Phrenic nerve involvement and respiratory muscle weakness in patients with Charcot-Marie-Tooth disease 1A

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Abstract
Diaphragm weakness in Charcot-Marie-Tooth disease 1A (CMT1A) is usually associated with severe disease manifestation. This study comprehensively investigated phrenic nerve conductivity, inspiratory and expiratory muscle function in ambulatory CMT1A patients. Nineteen adults with CMT1A (13 females, 47 ± 12 years) underwent spirometry, diaphragm ultrasound, and magnetic stimulation of the phrenic nerves and the lower thoracic nerve roots, with recording of diaphragm compound muscle action potentials (dCMAP, n = 15), transdiaphragmatic and gastric pressures (twPdi and twPgas, n = 12). Diaphragm motor evoked potentials (dMEP, n = 15) were recorded following cortical magnetic stimulation. Patients had not been selected for respiratory complaints. Disease severity was assessed using the CMT Neuropathy Scale version 2 (CMT-NSv2). Healthy control subjects were matched for age, sex, and body mass index. The following parameters were significantly lower in CMT1A patients than in controls (all \( P < .05 \)): forced vital capacity (91 ± 16 vs 110 ± 15% predicted), maximum inspiratory pressure (68 ± 22 vs 88 ± 29 cmH2O), maximum expiratory pressure (91 ± 23 vs 123 ± 24 cmH2O), and peak cough flow (377 ± 135 vs 492 ± 130 L/min). In CMT1A patients, dMEP and dCMAP were delayed. Patients vs controls showed lower diaphragm excursion (5 ± 2 vs 8 ± 2 cm), diaphragm thickening ratio (DTR, 1.9 [1.6-2.2] vs 2.5 [2.1-3.1]), and twPdi (8 ± 6 vs 19 ± 7 cmH2O; all \( P < .05 \)). DTR inversely correlated with the CMT-NSv2 score \( (r = -.59, P = .02) \). There was no group difference in twPgas following abdominal muscle stimulation. Ambulatory CMT1A patients may show phrenic nerve involvement and reduced respiratory muscle strength. Respiratory muscle weakness can be attributed to diaphragm dysfunction alone. It relates to neurological impairment and likely reflects a disease continuum.

KEYWORDS
Charcot-Marie-Tooth disease, diaphragm, motor evoked potentials, phrenic nerves, respiratory muscles
Charcot-Marie-Tooth disease (CMT) refers to a heterogeneous group of monogenetic neuropathies with a reported prevalence of 10-28:100,000.\(^1,2\) Distal muscle weakness and atrophy, foot deformities, gait disturbances, and sensory impairment are the clinical hallmarks of CMT.\(^2,4\) Disease classification is based on clinical and electrophysiological findings, the trait of inheritance, and the underlying genetic cause.\(^3,5,6\) Known candidate genes encode proteins essential for myelin formation or maintenance, and axonal function.\(^7\) Charcot-Marie-Tooth disease 1A (CMT1A) is the most common genetic subtype of CMT, caused by duplication of the peripheral myelin protein 22 (PMP22) gene.\(^5,7\) Although CMT1A is primarily demyelinating, axonal loss does occur over time and forms the basis of motor disability.\(^5,8\)

Phrenic nerve involvement and respiratory muscle weakness have been described in severely affected, mostly wheelchair-bound, patients with CMT.\(^9-14\) A limited number of studies conducted in larger cohorts suggest that respiratory muscle weakness (as reflected by vital capacity or maximum inspiratory pressure) may relate to overall neurological handicap.\(^15,16\) Furthermore, lung restriction may be worse in patients with concomitant spine deformities.\(^19\) However, non-volitional tests such as twitch transdiaphragmatic pressure (twPdi) following magnetic stimulation of the phrenic nerves have only been applied in two severely affected patients with genetically unspecified CMT.\(^12\)

Thus, morphological and electrophysiological evidence of diaphragm dysfunction is still limited, especially in ambulatory patients without clinically overt respiratory muscle weakness (ie, with no dyspnea on exertion and normal blood gases).

Diaphragm ultrasound has been proposed as an objective bedside test for assessment of diaphragm function.\(^20-22\) Specifically, the diaphragm thickening ratio (DTR) may relate to diaphragm strength.\(^21,22\) However, twPdi following magnetic stimulation of the phrenic nerves is still regarded as the diagnostic gold standard for directly measuring diaphragm strength.\(^23,24\)

Electrophysiological evaluation of phrenic nerve and inspiratory pathway function can be performed using surface electromyography (EMG) for recording of diaphragm motor evoked potentials (dMEP) following cortical magnetic stimulation (COMS), and compound action potentials (dCMAP) following cervical magnetic stimulation (CEMS).\(^24,25\)

Data on the potential involvement of expiratory muscles in CMT are limited and inconclusive, and again, only volitional tests have been applied in previous studies.\(^18,19\) Magnetic stimulation of the abdominal muscles along with invasive recording of the twitch gastric pressure (twPgas) overcomes the difficulties inherent in volitional tests of expiratory muscle strength,\(^26\) but this has never been studied in CMT patients.\(^26,27\)

This case-control study tested the hypothesis that diaphragm dysfunction is present in ambulatory patients with CMT1A even if symptoms of respiratory muscle weakness are not reported, and determined whether objective diaphragm dysfunction can be attributed to measurable motor neuropathy of the phrenic nerves.

### 2 | PATIENTS AND METHODS

#### 2.1 | Study design

This case-control study was conducted from November 2017 to March 2019. Ethical approval was obtained from the local ethics committee (Ethikkommission der Ärztekammer Westfalen-Lippe und der WWU Münster, Az. 2016-072-f-S). The present study was part of a wider project investigating respiratory muscle strength and function in neuromuscular disorders and chronic obstructive pulmonary disease (ClinicalTrials.gov Identifier: NCT03032562).

#### 2.2 | Study population and clinical assessment

Patients with CMT1A were recruited from our neuromuscular outpatient clinic. Thirty-seven adult patients were consecutively invited to participate in the study. Written informed consent was given by 19 fully ambulatory individuals with genetically proven CMT1A. In all patients, duplication of the PMP22 gene had been verified by our own genetic laboratory. The CMT Neuropathy Scale version 2 (CMT-NSv2)\(^28\) was used to assess clinical disease severity. This composite scale combines self-reported symptoms, motor signs and electrophysiological findings, yielding a sum score of 0-36, with higher scores reflecting more advanced disease. All patients also completed the Medical Research Council (MRC) Breathlessness Scale.\(^29\) Capillary blood gas analysis was performed as previously described or derived from clinical records.\(^30\) For patient recruitment, clinical signs or symptoms possibly suggesting respiratory muscle involvement were irrelevant. All but two patients underwent standard electroneurography of the right ulnar nerve. Nineteen healthy volunteers matched for age, sex, and body mass index (difference < 3 kg/m\(^2\)) served as controls. All patients and controls gave written informed consent to participate in the study. Figure 1 summarizes the study design.

#### 2.3 | Spirometry, maximum inspiratory and expiratory pressures

Lung function tests were performed according to standard recommendations using an electronic spirometer (Vitalograph 3000, Vitalograph, Hamburg, Germany).\(^23,31\) Participants were encouraged to perform a maximum effort towards their individual forced vital capacity (FVC) and forced expiratory volume in the first second (FEV\(_1\)) in the upright position. At least five consecutive tests were performed until the best result for FVC was achieved and showed <10% variation from the preceding test. FVC was expressed as percentage of the predicted value based on sex, height, and age.\(^32\) Spirometric measurements also included peak expiratory flow (PEF). Maximum inspiratory and expiratory pressures (PImax and PEmax, respectively) were obtained using a
handheld electronic manometer equipped with a large-diameter flanged mouthpiece (MicroRPM, Care Fusion, Baesweiler, Germany). All participants performed at least three tests, each requiring a 1-second plateau in a 3- to 4-second effort and a 25-second pause between tests. Subjects who failed to achieve <10% variability from the preceding test within the first three runs repeated the test until they were able to achieve more consistent results. PImax was measured first (from residual volume), and PEmax was measured second (from total lung capacity [TLC]), both with the subject sitting upright which was ascertained by using a standardized chair. Finally, the peak cough flow (PCF) was determined using a handheld peak flow meter. All measurements were performed using a nasal clip to prevent air leakage.32

2.4 | Diaphragm ultrasound

A standardized and comprehensive approach to evaluate diaphragm function by means of diaphragm ultrasound was used and based upon previous diaphragm ultrasound studies.22,23,26,28 A portable ultrasound machine (LOGIQ S8-XD clear, GE Healthcare, London, UK) with a 3.5 MHz convex transducer was used for assessment of diaphragm excursions in the subcostal view, and a 10 MHz linear transducer was used for evaluation of diaphragm thickness in the zone of apposition. Measurements were performed on the right hemidiaphragm in the supine position. All sonographic recordings were saved for later analysis. Measurements were performed three times and the average value for each parameter was calculated. For real-time evaluation of diaphragm excursions, the probe was positioned in the subcostal area between the mid-clavicular and anterior axillary line and directed cranially (Figure S1A). Diaphragm excursion amplitude was assessed during tidal breathing (Figure S1A), at TLC, and following a voluntary sniff maneuver. Diaphragm excursion velocity was assessed during tidal breathing and following a voluntary sniff maneuver only (Figure S1B). Excursion amplitude was defined as the upright-perpendicular distance from the minimum to the maximum point of diaphragm displacement, and excursion velocity was defined as the upright-diagonal distance from the minimum to the maximum point.

Diaphragm thickness was measured as the vertical distance between the pleural and peritoneal layer at both TLC and functional residual capacity (FRC) (Figure S1C,D). The 10 MHz probe was positioned in the posterior axillary line between the eighth and 10th intercostal space (Figure S1C). Diaphragm thickness was defined as the distance from the inner part of the pleural layer to the inner part of the peritoneal layer, measured at its thickest portion adjacent to the lung. The DTR was calculated as thickness at TLC divided by thickness at FRC.

2.5 | Cortical and posterior cervical magnetic stimulation

Diaphragm electrical activity was bilaterally recorded following both cortical and posterior CEMS according to current guidelines and previous studies.24,25,37 Surface EMG was recorded using a Dantec 2000 EMG device (Dantec Medical, Skovlunde, Denmark). Electrodes were placed in the seventh intercostal space approximately on the anterior axillary line with the reference electrodes positioned cranially to the xiphoid process (16-cm inter-electrode distance).24,25,37 The ground electrode was placed around the right wrist.24,25,37

Magnetic stimulation was performed with the subject in the seated position.24,25,37 After a 20-minute resting period with quiet breathing and no speaking, stimuli were delivered using a MagPro Compact magnetic stimulator equipped with a 12 cm C-100 circular coil (MagVenture, Willich, Germany). Square-pulse stimuli were 0.1 ms in duration.24,25,37 Stimulus conditions were standardized by using maximum magnetic flux density (2 Tesla) for all stimulations.24,25,37 For COMS, the magnetic coil was placed over Cz.24,25,37,38 For CEMS, the coil was first placed over C7 and then moved up towards C6 until the highest reproducible dCMAP was obtained.24,25,37,38 For each run of tests, at least five stimuli were delivered in order to achieve the highest possible dCMAP amplitude showing <10% variation from the preceding two stimulations. In order to avoid twitch potentiation, stimuli were separated by a pause of at least 40 seconds.24,25,37,38 Stimulation at FRC was determined by visual observation of abdominal movements after instructing subjects to breathe out and hold their breath until the magnetic stimulus was delivered (the stimulus was then deployed within 1 second after FRC had been reached). Figure S2 displays representative dCMAP values following CEMS at FRC.

2.6 | Invasive inspiratory muscle strength measurements

Twitch esophageal pressure (twPes) and twPgas were simultaneously obtained using balloon catheters (Cooper Surgical, Trumbull,
2.5 mL of air, respectively. To ensure constant filling, both balloons were deflated and refilled after every run of tests. Balloon catheters were connected to a differential pressure transducer (DPT-100, Utah Medical Products, Athlone, Ireland) and a carrier amplifier (ADIInstruments, Oxford, UK). Pressure data for twPgas, twPes, and twPdi (defined as twPes − twPgas) were continuously displayed using LabChart software (ADIInstruments) on a personal computer. Figure S3A shows representative tracings of twPdi following CEMS at FRC. In addition to non-volitional tests, all participants were instructed to perform consecutive maximum sniff maneuvers with a resting period of 5 minutes in between. Subjects were encouraged to achieve maximum deflection of the Pdi curve. After participants had learned and practiced the maneuver several times, five measurements were recorded for each and the highest value was used for analysis. Figure S3B shows representative pressure tracings following maximum voluntary sniff.

2.7 | Invasive expiratory muscle strength measurements

As initially proposed by Polkey and coworkers, the abdominal nerve roots were magnetically stimulated at the 10th vertebrae, with a clear instruction to go up and down the vertebral column (by no more than two vertebrae) to determine the optimal position yielding the highest reproducible dCMAP amplitude. Abdominal muscle CMAP was bilaterally recorded using surface electrodes placed in the anterior axillary line close to the lower costal margin. Stimulus duration was 0.1 ms. Stimulation intensity was 100% of the maximum magnetic output as previously described. Again, stimulation at FRC was determined by visual observation of abdominal movements. In addition, subjects were instructed to repeatedly perform a maximum voluntary cough with a resting period of 5 minutes between subsequent maneuvers. Gastric pressure following the cough maneuver and twPgas in response to magnetic stimulation of the abdominal muscles were recorded using a gastric balloon catheter and the same technical set-up as described above. Representative pressure tracings are shown in Figure S3C,D.

2.8 | Statistical analysis

All analyses were performed using Sigma Plot software (version 13.0, Systat Software Ltd, Erkrath, Germany). Assuming a two-sided significance level of .05 (α) and 80% power (β), a sample size of at least 11 subjects per group (of invasive measurements) was calculated for detection of a 25% difference in twPdi (this was considered clinically relevant based upon previous studies that showed that such decrease in twPdi is usually associated with worse functional status in patients with neuromuscular disorders; mean value and SD of twPdi in healthy individuals was derived from previous experiments in our laboratory). Data distribution was tested using the Kolmogorov-Smirnov test. Results are expressed as mean and SD for continuous variables, and the t-test for independent samples was used for between-group comparisons. For non-parametric data, the Mann-Whitney U test was used for comparison between groups as appropriate. For all analyses, a p-value of <.05 was considered statistically significant. For graphical illustrations GraphPad Prism 7 (GraphPad Software, San Diego, California) was used.

3 | RESULTS

3.1 | Subjects

Table 1 summarizes baseline demographic and anthropometric characteristics of study participants. All patients with CMT1A were fully ambulatory and the mean CMT-NSv2 sum score was 15.3 ± 4.9 (range: 8-21). The mean score on the MRC Breathlessness Scale was 1.53 ± 0.64. None of the patients showed hypercapnia.

| TABLE 1 | Demographic and basic lung function data in Charcot-Marie-Tooth disease 1A (CMT1A) patients and controls |
|-----------------------------------------------|
| CMT1A patients | Controls | P-value |
|----------------|----------|---------|
| Male, n (%)    | 6 (32)   | 6 (32)  | n.s. |
| Age, years     | 47.0 ± 12.1 | 46.9 ± 10.8 | n.s. |
| Body mass index, kg/m² | 28.0 ± 5.4 | 24.1 ± 2.0 | n.s. |
| Self-reported disease onset (age) | — | — | — |
| CMT-NSv2 score (0-36) | 15.3 ± 4.9 | — | — |
| CMAP amplitude ulnar nerve (mV) | 1.35 ± 1.17 | — | — |
| MRC breathlessness scale (0-5) | 1.53 ± 0.64 | — | — |

Lung function data

FVC, L 3.3 ± 0.8 4.2 ± 1.0 <.01
FVC, % predicted 91.0 ± 16.2 110.4 ± 15.4 <.01
FEV₁/FVC, % 82.5 ± 4.2 80.3 ± 5.6 n.s.
PEF, L/s 6.6 ± 2.0 8.3 ± 1.7 .01
PEF, % predicted 89.2 ± 18.5 110.3 ± 17.1 <.01
PCF 377.2 ± 135.0 491.6 ± 130.0 .01
Plmax, cmH2O 68.0 ± 22.1 87.6 ± 28.7 .03
PEmax, cmH2O 91.4 ± 23.3 122.6 ± 23.9 <.01

Note: Data are presented as mean ± SD, number of patients (%). Bold numbers indicate statistical significance, i.e. p-values < .05. Abbreviations: CMAP, compound muscle action potential; CMT-NSv2, CMT Neuropathy Scale version 2; FEV₁, forced expiratory volume in the first second; FVC, forced vital capacity; PCF, peak cough flow; PEF, peak expiratory flow; PEmax, maximum expiratory pressure; Plmax, maximum inspiratory pressure; MRC, Medical Research Council; n.s., not significant.
(PCO₂ ≥ 45 mm Hg) on capillary blood gas analysis. Motor nerve conduction studies (NCS) of the right ulnar nerve showed marked reduction of nerve conduction velocity (28.8 ± 12.5 m/s) and a mean CMAP amplitude of 4.6 ± 3.4 mV (median: 4.9; range: 0.028–13.4).

3.2 | Spirometry, maximum inspiratory and expiratory pressures

Spirometry and measurement of mouth occlusion pressures showed lower FVC (by 17%, 91.0 ± 16.2 vs 110.4 ± 15.4, P < .01), PImax (by 22%, 68.0 ± 22.1 vs 87.6 ± 28.7 cmH₂O, P = .03) and PEmax (by 25%, 91.4 ± 23.3 vs 122.6 ± 23.9 cmH₂O, P < .01) in CMT1A patients compared with healthy controls (Table 1 and Figure 2). In addition, both PEF and PCF were lower in patients with CMT1A vs controls (PEF: 6.6 ± 2.0 vs 8.3 ± 1.7 L/s; PCF: 377.2 ± 135.0 vs 491.6 ± 130.0 L/min; both P = .01). In patients with CMT1A, five (26%) had FVC <80% of the predicted value and seven (37%) had PImax <60 cmH₂O. There was an inverse correlation between FVC and the CMT-NSv2 sum score (r = −.61, P < .01) (Figure 3A), and a moderate positive correlation between PImax and ulnar nerve CMAP amplitude (r = .53, P = .03) (Figure 3B). PEmax and PCF were not correlated with disease severity, as reflected by the CMT-NSv2 score (both P > .05).

3.3 | Diaphragm ultrasound

Diaphragm excursion amplitude was lower in CMT1A patients compared with controls (by 33%, 5.3 ± 1.7 vs 7.8 ± 2.0 cm, P < .01), as was the DTR (by 23%, 1.9 [1.6–2.2] vs 2.5 [2.1–3.1], P < .01) (Table 2 and Figure 4). Excursion velocity during a voluntary sniff maneuver tended to be lower in CMT1A patients vs controls (by 13%, 5.8 ± 1.5 vs 6.7 ± 1.5 cm/s), but the difference did not reach statistical significance (P = .09). There was an inverse correlation between DTR and the CMT-NSv2 sum score (r = −.51, P = .03) (Figure 5). Ultrasound parameters did not correlate with FVC or PImax (all P > .05; data not shown).

3.4 | Diaphragm motor evoked potentials and phrenic nerve conduction studies

Reproducible dMEP and dCMAP amplitudes (showing <10% of variability from the preceding two stimulations) could be obtained in 15 CMT1A patients following COMS or CEMS, respectively. Accordingly, group comparisons for these parameters included 15 matched
Neither dMEP nor dCMAP showed any side-to-side difference in latency or amplitude (all $P > .05$; Table 3 and Figure 6). Therefore, only parameters derived from right-sided measurements are reported in the text, but Table 3 shows all values recorded from both sides.

Both dMEP and dCMAP latencies were prolonged in patients with CMT1A compared with control subjects (dMEP: by 35%, 24.0 ± 4.7 vs 17.7 ± 3.4 ms; dCMAP: by 35%, 2.1 [1.9-2.8] vs 2.0 [1.8-2.7] mV).

\[ r = -0.51 \quad p = 0.03 \]

**TABLE 2** Diaphragm ultrasound measures in CMT1A patients and controls

|                     | CMT1A patients (n = 19) | Controls (n = 19) | $P$-value |
|---------------------|-------------------------|-------------------|-----------|
| Diaphragm excursion |                         |                   |           |
| Amplitude during tidal breathing, cm | 1.7 ± 0.8 | 1.6 ± 0.6 | n.s.      |
| Velocity during tidal breathing, cm/s | 1.1 ± 0.5 | 1.0 ± 0.4 | n.s.      |
| Amplitude during voluntary sniff, cm | 2.0 [1.8-2.7] | 2.1 [1.9-2.8] | n.s.      |
| Velocity during voluntary sniff, cm/s | 5.8 ± 1.5 | 6.7 ± 1.5 | .09       |
| Amplitude during maximum inspiration, cm | 5.3 ± 1.7 | 7.8 ± 2.0 | <.01      |

| Diaphragm thickness |                     |                   |           |
|---------------------|---------------------|-------------------|-----------|
| At FRC, cm          | 0.21 [0.18-0.23]    | 0.21 [0.15-0.22]  | n.s.      |
| At TLC, cm          | 0.36 [0.33-0.45]    | 0.44 [0.38-0.65]  | .03       |
| DTR                 | 1.9 [1.6-2.2]       | 2.5 [2.1-3.1]     | <.01      |

Note: Data are presented as mean ± SD or median [interquartile range]. Abbreviations: CMT1A, Charcot-Marie-Tooth disease 1A; DTR, diaphragm thickening ratio; FRC, functional residual capacity; n.s., not significant; TLC, total lung capacity.

**FIGURE 4** Diaphragm thickening ratio in Charcot-Marie-Tooth disease 1A (CMT1A) patients and controls

**FIGURE 5** Association between diaphragm thickening ratio and the CMT Neuropathy Scale version 2 (CMT-NSv2) score

**TABLE 3** Phrenic nerve conduction studies in CMT1A patients and controls

|                     | CMT1A patients (n = 15) | Controls (n = 15) | $P$-value |
|---------------------|-------------------------|-------------------|-----------|
| Demographics and anthropometrics |                   |                   |           |
| Age, years          | 48.7 ± 12.3             | 48.3 ± 10.8       | n.s.      |
| Height, cm          | 171.4 ± 8.1             | 174.8 ± 8.5       | n.s.      |
| Body mass index, kg/m² | 26.5 ± 4.1             | 24.1 ± 2.2        | n.s.      |

| COMS at FRC |                     |                   |           |
|-------------|---------------------|-------------------|-----------|
| Right-sided latency, ms | 24.0 ± 4.7 | 17.7 ± 3.4 | <.01      |
| Right-sided amplitude, mV | 0.20 [0.10-0.40] | 0.80 [0.60-1.50] | .01       |
| Left-sided latency, ms | 24.5 ± 5.0 | 18.0 ± 3.0 | <.01      |
| Left-sided amplitude, mV | 0.20 [0.10-0.50] | 0.90 [0.60-1.00] | <.01      |

| CEMS at FRC |                     |                   |           |
|-------------|---------------------|-------------------|-----------|
| Right-sided latency, ms | 7.7 [6.0-9.5] | 4.6 [4.2-5.1] | <.01      |
| Right-sided amplitude, mV | 0.15 [0.09-0.20] | 0.20 [0.10-0.55] | n.s.      |
| Right-sided CMCT at FRC, ms | 17.1 ± 4.8 | 13.9 ± 3.4 | n.s.      |
| Left-sided latency, ms | 8.2 [7.7-9.8] | 4.9 [4.3-5.4] | <.01      |
| Left-sided amplitude, mV | 0.10 [0.10-0.20] | 0.25 [0.10-0.63] | n.s.      |
| Left-sided CMCT at FRC, ms | 17.0 ± 4.8 | 13.8 ± 3.1 | n.s.      |

Note: Data are presented as mean ± SD or median [interquartile range]. Abbreviations: CEMS, posterior cervical magnetic stimulation of the phrenic nerves; CMCT, central motor conduction time (latency after cortical stimulation – latency after cervical stimulation); CMT1A, Charcot-Marie-Tooth disease 1A; COMS, cortical magnetic stimulation of the diaphragm; FRC, functional residual capacity; n.s., not significant.
17.7 ± 3.4 ms, P < .01; dCMAP: by 75%, 7.7 [6.0-9.5] vs 4.6 [4.2-5.1] ms, P < .01). Central motor conduction time was similar in patients and healthy volunteers (P > .05; Table 3). Mean dMEP amplitude was lower in CMT1A patients than in controls (by 75%, 0.20 [0.10-0.40] vs 0.80 [0.50-1.60] mV, P < .01; Figure 6). Mean dCMAP amplitude was slightly lower in the CMT1A vs control group, but the difference was not statistically significant (0.15 [0.09-0.20] vs 0.20 [0.10-0.55], P = .23). Both, dMEP amplitude following COMS and dCMAP amplitude following CEMS correlated with DTR (r = .68, P = .01 and r = .51, P = .09, respectively).

3.5 | Invasive inspiratory muscle strength measurements

Transnasal insertion of balloon catheters was declined by seven patients with CMT1A, leaving 12 individuals in whom invasive pressure measurements could be performed. Therefore, the control group for this comparison included 12 matched subjects. Twitch Pdi was lower in CMT1A patients than in controls (by 71%, 7.8 ± 5.8 vs 19.5 ± 6.7 cmH2O, P < .01) (Table 4 and Figure 7) whereas Pdi following a voluntary sniff maneuver did not differ between the two groups (P > .05; Table 4).

3.6 | Invasive expiratory muscle strength measurements

Twitch Pgas values following magnetic stimulation of the lower thoracic nerve roots and Pgas following a voluntary cough maneuver were similar in CMT1A patients and healthy controls (Table 4).

4 | DISCUSSION

This study is the first to comprehensively apply volitional and non-volitional tests of respiratory muscle strength and function in a group of ambulatory CMT1A patients. Main findings of this study can be summarized as follows: Firstly, inspiratory muscle strength is reduced in patients with CMT1A, as reflected by both volitional (ie, PImax) and non-volitional tests (ie, twPdi), even if no or only mild symptoms of diaphragm weakness are present. Secondly, in CMT1A, magnetic phrenic NCS clearly reflect demyelinating neuropathy. Finally, patients with CMT1A also showed impaired expiratory strength that can be detected using volitional test such as PEmax and PCF.

4.1 | Review of the literature

Respiratory muscle involvement in patients with CMT has been described previously in original articles and case reports. One large study included 200 patients with CMT1A, of whom 40 reported respiratory symptoms and 15 showed possible respiratory muscle weakness, as roughly estimated by FVC <80% of predicted and maximal inspiratory pressure <40 cmH2O.17 Another study including 11 patients electrophysiologically demonstrated phrenic nerve involvement, even in the absence of respiratory complaints or abnormal FVC and mouth occlusion pressures. A third study documented altered phrenic nerve morphology on ultrasound and prolonged latencies on phrenic NCS in 16 patients with CMT1A; reduction of PImax and PEmax was present in 5 and 12 patients, respectively. Similarly, another study found a decrease in PImax in 6 out of 10 patients and reduced PEmax in 10 out of 10 patients with CMT1A (<60% of the predicted values). Against this background, the present study extends the spectrum of diagnostic methods to include diaphragm ultrasound and invasive measurement of twPdi, the latter having been applied only in one previous case report. Furthermore, our study is the first to apply magnetic stimulation of the lower thoracic nerve roots and invasive recording of twPgas to evaluate expiratory muscle function in CMT1A.

4.2 | Respiratory impairment and diaphragm dysfunction in CMT1A patients

The findings of this study are of both clinical and neurophysiological interest. They confirm previous evidence that phrenic nerve involvement is an inherent feature of CMT1A, and is associated with a measurable decrease in inspiratory muscle strength. In addition, impaired inspiration reduces the maximum TLC which can be achieved and negatively affects forced expiration. At the same time, abdominal muscle function following magnetic stimulation of the lower thoracic nerve roots appears to be normal. These observations can be made not only in patients with clinically apparent diaphragm weakness (ie, those who complain of significant dyspnea) but also those with no or only mild respiratory complaints. However, measures of diaphragm strength and function (PImax and DTR) are inversely related to disease severity, suggesting that respiratory impairment is more pronounced in patients with worse motor function. Thus, the present data underline that signs and symptoms of respiratory muscle involvement deserve...
special attention in patients with CMT1A, especially if advanced
disease is present.

4.3 | Phrenic nerve involvement in CMT1A

Neurophysiologically, our results allow several conclusions. Firstly,
phrenic nerve conduction abnormalities may be present in CMT1A
patients even if measures of respiratory muscle strength are still
normal. This is consistent with the fact that conduction slowing of
peripheral nerves in CMT1A is known to already be present in child-
hood, without notable change over time and showing no correlation
with motor impairment.43 Electrophysiological signs of demyelination
can usually be found in all peripheral nerves, including those that are
clinically unaffected (eg, cranial nerves).44 In CMT1A, it has been
shown that CMAP amplitudes of peripheral nerves directly correlate
with disease severity, and motor impairment is associated with axonal

| TABLE 4 | Invasively obtained inspiratory and expiratory muscle strength data in CMT1A patients and controls (n = 12 per group) |

| Patient No. (age, sex, CMT-NSv2 score) | Inspiratory muscle strength tests | Expiratory muscle strength tests |
|--------------------------------------|----------------------------------|----------------------------------|
|                                      | twPdi (cmH2O)                     | Sniff Pdi (cmH2O)                | Plmax (cmH2O)                     | twPgas (cmH2O) | coPgas (cmH2O) | PEmax (cmH2O) |
| Patients                             |                                  |                                 |                                 |               |                |               |
| 1 (55, M, 22)                        | 10.3                             | 85.7                            | 106                             | 15.2          | 120.6          | 134           |
| 2 (62, M, 20)                        | 4.3                              | 65.4                            | 54                              | 3.5           | 69.0           | 85            |
| 3 (63, F, 20)                        | 20.6                             | 58.2                            | 43                              | 21.8          | 86.1           | 101           |
| 4 (36, F, 13)                        | 6.6                              | 38.1                            | 76                              | 5.7           | 83.1           | 64            |
| 5 (42, M, 9)                         | 17.6                             |                                 | 111                             | 47.2          | 121.7          | 133           |
| 6 (60, F, 8)                         | 7.5                              | 86.3                            | 51                              | a             | 55.7           | 87            |
| 7 (25, F, 9)                         | 5.3                              | 68.1                            | 76                              | a             | 68.8           | 78            |
| 8 (41, F, 17)                        | 1.6                              | 84.1                            | 68                              | 5.9           | 124.0          | 74            |
| 9 (34, F, 19)                        | 7.7                              | 70.7                            | 68                              | 6.3           | 127.3          | 71            |
| 10 (51, F)                           | 3.6                              | 17.3                            | 20                              | a             | 60.2           | 61            |
| 11 (41, M, 14)                       | 4.7                              | 94.8                            | 88                              | 37.9          | 89.3           | 109           |
| 12 (41, F, 17)                       | 3.6                              | 105.9                           | 84                              | 9.4           | 103.0          | 70            |
| Mean                                 | 7.8                              | 70.4                            | 70.4                            | 17.0          | 92.4           | 88.9          |
| SD                                   | 5.8                              | 25.6                            | 26.0                            | 15.7          | 26.3           | 25.2          |
| Controls                             |                                  |                                 |                                 |               |                |               |
| 1 (60, M)                            | 27.1                             | 77.9                            | 68                              | 17.8          | 116.4          | 115           |
| 2 (64, M)                            | 25.5                             | 113.7                           | 81                              | 19.8          | 164.2          | 130           |
| 3 (58, F)                            | 11.0                             | 79.2                            | 97                              | 19.7          | 151.5          | 122           |
| 4 (37, F)                            | 16.0                             | 70.6                            | 57                              | 16.4          | 50.1           | 80            |
| 5 (43, M)                            | 6.7                              | (142.8)                         | 161                             | 14.4          | 96.0           | 168           |
| 6 (57, F)                            | 19.4                             | 83.5                            | 76                              | (14.1)        | 88.5           | 133           |
| 7 (26, F)                            | 30.1                             | 69.8                            | 60                              | (16.3)        | 140.6          | 116           |
| 8 (45, F)                            | 14.4                             | 51.5                            | 65                              | 21.1          | 68.5           | 110           |
| 9 (34, F)                            | 20.6                             | 68.5                            | 124                             | 31.8          | 106.1          | 145           |
| 10 (51, F)                           | 21.2                             | 84.3                            | 68                              | a             | 145.6          | 126           |
| 11 (42, M)                           | 20.4                             | 107.9                           | 74                              | 62.2          | 111.3          | 100           |
| 12 (39, F)                           | 21.4                             | 75.0                            | 100                             | 13.6          | 121.8          | 124           |
| Mean                                 | 19.5                             | 80.2                            | 85.9                            | 24.2          | 113.4          | 122.4         |
| SD                                   | 6.7                              | 17.6                            | 30.5                            | 14.5          | 34.2           | 22.0          |

P-value<sup>c</sup> <0.01 n.s. n.s. n.s. n.s. n.s. <0.01

Abbreviations: CMT-NSv2, CMT Neuropathy Scale version 2; coPgas, gastric pressure following maximum voluntary cough; F, female; M, male; n.s. not significant; Sniff Pdi, transdiaphragmatic pressure following sniff maneuver; twPdi, twitch transdiaphragmatic pressure following cervical stimulation of the phrenic nerve roots; twPgas, twitch gastric pressure following stimulation of the abdominal muscle nerve roots at the 10th vertebra.

<sup>a</sup>Missing value due to poor cooperation or insufficient reproducibility.
<sup>b</sup>Missing value due to incomplete electrophysiological data.
<sup>c</sup>For comparison of mean values in patients vs controls.
home ventilatory support. Given that capnographic data from patients with CMT1A and severe respiratory muscle involvement requiring chronic hypercapnic respiratory failure. Indication criteria that justify initiation of nocturnal non-invasive ventilation include clinical symptoms of sleep-disordered breathing and respiratory muscle weakness, marked reduction of FVC or PImax, and nocturnal hypercapnia. Only a few case reports have been published on patients with CMT1A and severe respiratory muscle involvement requiring home ventilatory support. Given that capnographic data from sleep studies in CMT1A patients are not yet available, sleep-related breathing has only been systematically evaluated with regard to intermittent upper airway collapse, that is, obstructive sleep apnea. Thus, current evidence suggests that severe diaphragm weakness is rare in CMT1A, which is in clear contrast with other genetic subtypes that may be associated with respiratory failure already in childhood. However, phrenic nerve involvement with variable impact on diaphragm function may be present as outlined above.

4.5 | Expiratory muscle function in CMT1A

Expiratory muscle function in patients with CMT1A has been shown to occur to a very variable extent. This might be related not only to the wide spectrum of disease severity in previous cohort studies but also to the volitional nature of PEmax testing. In fact, insufficient patient effort while performing a maximal expulsive maneuver would overestimate expiratory muscle weakness. In the current study, volitional measures of expiratory muscle strength were markedly decreased in CMT1A patients compared with control subjects. The present study combined measurement of twPdi following CEMS of the phrenic nerves with recording of twPg as in response to magnetic stimulation of the lower thoracic nerves. The latter method has been introduced for non-volitional testing of expiratory muscle strength but, to date, has only been applied in healthy subjects and patients with amyotrophic lateral sclerosis. In patients with CMT1A, twPdi was lower than in controls, clearly reflecting diaphragm weakness. At the same time, PEmax and PCF were impaired, but twPg as in response to magnetic stimulation of the abdominal muscles was normal. Thus, reduction of PEmax and PCF is likely to result from diaphragm dysfunction and impaired inspiration rather than abdominal muscle weakness. This finding is consistent with a previous report on two patients with CMT1A who had significant respiratory muscle weakness that was inspiratory and not expiratory in nature.

4.6 | Limitations of the current study

This study has several limitations which need to be addressed. Firstly, the sample size may limit the validity of our findings. Small sample size and high standard deviations might also explain why mean dCMAP amplitude was not significantly lower in CMT1A patients compared with controls. However, the present study was designed as a case-control study rather than a large-scale cohort study, and aimed to search for abnormalities that reach statistical significance even in a small group of patients. In addition, the technical complexity of the applied methods would render a much larger study extremely laborious. Secondly, this study did not specifically investigate whether the extent of phrenic nerve and respiratory muscle involvement relates to disease duration or disease progression, respectively. Here, further studies are necessary which either include long-term CMT-NSv2 scores prior to respiratory muscle assessment or prospectively monitor both phrenic nerve function and disease severity over a longer period of time. Thirdly, inter- and intra-observer variability may have affected magnetic NCS. We
aimed to address this by providing extensive training prior to the study, and by performing up to five tests per patient until variability was <10%, and by using a case-control study design. Fourthly, it may be considered a limitation that twPdi but not Sniff Pdi was significantly different between CMT1A patients and controls. However, this observation underlines that non-volitional evaluation of inspiratory muscle strength is more suitable to uncover subclinical abnormalities than voluntary test procedures. Ultimately, threshold testing for magnetic output was not performed. Instead, maximum magnetic flux density was used in each subject in order to achieve reproducible amplitudes in patients and matched controls.

5 | CONCLUSION

To conclude, phrenic nerve neuropathy may be present in ambulatory patients with CMT1A. Both volitional and non-volitional tests of respiratory muscle strength and function show that axonal neuropathy of the phrenic nerves is associated with inspiratory muscle weakness. Finally, diaphragm dysfunction is related to clinical disease severity even in patients who are not wheelchair-bound. Most likely, it reflects a disease continuum that already begins in patients free of respiratory symptoms.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

J.S. and M.B. designed the study. J.S. and C.H. were responsible for data collection. J.S., C.H. and M.B. performed the statistical analyses and prepared the manuscript which was critically revised and amended by M.B., T.B., P.Y., S.H., H.J.K. and W.R.

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