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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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. 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. 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.

SMN1 and SMN2 exon 7 specific probes were used to determine the SMN1 and SMN2 CN. The SMN1/2Δ7-8 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 SMN1/2Δ7-8 gene variant.

Statistical Analysis
We used R (version 3.6.1) to perform statistical analyses. SMN1, SMN2, and SMN1/2Δ7-8 CN in patients with MMN, PMA, and PLS were separately compared with controls using a χ² 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.
SMN1, SMN2, and SMN1/2Δ7-8 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 SMN1/2Δ7-8 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.
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 SMN2 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 SMN1/2Δ7-8 gene variant was found. Besides carrying 1 extra copy of

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### Table 2 SMN1, SMN2, and SMN1/2Δ7-8 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**         |          |     |         |             |     |         |             |     |         |             |
| 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* | 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* | 2.69 (1.43-4.91) | 7 (6.7) | 0.48 | 1.36 (0.50-3.14) |
| **SMN2**         |          |     |         |             |     |         |             |     |         |             |
| 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 | 1.85 (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) |
| **SMN1/2Δ7-8**   |          |     |         |             |     |         |             |     |         |             |
| 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**      | 3.48 (0.72) | 3.51 (0.78) | 0.68 | — | 3.50 (0.76) | 0.67 | — | 3.62 (0.89) | 0.11 | — |
| **SMN1/SMN2**    | 3.74 (0.64) | 3.76 (0.67) | 0.73 | — | 3.82 (0.75) | 0.15 | — | 3.84 (0.75) | 0.19 | — |
| **SMN1/SMN2/ SMN1/2Δ7-8** | 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.

* Values displayed as n (%).

* Values displayed as mean (SD).

* Statistically significant after Bonferroni p-value adjustment. Uncorrected p values are shown.
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

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[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.
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). Of interest, a recent study including >6,000 patients with ALS identified no association between ALS and SMN CNV. 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

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**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.
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limited to clinical signs of LMNs. However, up to 50% of the patients with PMA may show signs of corticospinal tract degeneration at autopsy. 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. 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. 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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**Table 3 Genetic Architecture of SMN1 Duplications in Controls and Patients With PMA**

| (SMN1:SMN2) (n, %) | Controls (n = 46) | PMA (n = 15) | p Value |
|--------------------|------------------|-------------|---------|
| 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)       |         |
| SMN1/2Δ7-8 (n, %)  |                  |             |         |
| 0                  | 36 (78)          | 14 (93)     | 0.26    |
| 1                  | 10 (22)          | 1 (7)       |         |
| NAI-P-5 (n, %)     |                  |             |         |
| 2                  | 11 (24)          | 5 (33)      | 0.51    |
| 3                  | 31 (67)          | 10 (67)     |         |
| 4                  | 4 (9)            | 0 (0)       |         |

Abbreviations: NAI-P-5 = NLR family apoptosis inhibitory protein exon 5; PMA = progressive muscular atrophy; SMN = survival motor neuron.
commercially available MLPA assays. W.L. van der Pol serves on the scientific advisory board for SMA Europe, is a member of the Branaplam data monitoring committee (DMC) for Novartis, provides ad hoc consultancy for Biogen and AveXis, and receives research support from the Prinse Beatrix Spierfonds, Vriendenloteijir, and Stichting Spieren voor Spieren. L.H. van den Berg serves on the scientific advisory boards for Orion, Biogen, and Cytokinetics; received an educational grant from Takeda; serves on the editorial boards of Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration and the Journal of Neurology, Neurosurgery, and Psychiatry; and receives research support from the Prinse Beatrix Spierfonds, the Netherlands ALS Foundation, and the Netherlands Organization for Health Research and Development (Vic Scheme, JPND [SOPHIA, STRENGTH, ALSCare]). Go to Neurology.org/NG for full disclosures.

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| Ewout J.N. Groen, PhD | UMC Utrecht | Drafting and revising the manuscript for intellectual content |
| Renske I. Wadman, MD PhD | UMC Utrecht | Drafting and revising the manuscript for intellectual content |
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| 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 Snetseelaar, PhD | MRC Holland | Design and conceptualization of the study, analysis and interpretation of data, and drafting and revising the manuscript for intellectual content |

Appendix (continued)

| Name | Location | Contribution |
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