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Short Report

Genotype–phenotype studies in infantile spinal muscular atrophy (SMA) type I in Germany: implications for clinical trials and genetic counselling

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We reviewed the natural history and assessed the *SMN2* copy number of 66 patients with infantile spinal muscular atrophy (SMA) type I born between 2000 and 2005 in Germany whose diagnosis was confirmed by a homozygous *SMN1* deletion in the first 6 months of life. After excluding patients who had received valproic acid, the median/mean age at disease endpoint was 6.1/7.3 months (range 0.0–34.0). Four (6.1%) patients with one *SMN2* copy had severe SMA type '0' with joint contractures and respiratory distress from birth. Median/mean age at onset (months) in 57 (86.3%) patients with two *SMN2* copies was 1.2/1.3, and 3.5/3.4 in 5 (7.6%) patients with three *SMN2* copies. Median/mean age at disease endpoint was 6.5/7.8 months (range 0.5–30) in patients with two *SMN2* copies. All patients with three *SMN2* copies were still alive at 10–55 months, two of them under permanent ventilation. Our data are relevant for prognostication and genetic counselling. The observed clinical variability, especially in the group with two *SMN2* copies, might be important for clinical trials in SMA I where a possible control group could be defined as follows: age at onset within 4–5 months, age at genetic diagnosis <6 months, two *SMN2* copies present, head control in less than 10%, no respiratory distress from birth, disease endpoint either age at death or age at permanent ventilation.

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Infantile spinal muscular atrophy (SMA) is characterized by a motor neuron degeneration of the spinal cord and brain stem, resulting in progressive muscle weakness and atrophy. Patients are classified into three types, SMA I–III, on the basis of the age of onset and clinical severity. More than 90% of all patients show homozygous absence of the *SMN1* gene, and clinical severity is modified by the number of *SMN2* gene copies.

Following encouraging results from cell culture experiments and animal models with substances that increase the SMN expression from the retained *SMN2* copies, international efforts have been concentrated on treatment studies in SMA (1). In Germany, studies have concentrated on valproic acid (VPA), a short-chain fatty acid histone deacetylase (HDAC) inhibitor, which increases the protein level of SMN2 through transcription activation and restoration of correct splicing in fibroblast cell lines from SMA patients (2, 3) and *in vivo* in SMA carriers and SMA patients as well as in SMA-like mice (4, 5). While placebo-controlled studies with HDAC inhibitors have been initiated in chronic SMA II and III (ClinicalTrials.gov

identifier: NCT00227266), ethical concern has been raised as to whether controlled studies can be justified in severe SMA I patients who might not live long enough to benefit after unblinding the study. It was suggested that an open study in SMA I might be feasible with life span as primary outcome measure, if a historical control group were available in lieu of the control arm of a blinded study. At the 134th ENMC consortium meeting (1), the participants agreed on the following criteria: SMA I phenotype (onset within 6 months of age; patients never achieve a sitting position) but excluding the neonatal onset form, homozygous *SMN1* deletion/mutation and known *SMN2* gene copy number. Patients with three *SMN2* copy numbers were predicted to have a milder form of SMA I and considered a separate cohort (1).

We reviewed the clinical picture of 66 patients with SMA I in Germany and analysed the *SMN2* gene copy numbers. In particular, we were interested in the following questions: (i) Which are the clinical characteristics in a strictly defined cohort of SMA I patients? (ii) Are there genotype–phenotype correlations according to *SMN2* gene copy numbers? (iii) What are the impacts of our results for clinical trials?

The importance of our study is underlined by the fact that in 2008 recruitment for an open multi-centre study (CarniVal Type I) using VPA and L-carnitine in SMA I has started at considerable costs and efforts (ClinicalTrials.gov Identifier: NCT00661453).

Patients and methods

We conducted a retrospective study on the natural history of SMA type I patients in Germany. Those patients were eligible who were diagnosed between 2000 and 2005 and whose diagnosis of SMA had been confirmed genetically in one of the participating laboratories (Aachen, Cologne, Würzburg) within 6 months of age. These laboratories provided nearly 90% of the molecular genetic tests for SMA in all parts of Germany in the corresponding period. All patients had a homozygous deletion of the *SMN1* gene. Recruitment was through the referring physicians of all eligible patients rather than waiting for parents to respond to a general invitation to participate in this study. In order to avoid a major ascertainment bias towards families who are more prone to take initiative possibly because their child is more or less severely affected, no patient support groups, parent support groups or patient registries were asked to participate. Study forms were provided via mail between April and October 2006. Of the 174 patients who met the inclusion criteria, parents of 66 (38%) patients agreed to participate, completed questionnaires, and consented to the review of medical reports and to further DNA studies. As many as 35 parents of 99 diagnosed patients in Aachen and Würzburg (35% response rate) and 31 parents of 75 patients diagnosed in Cologne (41% response rate) participated. Consent was obtained according to the Declaration of Helsinki. The Ethical Committees of the medical faculties RWTH Aachen and University of Cologne have approved the study.

All case studies were personally reviewed by one of the authors in Aachen or Cologne and electronically documented. The following items were documented: age at onset, life/disease span, motor milestones, loss of motor functions, hospitalizations, respiratory infections requiring antibiotic treatment, assisted ventilation, nutrition/tube feeding/gastrostomy, drug treatment, symptomatic treatment (physiotherapy, orthopaedic care).

Some important clinical features were studied in more detail: Head control was scored as present when the infant was able to lift the head in prone position. Pneumonias were counted for survival statistics only if clearly documented in the medical files of a patient. Patients who received nutrition via a nastrogastric tube or a gastrostomy were regarded as tube-fed from this age. Disease endpoint was defined by age at death or the age when either tracheostomy or permanent ($>$14 days) ventilation was performed. Kaplan-Meier survival analysis was calculated with SPSS version 14 for Windows; the statistical comparison of survival curves was performed with the log rank or Mantel-Haenszel test if applicable. A p value of <0.05 was regarded as significant.

Ten patients received VPA orally at some point during the disease course, one of whom had been started on individual treatment 2 months after initiation of permanent ventilation. Thus nine patients (13.6%) were counted as being VPA-treated. As it was not clear at the time of this writing whether VPA had a clinical effect, we analysed the total cohort irrespective of VPA treatment separately from the subgroup where VPA-treated patients were excluded from the beginning of VPA intake (non-treated group).

For *SMN1* and *SMN2* gene analysis, genomic DNA of the patients was isolated from peripheral lymphocytes by a simple salting out procedure. Polymerase chain reaction (PCR)-based testing for the homozygous *SMN1* deletion was performed (6). A homozygous deletion of *SMN1* was confirmed in all patients. Dosage analysis to determine the number of *SMN2* copies was undertaken by real-time PCR (7) and/or MLPA (8).

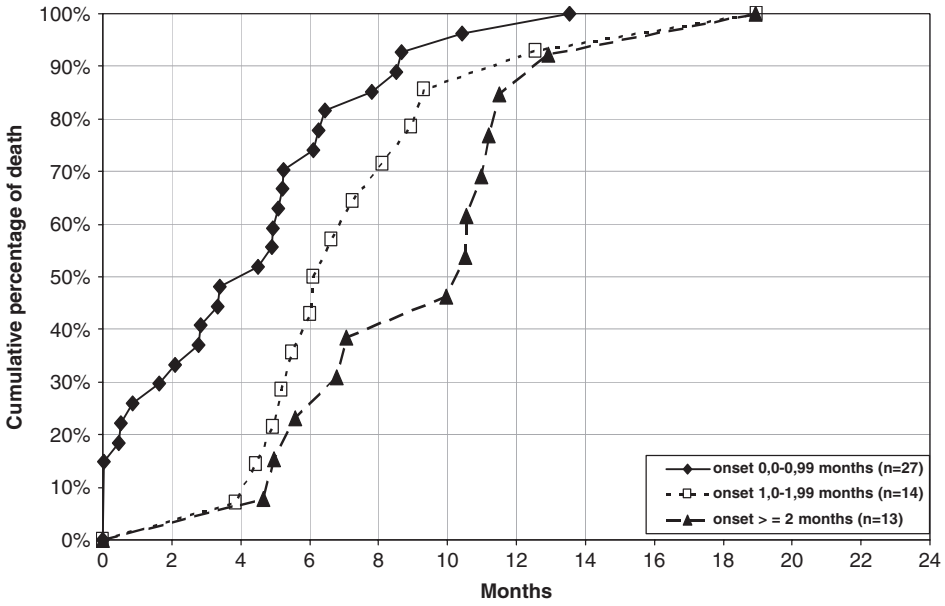

Fig. 1. Cumulative percentages of death in different age of onset groups for all patients who died or reached the functional disease endpoint.

Results

Clinical picture

Data were recruited for 66 SMA I patients; 35 boys and 31 girls; 62 unrelated patients and 2 sibships. Age at onset was within 4.5 months in all patients (median/mean 1.2/1.4 months).

Initial manifestation

Reduced fetal movements were recorded in 33% of pregnancies. In 16 patients (24%) onset of weakness was apparent in the first week of life. The leading initial symptoms were reduced motor activity (82%), followed by lack of head control (59%) and muscular hypotonia observed by the paediatrician (58%). Only a few patients (13.6%) achieved head control at some time in the development; no patient was able to maintain a sitting position – in agreement with the definition of SMA I.

Survival statistics

At the time of investigation, 57 (86.3%) patients were deceased. Five (7.6%) patients were still alive and four (6.1%) were permanently ventilated and had thus reached the disease endpoint according to the above definition. Life span until death or functional disease endpoint ranged from a few days to 55 months. There was a weak positive correlation (0.478) between age at onset and survival in the deceased patients, although a distinct onset did not allow to predict life span. The cumulative percentage of death in all patients who died or had reached their functional disease endpoint was studied for different age-at-onset groups (Fig. 1). The age when 50% of patients were deceased or permanently ventilated was 4.5 months in patients with an age at onset in the first month, 6.6 months in those with an age at onset of 1–2 months, and 10.6 months in patients with an age at onset after 2 months.

The Kaplan-Meier survival probabilities for the total cohort and after censoring of VPA treatment (non-treated cohort), respectively, are shown in Fig. 2. The mean age at death/disease endpoint was 9.0 months in both groups; the probability to survive the second birthday was 6% in the total and 3.8% in the non-treated group. The exclusion (censoring) of VPA-treated patients from the age of treatment resulted in a slight shift to the left of the survival curve from about 10 months of age (Fig. 2), the difference not being statistically significant. The 25% (all patients/non-treated patients: 4.7/4.4 months), 50% (6.6/6.1 months) and 75% (10.6/10.0 months) survival probabilities were very close in both groups but differed at the 20% probability level by 6 weeks with a better survival in all patients (10.1/11.4 months). There was no significant sex influence in the survival statistics between boys and girls.

Supportive treatment

Of those four patients who were permanently ventilated, all received invasive ventilation via tracheostomy ($n = 3$) or intubation ($n = 1$). Prior to invasive ventilation, non-invasive mask ventilation

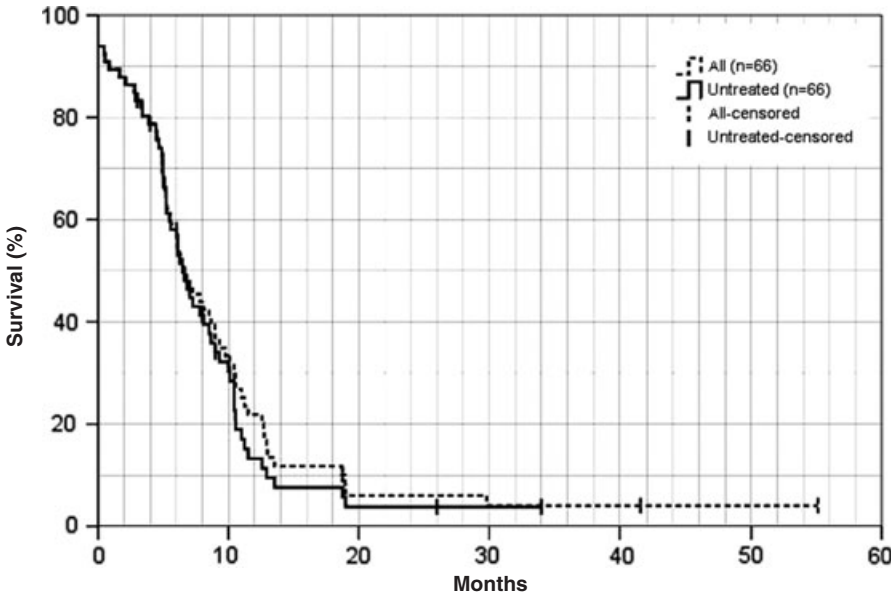

Fig. 2. Kaplan-Meier survival statistics. Comparison of the total cohort (dashed curve) *vs* the non-treated cohort (solid line). Those nine patients who were treated with valproic acid (VPA) were excluded (censored) from the survival analysis from the beginning of treatment in the non-treated cohort. Censored patients are depicted in small vertical dashes.

was applied over a period of 3 and 10 months in two patients. The period of invasive ventilation ranged from 7 to 47 months. Five patients (7.6%) were still alive at an age between 10 and 55 months (median age 18.8 months) without being permanently ventilated. Two patients received intermittent ventilation from 14 to 55 months and from 10 to 11 months; the remaining three had no respiratory problems at the time of recording.

Feeding and swallowing difficulties occurred in 60 (90.9%) patients and were treated with nasogastric tubes or gastrostomy in 51 (77.3%) patients. The period of tube feeding ranged from a few hours to 11 months until death or functional disease endpoint.

We addressed the question whether the number of pneumonias or nutritional interventions had an influence on the probability of survival. The survival analysis of patients who received antibiotic treatment because of pneumonia showed that severe lung infections are slightly more likely to occur in patients with a longer survival (Fig. 3a). Patients with respiratory distress (functional disease endpoint) from birth are included in the cohort without pneumonia and cause a parallel deviation of the curve in comparison to the cohort with at least one lung infection. This explains the non-significantly better ($p = 0.079$) probability of survival in the group of patients who had at least one pneumonia compared with none recorded (Fig. 3a). We also wanted to know whether tube feeding via nasogastric tubes ($n = 46$) or gastrostomy ($n = 3$) had an influence on life span compared with patients who had not received assisted nutrition ($n = 17$). There was no significant difference in the overall survival as both curves crossed (Fig. 3b).

Phenotype according to SMN2 copy numbers

In four (6.1%) patients, only a single copy of *SMN2* was seen. Fifty-seven patients (86.3%) had two and five patients (7.6%) had three *SMN2* copies (Table 1).

The clinical course of the four unrelated single copy patients was most severe with prenatal onset of muscle weakness, congenital contractures and respiratory distress from birth. Life span did not exceed a few months; all infants survived only upon mechanical ventilation (SMA type '0'). Three patients with a single *SMN2* gene copy had congenital heart defects (atrial or ventricular septal defects) that were not explained by intrauterine muscle weakness, respiratory insufficiency at birth or other causes (9).

Patients with two *SMN2* copies showed a variable disease course. Motor development was limited to head control in 7.0%. Median/mean age at onset was 1.2/1.3 months and median/mean age at disease endpoint was 6.5/7.8 months (range 0.5–30 months). After excluding seven patients who received VPA (non-treated group, $n = 50$) median/mean age at death was 6.1/6.7 months (range 0.5–19.0 months). Severity of patients with

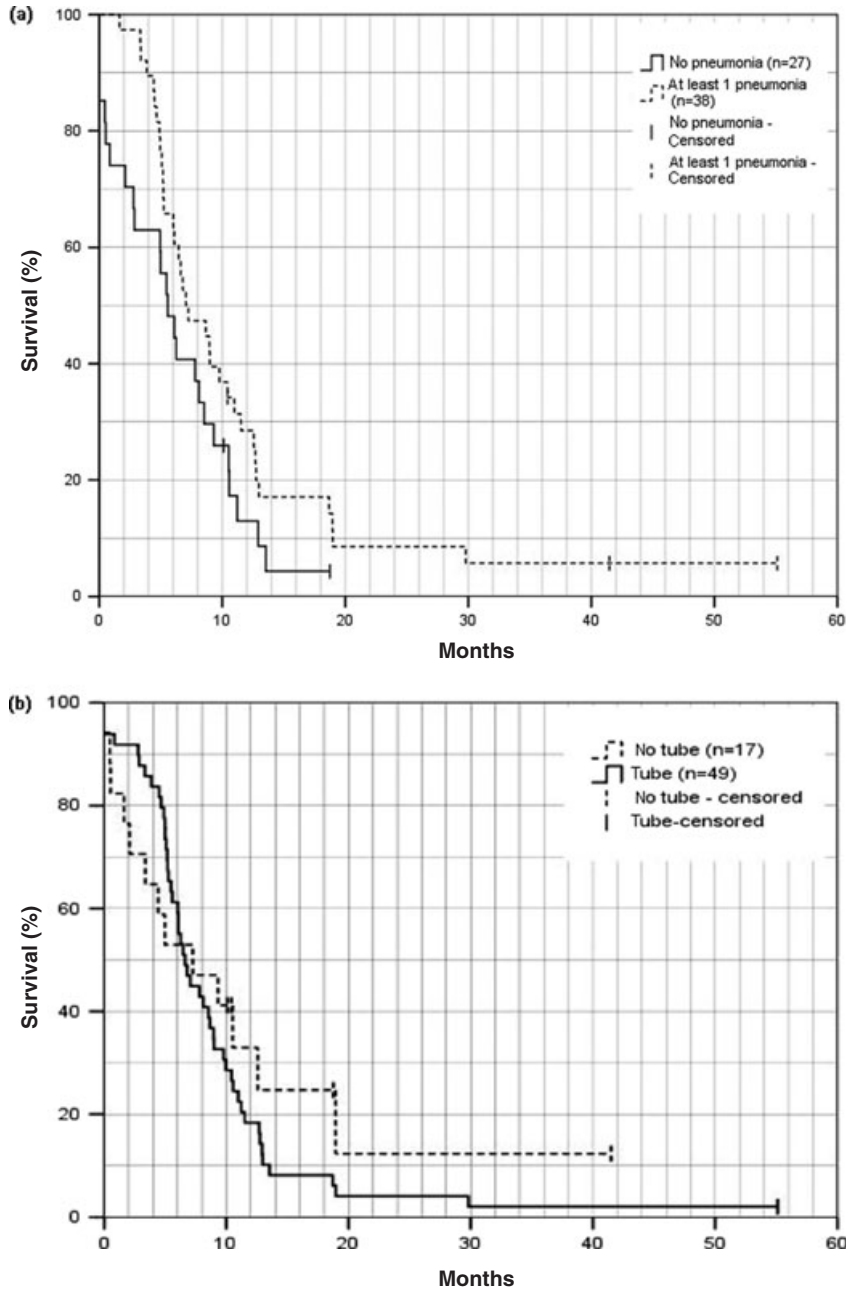

Fig. 3. Influence of supportive management on survival in the total cohort. **(a)** Survival statistics in 27 patients who had at least one recorded pneumonia (dashed line) compared with those who had not (solid line). In one patient, details regarding respiratory infections were not reported. **(b)** Survival statistics in 49 patients with assisted nutrition – nasogastric tube, gastrostomy–(solid line) compared with those without (dashed line).

two *SMN2* copies showed the full spectrum of all SMA I phenotypes including those with prenatal onset, neonatal respiratory distress and early death (Fig. 4a) and those with a stabilising course and longer survival (Fig. 4b) corresponding to patients who had three *SMN2* copies.

Those five patients who retained three *SMN2* copies of at least exon 7 had an onset at 2–4.5 months and a much better prognosis. All patients had achieved head control. One patient was ventilated from 19 months and was treated with VPA from 21–47 months (Fig. 5, patient 8); the others were alive at 10–55 months (median/mean age 30.1/31.4 months). One patient received nocturnal non-invasive ventilation from 15 months and was tube-fed from 3 years, his current age being 55 months. He received VPA from 34 months of age (Fig. 5, patient 9). The remaining three patients had no ventilatory or nutritional support at ages 10, 19 and 41 months, one of whom (Fig. 5,

Table 1. Clinical characteristics of the study group according to *SMN2* copy number

Item	One *SMN2* copy ($n = 4$)	Two *SMN2* copies ($n = 57$)		Three *SMN2* copies ($n = 5$)	
		All patients	Non-treated patients[a]	All patients	Non-treated patients[a]
Age at onset: median/mean (range) in months	0.0/0.0 (0.0–0.0)	1.2/1.3 (0.0–4.0)	1.2/1.3 (0.0–4.0)	3.5/3.4 (2.0–4.5)	3.5/3.4 (2.0–4.5)
Age at disease endpoint or current age: median/mean (range) in months	0.0/0.0 (0.0–0.0)	6.5/7.8 (0.5–29.8)	6.1/6.7 (0.5–19.0)	19.0/28.9 (10.1–55.1)	19.0/21.6 (10.1–34.0)
Probability of survival >2 years	0%	2%	0%	67%	40%
Head control (%)	0 (0%)	4 (7.0%)	4 (7.0%)	5 (100%)	5 (100%)

[a]This cohort includes 57 patients who did not receive VPA and 9 patients who were disregarded (censored) from the beginning of VPA intake.

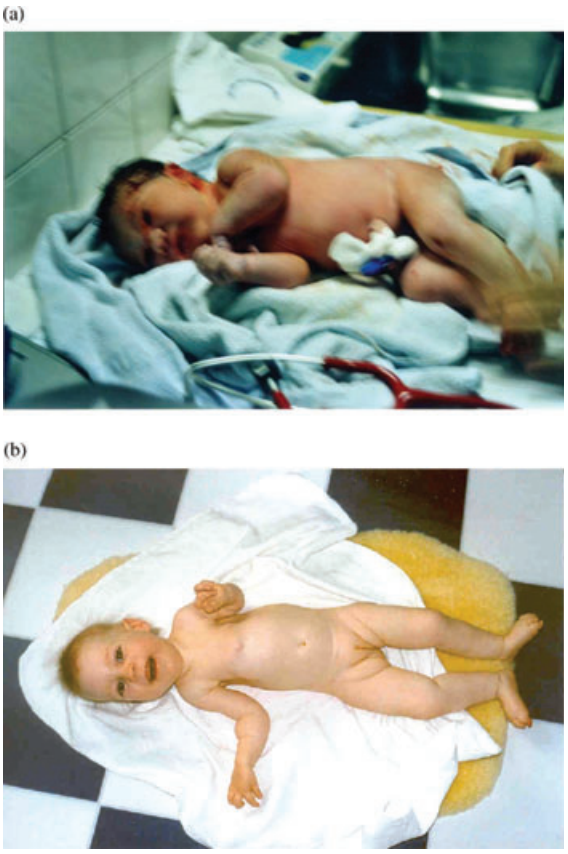

Fig. 4. Patients with two *SMN2* copy numbers displaying different degrees of severity. **(a)** Severe SMA type '0'; reduced fetal movements, severe muscular hypotonia at birth, congenital joint contractures, death at 14 days. **(b)** SMA I with prolonged survival; onset at 2 months, no motor functions, never ventilated, death at 30 months.

patient 10) took VPA from 26 months until the time of investigation (41 months). Thus, the presence of three *SMN2* copies was associated with better function and longer life span irrespective of VPA treatment (Table 1).

VPA treatment

Ten patients received VPA orally (liquid dose about 20 mg/kg) at some point during the disease course (Fig. 5), one of whom had been

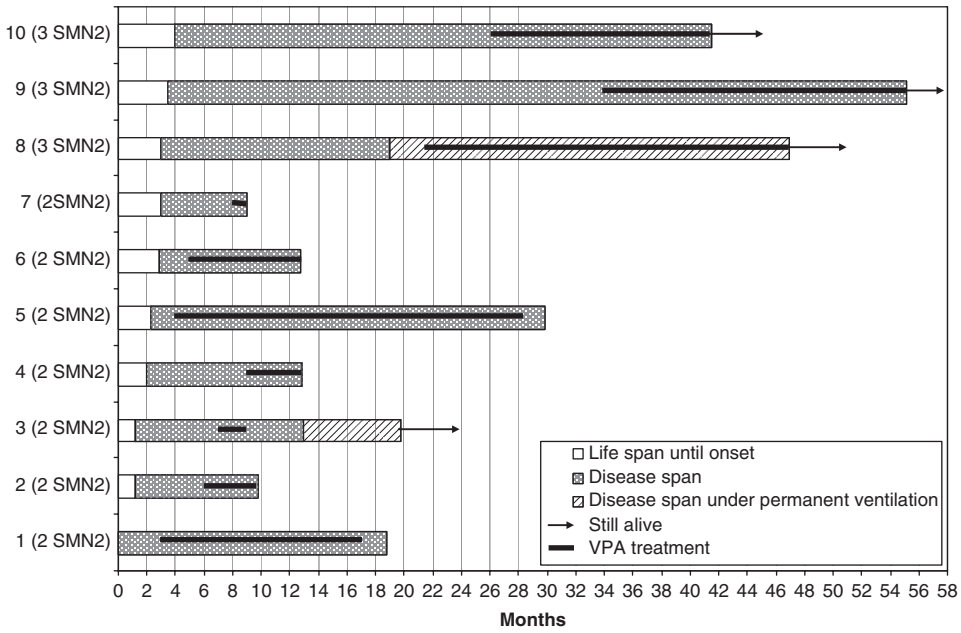

Fig. 5. Bar chart of 10 patients who were involved in individual curative trials with VPA at some point during the disease, arranged by increasing age at onset. The *SMN2* copy number is given in brackets. The right end of each bar indicates the age at death in six patients; four patients (arrows) were marked with their current ages as they were still living at the time of study entry.

started on individual treatment 2 months after initiation of permanent ventilation. From the nine patients (13.6%) counted as being VPA-treated, four had an onset at or after 3 months of age. Median/mean age at onset in the non-treated group was 1.0/1.3 months and 2.3/2.2 months in the treated group. Seven VPA-treated patients had two and two had three *SMN2* gene copies. There was a significant difference of Kaplan-Meier survival statistics in treated *vs* non-treated patients but this was related to the fact that treatment was preferentially offered to milder SMA I patients. Median/mean age of starting VPA treatment was 7.0/11.3 months, i.e. at an age when the majority of non-treated patients was already deceased (Table 2). Median/mean survival in the non-treated group was 6.1/6.8 months and 13.0/22.5 months in the treated group. Duration of VPA treatment was 1.0–25 months; six treated patients died at 9–30 months (median 12.7 months). Three patients under treatment were still alive at the time of investigation; one girl became respirator-dependent 5 months after initiation of VPA intake. The two living patients (41 and 55 months) who received VPA from 26 and 34 months had three *SMN2* copies. There was no evidence that the number of pneumonias or hospitalizations due to lung atelectasis was higher during VPA treatment compared with the disease period before or after treatment. In three patients VPA treatment was discontinued due to increased mucus production, respiratory insufficiency or infections. In one patient dosage had to be reduced because of hyperammonaemia.

Discussion

In this study we tried to systematically assess life span and functional items in SMA I in correlation with the number of *SMN2* gene copies. Because quantitative analysis of *SMN* copy numbers has been implemented in research testing only recently and should not be performed as part of diagnostic testing, *SMN2* copy number is as yet unknown in patients from larger studies on the natural history in SMA I with a few exceptions (7, 10).

We chose to focus on patients born after 2000 as standard of care and symptomatic treatment in SMA I underwent dramatic changes over the past decades. An increasing number of SMA I patients receives aggressive antibiotic treatment for respiratory infections, early nutritional and respiratory assistance and drug treatment (current recommendations, see ref. (11)) The respiratory management in SMA I patients varies from country to country and is still a highly controversial area. There is no doubt that survival is prolonged for years or even decades if assisted ventilation is applied in SMA I (12–14), and any kind of

Table 2. Clinical characteristics of the study group according to valproic acid (VPA) treatment

Item	All patients ($n = 66$)	Non-treated patients ($n = 66$)[a]	Patients receiving VPA ($n = 9$)
SMN2 copy number	1–3	1–3	2–3
Age at onset: median/mean (range) in months	1.2/1.4	1.0/1.3	2.3/2.2
	(0.0–4.5)	(0.0–4.5)	(0.0–4.0)
Age at starting VPA: Median/mean in months			7.0/11.3
Age at disease endpoint or current age: median/mean (range) in months	6.7/9.0	6.1/6.8	13.0/22.5
	(0.0–55.1)[b]	(0.0–34.0)	(8.9–55.1)*
Probability of survival >2 years	6%	3.8%	33%
Head control (%)	9 (13.6%)	9 (13.6%)	4 (44.4%)

[a]This cohort includes 57 patients who did not receive VPA and 9 patients who were disregarded (censored) from the beginning of VPA intake.

[b]Included are two patients who lived at the age of 41 and 55 months without permanent ventilation.

proactive care that is not standardized in clinical trials is likely to yield inclusive results. Therefore we chose to define the functional endpoint of the disease either by the age at death or the age when permanent ventilation was initiated.

As prospective studies in rare diseases are mostly limited by small patient numbers in a reasonable period of time, we decided for a retrospective design which is confined by several factors. The most important issue was to avoid any kind of ascertainment bias as outlined in Patients and Methods. We attempted to contact all families with SMA I born in the geographical areas where the three participating laboratories provide the *SMN1* gene testing. More than 80% of patients from all regions in Germany were diagnosed in the participating laboratories. Assuming an incidence of SMA I of 1 in 20,000 (15) and an annual birth rate of about 700,000 in Germany in the corresponding period, the total number of SMA I patients born between 2000 and 2005 was estimated to be 210. With 174 patients diagnosed in the participating laboratories, only a small number (about 5 per year) of SMA I patients were missing and were not part of our database. In conclusion, we tried to reduce to a minimum any potential selection of cases that might be caused by either clinical or geographical factors.

The main limitation is the small number of participating families, and it cannot be excluded that responders differ in clinical severity and management from non-responders. The proportion of eventually recruited patients was similar in Aachen (35%) and Cologne (41%), i.e. there was no selection bias between the two study centres. The reasons for no return included 'unknown', 'unknown contact details of family', 'parents or physicians emotionally upset by death of child'. The limitation given by inaccurate parental information does not play a major role in our study design as the hard clinical facts used for the statistics (onset, medical intervention, life span) were clearly documented in the medical files.

Survival analysis in SMA I differed in previous studies depending on the in- and exclusion criteria used and were difficult to compare with our study or with each other. Cumulative death rates were reported in the UK (16), Finland (17) and Germany (18). In a British series only those patients who never sat and died within 2 years were included (16). Interestingly, cumulative death statistics according to age-at-onset groups assessed in 99 patients (16) were very similar like ours (Fig. 1). Patients with an age at onset in the first 2 months, subdivided into onset groups 'at birth', '0–1 month' and '1–2 months' showed very uniform cumulative death statistics; 50% were deceased by 5–6 months of age. Patients with an age at onset of 3–6 months had a longer survival with a 50% death rate of 9 months. Nearly identical death rates were seen in the Finnish study (17). In Germany (18) death rates were only marginally better with 50% being deceased (without intervention) at the age of 7–8 months if age at onset was within the first 3 months. We conclude from these data that our current patient cohort reflects a representative group of SMA I patients without much bias towards a specific phenotype.

Kaplan-Meier statistics of our study group are well in accordance with the results obtained in a recent prospective study in the Netherlands (10). Median age at death was 176 days as compared to

185 days (6.1 months) in our non-treated group. The proportion of patients surviving more than 2 years was also identical with 6% reflecting a comparable impact of medical care. Survival probabilities were also calculated in larger patient samples including SMA I–III in Germany (19) and Hongkong (20). In the previous German study conducted in the 1980s and 1990s (19), all patients were regarded as SMA I who did not achieve a sitting position. This definition included 197 SMA patients with an onset in the first 9 months and resulted in a high proportion of long-term survivors (32% at 2 years). Using a similar approach, even higher survival probabilities were calculated in 22 Chinese SMA I patients (40% at 2 years) (20).

Data for a recent American study on the changing history in SMA I (14) were obtained from an international SMA patient registry which collects data through voluntary participation of patients and their families. Response rate of a mailed questionnaire was 49.1%.Two sample groups of SMA I patients were compared, 65 patients born between 1980 and 1994 and 78 patients born between 1995 and 2006. It was found that probability of survival was significantly higher in patients recruited after 1995 and that ventilation and gastrostomy tube feeding had independent effects on survival. In this particularly selected patient cohort, probability of survival until death or permanent ventilation at the age of 2 years was 17.5% in SMA I patients born between 1980 and 1994 and 47% in patients born between 1995 and 2006. Age at onset and age at diagnosis were significantly lower in the earlier born than in the later born group. From our view, a major ascertainment bias was responsible for the recruitment of patients with a better prognosis and active parents in the study by Oskoui et al. (14). It was suggested by the authors themselves that their sample might differ from the general SMA type I population.

Neither in our study (nor in others) we found any evidence that tube feeding prolongs survival of severe SMA I, as long as the same clinical entry criteria are used. Nonetheless, proactive nutritional supplementation is required as soon as inadequate oral intake is recognized (11). There is international consensus that gastrostomy feeding is the optimal method of feeding as it prevents the potential morbidity associated with prolonged use of nasogastric or nasojejunal tubes (11), but exposing severely affected infants to the risk of surgery is an important issue which has to be discussed by the caregivers on an individual basis.

In addition, the number of respiratory infections and corresponding interventions increased with longer disease span do not allow, of course, to conclude that pneumonias have a protective effect on SMA.

The question whether the number of *SMN2* copies is useful for the definition of a possible historical control group for clinical trials remains a matter of debate. With the introduction of quantitative methods for calculating *SMN* copy numbers, information about *SMN2* copy numbers and their influence on disease severity has become available in the past 10 years. The distribution of *SMN2* copy numbers in SMA I patients varies in the literature depending on the selection of patients. The proportion of patients with a single *SMN2* copy ranged from 0% in smaller series (21–23) to 7% (13/188) (7), 10% (3/29) (10) and 19% (3/16) (24). SMA I patients with three *SMN2* copies were observed in 4% (2/52) (25), 10% (3/29) (10) and 20% (37/188) (7) depending on the inclusion criteria of patients. SMA I patients with four *SMN2* copies have not yet been observed. The vast majority of SMA II patients harbours three *SMN2* copies, but our data and those of other studies clearly show that the presence of three *SMN2* copies can result in SMA I (with longer life span), SMA II or SMA III.

We believe that our approach by including patients with the genetic diagnosis latest by the age of 6 months is reasonable as setting the upper age limit for an onset within 6 months bears the risk of accidentally including SMA type II patients. This was seen in the prospective Dutch study where initially 47 patients with an onset within 6 months were recruited and 2 of them turned out to be SMA II (10).

It was also suggested to exclude patients with a ‘neonatal onset’ SMA, although this had not been further outlined by the members of the 134th ENMC meeting (1). This is clearly a critical point as the neonatal period lasts 28 days by definition, and 41% of the patients in this study had an onset in this period. This problem has also been addressed by Cobben et al. (10) who found a wide range in survival (0 and >450 days) in congenital onset cases. If most severe SMA type ‘0’ were to be excluded in a trial design, we suggest to exclude patients with respiratory distress from birth, i.e. patients who died within the first 7 days or are being permanently ventilated. In the present study, this group consisted of four patients with exclusively a single *SMN2* copy. The earliest age at disease endpoint in the group with two *SMN2* copies is the patient shown in Fig. 4a who died at 14 days. Our observations differ from those made in the Dutch study where the 3 patients with one *SMN2* copy showed a longer life span that was not significantly different from the 23 patients with

two *SMN2* copies (10). It was not communicated whether there was a period of respiratory distress prior to death or if some kind of respiratory management was applied in the Dutch cohort, as the results concentrated on survival. Therefore, the reason for this discrepancy should be addressed in larger studies.

From our data we conclude that in future, placebo randomized clinical trial patients should be stratified according to *SMN2* copy numbers between the placebo and the treated arm. In order to define a largely homogeneous patient population, the clinical characteristics of patients harbouring two *SMN2* copies might be used as baseline for a possible historical control group: age at onset within 4–5 months, age at genetic diagnosis <6 months, homozygous *SMN1* deletion and two *SMN2* copies present, head control possible in less than 10% of patients, unaided sitting not achieved, no respiratory distress from birth, functional disease endpoint either age at death or age at permanent ventilation (tracheostomy or non-invasive ventilation >14 days for >16 h a day). In this group, the probability to survive more than 2 years is only 2% as compared to 67% in patients with three *SMN2* copies (Table 1).

One has to bear in mind that even between SMA patients with similar ages at onset and similar motor functions, survival is variable and might be influenced by difference in standard of care and/or by the genetic background. A small but considerable proportion of severe SMA I patients has a life span of more than 2 years without medical intervention. This is in accordance with previous reports and has major implications regarding future trials in SMA I if life expectancy is considered the main outcome measure. Our data are insufficient to draw any conclusion on the efficacy of VPA in SMA I patients as they are confounded by the fact that treated patients tended to have later disease onset, better function and higher *SMN2* copy numbers than untreated patients. Individual treatment experience cannot be interpretable without randomization or concurrent controls, as this happened in Germany and in other countries of the world. Therefore the authors stress the importance of placebo-controlled clinical trials, although this may be hampered by parents' compliance.

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