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Electrophysiological features of *SYT2* mutations; a novel and treatable neuromuscular syndrome.

Roger G Whittaker PhD[1ɸ]*, David N Herrmann MBBCh[ɸ 2], Boglarka Bansagi MD[3], Bashar Awwad Shiekh Hasan PhD[1], Robert Muni Lofra BSc[3], Eric L. Logigian MD[2], Janet E. Sowden BSc[2], Jorge L. Almodovar MD[4], J. Troy Littleton MD, PhD[5], Stephan Zuchner MD, PhD[6], Rita Horvath MD[3#], Hanns Lochmüller MD[3#].

[1]Institute of Neuroscience, Newcastle University, Newcastle, UK. [2]Department of Neurology, University of Rochester Medical Center, Rochester, NY. [3]John Walton Muscular Dystrophy Research Centre, Institute of Genetic Medicine, Newcastle University, Newcastle, UK. [4]Department of Neurology, Dartmouth Hitchcock Clinic, Geisel School of Medicine, Hanover, NH. [5]The Picower Institute for Learning and Memory, Department of Biology and Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA. [6]Dr. John T Macdonald Department of Human Genetics and Hussman Institute for Human Genomics, University of Miami, Miller School of Medicine, Miami, FL.

*corresponding author : Dr Roger G Whittaker, Institute of Neuroscience, Henry Wellcome Building for Neuroecology, Newcastle University, Framlington Place, Newcastle upon Tyne. NE2 4HH, UK. Roger.whittaker@newcastle.ac.uk; Tel (0044) 191 2083543

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Author contributions:
Dr Whittaker: study design, acquisition of data, analysis of data, writing of manuscript.
Dr Herrmann: study design, acquisition of data, analysis of data, writing of manuscript.
Dr Bansagi: Acquisition of data, analysis of data.
Dr Hasan: Analysis of data.
Dr Lofra: Acquisition of data.
Dr Logigian: Acquisition of data, critical revision of the manuscript for important intellectual content.
Dr Sowden: Acquisition of data, analysis of data.
Dr Almodovar: Acquisition of data, analysis of data.
Dr Littleton: Critical revision of the manuscript for important intellectual content.
Dr Zuchner: Critical revision of the manuscript for important intellectual content.
Dr Horvath: Study design, acquisition of data, analysis of data, writing of manuscript.
Dr Lochmuller: Study design, acquisition of data, analysis of data, writing of manuscript.

[Φ] RGW and DH, and [#]RH and HL, contributed equally to this work.

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Abstract

Objectives: To describe the clinical and electrophysiological features of synaptotagmin 2 mutations, a novel neuromuscular syndrome characterised by foot deformities and fatiguable ocular and lower limb weakness, and the response to modulators of acetylcholine release.

Methods: We performed detailed clinical and neurophysiological assessment in two multi-generational families with dominant *SYT2* mutations (c.920T>G, p.Asp307Ala and c.923G>A, p.Pro308Leu). Serial clinical and electrophysiological assessments were performed in members of one family treated first with pyridostigmine and then with 3,4-diaminopyridine.

Results: Electrophysiological testing revealed features indicative of a presynaptic deficit in neurotransmitter release with post-tetanic potentiation lasting up to 60 minutes. Treatment with 3,4-diaminopyridine produced both a clinical benefit and an improvement in neuromuscular transmission.

Discussion: *SYT2* mutations cause a novel and potentially treatable complex presynaptic congenital myasthenic syndrome characterised by motor neuropathy causing lower limb wasting and foot deformities, with reflex potentiation following exercise and a uniquely prolonged period of post-tetanic potentiation.

Introduction

Congenital myasthenic syndromes (CMS) are a heterogeneous group of disorders caused by abnormal signal transmission between motor axons and skeletal muscle[1]. Mutations in an increasing number of presynaptic proteins, components of the synaptic basal lamina, proteins involved in endplate development and maintenance, and more recently in protein glycosylation[2] have been reported, causing novel and complex phenotypes. Since most CMS are treatable, diagnosing them is of utmost importance.

Signal transmission at the neuromuscular junction is mediated via the release of acetylcholine from synaptic vesicles.. This process is rendered calcium sensitive by members of the synaptotagmin protein family, which also play a role in vesicle priming and in reducing spontaneous transmitter release[4,5]. Synaptotagmin 2 (SYT2) is the major isoform expressed at the neuromuscular junction, and Syt2 knockout mice show markedly reduced calcium-evoked transmitter release[6]. Synaptotagmins interact with SNAP-25[7] and mutations in *SNAP25B* have been described in patients with myasthenia and additional CNS phenotypes [8].

Here, we describe the clinical and electrophysiological characteristics of two multi-generational families displaying a novel human motor syndrome caused by dominant *SYT2* mutations; c.920T>G (p.Asp307Ala) and c.923G>A, (p.Pro308Leu)[9]. These heterozygous missense mutations alter adjacent amino acids within the C2B calcium-binding domain of Syt2. Patients present with childhood onset foot deformities, lower limb weakness and wasting with areflexia. However, in some cases, additional fatigability of the eye and limb muscles is observed, and strength and reflexes improve with exercise. Furthermore, neurophysiological testing reveals features indicative of a presynaptic congenital myasthenic syndrome, with a uniquely prolonged period of post-tetanic potentiation.

Materials and methods

Patients were investigated at the peripheral neuropathy clinic at University of Rochester and at the inherited peripheral neuropathy and congenital myasthenia clinics in Newcastle upon Tyne. They were included in the study on the basis of a genetically confirmed autosomal dominant SYT2 mutation. Patients were assessed using the CMTNS2 peripheral neuropathy rating scale[10] and the Congenital Myasthenic Syndrome scale (CMSS) which is a modified version of the myasthenia gravis score[11] before, during and after medication discontinuation.

Standard Protocol Approvals, Registrations, and Patient Consents

The project received approval by local ethics committees. Patients gave informed consent for all clinical, electrophysiological and therapeutic studies.

Electrophysiological studies

Studies were performed on a Viking-Nicolet (USA) or a Dantec Keypoint G4 (UK) electromyography machine. Surface electrical stimulation was applied via either Carefusion ring electrodes or a hand-held Alpine Biomed bipolar stimulating electrode. Responses were recorded using Natus Neurology disposable disk electrodes (1cm diameter). Amplitudes were measured baseline to peak. Single fibre electromyography was performed using Natus Neurology disposable 30G concentric needles with a bandpass of 2-10kHz.

Repetitive nerve stimulation (RNS) was performed on: Abductor digiti minimus (ADM); abductor pollicis brevis (APB), and tibialis anterior (TA). Ten supramaximal stimuli were applied, with the percentage increment or decrement calculated between the first and fourth response. An amplitude increase or decrease of greater than 10% was regarded as significantly abnormal[12].

To assess post-tetanic potentiation, single supramaximal stimuli were applied at least 60 seconds apart to establish the baseline compound muscle action potential (CMAP) amplitude. Subjects were then asked to make a 10 second isometric maximum voluntary contraction against resistance. Single supramaximal CMAP responses were then recorded every 30 seconds for 5-10 minutes, with longer time intervals up to 60 minutes.

Results

Family 1 (US)

This family carried a heterozygous missense mutation c.920T>G (p.Asp307Ala) in the *SYT2* gene.

The proband (USA II.2), a 49 year old woman, was referred to the University of Rochester for putative Charcot-Marie-Tooth disease (CMT). She had high-arched feet and hammer toes since childhood. At age 44 she underwent surgery to correct hammer toe deformity and had delayed recovery following general anaesthesia, describing months of muscle fatigue and weakness. She continued to experience fatigue, weakness and exertional dyspnoea that improved with rest.

Physical examination showed pes cavus (Fig. 1a). Strength testing showed symmetric, proximal and distal MRC grade 4 weakness in upper and lower extremities that improved with muscle activation. Deep tendon reflexes were unobtainable at rest, but elicited at the knees and biceps following exercise. Acetylcholine receptor binding, blocking and modulating antibodies and voltage-gated calcium channel antibodies were negative. These were not checked in the other patients. No pathogenic *CACNA1A* gene mutations were found, and none of the patients underwent a muscle or nerve biopsy.

USA I.2; a 70 year old woman. She had high-arched feet and hammer toes since childhood. Examination showed pes cavus and hammer toes. Strength testing showed grade 4+ weakness of finger extensor, finger abductor, ankle dorsiflexion and hip girdle muscles. Deep tendon reflexes were absent, and did not facilitate after exercise. Gait was narrow-based with mild waddling. She could not tandem, toe or heel walk.

USA III.1; a 23 year old woman. Examination revealed slight pes cavus and hammer toes. Deep tendon reflexes were unobtainable at the ankles and in the arms, and hypoactive at the knees. The biceps reflex could be elicited after brief exercise. She had slight difficulty squatting and heel walking.

USA III.2; A 15 year old male. He experienced tripping and falls starting in elementary school. He participated in school sports, but described early muscle fatigue. Examination revealed pes cavus and hammer toes (Fig. 1a). Deep tendon reflexes were initially unobtainable, but could be elicited following brief exercise. The remainder of the neurological examination was normal, except for mild difficulty with tandem gait.

Family 2 (UK)

In this family, a heterozygous missense mutation of *SYT2* in the adjacent amino acid residue to that in the US family was found (c.923G>A, p.Pro308Leu).

UK III.2; The index patient is a 27 year old woman. She was diagnosed with congenital hip dysplasia after birth, and had foot deformities since childhood. At age 12 years she underwent bilateral foot surgery for her high arches. On general physical examination she had severe bilateral foot deformities (Fig 1.b). Neurological examination revealed fatigable eye movements, with slight ptosis after exercise and distal lower extremity muscle wasting, with normal power in proximal limb muscles, and grade 4+ weakness in hand intrinsic muscles, and grade 3 in ankle dorsiflexion and plantar flexion. Deep tendon reflexes were absent. Vibration and pinprick sensation was diminished in the feet and hands. Casual gait was non-ataxic, but aided by bilateral ankle foot orthotics and orthotic shoes.

UK IV.1; A 7 year old male. He was born by emergency Caesarian section for failure to progress. His motor milestones were delayed with poor balance and frequent falls. He had a mild language delay, but normal academic achievement. Physical examination showed joint hyperlaxity and pes planus. Neurological examination showed poor fine motor skills, reduced muscle tone, with normal muscle bulk and strength. All deep tendon reflexes were absent. Gait was unsteady, and he was unable to heel walk.

UK III.6; A 16 year old male. He was asymptomatic. General examination revealed pes cavus and clawing of his toes bilaterally, and mild wasting of distal lower limb musculature. Strength was normal but deep tendon reflexes were absent.

UK III.7; A 12 year old female. She was born with developmental hip dysplasia, and had bilateral foot deformity since early childhood. She complained of difficulty with sports participation and of muscle cramps and pain in her lower limbs with prolonged exertion. Examination showed bilateral foot deformities. Muscle tone and bulk were normal, with MRC grade 4 weakness of right ankle dorsiflexion. She had generalized areflexia. Sensation was intact. She was unable to heel walk.

UK III.3; (a 24 year old male) and UK III.8 (a 9 year old female) are unaffected with no neurologic symptoms or signs and no foot deformities.

UK II.2; A 44 year old woman. She had bilateral hip dysplasia, corrected with braces. She had a history of progressive weakness and wasting predominantly in the right lower extremity since early childhood. She had been diagnosed with a variant of localized morphea, contributing to loss of soft tissue bulk in the right thigh. She also complained of right leg pain, and abnormal sensory symptoms in her right foot and hand. She required surgery for bilateral foot deformity and complex reconstructive right knee surgery. General examination showed bilateral pes cavus, and reduced muscle bulk proximally and distally in the right lower extremity, and distally in the left lower limb. There was a slight ptosis and diplopia triggered by exercise. She had grade 4+ weakness in intrinsic hand muscles bilaterally, grade 4 weakness in proximal and distal right lower limb muscles and 4+ in left ankle plantar and dorsiflexion. Deep tendon reflexes were diffusely unobtainable. Sensation was mildly reduced to pin prick in the feet. She had a waddling gait, with bilateral foot drop, and was unable to heel or toe walk.

UK II.3; A 42 year old male. He had his first foot surgery at the age of 27 years for clawing of his toes. He described muscle cramps and pain in his lower limbs with sustained exertion. Examination demonstrated grade 4 strength in ankle dorsiflexion and plantar flexion. Upper limb strength was normal. Deep tendon reflexes were absent, but patella reflexes were obtainable after brief exercise. He was unable to heel or toe walk.

Repetitive nerve stimulation:

Because of the history of fatiguable weakness, we performed repetitive nerve stimulation in order to assess the reliability of neuromuscular transmission. RNS places the neuromuscular junction under stress, and a decrement of greater than 10% in the motor amplitude indicates a significant failure of transmission. Low frequency (0.5Hz) RNS of APB produced a decrement of -12% in the US proband (US II.2) and -18% in the UK proband (UK III.2). 0.5Hz RNS of TA in UK III.2 and III.6 produced decremental responses of -20% and -15% respectively.

Response to brief maximum voluntary contraction:

Brief (10 seconds) maximum voluntary contraction produced a significant amplitude increment in all of the muscles examined (Table 1, Figure 2A). In the US family the mean amplitude increase was +91.2% (range +21.3 to +200%). In the UK family, the mean amplitude increase was +87.2% (range +19.0 to +420%) with a larger increment in lower limb vs upper limb muscles (APB: mean +34%, range +21.3 to +119.5%, ADM mean +35%, range +20.5 to +200% , TA mean +149%, range +19 to +420%).

Time course of the incremental response:

To estimate the time course of this incremental response, the study was repeated in UK III.2, UK III.6, UK II.2 and US II.2 with more frequent time points (every 30 seconds after MVC for 10 minutes) (figure 2C). The initial increment varied between +270 and +19%, and in all subjects showed an initial decay over 2-3minutes followed by a persistent >10% potentiation for the entire 10 minutes.

A particularly striking response (+187%) was seen in the TA muscle in subject UK III.2. This subject also underwent a prolonged study in which CMAP responses were measured at baseline, after 10s MVC, and subsequently at intervals over the next 60 minutes (figure 2B). The potentiated response decayed over this period, but remained increased compared to baseline even after 60 minutes (+53%).

The decay for both US and UK families was best-modelled using a two-component exponential fit: $ae^{-bt} + ce^{-dt}$ where a and c are the weighting constants for both components and 1/b and 1/d are the corresponding time constants for decay. Using data pooled from both families, 1/b was found to be 28 seconds and 1/d equal to 24500 seconds (~ 408 minutes). (R-square was 0.3167).

Effects of treatment with pyridostigmine and 3,4-diaminopyridine:

Given the evidence of significant neuromuscular junction dysfunction, we treated both the UK proband (UK III.2) and her mother (UK II.2) with a trial of pyridostigmine (60mg tds). Neither reported any change in their muscle strength or daily activities and the CMSS showed no difference after 1 months of therapy (data not shown). Following a period of several days washout, they were commenced on 20 mg tds of 3,4-diaminopyridine (3,4-DAP). Both patients experienced slight improvement in exercise tolerance and in performing their daily activities. The CMSS confirmed an improvement in several indices, particularly eye muscle fatigability (from 26 and 45 seconds to 1 minute), improved strength in timed head lifting (from 45 seconds and 1 min 30 seconds to 2 minutes) and some more complex motor functions (Table e-1). Following discontinuation of 3,4-DAP for 14 days, the values returned to the original assessment before therapy.

Electrophysiological assessment during therapy:

Neither drug had a consistent effect on the initial CMAP amplitudes (figure 3A), or the degree of increment following 10s MVC (3B). We therefore performed single fibre electromyography in the TA muscle of the UK proband (UK III.2). This technique measures both the variability in the initiation of muscle fibre action potentials ('jitter') and failures of neuromuscular transmission ('blocking'), and is the most sensitive test of neuromuscular instability. Normal muscles show jitter and blocking in no more than 10% of fibres[13]. Before treatment with either agent, an increased jitter was seen in 16 of 16 pairs (100%), with intermittent blocking in 14 of these (88%). The mean consecutive difference was 193µs (upper limit 50µs) indicating a significant defect of neurotransmission.

Following treatment with pyridostigmine, the proportion of fibre pairs showing increased jitter fell to 87%, with blocking in 25% and a mean consecutive difference of 139µs (figure

3C, 3D). Following treatment with 3,4-DAP the proportion of fibre pairs showing increased jitter fell further to 70%, with intermittent blocking in 15% and a mean consecutive difference of 93μs. Whilst still abnormal, these indicate a significant improvement in neuromuscular transmission compared to baseline.

Discussion

We describe a novel human neuromuscular syndrome caused by dominant mutations in synaptotagmin 2 (*SYT2*), a protein known to be essential for synchronous vesicle release at the neuromuscular junction. The two families described have mutations in adjacent amino acids, both residing within the calcium-binding pocket of the C2B domain of Syt2. This domain has emerged as the key effector for driving synaptic vesicle fusion[14,15,16].

Both families present with a phenotype suggestive of a motor neuropathy. The proband of the US family was initially referred for the investigation of presumed CMT, while the UK proband had previously been diagnosed with a distal hereditary motor neuropathy (dHMN). Patients with dHMN typically present with symmetrical distal muscle wasting and weakness, and with foot deformities[17,18]. Distal reflexes are absent and do not recover with exercise. The observation in our patients of reflex potentiation following brief exercise suggested an additional reversible defect of neuromuscular transmission. This clinical sign is also seen in the Lambert Eaton Myasthenic Syndrome (LEMS). The classical electrophysiological features in LEMS are reduced amplitude CMAP responses with a decrementing response to low frequency (0.5Hz) nerve stimulation, but a marked incremental response at high frequency stimulation (50Hz) or following brief maximum voluntary contraction[19]. This pattern is the hallmark of presynaptic disorders in which there is a deficit of vesicle release. The patients described here exhibit the same pattern, likewise indicating a presynaptic deficit of transmitter release. In the case of LEMS, high frequency motor nerve depolarization is able to overcome the blockade of voltage gated calcium channels. In the case of *SYT2* mutations, the incremental response presumably occurs as a result of raised intracellular calcium overcoming the reduced affinity of the calcium-binding domain.

One notable feature in our study is the markedly prolonged time-course over which this incremental response decays back to baseline. In one subject this period of post-tetanic potentiation lasted at least 60 minutes, and all subjects showed a greater than 10% increase in CMAP amplitude after 10 minutes. Post-tetanic potentiation lasts approximately 2 minutes in normal subjects[20], but can be prolonged in LEMS with a decay time constant that is increased with cooling, implying a relationship to calcium clearance from the presynaptic terminal[21]. Post-tetanic potentiation can also last up to 21 minutes in infantile botulism[22], another cause of reduced presynaptic transmitter release; however our finding of potentiation lasting up to 60 minutes is to our knowledge unique.

As well as acting as calcium sensors for vesicle release, synaptotagmins have also been implicated in the tethering of vesicles to VGCCs[23]. Opening of VGCCs in response to membrane depolarization results in a transient and highly localized micro-domain of increased calcium concentration, and vesicle release occurs from a population of 'primed' vesicles within a few tens of nanometers of the VGCCs[24]. *SYT2* mutations could impact on vesicle tethering, resulting in changes in the replenishment of this population of vesicles. Whether such a redistribution of vesicles can lead to a potentiation lasting up to 60 minutes is unclear. An alternative mechanism could involve post-translation modification (such as phosphorylation) of SYT2 or another component of the fusion machinery that leads to a long-lasting increase in release probability. Further studies are ongoing to elucidate the mechanism of the prolonged post-tetanic potentiation and the molecular basis of the distal lower limb atrophy. This is of great clinical relevance since understanding this unique feature may identify a specific treatment strategy.

Given the evidence of significant neuromuscular junction dysfunction, we treated two of the UK family members with pyridostigmine and subsequently 3,4-DAP. Pyridostigmine potentiates the effect of acetylcholine by inhibiting acetylcholinesterase in the synaptic cleft, and has been previously been used in the treatment of LEMS[25], albeit with limited efficacy[25]. 3,4-DAP increases calcium entry to the presynaptic terminal via blockade of potassium channels, and reduces weakness in patients with LEMS[26] and other neuromuscular disorders[27]. Both our patients reported a reduction in fatigable weakness and several objective measures on the CMSS improved during 3,4-DAP treatment. Furthermore, neurophysiological testing revealed an improvement in synaptic transmission. These preliminary results provide an impetus for clinical trials, and emphasise the potential treatability of this disease. Interestingly, a patient with *SNAP25* mutation also benefitted from 3,4-DAP treatment, but not from pyridostigmine emphasizing the similarity in presynaptic pathophysiology[8].

We describe the clinical and neurophysiological features of a novel human neuromuscular syndrome caused by autosomal dominant mutations in the synaptic vesicle calcium sensor Synaptotagmin 2. The phenotype of this mutation is suggestive of a distal hereditary neuropathy. However, in some patients fatiguable ptosis and reflex facilitation following exercise is also found. Furthermore, neurophysiological testing demonstrates a decrementing response at low frequency repetitive nerve stimulation and an incrementing response following brief maximum voluntary contraction; features indicative of presynaptic congenital myasthenic syndrome. Prolonged testing reveals a uniquely prolonged period of post-tetanic potentiation. Crucially, patients improve with pharmacological intervention to improve neuromuscular transmission, although it remains to be seen whether it also improves motor nerve function.

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Subject	Nerve	Recording site	Baseline CMAP amplitude (mV)	% change following 0.5Hz RNS	% change following 10s MVC
US I.2	Ulnar	ADM	7.3		+27.4
	Median	APB	6.1		+21.3
	Peroneal	TA	2.3		+26.1
US II.2	Ulnar	ADM	7.8		+42.3
	Median	APB	4.1	-12	+119.5
	Peroneal	TA	1.8		+138.9
US III.1	Ulnar	ADM	4.7		+108.5
	Median	APB	5.5		+118.2
US III.2	Ulnar	ADM	3.3		+200
	Median	APB	5.3		+109.4
UK II.2	Ulnar	ADM	11.2		+20.5
	Median	APB	9.4		+23.4
	Peroneal	TA	2.1	-5	+19.0
UK III.2	Ulnar	ADM	4.6		+47.8
	Median	APB	3.8	-18	+52.6
	Peroneal	TA (R)	1.6	-20	+218.8
		TA (L)	0.5		+420.0
UK III.6	Ulnar	ADM	10.6		+30.2
	Median	APB	8.2		+41.5
	Peroneal	TA	2.0	-15	+55.0
UK IV.1	Median	APB	6.8		+30.9

Table 1: Motor nerve conduction studies before and after low frequency repetitive nerve stimulation and brief maximum voluntary contraction. Motor nerve conduction velocities are normal in all subjects. Baseline CMAP amplitudes are reduced and show greater than 10% decrement in 4 out of 5 muscles examined, and a greater than 10% increment following 10 seconds of maximum voluntary contraction (MVC) in all of the muscles examined.

Figure legends

Figure 1: Clinical presentation of dominant SYT2 mutations.

Family tree of the US and UK families showing autosomal dominant inheritance pattern. A. US family show foot deformity but no lower limb wasting. B. Lower limb wasting and foot deformities in UK family. Asymmetrical lower limb wasting in UK II.2. The subject had undergone multiple operations to correct foot deformities and a right knee replacement. Symmetrical distal wasting after bilateral foot operations in III.2. Mild weakness and atrophy in toes and feet was seen in the other affected subjects.

Figure 2: Neurophysiological testing reveals features consistent with a deficit of acetylcholine release at the neuromuscular junction.

A. Recording electrodes were placed over the APB muscle and supramaximal electrical stimulation applied to the median nerve at the wrist. Marked incremental response in compound muscle action potential amplitude was seen following brief maximum voluntary contraction. B. Prolonged time course in decay of the incremental response following maximum voluntary contraction in the TA muscle of UK III.2. The period of post-tetanic potentiation lasted at least 60 minutes. The incremental response was replicated by a second period of maximum contraction, indicating that the recording electrodes remained correctly positioned. Δ% = % change compared to baseline CMAP amplitude. Red bar = period of 10 seconds maximum voluntary contraction (mvc). C. Post-tetanic potentiation lasting at least 10 minutes was seen in all of the UK and US family members tested. Modelling of the decay was best fitted assuming a two-component exponential decay.

Figure 3: Effects of treatment with pyridostigmine and 3,4-diaminopyridine (3,4-DAP). A. Treatment with pyridostigmine or 3,4-DAP produced no consistent change in initial CMAP amplitude or B. percentage incremental response following 10seconds maximum voluntary contraction. Δ% = % change compared to baseline CMAP amplitude. C. Treatment with pyridostigmine (Pyrido) produced a reduction in the percentage of muscle fibre pairs showing jitter and blocking on single fibre EMG. Pre = baseline values before treatment. A greater effect was seen with 3,4-diaminopyridine **(**3,4-DAP). D. Both agents also produced a reduction in the mean consecutive jitter, with a greater reduction seen for 3,4-DAP than for pyridostigmine.

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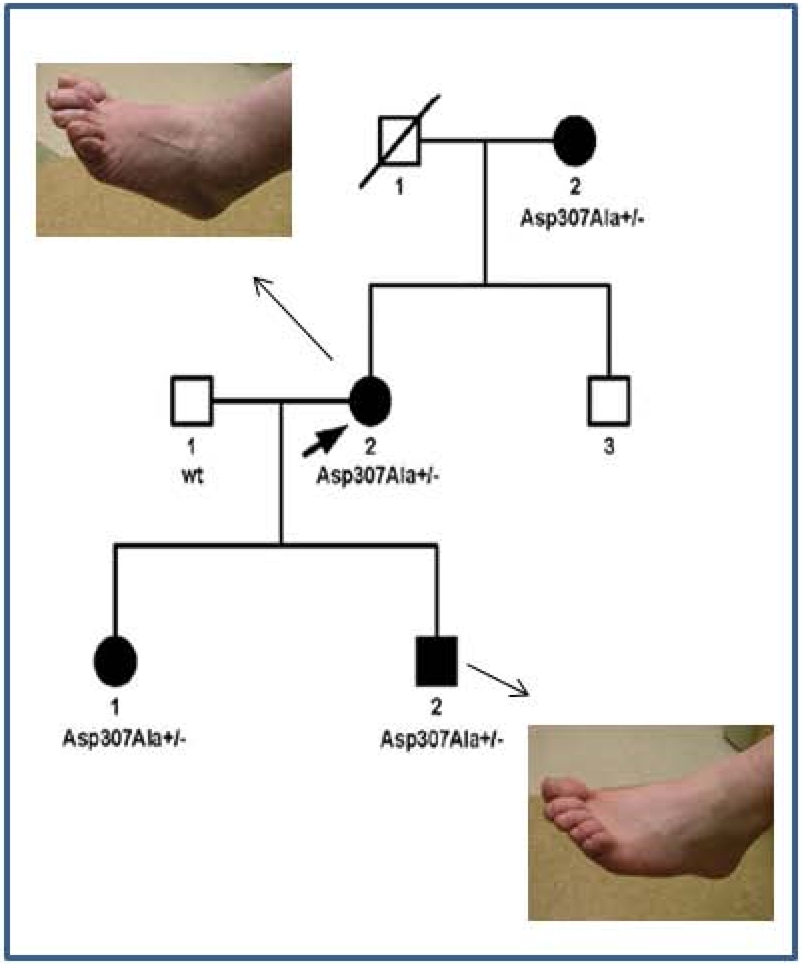
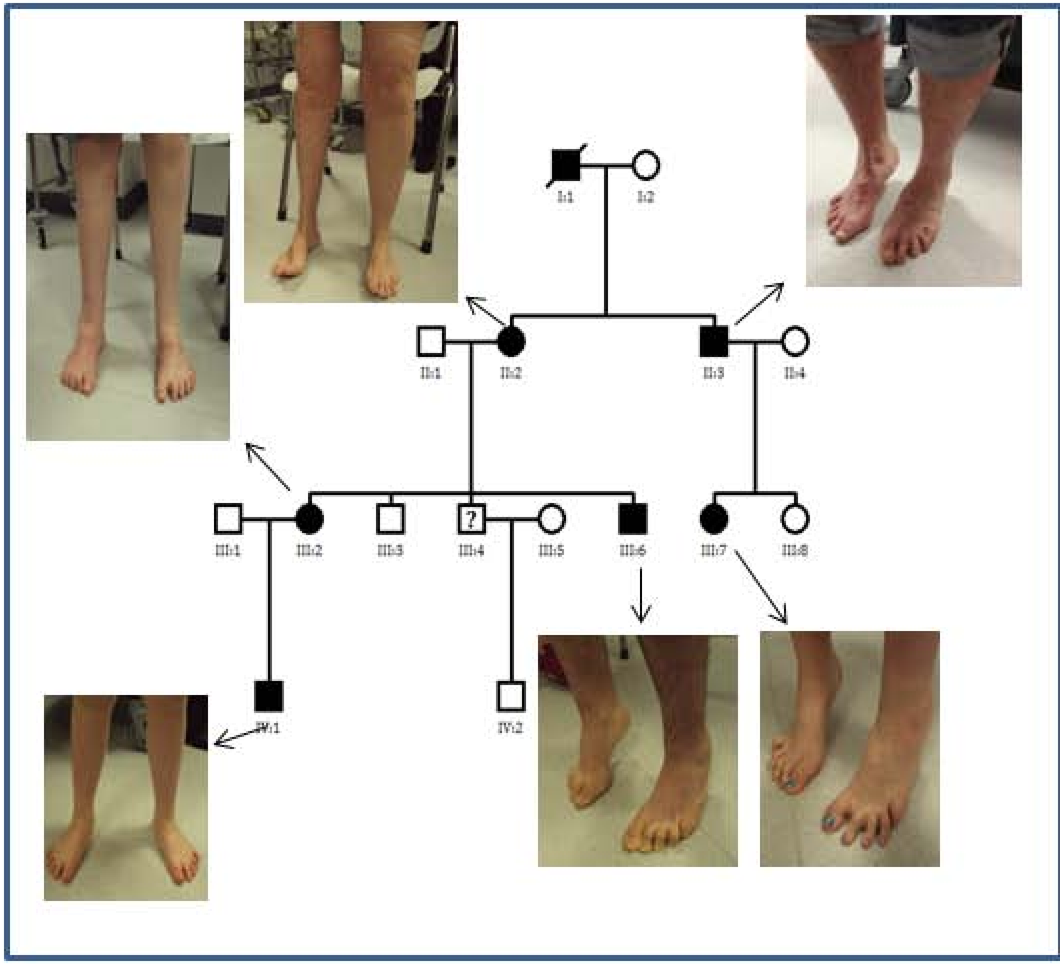
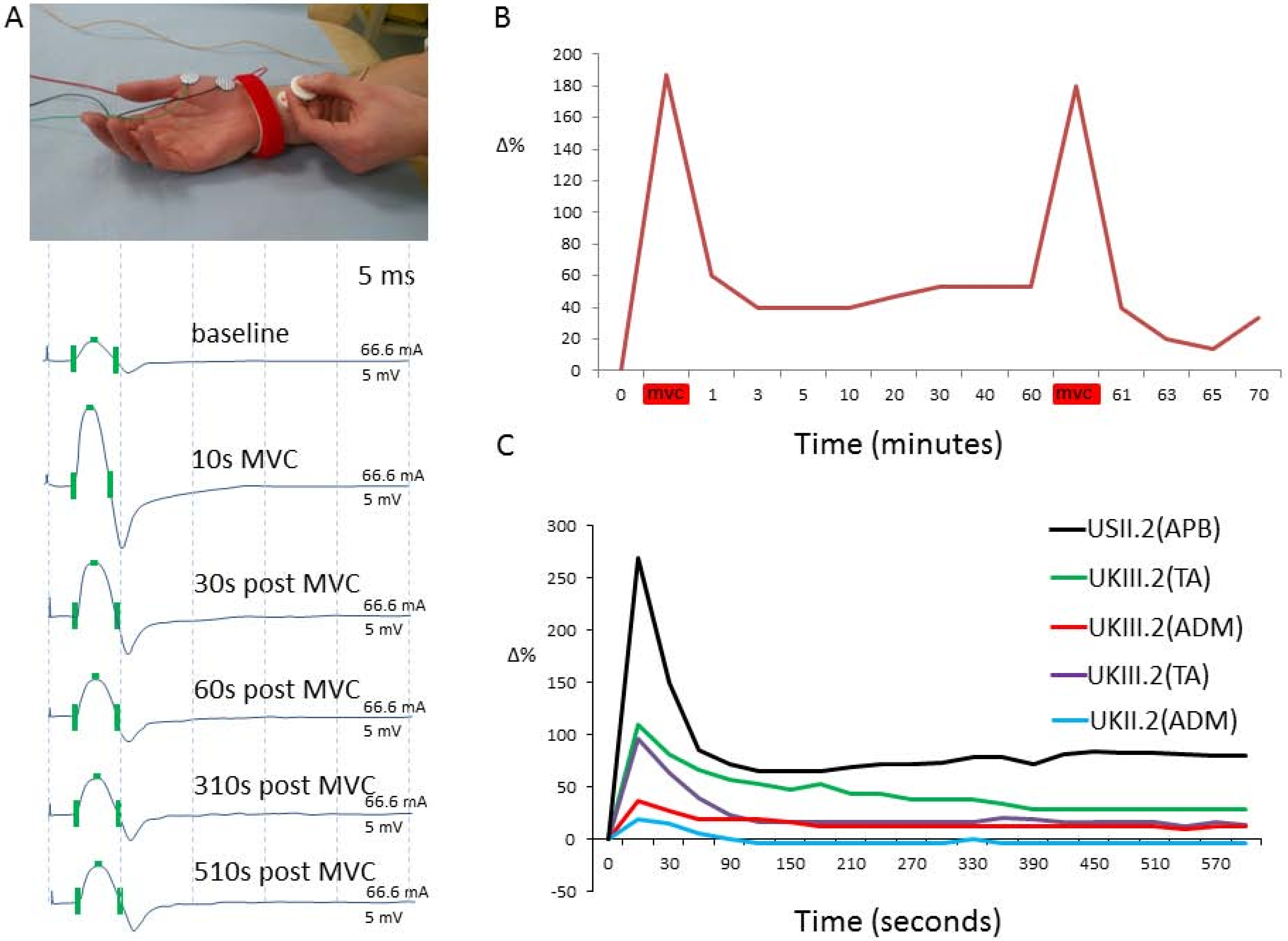
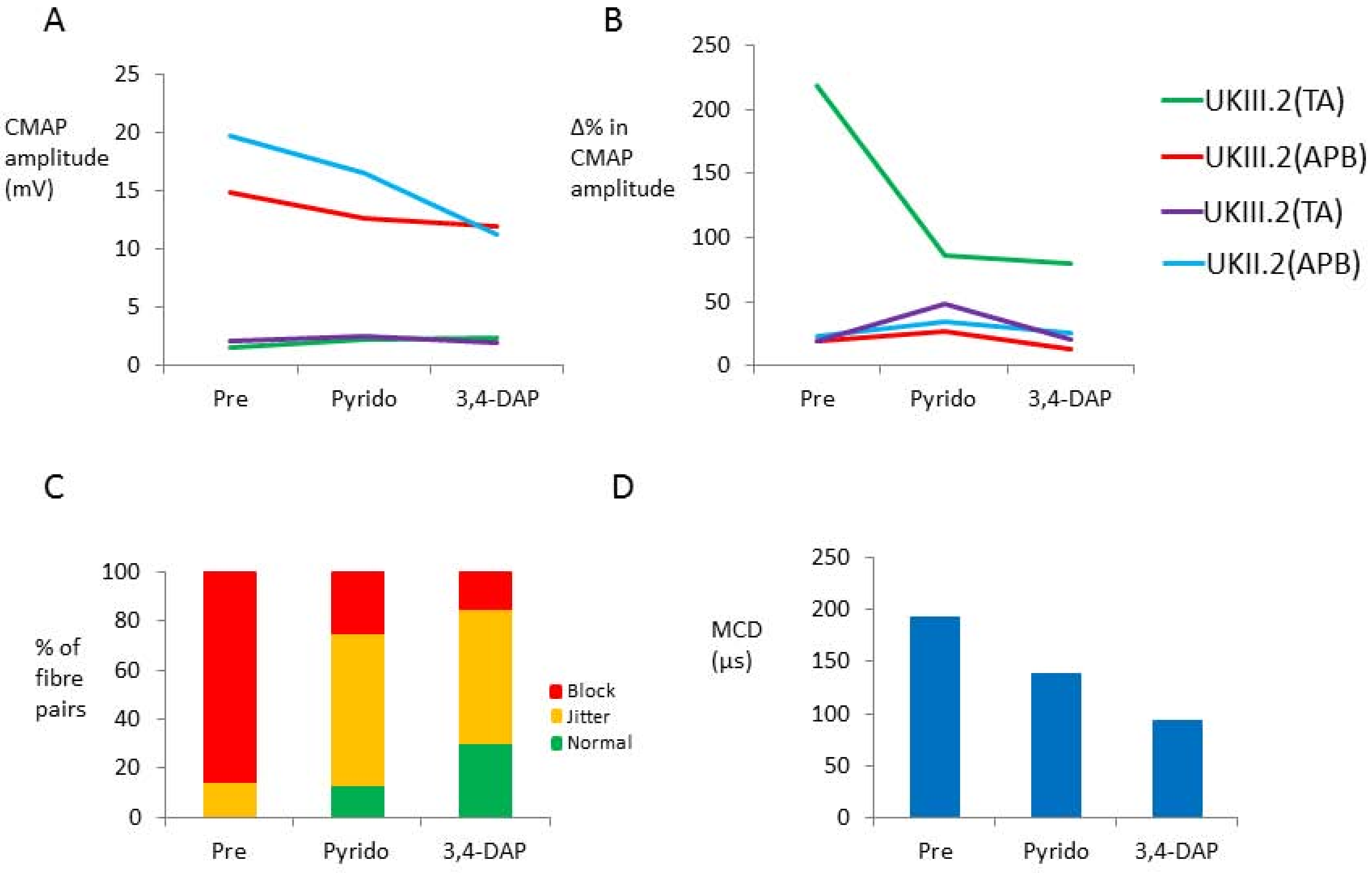
A) Family 1 (US)



B) Family 2 (UK)




A
5 ms
baseline
66.6 mA
5 mV
10s MVC
66.6 mA
5 mV
30s post MVC
66.6 mA
5 mV
60s post MVC
66.6 mA
5 mV
310s post MVC
66.6 mA
5 mV
510s post MVC
66.6 mA
5 mV
B
Δ%
200
180
160
140
120
100
80
60
40
20
0
0
mvc
1
3
5
10
20
30
40
60
mvc
61
63
65
70
Time (minutes)
C
Δ%
300
250
200
150
100
50
0
-50
0
30
90
150
210
270
330
390
450
510
570
Time (seconds)
USII.2(APB)
UKIII.2(TA)
UKIII.2(ADM)
UKIII.2(TA)
UKII.2(ADM)


A
CMAP amplitude (mV)
25
20
15
10
5
0
Pre
Pyrido
3,4-DAP
B
Δ% in CMAP amplitude
250
200
150
100
50
0
Pre
Pyrido
3,4-DAP
UKIII.2(TA)
UKIII.2(APB)
UKIII.2(TA)
UKII.2(APB)
C
% of fibre pairs
100
80
60
40
20
0
Pre
Pyrido
3,4-DAP
Block
Jitter
Normal
D
MCD (µs)
250
200
150
100
50
0
Pre
Pyrido
3,4-DAP