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SMN1 Duplications Are Associated With Progressive Muscular Atrophy, but Not With Multifocal Motor Neuropathy and Primary Lateral Sclerosis

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Abstract

Objective

To assess the association between copy number (CN) variation in the survival motor neuron (*SMN*) locus and multifocal motor neuropathy (MMN), progressive muscular atrophy (PMA), and primary lateral sclerosis (PLS) susceptibility and to determine the association of *SMN1* and *SMN2* CN with MMN, PMA, and PLS disease course.

Methods

In this monocenter study, we used multiplex ligation-dependent probe amplification to determine *SMN1* and *SMN2* CN in Dutch patients with MMN, PMA, and PLS and controls. We stratified clinical parameters for *SMN1* and *SMN2* CN. We analyzed *SMN1* and *SMN2* exons 1–6, intron 6, and exon 8 CN to study the genetic architecture of *SMN1* duplications.

Results

SMN1 and *SMN2* CN were determined in 132 patients with MMN, 150 patients with PMA, 104 patients with PLS, and 956 control subjects. MMN and PLS were not associated with CN variation in *SMN1* or *SMN2*. By contrast, patients with PMA more often than controls carried *SMN1* duplications (≥3 *SMN1* copies, 12.0% vs 5.0%, odds ratio 2.69 (1.43–4.91), p 0.0020). *SMN1* and *SMN2* CN status was not associated with MMN, PLS, or PMA disease course. In case of *SMN1* exon 7 duplications, exons 1–6, exon 8, and introns 6 and 7 were also duplicated, suggesting full *SMN1* duplications.

Conclusions

SMN1 duplications are associated with PMA, but not with PLS and MMN. *SMN1* duplications in PMA are balanced duplications. The results of this study highlight the primary effect of altered *SMN* CN on lower motor neurons.

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Glossary

ALS = amyotrophic lateral sclerosis; **CN** = copy number; **CNV** = CN variation; **LMN** = lower motor neuron; **MLPA** = multiplex ligation-dependent probe amplification; **MMN** = multifocal motor neuropathy; **MRC** = Medical Research Council; **OR** = odds ratio; **PAN** = Prospective ALS Study in the Netherlands; **PLS** = primary lateral sclerosis; **PMA** = progressive muscular atrophy; **SMA** = spinal muscular atrophy; **SMN** = survival motor neuron; **UMCU** = University Medical Center Utrecht; **UMN** = upper motor neuron.

The survival motor neuron (*SMN*) locus, including *SMN1* and the nearly identical *SMN2*, has gone through extensive duplication, deletion, and gene conversion events, causing variation in *SMN1* and *SMN2* copy number (CN).[1] Heterogeneity at this locus has been associated with motor neuron disorders. Homozygous *SMN1* deletions lead to lower motor neuron (LMN) degeneration in hereditary proximal spinal muscular atrophy (SMA), the severity of which is inversely correlated with *SMN2* CN.[2,3] *SMN1* duplications, by contrast, have been associated with amyotrophic lateral sclerosis (ALS), a neurodegenerative disease characterized by progressive loss of both upper MN (UMN) and LMN.[4,5] No study has systematically investigated the association of other motor neuron disorders with *SMN* CN variation (CNV).

Multifocal motor neuropathy (MMN) is a rare, slowly progressive inflammatory neuropathy, characterized by asymmetrical distal muscle weakness, which responds to immunoglobulin treatment.[6-8] MMN is associated with specific HLA haplotypes, suggesting genetic susceptibility underlying disease pathogenesis.[9] The selective motor neuron vulnerability seen in MMN is not fully explained. Progressive muscular atrophy (PMA) is characterized by progressive LMN degeneration, leading to muscular atrophy, muscle weakness, and, in some cases, premature death. Disease progression can be fast and reminiscent of ALS but is sometimes slow with patients surviving for decades.[5,10] In contrast with the LMN vulnerability in PMA and MMN, primary lateral sclerosis (PLS) is characterized by progressive degeneration of UMNs alone.[5]

Here, we hypothesized that the *SMN* locus modifies susceptibility for a range of motor neuron disorders and determined *SMN* CNV in 3 large cohorts of Dutch MMN, PMA, and PLS patients.

Methods

Study Population

Patients With MMN, Patients With PMA, Patients With PLS, and Controls

All patients with MMN were diagnosed and enrolled at the outpatient clinic of the University Medical Center Utrecht (UMCU), a tertiary neuromuscular referral center and national center of expertise for MMN, ALS, and SMA. All patients fulfilled the most recent diagnostic criteria for definite, probable, or possible MMN.[11] These criteria mostly rely on the combination of a typical clinical phenotype combined with conduction block found on nerve conduction studies or, in the absence of conduction block, on the basis of abnormal ancillary investigations and/or a response to treatment with immunoglobulins. All patients with MMN were Dutch.

Patients with PMA and PLS were enrolled through the Prospective ALS Study in the Netherlands (PAN), a Dutch population–based prospective case-control study.[12] All patients with PMA suffered from a progressive LMN loss without signs or symptoms consistent with UMN involvement, that is, no pseudobulbar affect, increased jaw jerk, muscle hypertonia, pathologic deep tendon reflexes, or pathologic signs including extensor plantar (Babinski) reflex or ankle clonus. Patients with PLS showed selective UMN loss, that is, pseudobulbar dysarthria, pseudobulbar affect, hypertonia, pathologic deep tendon reflexes, or pathologic signs including extensor plantar (Babinski) reflexes or clonus. None of the patients with PLS showed signs or symptoms of LMN degeneration, that is, no atrophy, fasciculations, or diminished or absent deep tendon reflexes.

Dutch population–based controls were also enrolled through the PAN.[12] Controls enrolled between January 2012 and August 2018 were included in this study. None of the controls were diagnosed with MND after inclusion in this study.

Clinical Data

For patients with MMN, PMA, and PLS, clinical data were drawn from the MMN or PAN database.[12,13] When needed, these data were supplemented with data from UMCU patient files.

For all patients, recorded data included sex, age at onset, diagnostic delay, site of onset, response to intravenous immunoglobulin treatment, presence of anti-GM1 IgM antibodies, and muscle strength testing on the first and last visit to the outpatient clinic. Results of nerve conduction studies were used to assess the presence of conduction block in patients with MMN. Survival data from patients with PMA and PLS were obtained by checking the last date the patient was known to be alive in the municipal population register (updated at quarterly intervals).

Onset of disease was defined as the first complaint of muscle weakness. Diagnostic delay was defined as the time between

the onset of muscle weakness and diagnosis. Anti-GM1 IgM antibody testing was performed using standardized ELISA.[14] Results of muscle strength testing on the first and last visit to the outpatient clinic were quantified on the 6-point Medical Research Council (MRC) scale. This scale ranges from 0 (no muscle contraction) to 5 (normal muscle strength against resistance). We documented the MRC scores for left and right shoulder abduction, elbow flexion and extension, wrist flexion and extension, finger flexion and extension, finger spreading, hip flexion, knee flexion and extension, and foot dorsal and plantar flexion. An MRC sum score was calculated by the summation of the MRC scores of all tested muscle groups (range 0–130). As an outcome measure in patients with MMN who were untreated at their first visit, a ΔMRC sum score/month was calculated by subtracting the MRC sum score at the first from that at the last visit, divided by the follow-up time.

In patients with MMN, nerve conduction studies were performed as described previously.[9,13] In short, motor and sensory function were tested bilaterally in the median, ulnar, radial, musculocutaneous, peroneal, and tibial nerves. In nerves with a distal CMAP of >1 mV, the presence of conduction blocks was assessed, where a definite block was defined as a CMAP reduction of at least 50% and a probable block as a reduction of 30%–50%.

Survival was defined as the time between the onset of muscle weakness and death or last date known alive.

DNA Samples

Genomic DNA was extracted from whole blood using standard DNA isolation methods. Samples from controls, patients with PMA, and patients with PLS were obtained upon participation in the PAN. Samples from patients with MMN were obtained during the Dutch national cross-sectional studies on MMN performed in 2007 and 2015.

SMN CNV Analysis

We used multiplex ligation-dependent probe amplification (MLPA) to assess CNV in the *SMN* locus. We used the SALSA MLPA P021-B1 SMA probe mix (MRC Holland, Amsterdam, The Netherlands). All analyses were performed as described previously.[2]

SMN1 and *SMN2* exon 7 specific probes were used to determine the *SMN1* and *SMN2* CN. The *SMN1/2*Δ7-8 gene variant CN was determined by subtracting the median CN of the 7 probes targeting *SMN1* and *SMN2* intron 6, as well as exons 7 and 8 from the median CN of the 10 probes targeting *SMN1* and *SMN2* exons 1–6. For example, when the median CN of the intron 6–exon 8 region of *SMN1* and *SMN2* was 3 and the median CN of *SMN1* and *SMN2* exons 1–6 was 4, this indicated the presence of 1 copy of the *SMN1/2*Δ7-8 gene variant.[15]

Statistical Analysis

We used R (version 3.6.1) to perform statistical analyses. *SMN1*, *SMN2*, and *SMN1/2*Δ7-8 CN in patients with MMN, PMA, and PLS were separately compared with controls using a χ^2 or Fisher exact test, as appropriate. Odds ratios and 95% confidence intervals were calculated.

Patients were stratified by the *SMN1* CN (1, 2, or ≥3 copies). To assess possible *SMN2* disease modification, clinical parameters within each *SMN1* group were stratified by the *SMN2* CN. We compared continuous variables using ANOVA or Kruskal-Wallis testing, as appropriate. In case of a p value of <0.05, a pairwise t test or Wilcoxon signed-rank test was performed. We used a prerequisite of at least 5 observations per group for pairwise comparisons.

Survival analyses within the PMA and PLS groups were performed using the "Survminer" package. Kaplan-Meier curves were drawn, stratified by the *SMN1* CN. At least 5 observations per stratum were required for survival analyses. The assumption of proportional hazard was tested, and hazard ratios with 95% confidence intervals were calculated. To assess possible *SMN2* disease modification, each *SMN1* group was stratified by the *SMN2* CN, and the same survival analyses were performed.

p-Value adjustment using the Bonferroni method was applied in case of multiple testing. A corrected p value of <0.05 was considered statistically significant.

Standard Protocol Approvals, Registrations, and Patient Consents

The locally appointed ethics committee of the University Medical Center Utrecht gave approval for this study (METC Utrecht, METC.NL.041). All included patients gave written informed consent before inclusion in this study.

Data Availability

The data that support the findings in this study will be available on reasonable request from the corresponding author.

Results

Study Population

We included 137 patients with MMN, 159 patients with PMA, 105 patients with PLS, and 981 controls. We obtained results from 132 patients with MMN (96.4%), 150 patients with PMA (94.3%), 104 patients with PLS (99%), and 956 controls (97.4%). Baseline characteristics are shown in table 1. Sex, age at onset, diagnostic delay, and site of onset were recorded in all patients. Clinical parameters of patients with MMN, that is, the first visit MRC sum score and the number of affected limbs at the first visit, are shown for patients who were treatment naive at their first visit (n = 102). Clinical parameters in the PMA group were recorded for all included patients. At the end of this study, 55% and 36% of patients with PMA and patients with PLS had deceased, of whom 47% and 13%, respectively, had died within 4 years after disease onset.

Table 1 Baseline Characteristics of Patients With MMN, PMA, and PLS

	MMN (n = 132)	PMA (n = 150)	PLS (n = 104)	Controls (n = 956)
Male sex (n, %)	102 (77)	119 (79)	57 (55)	592 (62)
Age[a]	42 (15)	62 (15)	58 (16)	66 (9)
Delay in mo[a]	42 (62)	17 (18)	34 (43)	
Site of onset (n, %)				
Bulbar	0 (0)	1 (1)	25 (24)	
Spinal	132 (100)	146 (97)	79 (76)	
Thoracic/respiratory	0 (0)	3 (2)	0 (0)	
EFNS diagnosis (n, %)				
Definite MMN	94 (73)	NA	NA	
Probable MMN	24 (19)	NA	NA	
Possible MMN	10 (8)	NA	NA	
Anti-GM1 IgM antibodies (n/N, %)	75/116 (65)	2/18 (11)	0/0 (0)	
IVIg response (n/N, %)	118/126 (94)	0/2 (0)	0/0 (0)	
IVIg treatment at the first visit (n/N, %)	27/129 (21)	0/150 (0)	0/0 (0)	
Disease duration at the first visit in mo[b]	31 (3–433)	13 (2–485)	28 (1–325)	
MRC sum score at the first visit[b]	122 (85–130)	122 (80–129)	130 (105–130)	
Limbs affected at the first visit				
1 (n, %)	35 (34)	32 (21)	13/98 (13)	
4 (n, %)	24 (24)	48 (32)	10/98 (10)	
Riluzole treatment (n, %)	0 (0)	130 (87)	89 (86)	
Follow-up data (n, %)	120 (91)	50 (33)	59 (57)	
Follow-up duration in mo[a]	101 (134)	22 (28)	31 (31)	
C9ORF72 repeat expansions (n, %)	NA	2/148 (1.4)	1/104 (1)	
Overall mortality	NA	83 (55)	37 (36)	
Death within 4 years of onset (n, %)	NA	39 (47)	5 (14)	

Abbreviations: IVIg = intravenous immunoglobulins; MRC = Medical Research Council; MMN = multifocal motor neuropathy; NA = not applicable; PLS = primary lateral sclerosis; PMA = progressive muscular atrophy.
Clinical parameters at the first visit are shown for treatment-naive MMN patients alone (n = 102).
[a] Values displayed as median (IQR).
[b] Values displayed as median (range).

CNV Analysis

SMN1, *SMN2*, and *SMN1/2*Δ7-8 CN are shown in table 2. In controls, we observed 48 *SMN1* duplications, of which 1 person had 4 and 1 person had 5 copies. All 11 patients with MMN with *SMN1* duplications had 3 copies. Eighteen patients with PMA had *SMN1* duplications, of which 3 had 4 copies. Seven patients with PLS had a single *SMN1* duplication.

Compared with controls, *SMN1* duplications were found more frequently in patients with PMA (12.0% vs 5.0%, odds ratio [OR] 2.69 (1.43–4.91), p 0.0020), but not in patients with MMN and PLS. This association is further exemplified by the fact that also double *SMN1* duplications (i.e., at least 4 *SMN1* copies) were found more frequently in patients with PMA (2% vs 0.2%, OR 11.0 [1.25–133.56], p 0.015). No associations with *SMN2* CNV were found, nor with the presence of the *SMN1/2*Δ7-8 gene variant. The average total *SMN* CN was comparable between the patient groups and controls, possibly indicating an *SMN2* deletion event accompanying *SMN1* duplications.

A detailed overview of the *SMN1*:*SMN2* configuration in controls and patients with PMA is included in supplementary table e-1, links.lww.com/NXG/A429.

Table 2 *SMN1*, *SMN2*, and *SMN1/2*Δ7-8 Copy Numbers in Controls (n = 956) and Patients With MMN (n = 132), PMA (n = 150), and PLS (n = 104)

	Controls	MMN	*p* Value	OR (95% CI)	PMA	*p* Value	OR (95% CI)	PLS	*p* Value	OR (95% CI)
SMN1[a]										
1	20 (2.1)	4 (3.0)	0.52	1.46 (0.36–4.46)	4 (2.7)	0.56	1.28 (0.31–3.90)	1 (1.0)	0.71	0.45 (0.01–2.90)
2	888 (92.9)	117 (88.6)	0.12	0.59 (0.32–1.16)	128 (85.3)	0.0028[c]	0.45 (0.26–0.79)	96 (92.3)	0.99	0.92 (0.42–2.28)
≥3	48 (5.0)	11 (8.3)	0.17	1.72 (0.78–3.47)	18 (12.0)	0.0020[c]	2.69 (1.43–4.91)	7 (6.7)	0.48	1.36 (0.50–3.14)
SMN2[a]										
0	78 (8.2)	11 (8.3)	1.00	1.02 (0.48–2.00)	11 (7.3)	0.85	0.89 (0.42–1.74)	9 (8.7)	0.85	1.07 (0.46–2.22)
1	401 (41.9)	56 (42.4)	0.99	1.02 (0.69–1.50)	74 (49.3)	0.11	1.34 (0.94–1.93)	35 (33.7)	0.13	0.70 (0.44–1.09)
2	451 (47.2)	59 (44.7)	0.66	0.91 (0.62–1.33)	60 (40.0)	0.12	0.75 (0.52–1.07)	54 (51.9)	0.41	1.21 (0.79–1.85)
3	24 (2.5)	6 (4.5)	0.29	185 (0.61–4.76)	5 (3.3)	0.58	1.34 (0.29–3.66)	4 (3.8)	0.35	1.55 (0.38–4.65)
4	2 (0.2)	—	—	—	—	—	—	2 (1.9)	0.05	9.31 (0.67–130)
***SMN1/2*Δ7-8**[a]										
0	718 (75.1)	102 (77.3)	0.66	1.13 (0.72–1.80)	104 (69.3)	0.16	0.74 (0.51–1.12)	83 (79.8)	0.34	1.31 (0.78–2.27)
1	230 (24.1)	27 (20.5)	0.42	0.81 (0.50–1.29)	45 (30.0)	0.14	0.35 (0.90–2.00)	20 (19.2)	0.33	0.75 (0.43–1.27)
2	8 (0.8)	3 (2.3)	0.14	2.75 (0.50–11.6)	1 (0.7)	1.00	0.80 (0.02–6.00)	1 (1.0)	0.61	1.15 (0.03–8.72)
Mean CN[b]										
SMN1/SMN2	3.48 (0.72)	3.51 (0.78)	0.68	—	3.50 (0.76)	0.67	—	3.62 (0.89)	0.11	—
***SMN1/SMN2/ SMN1/2*Δ7-8**	3.74 (0.64)	3.76 (0.67)	0.73	—	3.82 (0.75)	0.15	—	3.84 (0.75)	0.19	—

Abbreviations: CN = copy number; MMN = multifocal motor neuropathy; OR = odds ratio; PLS = primary lateral sclerosis; PMA = progressive muscular atrophy; SMN = survival motor neuron.
[a] Values displayed as n (%).
[b] Values displayed as mean (SD).
[c] Statistically significant after Bonferroni *p*-value adjustment. Uncorrected *p* values are shown.

Clinical Correlation

In all patient groups, age at onset and MRC sum score at the first visit were compared by *SMN1* group (1, 2, and ≥3 copies). Because of low numbers in groups with an *SMN1* deletion and duplication, the modifying potential of the *SMN2* CN was analyzed in the group with 2 *SMN1* copies alone. Results are shown in figure 1.

We did not find a positive association between *SMN1* and *SMN2* CN and median age at onset or median MRC sum scores at the first visit in patients with MMN, PMA, or PLS. In the MMN group, *SMN1* CN and *SMN2* CN within patients carrying 2 *SMN1* copies did not alter the rate of progression as expressed by ΔMRC sum score/month (p values 0.36 and 0.52, respectively, data not shown).

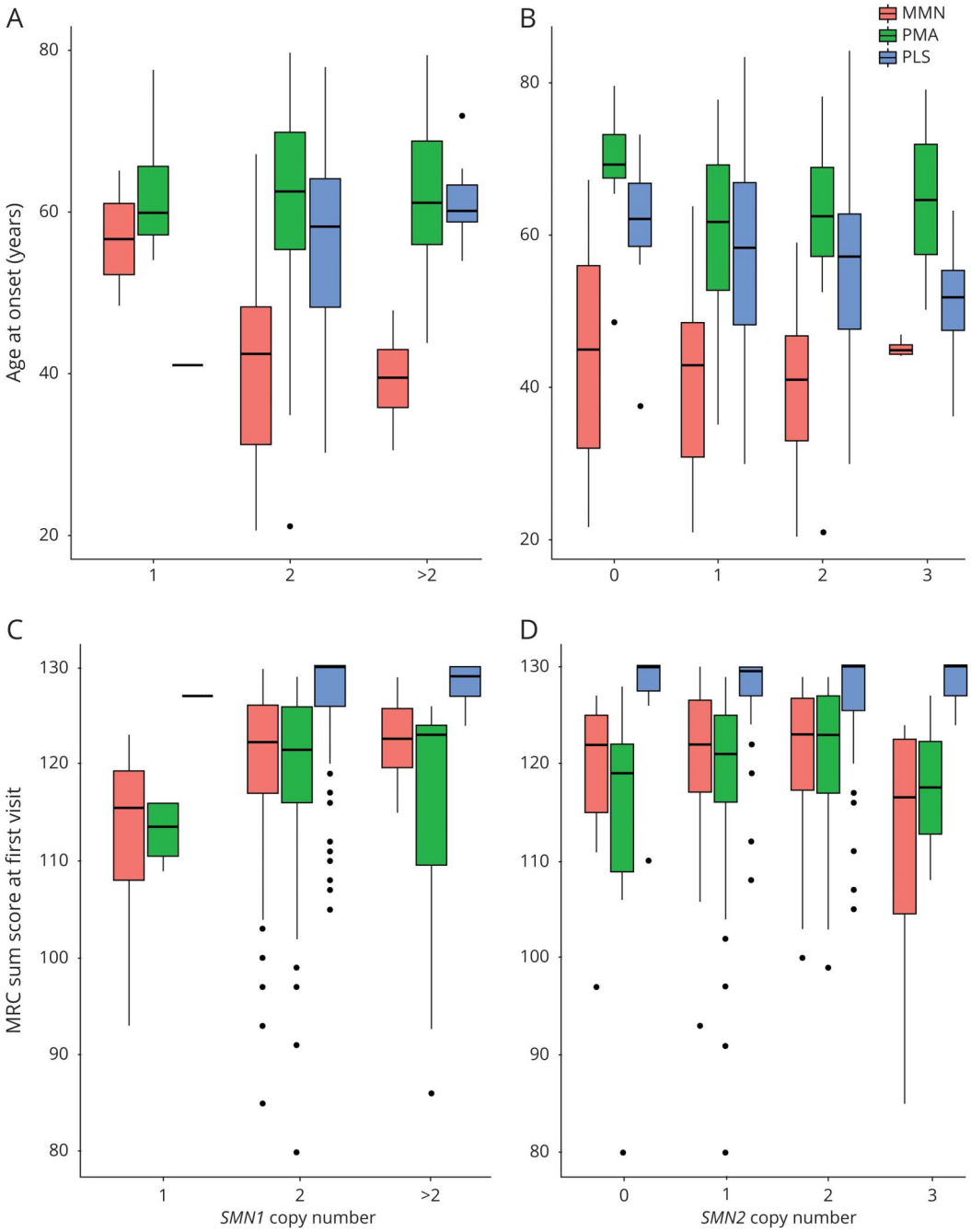
SMN1 duplications were not found more frequently in patients with PMA and a survival longer than 48 months (Fisher's exact test p 0.30, data not shown). Kaplan-Meier curves were drawn for all patients with PMA and PLS carrying 2 *SMN1* copies and *SMN1* duplications. Because of the low number of patients with an *SMN1* deletion, this group was excluded from survival analysis. Kaplan-Meier curves are shown in figure 2. Cox proportional hazard assumption was met in all analyses ($p > 0.05$). Although, compared with patients with PMA carrying 2 *SMN1* copies, survival in patients with PMA with *SMN1* duplications was lower, this was not statistically significant. *SMN2* CN did not affect survival in patients with PMA or PLS carrying 2 *SMN1* copies.

SMN1 Duplication Analysis

To gain further insight in the genetic architecture of the *SMN* locus in case of *SMN1* duplications, we further analyzed patients with PMA and controls with 3 *SMN1* copies (n = 15 and 46, respectively). Results are shown in table 3.

In all subjects carrying 1 *SMN1* duplication as expressed by the *SMN1* exon 7 CN, the *SMN1* exon 8 CN matched the *SMN1* exon 7 CN. The same accounts for *SMN2* exon 7 and 8 CN. In 36 controls (78%) and 14 patients with PMA (93%), the *SMN1* and *SMN2* exons 1–6 and intron 6 CN matched the combined *SMN1* and *SMN2* exon 7 CN, suggesting a balanced and full duplication of *SMN1*. In 10 controls (22%) and 1 patient with PMA (7%), one copy of the *SMN1/2*Δ7-8 gene variant was found. Besides carrying 1 extra copy of

Figure 1 Clinical Parameters Stratified by *SMN1* and *SMN2* Copy Number in Patients With MMN and PMA



Boxplots showing median age at onset in years and median MRC sum score at the first visit in patients with MMN, PMA, and PLS. A) Age at onset in years by the *SMN1* copy number. B) Age at onset by the *SMN2* copy number in patients carrying 2 *SMN1* copies. C) MRC sum score at the first visit by the *SMN1* copy number. D) MRC sum score at the first visit by the *SMN2* copy number in patients carrying 2 *SMN1* copies. No association between the clinical parameters and *SMN1* or *SMN2* copy number status was found in either disease group (all p values > 0.05). MMN = multifocal motor neuropathy; MRC = Medical Research Council; PLS = primary lateral sclerosis; PMA = progressive muscular atrophy; SMN = survival motor neuron.

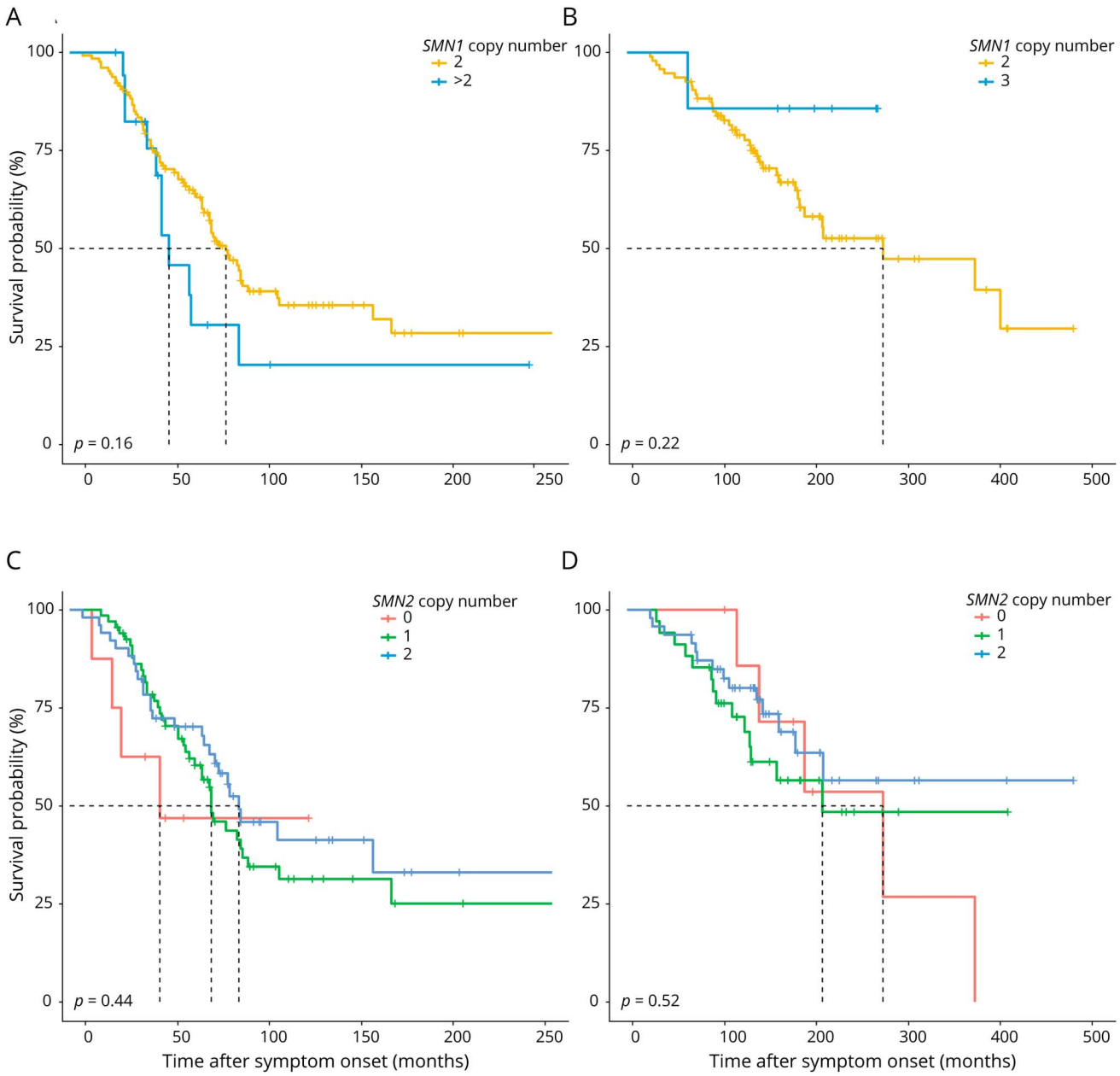
SMN1 and *SMN2* exons 1–6 and intron 6, these subjects all have a probable full *SMN1* gene duplication.

Regarding the *SMN1*:*SMN2* genetic architecture, there was a trend toward patients with PMA having a higher *SMN2* and thus lower *SMN1*/2Δ7-8 CN when compared with controls, although this was not statistically significant (Fisher's exact test p 0.64 and 0.26, respectively). Telomeric NAIP exon 5 duplications were found in 45 of 61 *SMN1* duplications (73%), which did not differ between patients with PMA and controls (Fisher's exact test p 0.51).

Discussion

In this study, we investigated the possible association between CNV in the *SMN* locus and the motor neuron disorders

Figure 2 Survival by the *SMN* Copy Number in Patients With PMA



Kaplan-Meier curves showing the probability of survival according to disease duration in patients with PMA (panels A and C) and PLS (panels B and D). Panels A and C show overall survival stratified by the *SMN1* copy number. Panels B and D show overall survival stratified by the *SMN2* copy number in patients carrying 2 *SMN1* copies. Because of low numbers, survival curves for patients with *SMN1* deletions and *SMN2* duplications are not shown. Survival did not differ between patients with PMA carrying 2 or 3 *SMN1* copies (log-rank test *p* 0.16, HR 1.6 [0.83–3.0], *p* 0.16), nor did the *SMN2* copy number have an effect on survival in patients carrying 2 *SMN1* copies (log-rank test *p* 0.44; HR 0 vs 2 copies: 1.7 [0.57–4.8], *p* 0.35; HR 1 vs 2 copies: 1.3 [0.76–2.1], *p* 0.38). In patients with PLS, the *SMN1* copy number was not associated with survival (log-rank test *p* 0.22, HR 0.31 [0.04–2.2], *p* 0.25), nor did the *SMN2* copy number affect survival in patients carrying 2 *SMN1* copies (log-rank test *p* 0.52; HR 0 vs 2 copies: 1.4 [0.51–4.1], *p* 0.49; HR 1 vs 2 copies: 1.4 [0.69–3.0], *p* 0.33). HR = hazard ratio; PLS = primary lateral sclerosis; PMA = progressive muscular atrophy; SMN = survival motor neuron.

MMN, PMA, and PLS, and the effect of *SMN1* and *SMN2* CN on MMN, PMA, and PLS disease course. We found that, compared with controls, *SMN1* and *SMN2* CN were not associated with MMN and PLS, nor their disease characteristics. PMA, but not its disease course, was associated with *SMN1* duplications (OR 2.69 [1.43–4.91], *p* 0.0020). These results indicate that CNV in the *SMN* locus is a novel genetic risk factor for PMA.

Previous studies have shown an association between ALS and *SMN1* duplications with a combined OR of 1.76 (95% CI 1.33–2.32).[4,17,18] Of interest, a recent study including >6,000 patients with ALS identified no association between ALS and *SMN* CNV.[19] To better understand these conflicting results and detail the relevance of CNV in the *SMN* locus for motor neuron disorders, we have taken a closer look at the extremes of the motor neuron disorder spectrum by analyzing large

Table 3 Genetic Architecture of *SMN1* Duplications in Controls and Patients With PMA

	Controls (n = 46)	PMA (n = 15)	*p* Value
[*SMN1*:*SMN2*] (n, %)			
3:0	6 (13)	2 (13)	0.64
3:1	20 (43)	4 (27)	
3:2	16 (35)	8 (53)	
3:3	4 (9)	1 (7)	
***SMN1/2*Δ7-8 (n, %)**			
0	36 (78)	14 (93)	0.26
1	10 (22)	1 (7)	
***NAIP*-5 (n, %)**			
2	11 (24)	5 (33)	0.51
3	31 (67)	10 (67)	
4	4 (9)	0 (0)	

Abbreviations: NAIP-5 = NLR family apoptosis inhibitory protein exon 5; PMA = progressive muscular atrophy; SMN = survival motor neuron.

cohorts of MNDs characterized by inflammation (MMN), predominant LMN (PMA), and UMN degeneration (PLS). We found an association between *SMN1* duplications and PMA, but not PLS and MMN. In PMA, we found a similar effect size identified in a previous study.[20] We hypothesize that our findings highlight the primary effect of altered *SMN* CN on LMNs.

PMA and PLS are considered part of a spectrum of MNDs that also includes ALS. In contrast to ALS and PLS, PMA is limited to clinical signs of LMNs.[5] However, up to 50% of patients with PMA may show signs of corticospinal tract degeneration at autopsy.[21] Whether PMA represents a distinct disease remains a topic of ongoing debate, and further research is warranted to further detail the clinical heterogeneity associated with PMA. We nevertheless think that our PMA cohort differs from a typical ALS cohort on various grounds. Based on detailed clinical and electrophysiologic examination, the patients included in this study showed exclusive progressive loss of LMNs, and almost none of the patients had a bulbar onset. Moreover, in 104 of 159 (65%) patients, survival was longer than 4 years, and 37 of 159 (23%) patients had a very long survival of at least 8 years (range 96–531 months). Analysis of available follow-up data of 54 of 159 (34%) patients showed a conversion to ALS in only 3 cases (i.e., upper motor neuron signs after the start of this study). Of these patients, none carried an *SMN1* duplication.

A central question in this study and in the aforementioned studies is what effect *SMN1* duplications have on LMN SMN protein levels. In our analysis, we showed that the genetic architecture of *SMN1* duplications in PMA does not differ from that of controls, and that about three-quarters of *SMN1* duplications are large, including the telomeric NAIP gene. Most importantly, we showed that *SMN1* duplications are probably balanced and could theoretically lead to a full-length SMN transcript. Although the transcriptional, translational, and posttranslational effects in individuals carrying 3 *SMN1* genes remain unknown, we cannot exclude the possibility that the association between *SMN1* duplications and PMA is caused by an excess of LMN SMN protein levels. Further supporting this hypothesis may be the fact that previous studies have not shown an association with *SMN1* deletions.[4,17,18] Alternative explanations may be found in the disruption of regulatory sequences by the large duplications in the *SMN* locus. Future research on SMN protein levels in, for example, induced pluripotent stem cell–derived motor neurons obtained from patients with different *SMN1* genotypes could further address this issue.

To the best of our knowledge, this is the first study reporting on CNV in the *SMN* locus in MMN and PLS. The size of the cohorts we included in our analyses is substantial, considering the low prevalence of these diseases. Clinically, patients with MMN were characterized in detail, and we had data from longer periods of follow-up for analysis. Our data virtually exclude the *SMN* locus as a susceptibility locus for inflammatory MNDs. Although our cohort of patients with PMA is also relatively large, it is not possible to draw definite conclusions about the association of *SMN* CNV and disease course because of a relatively small number of patients with *SMN1* duplications. *SMN* CN varies considerably among different ethnicities.[15,16] The results of our study therefore cannot readily be extrapolated to ethnicities other than that of the patients in this study, that is, Dutch.

In summary, our study shows that *SMN* CNV underlies motor neuron vulnerability in PMA, but not in MMN and PLS. This extends the role of *SMN* CNV in LMN diseases beyond SMA and supports the notion that the *SMN* locus is a general modifier for LMN disease susceptibility. The intriguing finding that, rather than loss of SMN as in SMA, gene duplications are a main genetic risk factor for PMA warrants further fundamental research to improve our understanding of the cellular mechanisms that underlie this susceptibility.

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Renske I. Wadman, MD PhD	UMC Utrecht	Drafting and revising the manuscript for intellectual content
Chantall A.D. Curial, BS	UMC Utrecht	Acquisition of data
Naomi N. Molleman, BS	MRC Holland	Acquisition of data, and analysis and interpretation of data
Marinka Zegers, BS	MRC Holland	Acquisition of data, and analysis and interpretation of data
Paul W.J. van Vught, PhD	MRC Holland	Design and conceptualization of the study, and drafting and revising the manuscript for intellectual content
Reinier Snetselaar, PhD	MRC Holland	Design and conceptualization of the study, analysis and interpretation of data, and drafting and revising the manuscript for intellectual content
Raymon Vijzelaar, PhD	MRC Holland	Design and conceptualization of the study, analysis and interpretation of data, and drafting and revising the manuscript for intellectual content
W. Ludo van der Pol, MD, PhD	UMC Utrecht	Design and conceptualization of the study, and drafting and revising the manuscript for intellectual content
Leonard H. van den Berg, MD, PhD	UMC Utrecht	Design and conceptualization of the study, and drafting and revising the manuscript for intellectual content

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