Axonal Ion Channel Dysfunction in C9orf72 Familial Amyotrophic Lateral Sclerosis

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IMPORTANCE Abnormalities of axonal excitability characterized by upregulation of persistent sodium (Na+) conductances and reduced potassium (K+) currents have been reported in sporadic amyotrophic lateral sclerosis (SALS) phenotypes and linked to the development of clinical features such as fasciculations and neurodegeneration.

OBJECTIVE To investigate whether abnormalities of axonal ion channel function, particularly upregulation of persistent Na+ conductances and reduced K+ currents, form the pathophysiological basis of chromosome 9 open reading frame 72 (c9orf72) familial amyotrophic lateral sclerosis (FALS).

DESIGN, SETTING, AND PARTICIPANTS This was a prospective study. Clinical and functional assessment, along with motor-nerve excitability studies, were undertaken in 10 clinically affected patients with c9orf72 FALS, 9 asymptomatic c9orf72 mutation carriers, and 21 patients with SALS from 3 hospitals and 2 outpatient clinics.

MAIN OUTCOMES AND MEASURES Axonal excitability variables were measured in patients with c9orf72 ALS and results compared with matched patients with SALS and healthy control participants.

RESULTS Strength-duration time constant (τSD) was significantly increased in the patients with c9orf72 FALS and those with SALS (mean [SD], c9orf72 FALS: 0.50 [0.02] milliseconds; SALS: 0.52 [0.02] milliseconds; P < .01) when compared with control participants (mean [SD], 0.44 [0.01] milliseconds). In contrast, there were no significant changes of τSD in asymptomatic c9orf72 mutation carriers (P = .42). An accompanying increase in depolarizing threshold electrotonus at 90 to 100 milliseconds (TEd 90-100 milliseconds) was also evident in the c9orf72 FALS (P < .05) and SALS (P < .01) cohorts. Mathematical modeling suggested that an increase in persistent Na+ conductances, along with reduced K+ currents, best explained the changes in axonal excitability. Importantly, these abnormalities in axonal excitability correlated with the motor amplitude (τSD: R = −0.38, P < .05 and TEd 90-100 milliseconds: R = −0.44, P < .01), muscle weakness (TEd 90-100 milliseconds: R = −0.32, P < .05), and the ALS Functional Rating Scale (TEd 90-100 milliseconds: R = −0.34, P < .05).

CONCLUSIONS AND RELEVANCE Findings from the present study establish that upregulation of persistent Na+ conductances and reduced K+ currents were evident in both c9orf72 FALS and SALS cohorts, and these changes in axonal excitability were associated with motor neuron degeneration.
Amyotrophic lateral sclerosis (ALS) is a rapidly progressive and universally fatal neurodegenerative disorder of the motor neurons. A genetic etiology has been identified in up to 60% of familial ALS (FALS) and 20% of apparently sporadic ALS (SALS) cohorts, with at least 21 genes and genetic loci implicated in ALS pathogenesis. An increased hexanucleotide repeat expansion (GGGGCC) in the first intron of the chromosome 9 open reading frame 72 (c9orf72) gene on chromosome 9p21 was reported to be the most common of the genetic mutations in ALS, underlining approximately 40% of FALS and 20% of SALS cases, although subsequent studies have established a c9orf72 mutation frequency of 4% to 8%. The c9orf72 phenotype may be characterized by ALS and frontotemporal dementia with psychiatric features, although the penetrance and expression of the genotype may vary within and between cohorts.

The c9orf72-associated ALS phenotype is clinically characterized by coexistence of upper motor neuron (UMN) and lower motor neuron (LMN) signs encompassing multiple body regions, with an earlier age at onset and shorter survival. Importantly, LMN signs are characterized by fasciculations and muscle wasting and weakness, clinically indistinguishable from the LMN features in SALS cohorts.

The pathophysiological mechanisms by which c9orf72 gene hexanucleotide expansion leads to the development of neurodegeneration and thereby the clinical features of ALS, particularly fasciculations, remain to be established. Wide-spread axonal ion channel dysfunction, including upregulation of persistent sodium (Na+) conductances and reduction of slow and fast potassium (K+) channel conductances, has been extensively documented in SALS, resulting in motor axonal hyperexcitability. Importantly, such changes in motor axonal excitability were postulated to underlie motor neuron degeneration and the development of clinical features of ALS, particularly fasciculations. In addition, motor axonal hyperexcitability, as reflected by upregulation of persistent Na+ conductances, was also reported in FALS cohorts secondary to mutations in the superoxide dismutase-1 (SOD-1) gene and linked to the process of neurodegeneration.

Threshold-tracking techniques may provide unique insights into nodal and internodal axonal membrane properties by sequentially assessing multiple excitability parameters. The strength-duration time constant (τSD), a measure of the rate at which the threshold current for a target potential declines as stimulus duration increases, appears to be a biomarker of persistent Na+ conductances. This notion is underscored by computer modeling studies of the human motor axon establishing that the τSD reflects the behavior of persistent Na+ conductances. In addition, depolarizing threshold electrotonus (TED) and hyperpolarizing threshold electrotonus (TEh), along with supereexcitability and late subexcitability, appear to be robust biomarkers of fast and slow K+ conductances, respectively. Consequently, the present study used axonal-excitability techniques to determine whether upregulation of persistent Na+ conductances and reduction of K+ currents was a feature of c9orf72 FALS and whether such changes in axonal excitability were linked to the processes of neurodegeneration, potentially informing the pathophysiological basis of c9orf72 FALS. In addition, the present study also aimed to determine whether axonal ion channel dysfunction was evident in asymptomatic c9orf72 mutation carriers.

**Methods**

Studies were undertaken on 10 clinically affected patients with familial c9orf72 ALS as defined by the Awaji criteria (6 men and 4 women; mean age, 63 years; age range, 41–78 years) and 9 asymptomatic c9orf72 mutation carriers (1 man and 8 women; mean age, 45 years; age range, 24–60 years). For comparison, 21 patients with SALS were studied (16 men and 5 women; mean age, 55 years; age range, 32–73 years). All patients with ALS (sporadic and familial) were clinically staged using the ALS Functional Rating Scale–Revised (ALSFRS-R). In addition, muscle strength was assessed using the Medical Research Council (MRC) score, with the following muscle groups assessed bilaterally yielding a total MRC score of 90: shoulder abduction; elbow flexion; elbow extension; wrist dorsiflexion; finger abduction; thumb abduction; hip flexion; knee extension; and ankle dorsiflexion. The degree of UMN dysfunction was assessed by a specific UMN score incorporating the following parameters: brisk jaw jerks (1 point); brisk facial reflex (1 point); pathologically brisk biceps, triceps, supinator, finger, knee, and ankle reflexes (1 point for each, assessed bilaterally); and extensor planter responses (1 point each and assessed bilaterally). The UMN score ranged from 0 (no UMN dysfunction) to 16 (severe UMN dysfunction).

Cognitive screening was undertaken only in the patients with c9orf72 FALS and asymptomatic mutation carriers using the Addenbrooke Cognitive Examination–Revised (ACE-R). Patients and carriers with diabetes mellitus or chronic renal failure were excluded from the study. None of the patients were taking medications that could affect the results. All participants gave written informed consent to the procedures, which were approved by the South East Sydney Area Health Service Human Research Ethics Committee.

Following clinical staging and phenotyping, the median nerve was stimulated at the wrist and the resultant compound muscle action potential (CMAP) recorded from the abductor pollicis brevis (APB) using surface electrodes. The active recording electrode was placed over the motor point of APB and the reference was placed 4 cm distally at the base of the thumb. Skin temperature was monitored close to the site of stimulation for the duration of each. Prior to excitability studies, CMAP amplitude and onset latency and F-wave latency and frequency were all measured. The neurophysiological index, a marker of peripheral disease burden in ALS, was derived according to a previously reported formula:

\[
\text{Neurophysiological index} = \text{CMAP amplitude (millivolt [mV])} \times \text{F-wave frequency/distal motor latency (milliseconds)},
\]

where F-wave frequency was expressed as the number of F responses recorded in 20 trials.

**Axonal Excitability**

All patients with ALS underwent axonal excitability studies on the median motor nerve, according to a previously described protocol. The median nerve was stimulated at the wrist using 5-mm nonpolarizable Ag-AgCl electrodes (ConMed) with the
cathode positioned over the skin crease and anode approximately 10 cm proximally over the lateral forearm. Stimulation was computer controlled and converted to current using an isolated linear bipolar constant current stimulator (maximal output ±50 mA; DS7, Digitimer). The CMAP responses were recorded from the APB muscle with the active (G1) electrode positioned over the motor point and the reference (G2) electrode placed at the proximal phalanx 4 cm away. Test current pulses were applied at 0.5-second intervals and combined with either subthreshold polarizing currents or suprathreshold conditioning stimuli, according to previously described protocols. The CMAP amplitude was measured from baseline to negative peak, with the target set to 40% of maximum for all tracking studies. Proportional tracking was used to determine the changes in threshold current required to produce and maintain a target response.34

Electrical stimuli were increased in incremental steps to generate the motor stimulus response curve. Subsequently, the \( t_{SD} \) a biomarker of persistent Na\(^+\) conductance, and rheobase, defined as the threshold current for a stimulus of infinitely long duration, were measured according to the Weiss formula.15,25,34,35

Following the determination of the stimulus strength-duration relationship, threshold electrotonus (TE) was determined using subthreshold polarizing currents of 100-millisecond duration, set to ±40% of controlled threshold current.21,28,34 Test stimuli of 1-millisecond duration were used to produce and maintain a target response of 40% of maximum CMAP amplitude. Three stimulus combinations were tested sequentially: test stimulus alone (measured control threshold current); test stimulus + depolarizing current; and test stimulus + hyperpolarizing current. Threshold was tested at 26 points before, during, and after the 100-millisecond polarizing pulse. The stimulus combinations were repeated until 3 valid estimates were recorded within 15% of target response.21 The following TE changes were recorded with subthreshold depolarizing currents: TE at 10 to 20 milliseconds; TE at 40 to 60 milliseconds; and TE at 90 to 100 milliseconds. In addition, changes in membrane threshold to hyperpolarizing currents at 10 to 20 milliseconds, TE at 10 to 20 milliseconds and at 90 to 100 milliseconds, and TE at 90 to 100 milliseconds were also measured.

The current-threshold relationship (I/V), a biomarker of inward and outward rectifying membrane currents,28 was assessed by tracking threshold changes following subthreshold polarizing currents of 200-millisecond duration, which were altered in ramp fashion from +50% (depolarizing) to −100% (hyperpolarizing) of controlled threshold in 10% steps. Conditioning stimuli were alternated with test stimuli until 3 valid threshold estimates were recorded. The following parameters were recorded: (1) resting I/V slope, calculated from polarizing currents between +10% to −10% and (2) hyperpolarizing I/V slope, calculated from polarizing current between −80% and −100%.

Lastly, the recovery cycle of axonal excitability was recorded, according to a well-established protocol.23,36 The following parameters were measured: (1) relative refractory period (in milliseconds), defined as the first intercept at which the recovery curve crosses the x-axis and a biomarker of transient Na\(^+\) channel function;28; (2) superexcitability (%), defined as the minimum mean threshold change of 3 adjacent points and a biomarker of paranodal fast K\(^+\) channel conduction;28; and (3) late subexcitability (%), defined as the largest increase in threshold following the superexcitability period and a biomarker of nodal K\(^+\) channel conduction.28

Recordings of CMAP responses were amplified and filtered (3 Hz–3 kHz) using a Nicolet-Biomedical EA-2 amplifier (Cardinal Health Viking Select version 11.1.0, Viasys Healthcare Neurocare Group) and sampled at 10 kHz using a 16-bit data-acquisition card (National Instruments PCI-MIO-16E-4). Data acquisition and stimulation delivery were controlled by QTRACS software (TROND-F, version 16/02/2009; Hugh Bostock, PhD, Institute of Neurology, London, England).

Mathematical Modeling of Axonal Excitability Changes

To model the axonal excitability changes evident in patients with c9orf72 FALS and those with SALS, mathematical simulations were undertaken using an established model of the human motor axon.13,37-40 Nodal transient and persistent Na\(^+\) currents were modeled using data from voltage-clamp and latent addition studies in human axons.24,41 This model incorporates slow and fast K\(^+\) currents at both the node and internode; the hyperpolarization-activated current \( I_h \) at the internode; and leak and pump currents at both the node and internode.42 The discrepancy between the model and the group data was calculated as the weighted sum of the squares of the error terms: \( (x_{m} - x)^2 \), where \( x_{m} \) is the model threshold and \( x \) is the mean threshold for the group data. The weights were 0.5 for the \( t_{SD} \) and 1 for each of the other threshold measurement types (ie, TE, current-threshold relationship, and the recovery cycle). The model was first adjusted to fit the normal control data using an interactive least-squares procedure, which minimized the discrepancy between the model and data.42

Statistical Analysis

Axonal excitability studies were compared with 34 age-matched control individuals (13 men and 21 women; mean [SD] age, 51.1 [2.2] years), with the t test used for assessing differences between means among patients, carriers, and control participants. All of the axonal excitability data were normally distributed as assessed by the Shapiro-Wilk test. Pearson correlation coefficients were used to examine the relationship between parameters. Because measures of excitability may vary with age and temperature, parameters were compensated for age, temperature, and sex, if required before statistical analysis, using the relations established previously in control individuals.43 The ACE-R score was nonparametrically distributed and a Mann-Whitney U test was used to compare differences between c9orf72 FALS and asymptomatic mutation carriers. \( P < .05 \) was considered statistically significant, and the probability values were corrected for multiple comparisons. Results were expressed as mean ± standard error of the mean (SEM) or median (interquartile range).

Results

Clinical Phenotype

The clinical features for the 10 clinically affected patients with FALS, 9 asymptomatic c9orf72 mutation carriers, and 21 pa-
Neurological examination findings were normal in all of the asymptomatic c9orf72 mutation carriers. Specifically, there was no clinical evidence of UMN or LMN dysfunction at the time of assessment. To date, all of the asymptomatic mutation carriers have remained well.

**Neurophysiology**

The mean (SEM) CMAP amplitudes were significantly reduced in the patients with c9orf72 FALS and SALS when compared with normal control participants (c9orf72 FALS: 3.4 [0.3] mV; SALS: 5.6 [0.8] mV; and control participants: 8.8 [0.4] mV; F = 17.9; P < .001). Importantly, there were no significant differences in CMAP amplitudes between the c9orf72 FALS and SALS cohorts (P = .11). In addition, the neurophysiological index was also significantly reduced in the patients with c9orf72 FALS and those with SALS when compared with control participants (mean [SEM], c9orf72 FALS: 1.1 [0.2]; SALS: 1.2 [0.2]; and control participants: 2.5 [0.1]; P < .01). While these findings indicate a significant degree of LMN dysfunction in the c9orf72 FALS and SALS cohorts, there was a trend for the CMAP amplitude to be lower in the patients with c9orf72 FALS, despite a comparable clinical disease burden. This discordant finding could be potentially explained by a greater sensitivity of neurophysiological techniques in detecting LMN dysfunction.44 Of further relevance, clinical and electrical evidence of fasciculations were evident in all the patients with c9orf72 FALS and SALS.

In contrast, there was no significant differences in the mean (SEM) CMAP amplitude (c9orf72 mutation carriers: 7.0 [0.6] mV and control participants: 8.8 [0.4] mV) and neurophysiological index (asymptomatic c9orf72 mutation carriers: 2.5 [0.3] and control participants: 2.5 [0.1]) between the c9orf72 mutation carriers and normal control participants, reaffirming the structural integrity of the peripheral nerves at the time of assessment in asymptomatic control participants.

**Strength-Duration Time Constant and Rheobase**

The τsd is a biomarker of persistent Na⁺ conductances at the node of Ranvier.15,24-27,45 The mean (SEM) τsd was significantly increased in the patients with c9orf72 FALS (0.5 [0.02] milliseconds; P < .05) and SALS (0.52 [0.02] milliseconds; P < .01) when compared with control participants (Figure 1A and B). In contrast, there were no significant differences in the τsd between asymptomatic c9orf72 mutation carriers and control participants (asymptomatic c9orf72 mutation carriers: 0.45 [0.04] and control participants: 0.44 [0.01]; P = .42; Figure 1A and B).

Of further relevance, rheobase was significantly reduced in both patients with c9orf72 FALS (mean [SEM], 1.61 [0.5] mA; P < .001; Figure 1A and C) and SALS (mean [SEM], 1.35 [0.10] mA; control participants: 2.27 [0.23] mA; P < .01; Figure 1A and C). In contrast, rheobase was slightly increased in the asymptomatic c9orf72 mutation carriers (mean [SEM], 2.64 [0.72] mA; Figure 1A and C) but again this increase was not significant.

**Threshold Electrotunus**

Threshold electrotunus provides insight into nodal and internodal membrane conductances. Previously, 2 types of abnormalities of TE have been described in SALS, namely the type I abnormality, in which there is a greater change in response to

| Table. Clinical Details for the Patients With C9orf72 FALS and SALS and AMCs |
|-----------------|----------------|-------------|
| **Clinical Features** | **C9orf72 FALS** | **SALS** | **AMCs** |
| Age at assessment, y | 63 (3.8) | 55 (2.5) | 45 (3.4) |
| Sex (M:F) | 6:4 | 16:5 | 1:8 |
| Disease duration, moa | 14.3 (3.9) | 12.5 (2.0) | NA |
| Site of disease onset, % | | | |
| Bulbar | 40 | 29 | NA |
| Limb | 60 | 71 | NA |
| ALSFRS-R scoreb | 39 (1.2) | 41.6 (1.4) | 48 |
| MRC scorec | Upper limb | 54 (2.2) | 53.8 (1.8) | 60 |
| Lower limb | 28 (0.7) | 28 (0.8) | 30 |
| Total | 82 (2.3) | 82 (1.5) | 90 |

Abbreviations: ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised; AMCs, asymptomatic c9orf72 mutation carriers; FALS, familial amyotrophic lateral sclerosis; MRC, Medical Research Council; NA, not applicable; SALS, sporadic amyotrophic lateral sclerosis; SEM, standard error of the mean.

a Disease duration refers to the period from symptom onset to date of testing.

b The patients were clinically graded using the ALSFRS-R, with a maximum score of 48 when there is no disability.

c Muscle strength was clinically assessed using the MRC, with an upper limb, lower limb, and total score being generated (see the Methods section).
a subthreshold depolarizing pulse, and the type II abnormality, in which there is a sudden decrease in membrane excitability marked by an abrupt increase in threshold.\(^\text{13}\) In the present study, the type I abnormality was evident in 40% of the patients with c9orf72 FALS and 29% of those with SALS, while the type II abnormality was not evident. Furthermore, neither the type I nor type II abnormalities were evident in the asymptomatic c9orf72 mutation carriers.

Group data analysis disclosed the presence of a significant difference in TE between patients with FALS and SALS, asymptomatic c9orf72 mutation carriers, and control participants (Figure 2A). Specifically, mean (SEM) TED (90-100 milliseconds) was significantly increased in both the c9orf72 FALS (51.0% [1.9%]; \(P < .05\)) and SALS (50.3% [1.4%]; \(P < .01\)) when compared with control participants (45.6% [0.6%]) (Figure 2B). Although there was a greater threshold change to hyperpolarizing subthreshold conditioning pulses in the patients with c9orf72 FALS and SALS (c9orf72 FALS: \(-128.7%\) [8.7%]; SALS: \(-122.5%\) [0.6%]); and control participants: \(-117.5%\) [3.2%]; \(P = .13\)) (Figure 2), this difference was not significant.

**Figure 1. Strength-Duration Time Constant in Amyotrophic Lateral Sclerosis (ALS)**

![Figure 1](https://www.jamanetwork.com/journals/jamaneurology/fullarticle/10.1001/jamaneurol.2014.5354)

The strength-duration time constant reflects nodal persistent Na+ channel conductances. A, The strength-duration time constant was significantly increased in patients with c9orf72 familial ALS (FALS, triangles) and sporadic ALS (SALS, circles) when compared with asymptomatic c9orf72 mutation carriers (AMCs, diamonds) and control participants (squares). B, Mean strength-duration time constant was significantly increased in patients with FALS and SALS. C, Rheobase was significantly reduced in patients with FALS and SALS. The error bars indicate standard errors of the mean.

**Figure 2.**

![Figure 2](https://www.jamanetwork.com/journals/jamaneurology/fullarticle/10.1001/jamaneurol.2014.5354)

**Current-Threshold Relationship**

The I/V relationship estimates the inward and outward rectifying properties of nodal and internodal axonal segments, with the hyperpolarizing I/V gradient reflecting conductances via inward rectifying currents.\(^\text{34,46}\) The hyperpolarizing I/V gradient was significantly increased in patients with c9orf72 FALS (mean [SEM], 0.42 [0.02]; \(P < .05\)) and SALS (mean [SEM], 0.41 [0.02]; \(P < .05\)) when compared with asymptomatic mutation carriers (mean [SEM], 0.34 [0.03]) and control participants (mean [SEM], 0.36 [0.01]).

**Recovery Cycle of Excitability**

The mean (SEM) relative refractory period duration was not significantly different when compared with control participants (c9orf72 FALS: 3.7 [0.2] milliseconds, \(P = .06\); SALS: 3.8 [0.3] milliseconds, \(P = .08\); asymptomatic c9orf72 mutation carriers: 3.2 [0.1] milliseconds, \(P = .36\); and control participants: 3.3 [0.1] milliseconds). In addition, there were no significant differences in superexcitability and late subexcitability between groups.

**Correlation With Clinical Parameters and Disease Duration**

Combining measures of axonal excitability, clinical assessment, and disease severity, it was evident that \(\tau_{SD} (R = -0.38; \text{Figure 3A})\) and TED (90-100 milliseconds) \(R = -0.44; \text{Figure 3B}\) in patients with c9orf72 FALS and SALS correlated with the CMAP amplitude, thereby suggesting that the increase in the \(\tau_{SD}\) and TED (90-100 milliseconds) were potentially linked to axonal degeneration. In addition, the TED (90-100 milliseconds) was significantly correlated with the ALS-FRS-R \(R = -0.34; \text{Figure 3A}\) and MRC upper limb scores \(R = -0.32; \text{Figure 3B}\). Taken together, these findings suggest that abnormalities of axonal excitability, particularly increased the \(\tau_{SD}\) and TED (90-100 milliseconds), are late features of c9orf72 FALS and linked to the process of neurodegeneration.

**Mathematical Modeling of Abnormal Excitability Properties**

To assist in interpreting the changes observed in axonal excitability, a mathematical model of the human motor axon was adjusted to provide a close match to the control group (Figures 4 and 5). The model was then used to explore which membrane parameter changes could reproduce the changes seen in the combined FALS and SALS case recordings. The axonal excitability data in c9orf72 FALS and SALS were best modeled by a 30% reduction in the nodal slow K+ conductance along with an increase in persistent Na+ conductances and increase in \(I_L\) current, reducing the overall discrepancy by 81.7%. The modeled changes resulted in a net depolarization of resting membrane potential of 0.8 mV.
Controls

Figure 2. Threshold Electrotonus (TE) Measures in Amyotrophic Lateral Sclerosis (ALS)

Discussion

The present study established a pattern of axonal excitability abnormalities in c9orf72 FALS that resembled changes observed in patients with SALS. Specifically, the τSD was significantly longer and TEd at 90 to 100 milliseconds significantly greater in the c9orf72 FALS and SALS cohorts. Mathematical modeling suggested that changes in axonal excitability were best explained by a reduction of slow nodal K⁺ currents along with an increase in persistent Na⁺ conductances, inward rectification, and Na⁺ pump activity. Importantly, the abnormalities of axonal excitability correlated with functional (ALS-FRS-R and MRC upper limb scores) and neurophysiological (CMAP amplitude) biomarkers of peripheral disease burden. In contrast, axonal excitability was normal in asymptomatic (CMAP amplitude) biomarkers of peripheral disease burden.

Threshold electrotonus reflects changes in membrane excitability in response to long-duration polarizing currents. Threshold reduction with depolarization is represented in an upward direction and hyperpolarization in a downward direction. A, Depolarizing TE (TEd) was significantly increased in c9orf72 familial ALS (FALS, triangles) and sporadic ALS (SALS, circles) when compared with asymptomatic c9orf72 mutation carriers (AMCs, diamonds) and control participants (squares). In contrast, hyperpolarizing TE was not significant (NS) between groups. B, Mean TEd at 90 to 100 milliseconds was significantly increased in c9orf72 FALS and SALS. The error bars indicate standard errors of the mean.

© References 10, 12, 15, 23, 25-28, 34, 45, 47

b, indicating that upregulation of persistent Na⁺ conductances contributed to the development of clinical features and neurodegeneration in SOD-1 FALS, a finding in keeping with transgenic SOD-1 mouse model studies.49

The present study establishes that prolongation of the τSD and thereby upregulation of persistent Na⁺ conductances, was a pathophysiological feature of c9orf72 FALS, a notion supported by mathematical modeling of axonal excitability. The correlation between prolongation of the τSD and biomarkers of peripheral disease burden (including the CMAP amplitude) suggests that persistent Na⁺ conductances may contribute to motor neuron degeneration in c9orf72 FALS. Importantly, findings from the present study imply the existence of a common pathogenic pathway in ALS, irrespective of the underlying genotype.

In addition to prolongation of the τSD, there were significant changes in TEd. Specifically, the type I abnormality of TE, in which there was a greater change in response to a subthreshold depolarizing pulse, was evident in the c9orf72 FALS cohort but not asymptomatic mutation carriers. In addition, the mean TEd (90-100 milliseconds) was significantly increased in c9orf72 FALS and SALS, correlating with peripheral biomarkers of peripheral disease burden, thereby suggesting that reduction of slow K⁺ currents contributed to the development of axonal hyperexcitability and clinical features in c9orf72 FALS. Importantly, mathematical modeling indicated that reduction of slow K⁺ currents in a large part accounted for the changes observed in axonal excitability, suggesting an important role for slow K⁺ channels in c9orf72 pathophysiology. This notion is further underscored by recent patch-clamp studies reporting that retigabine, a K⁺ channel activator, reduces axonal hyperexcitability and improves motor neuron survival in pluripotent stem cells derived from patients with c9orf72 FALS, thereby implying a therapeutic potential for retigabine.49 Of further relevance, a similar degree of reduction in slow K⁺ currents in the...
patients with \textit{c9orf72} FALS and SALS underscores the existence of common pathogenic pathways.

It could also be argued that the observed reduction in slow $K^+$ conductances contributed to the increase in the $\tau_{SD}$ through depolarization of the resting membrane potential. Such a hypothesis cannot be discounted given that modeling studies suggested that reduction in slow $K^+$ plays a bigger role in reducing the discrepancy in the measure of the $\tau_{SD}$ than the increase in fraction of persistent Na$^+$ channels. Given that axonal excitability parameters, such as superexcitability and TE, which are reduced with resting membrane potential depolarization,\textsuperscript{20} remained unchanged argues

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**Figure 3. Correlation of Axonal Excitability Parameters With Motor Amplitudes**

\begin{align*}
\text{A} & \quad \text{Strength-duration time constant and CMAP} \\
\text{B} & \quad \text{TEd at 90-100 ms and CMAP}
\end{align*}

The strength-duration time constant (A) and depolarizing threshold electrotonus (TEd) at 90 to 100 milliseconds (B) significantly correlated with the compound muscle action potential (CMAP) amplitude, thereby suggesting that strength-duration time constant and TEd at 90 to 100 milliseconds were linked with the process of axonal degeneration. FALS indicates familial amyotrophic lateral sclerosis; SALS, sporadic ALS.

**Figure 4. Mathematical Modeling of Axonal Excitability Parameters in Amyotrophic Lateral Sclerosis**

\begin{align*}
\text{A} & \quad \text{Threshold electrotonus recordings} \\
\text{B} & \quad \text{Modeled threshold electrotonus model} \\
\text{C} & \quad \text{Current-threshold relationship} \\
\text{D} & \quad \text{Modeled current-threshold relationship}
\end{align*}

Comparison of group data (control participants, open circles; combined \textit{c9orf72} and sporadic ALS, filled circles) and mathematical model (open triangles, normal model; \textit{c9orf72} and sporadic ALS model, filled triangles). A, Threshold electrotonus group data. B, Modeled threshold electrotonus. C, Current-threshold relationship. D, Modeled current-threshold relationship.
against the notion that membrane depolarization solely accounted for the increase in the \( \tau_{SD} \).

In contrast to previous studies in patients with SALS, \(^{9,10}\) paranodal fast K\(^+\) channel function appears to be preserved in patients with \( c9orf72 \) FALS and the current SALS cohort. Specifically, superexcitability and TEd (10-20 milliseconds), both biomarkers of paranodal fast K\(^+\) channels, \(^{28}\) were not significantly altered in the \( c9orf72 \) FALS and SALS cohorts. Importantly, paranodal fast K\(^+\) channel dysfunction appears to evolve with disease progression and seems most pronounced immediately prior to motor axonal loss. \(^{30}\) Consequently, the discordant findings between the current and previous ALS cohorts may relate to differences in the extent and stage of motor axonal loss, reflecting the membrane properties of surviving motor axons at the time of assessment.

An increase in the hyperpolarizing I/V gradient was also evident in the \( c9orf72 \) FALS and SALS cohorts and is best explained by an increase in inward rectifying currents (I\(_h\)). The increase in I\(_h\) most likely represents an adaptive response to the increased demands on surviving motor axons, perhaps secondary to axonal hyperexcitability due to reduction in slow K\(^+\) currents and upregulation of persistent Na\(^+\) conductances.

In contrast to abnormalities of axonal excitability evident in the patients with \( c9orf72 \) FALS and SALS, there were no significant changes in asymptomatic \( c9orf72 \) mutation carriers. This finding is in keeping with previous studies in SOD-1 asymptomatic mutation carriers, \(^{48}\) implying that factors other than inheritance of the genetic mutation may be required to trigger the disease process.

**Conclusions**

The mechanisms by which axonal ion channel dysfunction leads to neurodegeneration and adverse survival in ALS\(^{39}\) remain to be elucidated, although it has been postulated that an influx of Na\(^+\) ions results in reverse operation of the Na\(^+\)/calcium (Ca\(^{2+}\))-exchanger, intra-axonal accumulation of Ca\(^{2+}\), and ultimately activation of Ca\(^{2+}\)-dependent enzyme pathways leading to motor neuron degeneration. \(^{52-56}\) In addition, reduction of slow K\(^+\) currents and upregulation of persistent Na\(^+\) conductances would increase the depolarizing drive, thereby leading to axonal hyperexcitability and the development of cramps and fasciculations, both prominent symptoms in ALS. \(^{1,57-62}\)


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