- SUPPLEMENTARY FILE -

Methods and additional data on clinical characteristics and motor development

Association of motor milestones and SMN2 copy and outcome in spinal muscular atrophy types 0–4

PATIENTS AND METHODS

We enrolled patients with SMA types 1-4 between September 2010 and August 2014. Patients were informed and recruited through the Dutch patient organisation for neuromuscular diseases (www.vsn.nl), (paediatric) neurologists, paediatricians, rehabilitation physicians, the five Dutch Centres for Chronic Respiratory Ventilation and through patient communities on the internet. Inclusion criteria were a clinical diagnosis of SMA type 0, 1, 2, 3 or 4. There was no age restriction for inclusion. Informed consent was obtained from each subject and/or their parents in children younger than 18 years of age. All patients who agreed to participate visited the outpatient clinic for (paediatric) neurology at the University Medical Centre Utrecht, The Netherlands. The study protocol was approved by the Medical Ethical Committee of the University Medical Centre Utrecht. This study was registered at the Dutch registry for clinical studies and trials (http://www.ccmo-online.nl).

We used age at onset and acquired motor skills to define SMA types according to the diagnostic criteria defined by the SMA Consortium.[1] In case of discrepancy between age at onset and reached motor milestones, the latter determined the final diagnosis. SMA type 0/1a was defined by a prenatal onset of the disease with symptoms of hypotonia and respiratory insufficiency directly after birth with early neonatal death.[2,3] SMA type 1 (abc) was defined by an onset before 6 months and the inability to sit independently at any time. Patients with type 0/1a shows signs in the neonatal period with no head control ever achieved. Type 1b show signs of hypotonia after the neonatal period and will never have head control or will ever be able to roll. Type 1c are patients who meet the criteria of type 1, and not type 2, but show a relative better performance in motor skills (e.g. head control, roll over) or pulmonary function (no pulmonary complications before the age of 2). Type 1c with survival into childhood or adulthood with or with respiratory support after the age of 24 months has been reported in previous publications.[4-6]

Patients with SMA type 2 had onset between the age of 6 and 18 months and learned to sit or even stand (but not walk) for a brief period independently. Patients with SMA type 3 developed weakness after the age of 18 months and learned to walk independently. Patients with type 3 were further divided into SMA type 3a (disease onset before the age of 3) and type 3b (disease onset after the age of 3).[7] SMA type 4 was defined by an onset after the age of 30 in ambulatory patients.[2,7]
We used a systematic questionnaire according to the NINDS CDE guidelines (http://www.commondataelements.ninds.nih.gov) about present clinical situation, age at acquiring or losing motor milestones (head control, lifting head in prone position, roll over, sit, stand, walk, run and/or walking stairs),[8] medical and family history and performed neurological examination.

GENETIC ANALYSIS

All patients were tested for a homozygous deletion of exon 7 of the SMN1 gene or a hemizygous SMN1 deletion and a point mutation. The total number of SMN1 and SMN2 gene copies was determined by Multiplex Ligation-dependent Probe Amplification (MLPA) analysis using SALSA MLPA kit P060 version B2, according to the manufacturer's protocol (www.mlpa.com; www.mrcholland.com). SMN2 copy number analysis was performed twice to confirm copy number by two certified laboratories at the Departments of Genetics (Utrecht and Groningen, the Netherlands). In case of a heterozygous deletion of SMN1, we searched for point mutations using Sanger sequencing (SMN1 reference sequence NM_000344.3) with customized primers. The pathogenic character of point mutations was confirmed using mRNA analysis.

STATISTICAL ANALYSIS

Univariate and multivariate tests including dichotomous data were performed using (penalised-likelihood) logistic regression. We used Kruskal-Wallis or Chi-square/Fisher Exact analysis to compare data between SMA types and survival curves and log rank tests to analyse differences in age at the time of loss of ambulation, scoliosis surgery or start of ventilation. P values of <0.05 were considered significant. We used SPSS (IBM SPSS Statistics version 21, Inc., Chicago, IL) and R64 (R 2.14.2 GUI 1.50 Leopard build; R Foundation for Statistical Computing 2012) for analysis.

FIGURE

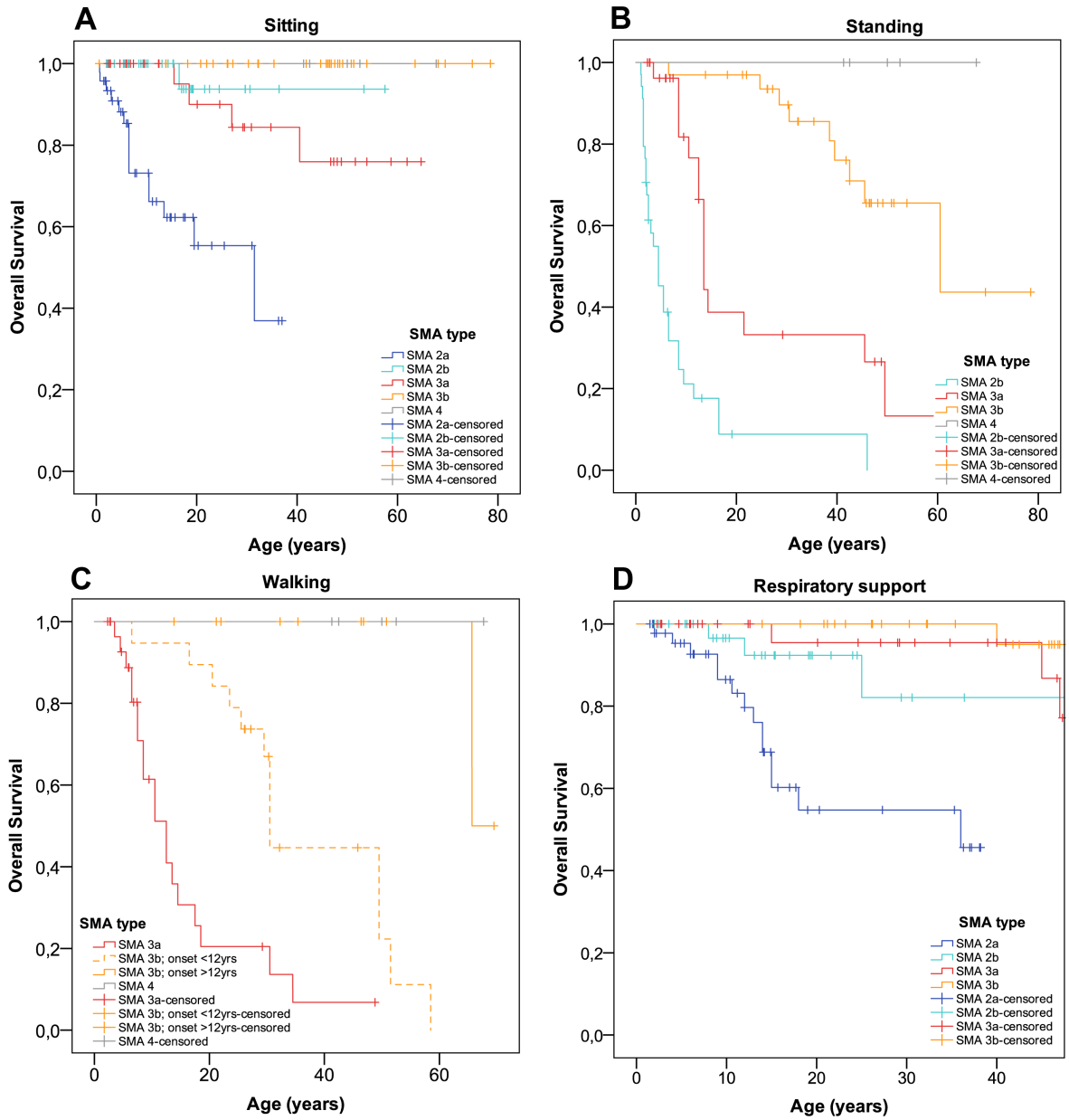

FIGURE LEGEND

Figure 1ABCD. Kaplan-Meier curves of sitting, standing, walking and respiratory function

Probability of the age at time of loss of the ability to sit (A), stand (B) or walk (C) in SMA types 2-4 and the age at time of start of respiratory support (D). SMA type 2a: patients who learned to sit independently, but never stand or walked. SMA type 2b: patients who learned to sit independently, and have learned to stand or walk with support. Censored patients are patients who had not lost the motor ability or did not use respiratory support at time of inclusion. C. Loss of dependent ambulation differs per age group (log rank p<0.001). Seventy percent of patients with types 3a, 3b onset <12

years and 3b onset >12 years had lost ambulation by the age of 8, 20 and >30 years, respectively. D. Age at start of respiratory support at night differed in patients with SMA types 2a, 2b or 3a (log rank $p<0.01$). Fifty percent of patients with SMA type 2a used respiratory support before the age of 20 years, whereas only 20% of patients with SMA types 2b and 3a needed respiratory support around the age of 25 and 50 years, respectively. Three of five patients with SMA type 3a used non-invasive respiratory support because of obstructive sleep apnea. Two patients with type 3a needed invasive respiratory support after a severe pneumonia.

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