Journal of Neuromuscular Diseases 9 (2022) 389–396
DOI 10.3233/JND-220789
IOS Press

Research Report

Spinal Muscular Atrophy – Is Newborn Screening Too Late for Children with Two *SMN2* Copies?

Oliver Schwartz[a], Heike Kölbel[b], Astrid Blaschek[c], Dieter Gläser[d], Siegfried Burggraf[e], Wulf Röschinger[e], Ulrike Schara[b], Wolfgang Müller-Felber[c] and Katharina Vill[c,*]
[a]*Münster University Hospital, Department of Pediatric Neurology, Münster, Germany*
[b]*Department of Pediatric Neurology, Developmental Neurology and Social Pediatrics, University of Essen, Essen, Germany*
[c]*Department of Pediatric Neurology and Developmental Medicine and LMU Center for Children with Medical Complexity, Dr. von Hauner Children's Hospital, LMU Hospital, Ludwig-Maximilians-University, Munich, Germany*
[d]*Genetikum®, Center for Human Genetics, Neu-Ulm, Germany*
[e]*Labor Becker und Kollegen, Munich, Germany*

Pre-press 12 April 2022
Published 6 May 2022

Abstract.
Background: Prompt treatment after genetic NBS for SMA substantially improves outcome in infantile SMA. However, deficiency of SMN-protein can cause damage of motor neurons even prior to birth.
Objective: To describe the neurological status at the time of NBS and the reversibility of neurological deficits in a cohort of patients with only two copies of the *SMN2* gene.
Methods: We present motor, respiratory, and bulbar outcomes of 21 SMA patients identified in newborn screening projects in Germany. Inclusion criteria was initiation of SMN targeted medication at less than 6 weeks of age and a minimum age of 9 months at last examination.
Results: Twelve patients (57%) developed completely normally, reaching motor milestones in time and having no bulbar or respiratory problems. Three children (14.5%) caught up after initial delay in motor development. Six patients (29%) developed proximal weakness despite early treatment: Three of them (14.5%) achieved the ability to walk with assistance and the other three (14.5%) showed an SMA type 2 phenotype at the age of 16–30 months. One patient (4.8%) had respiratory problems. Three children (14.5%) had mild chewing problems and two individuals (9.5%) needed feeding via gastrotube. Initial CHOP-INTEND values below 30 could be indicative of a less favourable outcome, whereas values above 50 could indicate a good outcome, however in-depth statistic due to the small case number is not predictive.
Conclusion: More than 70% of SMA patients with two *SMN2* copies can achieve independent ambulation with immediate initiation of therapy. However, caregivers and paediatricians must be informed about the possibility of less favourable outcomes when discussing therapeutic strategies.

Keywords: Spinal muscular atrophy, 2 SMN2 copies, outcome, CHOP INTEND, newborn screening, motor milestones

*Correspondence to: PD. Dr. Katharina Vill, Dr. von Hauner-sches Kinderspital, Abteilung für Neuropädiatrie, Entwicklungsneurologie und Sozialpädiatrie Lindwurmstraße 4, 80337 München. Tel.: +49 89 4400 55110; Fax: +49 89 4400 55111; E-mail: Katharina.vill@med.lmu.de.

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ABBREVIATIONS

SMA	Spinal Muscular Atrophy
NBS	Newborn Screening
CHOP INTEND	The Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders
HINE-2	Hammersmith Infant Neurological Examination – Section 2
SMN	Survival Motor Neuron
CMAP	Compound Muscle Action Potential

INTRODUCTION

Spinal muscular atrophy (SMA) is the most common neurodegenerative disease in childhood. In more than 95% of cases it is caused by a homozygous deletion of exon 7 of the *SMN1* gene, encoding the survival motor neuron (SMN) protein, which is involved in a variety of cellular processes (RNA biogenesis and splicing, translation, transcription, apoptosis, endocytosis, DNA damage repair, etc.) [1–3]. When there is a critical deficiency of SMN protein, there is a rapid degeneration of bulbar and spinal motor neurons [4].

SMA-specific therapies, that can correct SMN deficiency, have been available for a few years. Considering the irreversibility of motor neuron damage, there is wide consensus concerning the need for newborn screening to allow treatment in the first weeks of life. The dramatic benefit of NBS on the motor outcome of most patients has already been clearly demonstrated [5–8].

As a species-specific attribute, humans have a second, almost identical copy of the *SMN1* gene, called *SMN2*. *SMN2* differs from *SMN1* only by the exchange of five nucleobases. A critical C to T transition in exon 7 leads to an altered splicing of *SMN2* and thus to a significantly reduced production of functional SMN protein from the *SMN2* gene. The amount of SMN-protein, which is produced by *SMN2*, is usually insufficient to prevent disease onset in SMA patients. Nevertheless, increasing copies of *SMN2* is generally associated with a milder phenotype of SMA [9, 10]. Complete absence of the *SMN1* and *SMN2* gene leads to embryonic lethality and is incompatible with life. The gene dose of *SMN2* currently represents the major disease-modifying factor, apart from other genetic modifiers that have been previously described [1]. Patients with one *SMN2* copy are extremely rare. They show a severe clinical picture called SMA type 0 with respiratory failure at birth, and potentially, arthrogryposis. The majority of infants with two *SMN2* copies are expected to develop Werdnig-Hoffmann's disease (SMA type 1). Early initiation of disease specific therapy is critical. Previously, our group has reported that a relevant proportion of SMA patients with two *SMN2* copies must be considered "early symptomatic," (i.e., already showing an active disease process with a decline of motor neurons evidenced shortly after birth). This is best explained by the time-dependent, pivotal role of the SMN protein in the development of the peripheral nervous system in the last months of pregnancy and in the first months of life [11, 12].

SMA screening was implemented as part of the national newborn screening program in Germany in October 2021. SMA newborn screening has been launched in the majority of states in the U.S., and is expected to become a standard nationwide procedure in other European countries in the near future. The aim of this work, is to characterize the prognosis of the extremely vulnerable group of SMA patients with two *SMN2* copies identified through newborn screening and provided with timely initiation of treatment.

PATIENTS AND METHODS

The description of the NBS pilot projects, the method for screening for SMA, the confirmation of homozygous deletion of *SMN1* and *SMN2* quantification at neuromuscular referral centers were previously published [5, 6, 13, 14].

Twenty-one children born between January 2018 and January 2021 were included in this study. Inclusion criteria were detection by NBS, two copies of the *SMN2* gene, treatment start with Nusinersen or Onasemnogene abeparvovec within the first 6 weeks of life (range 10–39 days, median 17 days, mean 20.3 days), and a minimum of 9 months of age at last examination. Data cut was in November 2021. The follow-up period of individual patients ranged from 10 months to 3.5 years of age. Data collection was performed as part of a prospective cohort study [6]. The local ethics committee of the participating universities (Project No. 18–269) approved the study.

Twenty of 21 children were initially treated with the antisense oligonucleotide Nusinersen since gene replacement therapy with Onasemnogene abeparvovec, which has been approved for early treatment since summer 2020, was not yet available. In 9 of

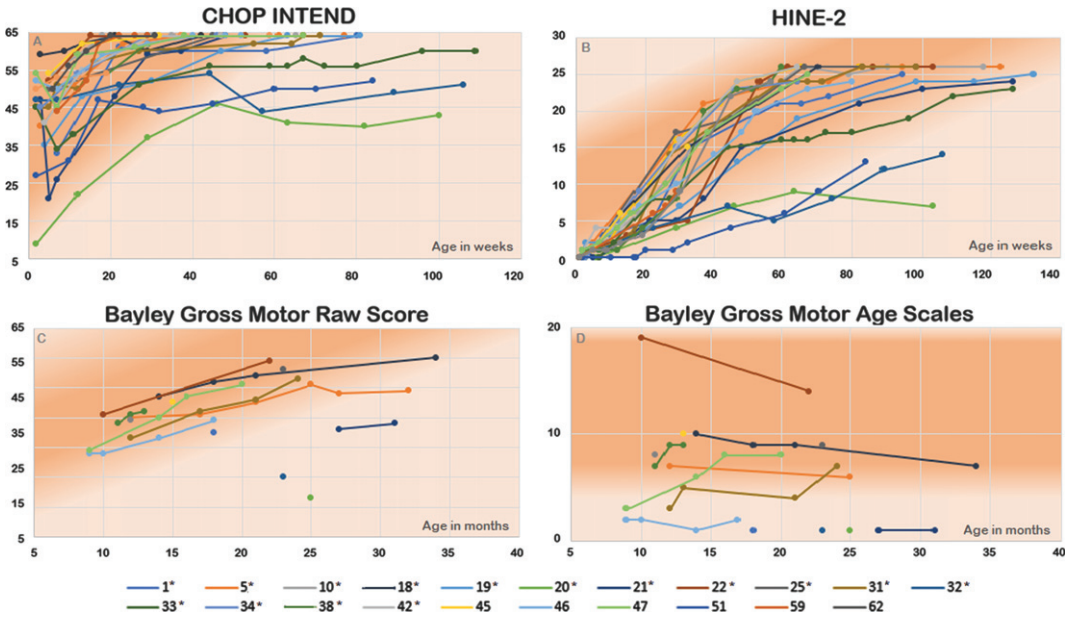

Fig. 1. (A) Course of CHOP INTEND scores, (B) Course of HINE-2 scores. X-axis: Age in weeks, y-axis: scoring results. (C) Bayley gross mwallowingw score and (D) Bayley gross motor age scales. X-axis: Age in months, y-axis: scoring results. The more intensely colored area represents the norm range of healthy children. *Data from HINE-2 and CHOP INTEND from patients 1-43 have already been published in our previous manuscripts [5, 6].

20 initially Nusinersen-treated patients, therapy was switched to Onasemnogene abeparvovec during the course (age 5–28 months, mean 14.7 months, median 13 months). The indication for gene replacement therapy was mostly the parents' wish to avoid the need for sedation associated with Nusinersen treatment. None of the children had therapy complications that may have had an impact on motor development. During the observation phase of this study, no child had switched treatment from Nusinersen to Risdiplam, which is a small molecule that is available orally and was approved by the EMA in March 2021 for SMA patients aged two months an older.

The CHOP INTEND, a reliable measure for patients with SMA, albeit with a strong "ceiling effect", comprises 16 items for evaluation of motor skills [15] and was regularly performed on all children. The first examination consistently took place before the start of treatment, the latest time being at 5 weeks of age in patient 10 (Fig. 1A). For infants with repeated maximum CHOP INTEND scores of 64, the test was discontinued. The Hammersmith Infant Neurological Examination Section 2 (HINE-2) is an additional assessment tool for evaluating motor milestones in infants with SMA. It consists of eight sections [16] and was also administered until the maximum score of 26 was repeatedly reached. Depending on the motor skills, endurance of patients, and the patient's understanding of the tasks, the Gross Motor Scale of the Bayley Scales of Infant and Toddler Development [17] was performed at age 12–24 months.

Measurement of ulnar CMAPs was done according to standard procedures. Ulnar CMAPs were performed in the first weeks of life and at different intervals and are available from all patients.

RESULTS

Presentation at first visit

Neurological exam was normal in 17 of 21 patients. Four of 21 children were hypotonic with reduced movements mainly of the legs and had CHOP INTEND scores of </=35 points. Within the first 4 weeks of life, three more children showed weakness of the legs, two of them with a significant decline of their CHOP INTEND score. In summary, 33% showed signs of muscle weakness within the first 4 weeks of life.

Swallowing and sucking were normal in all patients at first presentation (17/21 were breast-fed, two were fed with formula nutrition from maternal indication, and in two children this information is

missing), and maintained without problems during the newborn period.

One of 21 patients had a diaphragmatic pattern of breathing but no respiratory distress; the rest had no respiratory symptoms during the first weeks of life.

Deep tendon reflexes were present in 14 children, reduced in three children, absent in four children and undocumented in two children.

Ulnar CMAPs ranged between 0.4–6.4 mV at first examination (median 1.2 mV, mean 2.1 mV). Seven children had amplitudes < 1 mV and three children had amplitudes between 1 and 1.5 mV.

Motor outcome

CHOP INTEND

Seventeen of 21 patients (81%) achieved the maximum score of 64 points. Four patients slowly increased their CHOP INTEND values to a score of 42–60 points at an age of > 15 months. Two children with low CHOP INTEND scores (9 and 27 points) at baseline, finally improved to 42–51 points but were not able to sit unassisted during their entire follow-up period. Two children with initial scores of 35 and 33 finally reached 64 points and learned to walk independently. The two children with a decline from scores above 40 to scores below 35 within the first 2–3 weeks of life never reached a maximum score in the CHOP INTEND and currently exhibit an SMA type 3 phenotype. Patient 32 started with 47 points, presenting with a very slow increase during the first months. He deteriorated at age 10 months despite continuous SMA therapy when he suffered a severe, first pneumonia and attained an upper limit of 42 points only. All children with CHOP values above 50 had no deterioration and all reached 64 points (Fig. 1A).

Motor WHO-milestones

Fifteen of 21 children (71%) within the first 9 months of life eventually reached the WHO milestone „sitting unassisted“. Four more patients (10%) presented with a delay in acquiring this motor milestone at the age of 10–17 months. Two children (9.5%) have not yet learned to sit alone (age at last examination 16 months and 30 months, respectively).

Twenty of 21 children were at least 15 months old at the last visit (or had already reached this milestone earlier) and could be assessed for their ability to walk with assistance. Twelve of 20 (60%) reached this motor milestone in an age-appropriate time frame, while five children (25%) were delayed and ambulated with assistance at age 17–23 months. Three children (15%) have so far not achieved this milestone (18, 30 and 26 months at last examination).

Nineteen of 21 children were at least 18 months old or could already walk independently at the time of last examination, so the WHO milestone “walking alone” could be assessed. Ten of 19 children (53%) reached this milestone within the normal age range of 18 months. Three more patients gained independent walking at the age of 20–23 months. Six children are not yet able to walk unassisted despite being 18–30 months of age. Three of these children have progressed to ambulation with assistance (Fig. 2).

HINE-2 score

HINE-2 testing was performed in all children to describe motor functions not included in the WHO motor milestones such as head control and motor skills in prone position. Thirteen of 21 children (62%) showed normal and age appropriate motor development. Six children (38%) did not reach the maximum score, displaying heterogeneous underperformance in the HINE-2 score (7–25 points), with four of them (19%) continuously approaching the maximum and three children (14%) far behind (Fig. 1B). The two mildly affected children with HINE scores of 24 and 25 at > 2 years of age have proximal weakness only and cannot walk or stand independently. All children with HINE scores of 23 or lower at the corresponding age have proximal and axial weakness with difficulties also in a prone position.

Gross motor Bayley scores

In fourteen patients, a Bayley motor test was administered at least once. Seven children (50%) who attained all motor milestones within normal limits, had normal Bayley test scores. Two children (14%) started with below-average values but improved to test results consistent with their age; both achieved the ability to walk independently (one with an insignificant delay). Five of 14 children (36%) significantly underperformed on Bayley testing, all of them with delayed attainment of milestones (Fig. 1 C/D).

Respiratory outcome

Twenty of 21 patients (95%) had no relevant respiratory issues. One child (4.8%) is supported by non-invasive ventilation (NIV) during sleep, has had recurrent pneumonias and uses a cough assist device.

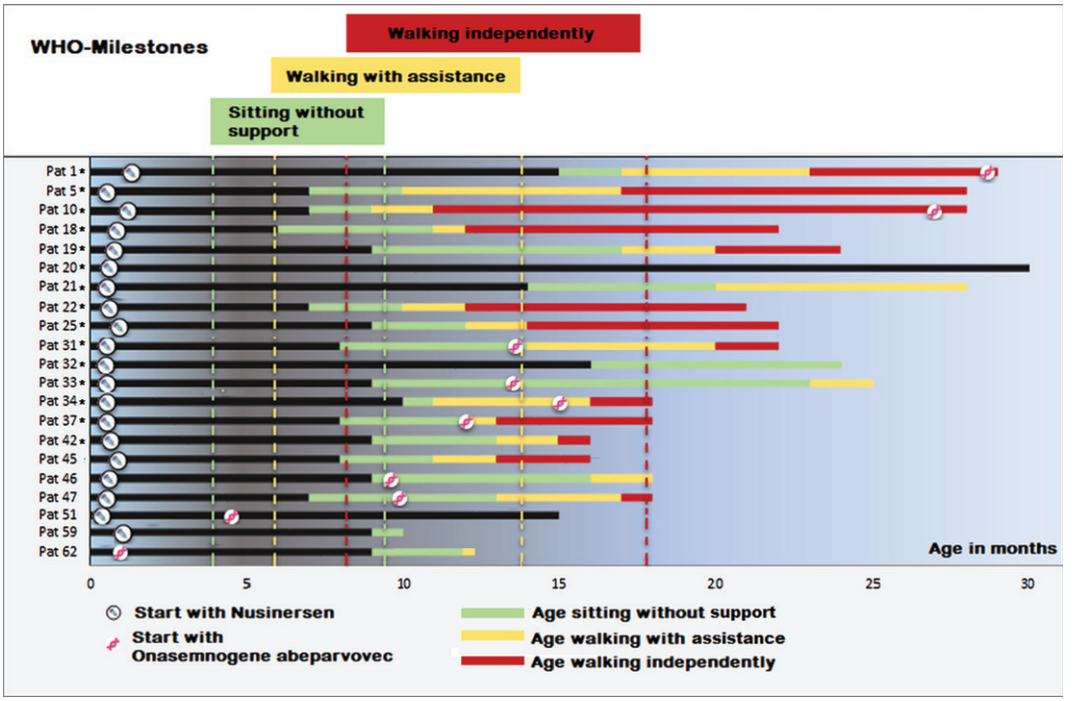

Fig. 2. Age at achievement of WHO-Milestones. Green: Sitting without support, yellow: walking with assistance, red: walking independently. Sign "syringe": Start of therapy with Nusinersen. Sign "double helix": Treatment with Onasemnogene abeparvovec. *Data from patients 1-43 have already been published in our previous manuscripts [5, 6].

Outcome on swallowing and feeding

Sixteen of 21 patients (76%) have no problems swallowing or chewing. Three of 21 children (14%) display mild problems with chewing. These patients prefer softer foods, but nevertheless have gained weight normally thus far. Two patients (9%) suffer from significant dysphagia and underwent gastrojejunostomy at 12 and 30 months of ages, respectively.

These two are also the patients with the least favourable motor outcomes. All patients with mild problems in bulbar function also showed delayed motor milestones.

Correlation of results with initial findings of deep tendon reflexes and CMAP amplitudes

Overall outcome of children with diminished or absent deep tendon reflexes encompassed the entire phenotypic spectrum from normal development to SMA type 2, and the child with the most undesirable outcome had a preserved deep tendon reflex at the first examination.

Children with baseline CMAPs above 3 mV developed normally throughout, reached milestones within the normal age-range and did not show any symptoms of SMA. All children with values below 1 mV showed motor delay; however, the outcome was heterogeneous, ranging from children with an SMA 2 phenotype to patients with the ability to walk independently. The most severely affected patient had an initial amplitude of 1.2 mV.

DISCUSSION

In this study, we investigated the outcome of children diagnosed with SMA in NBS, focussing on children with only two copies of the *SMN2* gene and who received SMN targeted treatment within the first 6 weeks of life. In summary, 57% of the patients developed without any signs of a motor neuron disease. This implies the achievement of motor milestones within the WHO predefined age ranges, the absence of dysphagia or respiratory problems as well as normal results on the Bayley motor tests. Another 14% with initial delay, progressed to a near normal or normal development. Despite early diagnosis and treatment, 29% of patients developed manifest proximal weakness, half of which were type 2 or type 3 SMA, respectively. One patient (4.8%) developed

respiratory problems. 14% have mild chewing problems and two patients (9%) are fed via gastrotube. All patients with respiratory or feeding problems had additional muscle weakness.

A key question to be answered is whether clinical or neurophysiological findings at the first examination following NBS, usually at ages 7–10 days, are predictive of children's outcomes. These data would facilitate adequate counselling of families, especially regarding treatment expectations.

We found that the CHOP INTEND might give some indicative information at rather low or rather high scores: in this study cohort, values below 30 were related with a less favourable motor outcome whereas values above 50 were associated with a good prognosis. Values between 30 and 50 were not associated with outcome. Some children in this range may deteriorate later despite medical treatment. However, predictions should be treated with caution because the number of cases is too small for in-depth statistics.

Absence or presence of deep tendon reflexes are ineffectual as clinical prognostic markers. Ulnar CMAP amplitudes values above 3 mV might indicate a favourable course. Low CMAPs may predict a low *SMN2* copy number at initial presentation and suggest urgency of therapy, but a low CMAP amplitude does not necessarily predict poor motor outcome.

It should be noted, that children from this study with poorer outcomes did not have a longer duration to initial treatment onset than patients with better outcomes.

This study supports the hypothesis [5] of an already intrauterine onset of the disease for the potentially worse prognosis of children with 2 *SMN2* copies compared with children with more copies. CMAPs are frequently used as a surrogate parameter for the stratification of SMA patients in clinical studies. Decrease of CMAPs precedes loss of motor function [19]. In this study, patients with 2 *SMN2* copies showed a median value of 1.2 mV on ulnar motor studies at first presentation. This was far below the values which were found in patients with 3 or more *SMN2* copies, indicating that, in the group of patients with 2 *SMN2* copies, some degree of denervation already occurred during pregnancy.

Our results are also consistent with the NURTURE and SPR1NT studies [8, 20] respectively. In the Nurture study, which investigated early Nusinersen treatment in presymptomatic children, a cut-off of the ulnar CMAP of > 1 mV was used [21]. In the SPR1NT study, which investigated presymptomatic children, undergoing early gene replacement therapy, a cut-off of 2 mV was used.

In both studies, a smaller proportion of patients with two *SMN2* copies were found to have relevant signs of motor neuron loss. This supports our conclusion that an unremarkable clinical impression at initial presentation is no guarantee of later absence of symptoms. Notwithstanding the fact that ulnar CMAPs above 2 mV were used as an inclusion criterion in the SPR1NT study, a small proportion of children with 2 *SMN2* copies became symptomatic with signs of motor developmental delay or dysphagia.

This work addresses the question of outcome of children with two *SMN2* copies who received a diagnosis of SMA through newborn screening and were treated either with SMN2-targeted treatment (Nusinersen) or gene replacement therapy (Onsamnogene abeparvovec) at 10–39 days of age. In this cohort, 20 of 21 children received Nusinersen as first therapy due to the fact that gene replacement therapy with Onasemnogene abeparvovec, was only approved in Germany in the summer of 2020 and the children presented in this study were at least 10 months old at the time.

This study produced comparable results to NURTURE and SPR1NT both of which were conducted in children diagnosed shortly after birth. As the outcomes reported in these studies are not significantly dependent on drug choice, we assume that the data generated from this patient cohort are independent of the initial type of SMN-targeted therapy [8, 20].

In conclusion, although NBS has significantly improved outcome, parents of children with SMA and 2 *SMN2* copies detected by NBS should be counselled that there is no guarantee for a cure.

A minority of 15% may still be at risk of significant physical disability despite early treatment and up to 30% of cases present with delayed motor development, which may correspond to mild proximal weakness along the child's developmental trajectory. CHOP-INTEND-scores and CMAP might facilitate outcome prediction, but a larger number of cases is needed to confirm this.

LIMITATIONS

This is a descriptive study in which the limited number of patients prevents a more in-depth statistical analysis, or renders such an analysis meaningless. The small numbers are a limitation as well as that only

one child was treated with Onasemnogen abeparvovec.

DECLARATIONS

Ethics approval and consent to participate

The local ethics committees of the participating universities (Ludwig-Maximilians-University of Munich, University of Munster and University of Essen, project no. 18–269) have approved the study. Compliance with guidelines on human experimentation was assured.

Consent for publication

Informed consent for prospective follow-up and publication was obtained from the participating families.

Availability of data and materials

Detailed data from CHOP INTEND, HINE-2, Bayley motor function scores and electrophysiology studies are available from the corresponding author's institution in Munich, Dr. v. Haunersches Kinderspital, Lindwurmstr. 4, 80337 München, Germany, upon reasonable request.

DISCLOSURES

All authors have indicated that they don't have any non-financial conflicts of interest.

Financial disclosure

Astrid Blaschek has received travel expenses and speaker fees from Roche, Novartis, Sanofi Genzyme.

Siegfried Burggraf, Wulf Röschinger, are employed at a commercial entity (Laboratory Becker and colleagues MVZ GbR, Führichstraße 70, 81871 Munich, Germany).

Dieter Gläser is the co-owner of a commercial entity (Genetikum®, Wegenerstr. 15, 89231 Neu-Ulm, Germany).

Katharina Vill has received travel expenses and speaker fees from Biogen and Santhera.

Oliver Schwartz has served as a member of a scientific advisory board for Avexis and received travel expenses and speaker fees from Biogen.

Heike Kölbel has served as a member of a scientific advisory board for Avexis and received travel expenses and speaker fees from Biogen and Sanofi-Aventis.

Ulrika Schara has served as a member of a scientific advisory board and data safety monitoring board for Biogen, Avexis and Novartis and received speaker fees from Biogen, Avexis, PTC and Sanofi-Aventis.

Wolfgang Müller-Felber has served as a member of a scientific advisory board for Biogen, Avexis, PTC, Sarepta, Sanofi-Aventis, Roche and Cytokinetics and received travel expenses and speaker fees from Biogen, Avexis, PTC, Roche, Sarepta and Sanofi-Aventis.

FUNDING SOURCES

Not applicable.

AUTHOR'S CONTRIBUTION

Oliver Schwartz conceptualized and designed the clinical study, collected clinical and electrophyisological data and co-drafted the manuscript.

Heike Kölbel conceptualized and designed the clinical study, collected clinical and electrophyisological data and reviewed the manuscript.

Astrid Blaschek and Ulrike Schara collected clinical and electrophyisological data and reviewed the manuscript.

Dieter Gläser performed the genetic confirmation and the *SMN2* copy number determination and reviewed the manuscript.

Siegfried Burggraf developed the screening method of the newborn screening from dried blood spots on behalf of the respective project owner.

Wulf Röschinger performed the newborn screening from dried blood spots on behalf of the respective project owner.

Wolfgang Müller-Felber conceptualized and designed the clinical study, collected clinical and electrophyisological data, and reviewed and revised the manuscript.

Katharina Vill conceptualized and designed the clinical study, collected clinical and electrophyisological data, co-drafted the initial manuscript, and reviewed and revised the manuscript.

All authors approved the final manuscript as submitted and agreed to be accountable for all aspects of the work.

ACKNOWLEDGMENTS

The authors thank all the parents of the infants who participated in this study; they also thank the physiotherapists Barbara Andres, Stefan Grziwa, Karin Hoffmann, Maren Nitzsche, Elisabeth Rothenfußer, Birgit Warken, and Therese Well who carried out the CHOP INTEND Scores, and the Cystinosis Foundation for its initial support.

The Cystinosis Foundation initiated, designed, and conducted the pilot project for genetic newborn screening for SMA and cystinosis in Germany in 2017. Within this pilot project (in the period from January 2018 to May 2019) 200,901 newborns were tested and a total of 29 newborns with a homozygous deletion in the *SMN1* gene were identified [13]. The authors also thank Biogen, Novartis, Roche and the German Muscle Society for their financial assistance in continuing SMA screening between October 2019 and September 2021. No screening funder had any impact on the study design, interpretation and publication of clinical data.

REFERENCES

[1] Wirth B, Karakaya M, Kye MJ, Mendoza-Ferreira N. Twenty-Five Years of Spinal Muscular Atrophy Research: From Phenotype to Genotype to Therapy, and What Comes Next. Annu Rev Genomics Hum. Genet 2020.

[2] Groen EJN, Talbot K, Gillingwater TH. Advances in therapy for spinal muscular atrophy: Promises and challenges. Nat Rev Neurol. 2018;14:214-24.

[3] Singh RN, Howell MD, Ottesen EW, Singh NN. Diverse role of survival motor neuron protein. Biochim Biophys Acta. 2017;1860:299-315.

[4] Foust KD, Wang X, McGovern VL, et al. Rescue of the spinal muscular atrophy phenotype in a mouse model by early postnatal delivery of SMN. Nature Biotechnology. 2010;28:271-4.

[5] Vill K, Kolbel H, Schwartz O, et al. One Year of Newborn Screening for SMA - Results of a German Pilot Project. J Neuromuscul Dis. 2019;6:503-15.

[6] Vill K, Schwartz O, Blaschek A, et al. Newborn screening for spinal muscular atrophy in Germany: Clinical results after 2 years. Orphanet Journal of Rare Diseases. 2021;16:153.

[7] De Vivo DC. Nurture Daten Präsentation. MDA meetingg 2018 Arlington 2018.

[8] Kevin A. Strauss; Michelle A. Farrar PFMKSJRMLSHJMRFKJS. Onasemnogene Abeparvovec for Presymptomatic Infants with Spinal Muscular Atrophy and Two Copies of SMN2: A Phase III Study. Presented at EAN 2021.

[9] Calucho M, Bernal S, Alias L, et al. Correlation between SMA type and SMN2 copy number revisited: An analysis of 625 unrelated Spanish patients and a compilation of 2834 reported cases. Neuromuscular disorders : NMD. 2018;28:208-15.

[10] Feldkotter M, Schwarzer V, Wirth R, Wienker TF, Wirth B. Quantitative analyses of SMN1 and SMN2 based on real-time lightCycler PCR: Fast and highly reliable carrier testing and prediction of severity of spinal muscular atrophy. American Journal of Human Genetics. 2002;70:358-68.

[11] Ramos DM, d'Ydewalle C, Gabbeta V, et al. Age-dependent SMN expression in disease-relevant tissue and implications for SMA treatment. The Journal of Clinical Investigation. 2019;129:4817-31.

[12] Tizzano EF, Zafeiriou D. Prenatal aspects in spinal muscular atrophy: From early detection to early presymptomatic intervention. European Journal of Paediatric Neurology : EJPN : Official Journal of the European Paediatric Neurology Society 2018.

[13] Hohenfellner K, Bergmann C, Fleige T, et al. Molecular based newborn screening in Germany: Follow-up for cystinosis. Mol Genet Metab Rep. 2019;21:100514.

[14] Czibere L, Burggraf S, Fleige T, et al. High-throughput genetic newborn screening for spinal muscular atrophy by rapid nucleic acid extraction from dried blood spots and 384-well qPCR. European Journal of Human Genetics : EJHG 2019.

[15] Glanzman AM, Mazzone E, Main M, et al. The Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND): Test development and reliability. Neuromuscular Disorders : NMD. 2010;20:155-61.

[16] Ramsey D, Scoto M, Mayhew A, et al. Revised Hammersmith Scale for spinal muscular atrophy: A SMA specific clinical outcome assessment tool. PloS one. 2017;12:e0172346.

[17] Bayley N. Bayley scales of infant and toddler development: Administration manual. 2006.

[18] Mendell JR, Al-Zaidy SA, Lehman KJ, et al. Five-Year Extension Results of the Phase 1 START Trial of Onasemnogene Abeparvovec in Spinal Muscular Atrophy. JAMA Neurol. 2021.

[19] Swoboda KJ, Prior TW, Scott CB, et al. Natural history of denervation in SMA: Relation to age, SMN2 copy number, and function. Annals of Neurology. 2005;57:704-12.

[20] De Vivo DC, Bertini E, Swoboda KJ, 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. Neuromuscular Disorders : NMD. 2019;29:842-56.

[21] De Vivo DC, Bertini E, Swoboda KJ, 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. Neuromuscular Disorders : NMD. 2019;29:842-56.