint clinic in our hospital.

ETHICS STATEMENT

This study was approved by the Institutional Review Board of National Taiwan University Hospital (NTUH) (201308058RIN and 201702057RINB), and the parents of all participants signed informed consent forms.

RESULTS

From November 2014 to December 2019, 364,000 newborns were screened for SMA. Among them, 21 were confirmed to have *SMN1* dysfunction, including 20 with an *SMN1* homozygous deletion (95%) and one who had a false negative screening test with an *SMN1* heterozygous deletion (5%). This patient presented with hypotonia since 5 months of age. When she was diagnosed at 12 months of age, she was profoundly hypotonic, could not sit without support, and had difficulty swallowing. A physical examination revealed tongue fasciculation, an absence of deep tendon reflexes. One copy of her *SMN1* gene was deleted. She also harbored a c.373G>A (V125M) variant, a novel variant predicting to be damaging, on her *SMN* gene. The incidence of SMA in our cohort was ~1 in 17,000 (95% CI: 11 350–26,530) newborns. *SMN2* copy-number analysis showed two *SMN2* copies in nine infants (43%), three copies in six infants (29%), and four copies in six infants (29%) (Table 1). None of these 21 patients had the *SMN2* c.859G>C modifier.

A total of 17 infants were followed, of whom 12 (70%) developed SMA1 (Figure S1). Nine (75%) of these SMA1 infants (five with two *SMN2* copies and four with three *SMN2* copies) were treated either with nusinersen or AVXS-101, and the other three (all with two *SMN2* copies) died without treatment (Table 1). All eight infants with two *SMN2* copies followed became symptomatic before the age of 1 month, while five (63%) showed symptoms at birth. Some of the subtle symptoms at birth improved transiently, followed by rapid deterioration. Five of the six infants with three *SMN2* copies became symptomatic before the age of 1 year, of whom four developed SMA1 (80%) defined by age of onset, and one developed SMA type 2a. The other infant (patient 11) was treated presymptomatically, and so the clinical type was undefined. Only one of the four followed infants with four *SMN2* copies became symptomatic at the age of 37 months. The age of symptom onset was significantly different among the infants with different *SMN2* copies ($p < 0.001$, Figure S2).

CMAP amplitude values of the ulnar nerve of 12 infants were available, including six who were subsequently treated with nusinersen, four who were not treated, and two before entering an AXVS-101 trial. The initial CMAP amplitudes were much lower in the infants with two *SMN2* copies (1.39 ± 0.79 mV) than in those with three copies (3.70 ± 0.59 mV) (Fig. 1). CMAP amplitude decreased rapidly in all SMA1 infants. In the infants with three *SMN2* copies (patients 13–15), the ulnar CMAP amplitudes dropped significantly when the muscle power of their upper extremities was still normal. In the infants with type 2 and 3, a decline in CMAP amplitude was also correlated with symptom onset, although the speed of decline was slower than in those with SMA1.

CMAP data from six infants who were treated with nusinersen were available. The pretreatment CMAP amplitudes were 0.89 ± 0.76 mV and 2.36 ± 1.71 mV in the patients with two and three *SMN2* copies, respectively, compared with 1.33 ± 0.22 mV and 3.69 ± 2.84 mV 6 months after treatment. In those with a pretreatment CMAP amplitude ≥2 mV (patients 14 and 15), CMAP increased significantly after treatment, accompanied by normal motor development. In those with a pretreatment CMAP amplitude <2 mV (patients 3, 4, and 13), the increase in CMAP after treatment was small and slow, although still accompanied by functional improvements. The only outlier was patient 7 (with two *SMN2* copies) whose pretreatment CMAP amplitude was <1 mV and who showed significant improvements in CMAP after treatment. He had mild motor developmental delay, while he could sit independently at the age of 9 months and walk holding on at the age of 15 months.

DISCUSSION

The incidence of SMA in our population was around 1 in 17,000, which is lower than expected because of carrier screening and prenatal diagnosis.[10,11] Very recently, a study of population-based newborn screening showed that the SMA incidence in New York State, due to the uptake of carrier screening, is also low with 1 in 28,137.[12] In this study we prospectively observed the onset of symptoms in SMA patients identified by newborn screening. The high percentage (70%) of SMA1 in our cohort suggests that compared with historical experience with type 1, fewer patients died early and that the symptoms of type 1c were probably detected earlier than others.[13] Studies of SMA newborn screening from Australian and German groups also revealed that all patients with two *SMN2* copies had early symptom onset at the first visit or before the age of 3 months if presymptomatic therapies were not applied.[14,15] All patients with two or three *SMN2* copies in our cohort, who have not received treatment or before treatment, developed type 1 ($n = 13$) or 2a ($n = 1$); thus, our results further support that newborn babies with two or three *SMN2* copies should be treated immediately.[16] With regard to newborns with four copies, immediate presymptomatic treatment has not been used in Taiwan or in Australia. Therefore, those cases with four copies were not reported as screening positives in one Australia research pilot study.[14]

Definitions of the clinical types of SMA may need to be revised when prospective symptom monitoring and disease-modifying treatments both become common.[17] For example, the symptom onset time described in our prospective study was defined by neurologists, which should be earlier than that

Table 1 Clinical and electrophysiological findings, treatment and follow-up of 21 patients diagnosed by newborn screening between November 2014 and May 2020.

Patient (sex/ current age)	Condition at diagnosis	Onset age	Symptoms at onset	Baseline ulnar CMAP, mV (age)	Treatment from age	Current condition	Respiratory involvement at last visit	Bulbar involvement at last visit	Scoliosis at last visit	Regularly multidisciplinary care
2 copies of *SMN2*										
1 (F, 3 months, died)	Symptomatic	0 days	Floppy infant with respiratory distress and poor swallowing	NA	Untreated	Died at age 3 months				
2 (F, 3 months, died)	Unaffected	1 month	Leg weakness	2.2 (18 days)	Untreated	Died at age 3 months				
3 (M, 4.0 years)	Unaffected	3 weeks	Floppy infant with frog leg	1.7 (3 weeks)	ISIS 396443 from 3 weeks	Walker (waddling)	Cough assist	Gastrostomy	Mild	+
4 (F, 3.3 years)	Symptomatic	3 days	Floppy infant with respiratory distress	0.8 (3 months)	Nusinersen from 3 months	Sitter (supported stand)	Cough assist, night BiPAP <12 hours per day	Gastrostomy	-	+
5 (F, 3.1 years)	Unaffected	<6 months	NA	NA	Nusinersen in another hospital	NA	NA	NA	NA	NA
6 (M, 4 months, died)	Symptomatic	0 days	Floppy infant with weak crying	NA	Untreated	Died at age 4 months				
7 (M, 1.5 years)	Symptomatic	0 days	Floppy infant with respiratory distress	0.2 (12 days)	Nusinersen from 12 days	Sitter (supported walk)	-	-	-	+
8 (M, 1.0 years)	Symptomatic	0 days	Floppy infant with respiratory distress	0.1 (5 weeks)	AVXS-101 at 5 weeks[a]	NA	NA	NA	NA	+
9 (F, 0.4 years)	Unaffected	2 weeks	Leg weakness	1.0 (3 weeks)	AVXS-101 at 3 weeks[a]	NA	NA	NA	NA	+
3 copies of *SMN2*										
10 (M, 5.3 years)	Unaffected	11 months	Leg weakness and not bearing weight since age 11 months	4.5 (18 days)	Untreated	Sitter	Night BiPAP <6 hours per day, cough assist	-	Mild	+
11 (M, 3.8 years)	Unaffected	NA[b]	NA	NA	ISIS 396443 in other hospital	NA	NA	NA	NA	NA

Table 1 continued

Patient (sex/ current age)	Condition at diagnosis	Onset age	Symptoms at onset	Baseline ulnar CMAP, mV (age)	Treatment from age	Current condition	Respiratory involvement at last visit	Bulbar involvement at last visit	Scoliosis at last visit	Regularly multidisciplinary care
12 (F, 3.2 years)[c]	Unaffected	5 months	Floppy and tongue fasciculation	NA	Nusinersen in another hospital	NA	NA	NA	NA	NA
13 (M, 2.4 years)	Unaffected	4 months	Floppy infant with decreased leg kicking	0.7 (6 months)	Nusinersen from 6 months	Sitter (supported walk)	-	-	-	+
14 (M, 2.0 years)	Unaffected	4 months	Decreased leg kicking and tongue fasciculation	2.7 (4 months)	Nusinersen from 4.8 months	Walker after 1 year 4 months	-	-	-	+
15 (M, 0.9 years)	Unaffected	2.5 months	Decreased leg kicking and tongue fasciculation	3.6 (3 months)	Nusinersen from 3 months	Sitter (supported stand)	-	-	-	+
4 copies of *SMN2*										
16 (M, 5.4 years)	Unaffected	-	-	1.3 (10 days)	-	Unaffected				
17 (F, 4.5 years)	Unaffected	NA	NA	NA	-	NA	NA	NA	NA	NA
18 (F, 3.4 years)	Unaffected	37 months	Difficulty in supine-to-stand position	5.3 (5 months)	Untreated	Walker	-	-	-	+
19 (F, 3.1 years)	Unaffected	NA	NA	NA	-	NA	NA	NA	NA	NA
20 (M, 3.0 years)	Unaffected	-	-	NA	-	Unaffected				
21 (F, 2.4 years)	Unaffected	-	-	NA	-	Unaffected				

BiPAP biphasic airway pressure, *CMAP* compound motor action potential, *NA* not available.
[a]They were enrolled in the AVXS-101 trial and the current clinical characteristics are confidential.
[b]The patient was treated presymptomatically and so the clinical type was undefined.
[c]A patient with false negative screening at birth and further confirmed to have point variants of the *SMN1* gene.

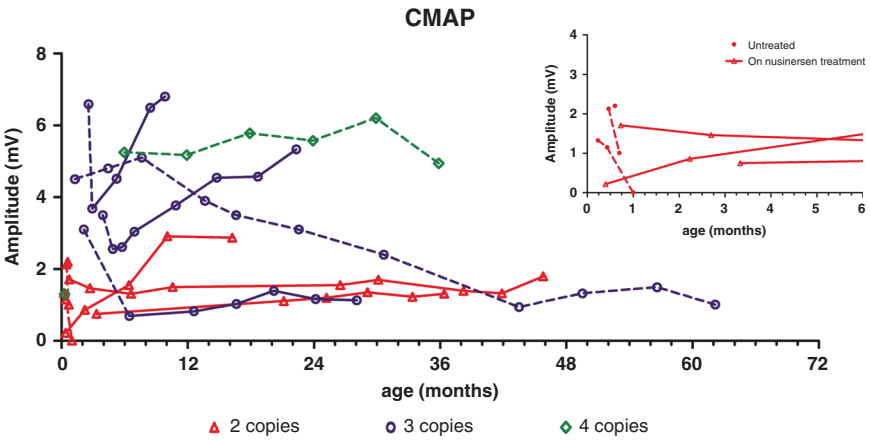

Fig. 1 Changes in compound motor action potential (CMAP) amplitude (amp) of the ulnar nerve against age in 12 infants with spinal muscular atrophy (SMA) identified through a newborn screening program. The infants are grouped into those with two (red), three (blue), and four (green) copies of *SMN2*. Changes in the pretreatment periods are connected by dashed lines, and changes after nusinersen treatment are connected by solid lines. The upper right insert is an enlargement of the infants with two copies of *SMN2* at age 0–6 months, to better show the rapid decline in CMAP amplitude in the first month of age.

detected by parents. In addition, because of the effective treatment, we could only use the disease onset time for classification but not the maximal motor function achieved. For example, our patient 14 was type 1c but can now walk, suggesting that interventions may modify the phenotypes of patients. Moreover, interventions may lead to a new phenotype instead of changing from type 1 to 2 or 3, which should further remind clinicians to carefully describe the phenotypes in treated patients. Taken together, these findings suggest that the scenario of complication management and standard of care in SMA will need to be changed in the future.[18]

In this report we provide evidence that CMAP amplitude can serve as a sensitive method to earlier detect the onset of disease from presymptomatic to symptomatic. Our results revealed that the CMAP values were lower and decreased faster in the infants with two *SMN2* copies than in those with three or four copies, similar to the findings of Swoboda et al. with regard to the natural history of denervation in SMA.[8] We also demonstrated the rapid decrease of CMAP amplitude in the very beginning of clinical or subclinical symptoms and signs in our type 1 patients, both two and three copies, suggesting the use of rapid decline in CMAP amplitude to earlier detect infantile-onset SMA for prompt treatment.[17]

We also correlated CMAP to treatment. The NURTURE study demonstrated a trend regarding higher baseline CMAP values, post-treatment D64 CMAP values, and better prognosis.[3] In our patients with two copies, although patient 7 had even lower pretreatment CMAP values, he still demonstrated a late increase in CMAP values 9 months after treatment. Interestingly, patients with three copies of *SMN2* (patients 14 and 15) exhibited a rapid decline in CMAP upon symptom onset, but had a rapid recovery of CMAP after the initiation of treatment. It is likely that, under the current follow-up protocol, motor neurons can still be rescued even after the occurrence of symptoms. Our results further clearly indicated that pretreatment CMAP and a rapid reversal or increase in CMAP after treatment were closely related to the developmental outcomes. Therefore, CMAP appears to have great potential in clinical application and in the evaluation of therapeutics for infantile-onset SMA patients. Still, the application of CMAP in evaluating therapeutics for later-onset SMA patients with four *SMN2* copies needs further investigations.

In conclusion, this report demonstrates the incidence of SMA and its types, and the importance of pretreatment CMAP amplitude as well as the change of post-treatment CMAP with regard to disease presentation and also treatment outcomes. CMAP may have great potential in clinical application and in the evaluation of therapeutics in SMA patients. However, there are several limitations to this study, including the small sample size of treated patients and a lack of detailed clinical and CMAP information of the patients with four *SMN2* copies. Considering the latest treatment recommendations,[19] further studies regarding the natural history and application of CMAP amplitude in treatment, especially in patients with four *SMN2* copies, are needed.

SUPPLEMENTARY INFORMATION

The online version of this article (https://doi.org/10.1038/s41436-020-00987-w) contains supplementary material, which is available to authorized users.

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DISCLOSURE

W.C.W. and W.L.H.: consultant on advisory boards, research and/or speaker honoraria from for Biogen. Y.H.C.: consultant on advisory boards, research and/or speaker honoraria from/for Biogen, Avexis/Novartis. Y.H.C.: consultant on advisory boards, research and/or speaker honoraria from/for Biogen, Avexis/Novartis.

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