Cathepsin D as biomarker in cerebrospinal fluid of nusinersen-treated patients with spinal muscular atrophy

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Abstract

Background and purpose: The therapeutic landscape of spinal muscular atrophy (SMA) has changed dramatically during the past 4 years, but treatment responses differ remarkably between individuals, and therapeutic decision-making remains challenging, underlining the persistent need for validated biomarkers.

Methods: We applied untargeted proteomic analyses to determine biomarkers in cerebrospinal fluid (CSF) samples of SMA patients under treatment with nusinersen. Identified candidate proteins were validated in CSF samples of SMA patients by Western blot and enzyme-linked immunosorbent assay. Furthermore, levels of peripheral neurofilament heavy and light chain were determined.

Results: Untargeted proteomic analysis of CSF samples of three SMA type 1 patients revealed the lysosomal protease cathepsin D as a candidate biomarker. Subsequent validation analysis in a larger cohort of 31 pediatric SMA patients (type 1, n = 12; type 2, n = 9; type 3, n = 6; presymptomatically treated, n = 4; age = 0–16 years) revealed a significant decline of cathepsin D levels in SMA patients aged ≥2 months at the start of treatment. Although evident in all older age categories, this decline was only significant in the group...
INTRODUCTION

Spinal muscular atrophy (SMA) is predominantly caused by homozygous deletions and less frequently by point mutations in the SMN1 gene (survival of motor neuron 1) on chromosome 5 (5q-SMA) [1,2], resulting in functional loss of SMN protein. The neighboring and paralogous gene SMN2 is present in a variable copy number and encodes an unstable and dysfunctional protein lacking exon 7 (SMN∆7) [3]. Smaller amounts of functional SMN protein can be produced via SMN2 so that the amount of SMN2 copies correlates inversely with the severity of the phenotype [4]. This mechanism is targeted by the SMN2-directed splicing modifier nusinersen, an antisense oligonucleotide that is delivered intrathecally following a designated dosing scheme (12 mg for all age and weight groups at Days 0, 14, 28, 63, 180, and 300, then every 4 months). Experience from both clinical trials [5,6] and real-world data [7–9] showed that benefit is greatest when treatment is initiated early and that nusinersen treatment can determine new and unprecedented SMA phenotypes, but that treatment effects also can vary substantially between patients. The latter emphasizes the need for prognostic biomarkers predicting motor response to allow for optimal clinical decision-making [10]. Apart from the SMN2 copy number and less frequent genetic modifiers [11,12], only few laboratory biomarkers such as neurofilaments (peripheral neurofilament light chain [pNF-L], peripheral neurofilament heavy chain [pNF-H]) are available for SMA with early onset [13]. No longitudinal changes under nusinersen treatment were identified by extensive analyses of the metabolome in urine, serum, and cerebrospinal fluid (CSF) [14,15].

Cathepsin D is a lysosomal aspartyl protease that is highly expressed in the central nervous system as well as skeletal and cardiac muscle (Figure S1) and is involved in lysosomal degeneration of proteins [16]. Cathepsin D serves as part of the neuronal “garbage disposal” [17] and is involved in pathophysiology of different neurodegenerative diseases [16]. Mutations cause congenital neuronal ceroid lipofuscinosis type 10 [18]. In the etiology of Alzheimer disease, cathepsin D is suspected to play a major role, being involved in the degradation of the amyloid precursor protein and thus reduction of beta amyloid deposits [19,20]. In murine models of synucleinopathies like Parkinson disease, overexpression of cathepsin D reduces the aggregation of α-synuclein [21]. On the other hand, murine microglial cells overexpress cathepsin D after induction of neuroinflammation, and cathepsin D knockout inhibits the NF-κB signaling pathway and thus protects dopaminergic neurons from apoptosis in vitro [22] and in vivo [23]. In postmortem analysis of spinal cord specimens of patients with sporadic amyotrophic lateral sclerosis, cathepsin D is increased, when compared to age-matched nonneurological controls [24]. In SMA, cathepsin D levels in plasma trended to be lower in a cohort of infants with SMA than in age-matched controls in baseline results of the NeuroNEXT biomarker study [25]. To our knowledge, no further cathepsin D results in SMA cohorts have been published to date.

Facing the emerging and yet available treatment options for SMA and thus unprecedented challenges in making treatment decisions [12,26,27], we analyzed CSF of SMA patients before and under nusinersen treatment to unravel possible prognostic biomarkers. Based on the findings of an initial untargeted proteomic profiling approach on a small cohort, we hereby focused on cathepsin D as a promising biomarker predicting response to treatment.

METHODS

Design and patients

We performed a bicentric, prospective study to determine possible biomarkers in CSF samples of SMA patients under intrathecal treatment with nusinersen. The study was realized at the
university hospitals of Freiburg and Essen in Germany, approved by the corresponding ethics committee (Freiburg, 66/20; 18 February 2020), and registered at the German registry for clinical trials (DRKS00019834). Inclusion criteria were (i) genetic diagnosis of a 5q-related SMA of all subtypes, (ii) available CSF samples from medically indicated lumbar puncture (e.g., treatment with nusinersen), and (iii) written consent of patients or caregivers for the scientific use of available biomaterials. Presence of intracranial or intraspinal malignancy was defined as the only exclusion criterion.

A two-step approach was chosen. First, samples of treatment Day 1 (start of therapy), Day 14, and Day 180 of a small cohort of SMA type 1 patients (n = 3) were analyzed in a discovery phase by untargeted proteomic analysis. Samples of treatment Days 1, 60, and 300 of a larger cohort of SMA patients (n = 31) were used for analyses in the subsequent validation studies.

Clinical data

Clinical data and data regarding motor function and physiotherapeutic assessments of patients were retrieved from the SMARTcare database (www.smartcare.de) [28] or respective documentation of the German newborn screening project [29,30]. Standard motor function assessment included performance of the (i) Children’s Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND) in children <2 years of age and children ≥2 years of age who were not able to sit independently and (ii) Hammersmith Functional Motor Scale Expanded (HFMSE) in children ≥2 years of age who were able to sit independently or achieved ≥50 points on CHOP-INTEND. Additionally, (iii) the functional status of younger patients was assessed by the Hammersmith Infant Neurological Evaluation (HINE2). Patients were considered to have a "general treatment response" under nusinersen treatment in case of an increase of ≥4 points in the CHOP-INTEND score or an increase of ≥3 points in the HFMSE score. If no data for the mentioned assessments were available, HINE2-based response criteria were applied for stratification as established earlier [5].

Proteomic analyses

Information regarding sample preparation, digest quality control, liquid chromatography–tandem mass spectrometry analysis, and data analysis is given in detail in the Appendix S1.

Validation of proteomic results

Information regarding analysis of protein concentration, Western blot, and enzyme-linked immunosorbent assay (ELISA) as well as staining of SMA-derived muscle biopsies is given in detail in the Appendix S1.

Statistical analysis

We analyzed clinical data descriptively and correlated those with absolute frequencies and percentage values. For statistical analysis, we used SPSS (v26.0). Normal distribution of data was controlled using the Shapiro–Wilk test. We calculated paired two-tailed Student t-test (normally distributed data) and Wilcoxon test (nonnormally distributed data) for differences of two variables. If not mentioned otherwise, all data are represented as mean ± standard error. Three levels of statistical significance were considered: p ≤ 0.05, p ≤ 0.01, and p ≤ 0.001. For correlation analysis, a two-sided approach was used (Spearman rho for ordinal-scaled parameters, Pearson for metric parameters). Given the exploratory design of this study, no correction for multiple testing was conducted.

RESULTS

Discovery phase

Untargeted proteomic profiling was performed in CSF samples at Days 1, 14, and 180 during nusinersen treatment of three selected patients with SMA type 1 (age at start of therapy = 3, 6, and 7 months) and comparable motor response in CHOP-INTEND scores (Table S1). The proteomic analysis of CSF revealed five proteins, which showed significant dysregulation in longitudinal course of treatment (Table S2). When samples of the different treatment days were compared to non-age-matched (adult) controls, a variety of different proteins showed significant up- or downregulation (Day 0 vs. controls: 55 up- and seven downregulated; Day 180 vs. controls: 71 up- and 18 downregulated). Neurofilaments were not among the proteins identified in CSF. As expected, SMN was not identified in CSF by untargeted proteomic analysis [31].

Two upregulated proteins revealed a possible link to amyloid-associated pathophysiology: cathepsin D and cyclophilin A, which were selected for subsequent validation analysis (Table 1 and Figure 1). Insulinlike growth factor-binding proteinlike 1 also showed significant dysregulation and was also included in further validation studies, as different growth factors are known to trigger the promoter of the CTSD gene, encoding cathepsin D [32].

Validation phase

Patients and clinical data

CSF samples of 31 SMA patients (age range = 0–16 years) were included in further analyses (12 patients with SMA type 1, nine patients with SMA type 2, six patients with SMA type 3, and four treated with nusinersen presymptomatically after identification via the German newborn screening pilot project [29,30]). Clinical data of the included patients are summarized in Table 2. Physiotherapeutic assessments were available for 30 patients, but not included in the
and patients ≥2 months were analyzed separately in subsequent statistical analyses. Based on these findings, results of patients treated in infants with SMA treatment response,” decline of pNF-H and pNF-L between Days 1 and 300 of nusinersen treatment was statistically significant in “responders” and “nonresponders.” Higher Day 1 levels of neurofilaments were evident in the “responder” cohort (pNF-H, p = 0.034; pNF-L, p = 0.003), and the mean decline was significantly higher than in the “nonresponder” cohort (analysis of variance; pNF-H, p = 0.034; pNF-L, p = 0.002). Patients in the “responder” cohort were younger at start of treatment (mean age at start of treatment = 20.5 ± 44.7 months vs. 81.9 ± 62.7 months). See Figure S4 for detailed stratification of neurofilament results.

**Protein concentration in CSF stabilizes during the first months of life**

As expected by increased permeability of the blood–brain barrier during the first months of life, the overall protein concentration in CSF was substantially higher in CSF samples of infants <2 months of age (n = 4; Day 1: 1.23 ± 0.23 µg/µl) and showed a significant decline during the first months of life. The total protein concentration in CSF was stable in older patients and did not differ significantly from samples of non-SMA patients (n = 27; Day 1: 0.65 ± 0.23 µg/µl; see Figure S3). Based on these findings, results of patients <2 months and patients ≥2 months were analyzed separately in subsequent statistical analyses.

**pNF-H and pNF-L in CSF decrease with age and treatment in infants with SMA**

Correlating with previous studies [13], we identified highly elevated pNF-H and pNF-L values in CSF samples from Day 1 derived from SMA patients <2 months of age, and a subsequent decline of both parameters (Figure 2). The same was found, but clearly less pronounced, in patients ≥2 months of age. When stratified for “general treatment response,” decline of pNF-H and pNF-L between Days 1 and 300 of nusinersen treatment was statistically significant in “responders” and “nonresponders.” Higher Day 1 levels of neurofilaments were evident in the “responder” cohort (pNF-H, p = 0.034; pNF-L, p = 0.003), and the mean decline was significantly higher than in the “nonresponder” cohort (analysis of variance; pNF-H, p = 0.034; pNF-L, p = 0.002). Patients in the “responder” cohort were younger at start of treatment (mean age at start of treatment = 20.5 ± 44.7 months vs. 81.9 ± 62.7 months). See Figure S4 for detailed stratification of neurofilament results.

**Cathepsin D in CSF of SMA patients declines during nusinersen treatment**

In Western blot studies of CSF, cathepsin D shows a double band, one band representing the pro-cathepsin D precursor single chain (53 kDa) and one band representing the active single chain intermediate (48 kDa). The mature double band (31 kDa) that can be found in human plasma was not present in CSF. Other than expected by our results of the proteomic discovery phase, quantification of the cathepsin D double band (53 kDa and 48 kDa) showed a nonsignificant reduction under nusinersen treatment in SMA types 1, 2, and 3, but a nonsignificant increase in patients treated presymptomatically (Figures 3 and S5). When patients were classified by SMA type and age, the lower band of cathepsin D (active single chain intermediate) declined from Day 1 to Day 300 in SMA type 3 patients (Wilcoxon rank test, p = 0.046) and trended to decline in patients ≥2 months of age at start of treatment (p = 0.075). In SMA patients <2 months of age at start of treatment, we observed a nonsignificant opposite trend.

We further quantified the cathepsin D levels by ELISA (Figure 4). Baseline cathepsin D levels were comparable in SMA patients and controls when adjusted to age (Figure 4b). ELISA showed a significant regulation of cathepsin D concentrations with opposite direction depending on the age at start of therapy, with a cutoff at 2 months of age. Patients aged <2 months at start of treatment (n = 4) showed a significant increase of cathepsin D levels from Day 1 to Day 300 of nusinersen treatment, whereas a significant decrease was seen in older patients (n = 27; Figure 4c). Interestingly,
this decrease persisted also in older patients, in whom treatment was initiated at the age of 6 years and older (paired t-test: Day 1 vs. Day 63, \( p = 0.016 \)).

**Cathepsin D values at baseline and the subsequent decline are associated with treatment response**

When stratified for treatment response, baseline cathepsin D values trended to be lower in the cohort of “general treatment responders” than in the cohort of “nonresponders” (126.0 ± 25.4 ng/ml vs. 150.3 ± 38.3 ng/ml, \( p = 0.082 \)). When only response criteria of the CHOP-INTEND score were applied, this difference reached statistical significance (124.4 ± 26.7 ng/ml vs. 167.1 ± 27.1 ng/ml, \( p = 0.015 \)). Cathepsin D decrease over 300 days of nusinersen treatment was significant only in the group of treatment “responders,” although a less pronounced trend was also seen in the group of “nonresponders” (Figure 4g). The extent of the decline in cathepsin D levels was comparable in both cohorts (delta Day 1 to Day 300: responders, −18.9 ± 18.8 ng/ml; nonresponders, −16.8 ± 36.1 ng/ml). This pattern was visible not only in the category of “general treatment response” but consistently when response criteria were applied to the different underlying outcome measures. Correlation analysis showed an inverse association between changes in cathepsin D levels and changes in the HINE2 score, not achieving statistical significance (Pearson coefficient = −0.404, \( p = 0.069 \)).

We identified a positive correlation between cathepsin D and pNF-L decrease over 300 days of treatment. Interestingly, this was...
only significant in patients aged >12 months at start of treatment who were classified as "motor responders" (Spearman rho = 0.9, p = 0.037) but not in "nonresponders." There was no significant correlation between the observed changes in cathepsin D and changes in pNF-H.

**Cathepsin D is downregulated in human SMA muscle**

We stained available muscle biopsies of seven treatment-naïve human SMA patients (age range = 2-15 years) and identified a downregulation of cathepsin D as a consistent pattern in all samples.

### TABLE 2 Clinical data of included SMA patients (validation phase)

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<td>M: n = 1 (25.0%)</td>
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<td>0.6 ± 0.8; 0.2</td>
<td>4.4 ± 3.2; 1.10</td>
<td>12.5 ± 3.3; 7.16</td>
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<td>4.0 ± 6.7; 0.14</td>
<td>11.5 ± 9.8; 0.34</td>
<td>57.0 ± 38.1; (12.15)</td>
<td>157.2 ± 39.7; (87.197)</td>
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<td>4.8 ± 2.6; 1.10</td>
<td>9.4 ± 3.7; 4.16</td>
<td>41.1 ± 31.1; 14.96</td>
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Abbreviations: F, female; M, male; max, maximum; min, minimum; NA, not applicable; SMA, spinal muscular atrophy.

aAtrioventricular block in one patient.

bAllergies in one patient.

cInhalative salbutamol in four patients; other medication included vitamin D supplementation and laxatives.
in comparison to normal controls. Given that cathepsin D acts as a lysosomal aspartyl protease, further immunostaining of LC3B, a protein involved in formation of autophagosomal vacuoles, was performed as control, revealing no changes in muscle cells of SMA patients (Figure 5).

Regulation of cyclophilin A and IGFBPL1 in CSF under nusinersen treatment

Western blot results of both proteins are displayed in Appendix S1 (Figure S6).

CONCLUSIONS

In SMA patients ≥2 months of age at start of nusinersen treatment, the course of 300 days of nusinersen treatment was identified. This decrease was evident in all SMA subtypes and in all age categories of ≥2 months, although statistical relevance was reached only in certain subgroups. Of major interest, we identified a significant decline of cathepsin D values in patients who were categorized as having a positive “general treatment response,” whereas “nonresponders” had a less pronounced, nonsignificant decline. The early increase of cathepsin D levels in non-SMA individuals appeared to be diametrically opposite to the overall protein concentration and likely reflects an increased need for degradation of proteins during early growth and neuronal maturation. SMA patients treated early at <2 months of age exhibited a similar trend.

No physiological inhibitor of cathepsin D exists in eukaryotic cells [33]; hence, the observed decline in patients ≥2 months of age is more likely to be secondary to changes in neuronal protein hemostasis than to yet unidentified other regulatory mechanisms. We hypothesize that cathepsin D serves as a biomarker of neurodegeneration and disease activity, which decreases slowly in natural
history (Figure 6). The group of “nonresponders” mainly consisted of older patients, who likely had lost a considerable proportion of motor units at start of treatment. We did not assess the compound muscle action potential in our patients, which would have been helpful to interpret our findings regarding disease progression. Second, we assume that the more pronounced decrease of cathepsin D under therapy reflects a diminished need for clearance of protein detritus, secondary to a treatment-dependent reduced grade of neurodegeneration.

The discrepant results of discovery phase and validation analysis are most likely attributable to the rather narrow age range of the three included patients, the overall younger age of the included patients, and the different chosen time points (Day 180 in discovery phase vs. Day 300 in validation phase). Regarding the corresponding results of Western blot and ELISA, we consider our results robust, despite the rather small cohort of included patients. Results of a study examining the proteome of CSF samples derived from 10 adult SMA patients undergoing nusinersen treatment were published recently by Kessler and coworkers [34]. No single protein appeared to be significantly regulated longitudinally over the course of 10 months. However, we reanalyzed the proteomic raw data (made available by the authors) and found that seven of the 10 analyzed adult patients also showed a decrease in cathepsin D levels, whereas three patients showed an increase (intensity and Label-free quantitation intensity). These three patients exhibited low initial HFMSE scores (0, 6, and 10) and did not achieve additional points during treatment. Of the seven patients exhibiting a decrease in cathepsin D intensities, six had higher HFMSE baseline scores (15–56) and achieved 2–12 additional points during treatment. The remaining patient also showed a decrease in cathepsin D levels, whereas three patients showed an increase and 10) and did not achieve additional points during treatment. Of the seven patients exhibiting a decrease in cathepsin D intensities, six had higher HFMSE baseline scores (15–56) and achieved 2–12 additional points during treatment. 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As changes of cathepsin D were identified also in serum and plasma in other neurodegenerative disorders [35,36] it may further serve as a biomarker applicable across different treatment regimes, including risdiplam or onasemnogene abeparvovec. We included analysis of longitudinal cathepsin D changes as a biomarker also in adult cohorts.
phenotypic features [37]. Furthermore, studies in human embryonic stem cell-derived SMA myoblasts revealed an impairment of myogenic development [38]. Our immunofluorescence studies suggest that cathepsin D might also serve as a tissue marker in SMA. Given that blood serum and plasma are exposed to metabolic pathways from other body compartments such as the musculoskeletal system, the decreased plasma cathepsin D levels previously identified in the context of the NeuroNEXT baseline study [25] might accord with the identification of decreased cathepsin D abundance in skeletal muscle derived from SMA patients.

Validation analyses of cyclophilin A and IGFBPL1 were performed because of promising results of prior proteomic analysis.

FIGURE 4 Results of cathepsin D quantification by enzyme-linked immunosorbent assay (ELISA; validation phase). Two-tailed Student t-test was performed for statistical analysis of all respective data. *p ≤ 0.05, **p ≤ 0.01. (a) Comparison of cathepsin D levels of non-SMA patients (gray) and SMA patients over treatment Days 1, 63, and 300 (colors). A nonsignificant decrease of cathepsin D levels over 300 days of nusinersen treatment can be seen. (b) Cathepsin D results according to age categories for non-SMA individuals (left) and SMA patients over 300 days of nusinersen treatment (right). (c) Comparison of patients <2 or ≥2 months old at start of treatment showing opposing dynamics with an increase in patients <2 months of age and a decline in patients ≥2 months of age. (d) Indication of cathepsin D levels according to general “treatment response.” A significant decrease is seen in the “general responder” cohort but not in “nonresponders” (left). Respective analyses of cathepsin D changes according to Children’s Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND), HINE2, and Hammersmith Functional Motor Scale Expanded (HFMSE) response show similar findings. CHOP responders show significantly higher cathepsin D levels than nonresponders.
Summarizing, results do not indicate suitability as prognostic biomarkers in SMA.

Neurofilaments are secreted to plasma and CSF during axonal damage and thus serve as nonspecific markers of neurodegeneration in different diseases [39,40]. Neurofilament heavy chains are increased in plasma of younger patients with SMA type 1 and 2 and decrease faster during nusinersen treatment than in untreated patients [13]. However, significant changes are mostly seen in patients <1 year of age, limiting the use in clinical routine. Analysis of neurofilaments in CSF of SMA patients undergoing nusinersen treatment likewise showed a decline in a smaller cohort of young type 1 patients [41] but no significant correlation with motor response in older patients [42–44]. Findings of neurofilament analyses are in line with published data [41–43,45,46] and validated the quality and validity of our cohort. We observed a consistent and significant decline of pNF-L not only in infants <4 years old as demonstrated earlier [41], but also in older patients with an age range from 6 to 15 years at start of treatment. These data may help to narrow down the age range at which pNF-L is applicable as a CSF biomarker in SMA. Very interestingly, the decrease of cathepsin D and pNF-L levels was significantly correlated in patients >12 months old at start of treatment who were classified as “motor responders.” Combined analysis of pNF-L and cathepsin D therefore might be suitable as a biomarker of motor response in patients from 12 months of age to adolescence.

Our study has some obvious limitations. First, our cohort of SMA patients was relatively small and heterogeneous. This is in part compensated by detailed clinical outcome measurements at multiple time points over up to 3 years of duration. Second, included patients younger than 6 months were exclusively classified as having a “general treatment response,” thus complicating the differentiation between age dependency and treatment response. Third, the classification of patients as having a “general treatment response” using different assessments is difficult. For the purpose of this study, it was necessary to categorize patients as “responders” and “nonresponders.”

In conclusion, we found that cathepsin D levels declined in CSF samples in our cohort of SMA patients under nusinersen treatment. This decline appeared to be present in all SMA subtypes and age categories of ≥2 months and was found to be more pronounced in patients who showed a positive motor response to treatment. Although our cohort was too small to compare age groups and SMA subtypes within the group of “treatment responders,” we believe these results to be very promising, as they indicate suitability of cathepsin D levels to serve as a biomarker in SMA also in older patients, in combination with analysis of pNF-L in adolescents or alone in adult patients. However, further validation studies in larger cohorts and in easier to obtain biomaterials like serum samples will be needed to validate the applicability across different treatment regimens.

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CONFLICT OF INTEREST
D.C.S. has participated in workshops sponsored by Biogen and Roche, and has received compensation for consulting services from Pfizer. H.K. serves on a scientific advisory board for AveXis and has received travel and received speaker honoraria from Biogen,
Pfizer, Roche, and Sanofi-Aventis. A.P. has received compensation for advisory boards, training activities, and research grants from Novartis Gene Therapies and Biogen. B.W. is chair of the scientific advisory board of SMA Europe, and has received speaker honoraria from Biogen and Novartis/Apexis for scientific talks at workshops, seminars, and video conferences. J.K. has received research funding and/or compensation for presentations and consulting services from Apexis, Biogen, Novartis, and Roche. U.S.-S. has received honoraria for presentations and consulting services from Apexis, Novartis, Biogen, and Roche. H.L. has received compensation for consultancy and financial support for research projects and clinical trials from Amplo Biotechnology, AMO Pharma, Biogen, Desitin, Fulcrum Therapeutics, GW Pharma, KYE Pharmaceuticals, Milo Biotechnology, Pfizer, PTC Therapeutics, Hoffman-La Roche Limited, Sanofi-Genzyme, Santhera, Sarepta, Satellos, and Ultragenyx. A.R. has received compensation for presentations and training activities from Sanofi-Genzyme, and has received research funding from AMO Pharma. None of the other authors has any conflict of interest to disclose.

**AUTHOR CONTRIBUTIONS**

David C. Schorling: Conceptualization (equal), formal analysis (lead), investigation (lead), methodology (equal), validation (equal), visualization (lead), writing—original draft (lead), writing—review & editing (equal). Heike Kölbel: Conceptualization (equal), investigation (equal), supervision (equal), writing—review & editing (equal). Andreas Hentschel: Formal analysis (equal), investigation (equal), software (equal), visualization (equal), writing—review & editing (equal). Astrid Pechmann: Conceptualization (equal), writing—review & editing (equal). Nancy Meyer: Conceptualization (equal), formal analysis (equal), investigation (equal), supervision (equal), writing—review & editing (equal). Brunhilde Wirth: Methodology (equal), supervision (equal), writing—review & editing (equal). Roman Rombo: Supervision (equal), writing—review & editing (equal). SMArtCare consortium: Resources (equal). Albert Sickmann: Formal analysis (equal), methodology (equal), software (equal), writing—review & editing (equal). Janbernd Kirschner: Conceptualization (equal), funding acquisition (equal), investigation (equal), writing—review & editing (equal). Ulrike Schara-Schmidt: Conceptualization (equal), investigation (equal), supervision (equal), writing—review & editing (equal). Hanns Lochmüller: Conceptualization (equal), formal analysis (equal), investigation (equal), supervision (equal), writing—review & editing (equal). Andreas Roos: Conceptualization (lead), formal analysis (equal), funding acquisition (lead), investigation (equal), methodology (equal), project administration (lead), resources (lead), validation (equal), writing—review & editing (equal).

**DATA AVAILABILITY STATEMENT**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**REFERENCES**


CATHEPSIN D IN SPINAL MUSCULAR ATROPHY


SUPPORTING INFORMATION
Additional supporting information may be found in the online version of the article at the publisher’s website.

APPENDIX 1

SMARTCARE STUDY GROUP [PRENAME, SURNAME]:

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PD: Parkinson’s Disease

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