

# Expanding the phenotypic spectrum associated with mutations of *DYNC1H1*

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## Abstract

Autosomal dominant mutations of *DYNC1H1* cause a range of neurogenetic diseases, including mental retardation with cortical malformations, hereditary spastic paraplegia and spinal muscular atrophy. Using SNP array, linkage analysis and next generation sequencing, we identified two families and one isolated proband sharing a known spinal muscular atrophy, lower extremity predominant (SMALED) causing mutation *DYNC1H1* c.1792C>T, p.Arg598Cys, and another family harbouring a c.2327C>T, p.Pro776Leu mutation. Here, we present a detailed clinical and pathological examination of these patients, and show that patients with *DYNC1H1* mutations may present with a phenotype mimicking a congenital myopathy. We also highlight features that increase the phenotypic overlap with *BICD2*, which causes SMALED2. Serial muscle biopsies were available for several patients, spanning from infancy and early childhood to middle age. These provide a unique insight into the developmental and pathological origins of SMALED, suggesting *in utero* denervation with reinnervation by surrounding intact motor neurons and segmental anterior horn cell deficits. We characterise biopsy features that may make diagnosis of this condition easier in the future.

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## 1. Introduction

Spinal muscular atrophies (SMAs) are a group of hereditary disorders caused by aberrant development and/or early loss of spinal cord motor neurons [1]. The major cause of SMA is the 'classical' autosomal recessive 5qSMA, caused almost exclusively by homozygous deletion of exons 7 and 8 of the survival of motor neuron 1 (*SMN1*) gene on chromosome 5q [2,3] (OMIM 253300, 253550, 253400, and 271150). Less common and less

well-characterised are the autosomal dominant forms of SMA, which are milder than 5qSMA, and static or slowly progressive rather than rapidly progressive [4]. One subtype of non-5qSMA is SMA, lower extremity predominant (SMALED), which is characterised by congenital onset of static or slowly progressive muscle weakness and atrophy in the lower limbs, delayed gross motor milestones, and little to no sensory impairment [5,6]. Mutations in dynein, cytoplasmic 1, heavy chain 1 (*DYNC1H1*, OMIM #600112) were the first described cause of SMALED (OMIM #158600) [6–9], shortly followed by bicaudal D homolog 2 (*Drosophila*) (*BICD2*, OMIM #609797), causing SMALED2 (OMIM #615290) [10–13]. These genes encode interacting proteins that form part of the microtubule transport system

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[14]. The challenge of the small neuronal cell body sustaining a large cell compartment makes neurons heavily reliant on the microtubule transport system, which functions in neuron development, morphology and survival [15,16]. Several genes involved in microtubule transport are known to cause neurological diseases with varying degrees of phenotypic overlap [10,14,17].

DYNC1H1 forms a dimer that is the core of the dynein motor complex, which traffics cargo along microtubules in a retrograde fashion [16,18]. The long tail domain at the N-terminus interacts with intermediate-light and light dynein chains to form a cargo-binding complex [19], and contains the dimerisation domain. The C-terminus holds six ATPase domains and a microtubule-binding stalk [18]. The majority of ‘pure’ SMALED causing mutations are in the dimerisation domain (amino acids 300–1140), although SMALED with cortical malformations can be caused by mutations in the stem, neck and motor domains (p.Arg264Gly, p.Arg1603Thr and p.Glu3048Lys respectively [9,20]), and SMALED with learning disabilities by the motor domain mutation p.Glu2616Lys [17]. *BICD2* encodes an adaptor protein, which links a variety of cellular cargos to the dynein–dynactin motor complex [21]. Recently, two studies showed evidence that *BICD2* also promotes activation of the dynein motor [22,23].

Next generation sequencing (NGS) provides an unbiased method for genetic diagnosis in neuromuscular disease, as opposed to the previous candidate gene based approach. This technique enables new phenotypes to be associated with known disease genes, expanding phenotypic spectra and blurring the boundaries of disease classification [24]. This applies to *DYNC1H1* and *BICD2*. *DYNC1H1* was first associated with disease in 2011 [7]. SMALED is now known to variably include cognitive impairment, attention deficit hyperactivity disorder (ADHD), cortical malformations [9] and arthrogryposis [17,20]. Additional phenotypes include hereditary spastic paraplegia (HSP) with thin corpus callosum [25], cortical malformations including polymicrogyria [9,26,27], and congenital cataracts with gut

dysmotility [28]. Similarly, *BICD2* mutations were first described to cause SMALED and HSP [10–12], but are now known to also cause SMA with cerebellar developmental disorder, arthrogryposis multiplex congenita and polymicrogyria [14], and distal myopathy [29]. The phenotypic spectra associated with *BICD2* and *DYNC1H1* have therefore been increasingly seen to overlap. With this cohort, we further the phenotypic expansion of *DYNC1H1* mutations.

We describe two families (AUS1 and TUR1) and one isolated proband (P4) with neuromuscular disease caused by a previously reported *DYNC1H1* mutation (c.1792C>T, p.Arg598Cys) [17,20,25,30], and one family (AUS2) with a previously reported *DYNC1H1* c.2327C>T, p.Pro776Leu mutation [31]. Families AUS1 and AUS2 and the isolated proband P4 are from Australia. AUS1 is a large multi-generational family. Family TUR1 is from Turkey. Families AUS1 and TUR1 had an initial diagnosis of congenital myopathy based on clinical and pathological signs, while AUS2 and P4 were diagnosed with distal arthrogryposis. Diagnosis by sequencing [32,33] caused a re-evaluation of the clinical and pathological features of these families. Muscle biopsies had been obtained from several family members over multiple time points from family AUS1, providing unprecedented characterisation of the pathology of this disease. We also present features from the earliest SMALED patient biopsy to date, taken at 1 year old.

## 2. Patients and methods

This study was approved by the UWA Human Research Ethics Committee and all patients gave informed consent.

### 2.1. Patient details

AUS1 is a non-consanguineous Caucasian family from Australia with ten affected individuals (aged 1 to 69 years) over three generations (Fig. 1A). Muscle biopsies were taken from four family members at various ages (AUS1:II:2, AUS1:III:2, AUS1:IV:7, AUS1:IV:8), as shown in Fig. 2.

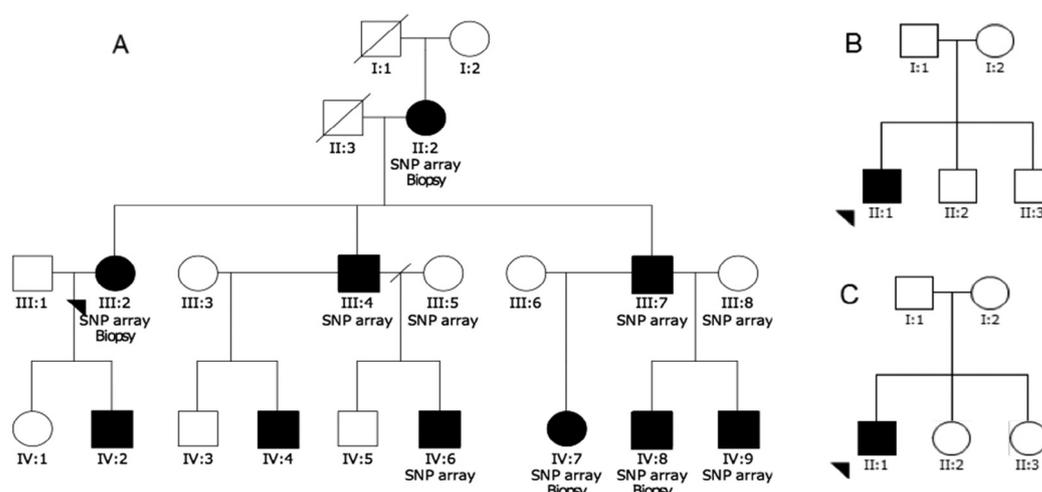


Fig. 1. Pedigrees of three families described in this study showing segregation of the *DYNC1H1* mutations. (A) Pedigree of family AUS1, a multi-generational Australian family in which affected individuals harboured the c.1792C>T, p.Arg598Cys substitution. Individuals in which a muscle biopsy was performed, or for whom a SNP-array was performed, are indicated. (B) Pedigree of family AUS2, an Australian family with the c.2327C>T, p.Pro776Leu mutation. (C) Pedigree of family TUR1, a Turkish family with one affected male patient harbouring c.1792C>T, p.Arg598Cys.

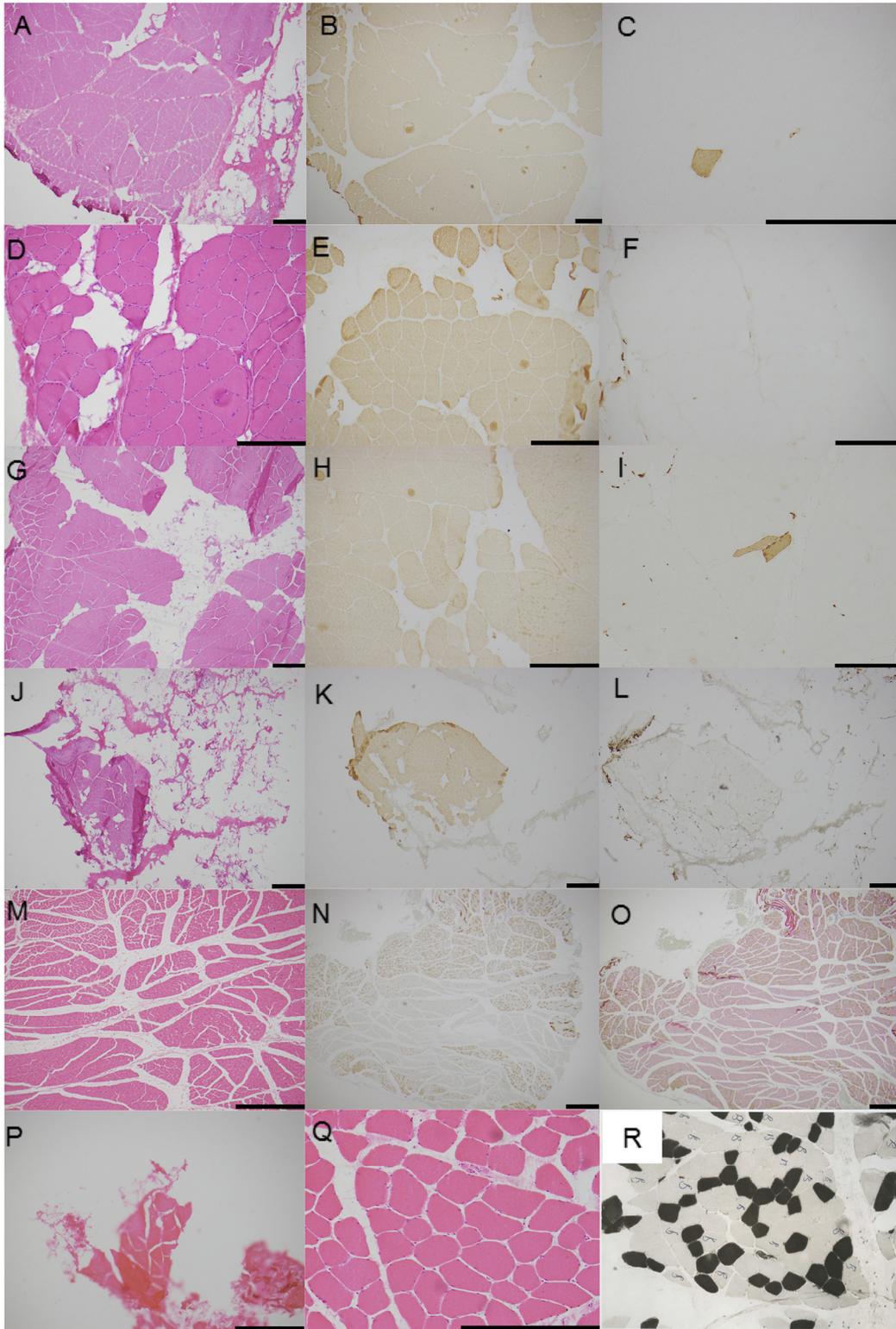


Fig. 2. A comprehensive overview of the muscle pathology identified in affected individuals from family AUS1. (A–C) AUS1:III:2 quadriceps biopsy at age 3, showing H&E, slow myosin and fast myosin respectively. They show predominantly uniform myofibre size and type I myofibre dominance with occasional tiny peripheral type II myofibres. (D–F) AUS1:III:2 vastus lateralis biopsy at age 16, showing H&E, slow myosin and fast myosin respectively. This shows islands of uniform myofibres 105  $\mu\text{m}$  average size, all type I. (G–I) AUS1:III:2 ‘thigh’ biopsy at age 39 showing H&E, slow myosin and fast myosin respectively. Average myofibre size is 107  $\mu\text{m}$  with pseudomyopathic pathology and type I myofibre dominance. (J–L) AUS1:IV:7 quadriceps biopsy at age 7 showing H&E, slow myosin and fast myosin. Exhibits islands of normal sized type I dominant myofibres with adjacent fat and very rare atrophic myofibres. (M–O) AUS1:IV:8 adductor longus biopsy at age 1 showing H&E, slow myosin and fast myosin. Myofibre sizes are normal, with central area of type I dominance and a normal peripheral pattern of myofibre typing. (P) AUS1:II:2 quadriceps biopsy age at 36, H&E, showing minimal residual muscle in a severely atrophic, fat replaced muscle. (Q–R) AUS1:II:2 deltoid biopsy at age 36 showing H&E and previously produced photograph of ATPase 4.3. Myofibres are in normal size range, with normal myofibre typing. Scale bar is 400  $\mu\text{m}$  in all images.

AUS2 is a non-consanguineous Caucasian family from Australia. The proband is a 14-year-old male with two healthy brothers and healthy parents (Fig. 1B). A muscle biopsy was taken from the proband at age 3.

TUR1 is a non-consanguineous family from Turkey. The proband is a 13-year old male, with two healthy sisters and healthy parents (Fig. 1C). A muscle biopsy was taken from the proband at age 3.

Patient P4 is a 49-year-old male from Australia. As he was adopted, there is no information about family history or pregnancy. No biopsy was taken.

## 2.2. Genetic analysis

### 2.2.1. Neuromuscular panel

In 2015, patient AUS1:IV:8 was screened on a targeted sub-exomic sequencing panel of 464 neurogenetic disease genes [34] in the Diagnostic Genomics Laboratory at PathWest, Department of Health, Western Australia. The data were analysed using the Cartegenia software package (Agilent Technologies). Variants with a minor allele frequency of >2% in control population databases (ExAC [35], 1000 genomes [36], dbSNP [37]) were bioinformatically excluded. Due to the clinical diagnosis of congenital myopathy, genes not associated with this phenotype were bioinformatically excluded from analysis.

The probands from families AUS2 and TUR1, and patient P4 were run on a previous iteration of the targeted sub-exomic sequencing panel, with 336 neurogenetic disease genes [38]. The data were annotated and filtered for rare variants as previously described [38]. As these patients had a diagnosis of distal arthrogryposis (AUS2 and P4) or myopathy (TUR1), genes not associated with these phenotypes were bioinformatically excluded from analysis.

### 2.2.2. SNP-array and linkage analysis

Whole genome linkage analysis was performed using HumanCytoSNP-12 (Illumina) data from 10 individuals in family AUS1 as indicated in Fig. 1. The output data files were prepared for linkage analysis using LINKDATAGEN [39] (<http://bioinf.wehi.edu.au/software>). Parametric multipoint linkage analysis was performed with Merlin software v1.1.2 [40], using a fully penetrant dominant disease model with a disease allele frequency of 0.001.

### 2.2.3. Whole exome sequencing

We performed Illumina whole exome sequencing (SureSelectXT V5+UTR, at TheraGenEtex, Korea) on AUS1:IV:8. The processing from FASTQ to VCF was performed as per the GATK best practice guidelines version 3.6 (<https://software.broadinstitute.org/gatk>). Variant annotation was performed as previously described [38], with the additional filter of a minor allele frequency of <1% in ExAC [35].

### 2.2.4. Sanger sequencing

Bi-directional Sanger sequencing [41] was performed using standard methods to confirm the variants segregated with disease. From family AUS1, all members who provided DNA for the SNP array had Sanger sequencing performed. For the remaining families, the proband and parents were sequenced.

Patient P4 was also sequenced, but no DNA was available from other family members.

## 2.3. Muscle biopsy and histology

Multiple muscle biopsies from four affected members of family AUS1 (Fig. 1A) had been taken over many years for diagnostic investigations (Fig. 2). For all samples except AUS1:IV:8, a portion of the biopsy was oriented for cross sectional histology and frozen at  $-80^{\circ}\text{C}$ . Haematoxylin and eosin (H&E) staining was performed on all samples as previously described [42]. The biopsy of AUS1:IV:8 was fixed in 10% buffered formalin and processed into paraffin. Individual AUS1:III:2 had muscle taken at ages 3, 16 and 39 from the quadriceps, vastus lateralis and ‘thigh’ respectively. Patient AUS1:IV:7 had a biopsy from the quadriceps at age 7. On these samples, retrospective myofibre-typing was performed in cryosections by immunoperoxidase staining for slow myosin heavy chain and fast myosin heavy chain. Patient AUS:IV:8 had a biopsy of the adductor longus at age 1, but no frozen tissue was available. H&E stained sections were already available, but slow myosin (red) alkaline phosphatase was performed on paraffin embedded tissues for myofibre-typing. Patient AUS1:II:2 had biopsies of the quadriceps and deltoid at age 36. Images of deltoid myofibre-typing with ATPase 4.3 were available from previous analyses.

AUS2:II:1 had a biopsy taken from the left vastus lateralis at age 3 for diagnostic purposes. This was stained with H&E and ATPase (9.5). Immunohistochemistry was performed for spectrin, merosin, dystrophin, sarcoglycans, caveolin 3 and oxidative enzymes. Images were not available for publication. A biopsy was taken from TUR1:II:1 vastus lateralis at age 3.

## 3. Results

### 3.1. Clinical findings

#### 3.1.1. Family AUS1

The clinical phenotype variably includes delayed motor development, congenital hip dislocation, talipes equinovarus, proximal greater than distal lower extremity weakness, preserved facial and extraocular movements, and absent tendon reflexes at the knees and sometime ankles. Brain and spine MRI on a number of individuals was normal. Creatine kinase levels were normal on all tested individuals and there is no cardiomyopathy. One individual (AUS1:IV:8) requires the use of a wheelchair whilst all others are ambulant. Patients AUS1:III:2 and AUS1:IV:7 had present reflexes, normal EMG and nerve conduction studies at ages 3 and 7 respectively. However, when AUS1:III:2 was re-examined at age 39, her nerve conduction study showed features of a right sural neuropathy, while her concentric EMG examination showed a chronic neurogenic picture with occasional myopathic potentials, strengthening the possibility of SMA. Detailed clinical information is available for AUS1:III:2 and AUS1:IV:7, which is described in greater detail in Table 1.

#### 3.1.2. Family AUS2

The 14-year old male proband AUS2:II:1 was active *in utero*, with no abnormalities noted on ultrasound. At birth, his right

Table 1  
Clinical information summary.

Patient/Family/ Mutation	Onset	Muscle atrophy	Muscle weakness	DTR	Joint contractures	Orthopaedic deformities	NCS	Other
AUS1:III:2, c.1792C>T, p.Arg598Cys	2 years old	Mild LL posterior compartment wasting	Weakness of HF, HE, HAb << HAd, KF, KE. Ankles normal. Trunk weakness++	UL 2+, knees 1+, ankles absent, plantars flexor	–	Mild kyphosis of lower back	Right sural neuropathy	Intermittent painful cramps with moderate exercise, history of malignant hyperthermia
AUS1:IV:2, c.1792C>T, p.Arg598Cys	Birth	No significant wasting	Selective weakness of HF, KF and ankle dorsiflexion	Preserved and briskly obtained in UL and LL, plantars flexor	Achilles contractures at age 16, contractures of ankle and feet++	High arched feet, shortened forefoot, hammering of toes	3 years old – normal	Possible seizures – Episodes of staring, unawareness lasting <=5 minutes, can include lip biting, no loss of postural tone. Possible ADHD
AUS2:II:1, c.2327C>T, p.Pro776Leu	Birth	LL atrophy	LL weakness	Reduced LL	Born with feet dorsiflexed to shin. Knee contractures	–	NA	–
TUR1:II:1 c.1792C>T, p.Arg598Cys	Birth	Distal LL atrophy	UL flexors MRC 4/5, LL proximal MRC 3/5, Gowers sign	Absent in LL	–	Pes cavus, scapular winging, lordosis, high-arched palate	NA	Myopathic face
P4, c.1792C>T, p.Arg598Cys	Birth	Severe distal LL wasting	Quadriceps and hamstrings moderately weak, hip strength normal	Absent in LL, normal in UL	–	Ankles fused in the neutral position	Low amplitude in the tibial nerve response	–

UL = upper limb; LL = lower limb; HF = hip flexion; HE = hip extension; HAb = hip abduction; HAd = hip adduction; KF = knee flexion; KE = knee extension; NA = not assessed.

foot dorsiflexed so his toes were against his shin, but a few months of physiotherapy allowed the foot to gradually be brought to the neutral position. He experienced delayed gross motor milestones. He did not sit until 9 months, and his legs always “crumpled” when he stood up. He commando crawled at 11 months. Once he got splints at 20 months to support his ankles, he started walking. At age 3 he experienced postural talipes, a waddling gait and proximal weakness more prominent in the legs. Examination has never changed, indicating a static disease. He has normal face, arms and back musculature but poor muscle bulk in legs, contractures of knees, very weak muscles and reduced deep tendon reflexes. He has normal sensation and normal bladder and bowel function.

### 3.1.3. Family TUR1

The proband (Fig. 3) had normal movement during pregnancy, but hyperlaxity and hyperextension at birth. Motor milestones were delayed. He sat with support at 7 months, then without support at 1 year. He achieved independent walking at age 2, but had difficulty in climbing stairs from 2.5 years old and has never been able to run well or hop. His condition is static according to parents, but with a gain of 10 kg in the last year he had more prominent difficulties in climbing stairs and running. Mental development is normal. On physical examination at age 13, he had a myopathic face, high arched palate and distal atrophy with *pes cavus* deformity. There was absence of deep tendon reflexes, but no facial and cranial nerve involvement. His upper extremity flexors were mildly affected. He had proximal

weakness in the lower extremity and pelvic girdle muscles. Gower’s sign was positive. He had no distal weakness. He had mild scapular winging, waddling gait, increased lordosis. He had less frequent falls compared to early childhood, but had difficulty in walking on his heels and tiptoes.

### 3.1.4. Patient 4

This patient was born with weak legs, but was ambulant until a severe fall at age 40. He used to lock his knees to walk, and then used elbow crutches. On examination at age 49, he had marked muscular atrophy below the knees, and his ankles were fused in the neutral position. Above the knee he had moderate weakness of quadriceps and hamstrings, but was strong about the hip. His arms were very strong with no abnormalities. Reflexes were absent in the legs but normal in the arms. Sensation was normal in arms and legs. Nerve conduction studies showed low amplitude in the tibial nerve response, but were otherwise normal. There had been no deterioration from birth.

### 3.2. Genetic results

The interrogation of AUS1:IV:8 on the neuromuscular gene panel with a myopathy gene filter did not reveal any reportable pathogenic (Class 5/Class 4) mutations in known myopathy genes, as prescribed by American College of Medical Genetics and Genomics (ACMG) guidelines [43]. The linkage analysis performed for 10 individuals in family AUS1 (see Fig. 1A) yielded a peak LOD score of 1.8046 in two regions of the



Fig. 3. Images showing the clinical features of the proband from family TUR1. (A–D) General appearance of patient 3 at the age of 13 years, mild scapular winging (C), distal atrophy (E), *pes cavus* deformity (F).

genome: Chr9:341,063–2,165,758 and Chr14:97,173,393–107,168,915. We then performed exome sequencing on AUS1:IV:8. Overlaying the regions with high LOD scores with the exome data provided 15 rare variants, but only *DYNCH1* c.1792C>T, p.Arg598Cys was previously described as disease-causing by multiple sources [17,20,25,30]. Sanger sequencing confirmed this variant segregated with disease (data not shown). *DYNCH1* mutations were also identified in the targeted sub-exomic sequencing panel data for P4, TUR1:II:1 (p.Arg598Cys) and AUS2:II:1 (p.Pro776Leu). Sanger sequencing showed that the mutation was *de novo* in the probands from families AUS2 and TUR1. The parents of P4 were not available for testing.

### 3.3. Pathology results

#### 3.3.1. Family AUS1

Longitudinal muscle biopsies were available for AUS1:III:2. At 3 years there was type I predominance (myofibre size 15–80  $\mu\text{m}$ ) very scant severely atrophic myofibres, increased internal nuclei, uneven oxidative enzyme staining and a central core. At age 16, almost all myofibres were type I with slight myofibre hypertrophy (95–125  $\mu\text{m}$ ). Fatty infiltration was increased and there were very rare atrophic myofibres. In conjunction with the clinical findings suggesting a congenital myopathy, the pathologic diagnosis suggested a congenital myopathy although anterior horn re-innervation was also raised as a possibility. At 39 years of age, type I myofibre predominance persisted as did the myofibre hypertrophy (mean diameter

107  $\mu\text{m}$ ). Angulated atrophic myofibres were present within adipose tissue. Abundant internal nuclei were suggestive of muscle regeneration giving the appearance of a pseudomyopathic presentation due to an underlying chronic neuropathic process.

Further biopsies on the family included the following: AUS1:IV:7 at age 7 showed islands of normal sized type I myofibres (range 20–55  $\mu\text{m}$ ) with adjacent fat. There were very rare atrophic myofibres. The pathology resembled that of incompletely formed fascicles within a single motor unit. AUS1:IV:8 at age 1 showed myofibres of normal size with a central area composed entirely of type I myofibres with a normal peripheral pattern of myofibre typing suggesting reinnervation. For AUS1:II:2 at age 39, a quadriceps muscle showed end-stage muscle with minimal residual atrophic myofibres and extensive fatty replacement. No conclusion could be reached. Further studies could not be performed. A deltoid muscle biopsy taken at the same time showed normal myofibre size and no significant pathology.

#### 3.3.2. Family AUS2

Muscle biopsy was taken from the left vastus lateralis of AUS2:II:1 at age 3. There was moderate variation in myofibre size, with very strong predominance of type I myofibres. Small groups of atrophic myofibres were seen with increased endomysial collagen. These were exclusively type II. No atrophic myofibres were type I. Scattered small angular dark myofibres were seen. Approximately 3% of myofibres were centrally nucleated. There were moth eaten myofibres and occasional vague core-targetoid myofibres. There was a focal increase in fat. A chronic neurogenic

process was raised as a possibility, but given the type I myofibre predominance and type II atrophy, congenital myopathy was thought to be more likely. Images were not available for publication.

### 3.3.3. Family TUR1

Muscle biopsy was performed from right vastus lateralis at age 3. H&E and ATPase (9.5) stains were performed, showing marked fibrosis, variation in myofibre size, and atrophic myofibres. An ATPase 9.5 showed type I myofibre predominance, with atrophic myofibres being mostly type II. Routine immunohistochemistry (spectrin, merosin, dystrophin, sarcoglycans, collagen VI) and oxidative enzyme stains were normal. Due to artefact in the biopsy, the images are not shown.

## 4. Discussion

In all our patients, the disease was static or very slowly progressive, and sensory examination was normal. Apart from AUS1:IV:8, no patients lost ambulation until late in life, if at all. Upper limb involvement was seen in only one patient (TUR1:II:1) and was relatively mild. Similar to the findings from Scoto et al. [20], lower limb contractures were common in our cohort, being present in three of five patients. Although the majority of features of our cohort were in keeping with the typical phenotypic range of *DYNC1H1* SMALED, there were also some unusual features. The scapular winging, myopathic face and high-arched palate seen in TUR1:II:1 are completely novel in the *DYNC1H1* spectrum. AUS1:III:2 showed pronounced trunk weakness, which has not been explicitly described before. Additionally, the distal lower limb atrophy in P4 is unusually severe. AUS1:III:2 exhibited cramping with exercise and a previous episode of malignant hyperthermia. Although leg cramping was described in a patient with *DYNC1H1* p.V612M [20], further testing is required to establish if the additional symptoms in III:2 were caused by the *DYNC1H1* mutation, or if they may have a secondary cause such as an *RYR1* mutation [44]. Interestingly, an *RYR1* mutation was considered due to the single central core [44] on her biopsy at age 3. Although intellectual impairment was absent from the cohort, AUS1:IV:2 reported possible seizures and was diagnosed with possible ADHD and treated with dexamphetamine. The significance of the seizures is unknown. ADHD has not been described with the p.Arg598Cys mutation previously [17,20,25,30]. However, Scoto et al. reported a higher prevalence of ADHD in their cohort of 30 patients with *DYNC1H1* mutations than the general community [20]. Our finding of another individual may indicate that ADHD is more common in SMALED than previously realised.

Here, we expand the phenotype of *DYNC1H1* mutations to include a clinical myopathic presentation in families AUS1 and TUR1. The present reflexes, normal EMG and nerve conduction studies of patients AUS1:III:2 and AUS1:IV:7 at ages 3 and 7 respectively, and normal EMG of TUR1:II:1 at age 3, were suggestive of a congenital myopathy rather than a neuronopathy, although it is recognised that EMGs are more difficult to perform in young children and that a neurogenic process may be more easily detected as the patient ages. The

early clinical features, particularly the scapular winging, myopathic face and high-arched palate seen in TUR1:II:1, also suggested a myopathic presentation. The predominance of type I myofibres in all families supported a diagnosis of congenital myopathy. However in retrospect, in families AUS1 and AUS2, emphasis could have been placed on the pathologists' comments about the type I dominance also reflecting a possible neurogenic/anterior horn cell pathology. Retrospective review of the early age biopsies also highlighted the presence of type II atrophic myofibres in adjacent fatty/endomysial connective tissue (AUS1 and AUS2) and occasional type II myofibres (AUS1), which further supported the pathologic diagnosis of neurogenic/anterior horn cell pathology in these initial biopsies, although not the more classic pattern of an SMA. This reflects the difficulty in pathologically distinguishing congenital myopathy from mild, slowly evolving forms of infantile SMA, and the distinction rests mainly on clinical, genetic and electromyographic grounds. When AUS1:III:2 was re-examined at age 39, her nerve conduction study showed features of a right sural neuropathy, while her concentric EMG examination showed a chronic neurogenic picture with occasional myopathic potentials, strengthening the possibility of SMA. This suggests that an apparent lack of neurogenic signs in young children does not exclude a diagnosis of a *DYNC1H1* mutation, and clinical myopathic features are compatible with *DYNC1H1*. This continues the trend of blurring the boundaries between clinical entities which were once considered distinct [24].

The p.Arg598Cys substitution arose *de novo* in two of our families and in the family described by Punetha et al. [30]. We also suspect that it arose *de novo* in the first affected member (AUS1:II:2) of family AUS1 as her parents were reported to be unaffected. The relatively common occurrence of *de novo* events suggests this disorder be considered even if there is no family history.

Each dominant SMA has a particular pattern of muscle weakness [4]. This specificity may be caused by segmental involvement of one or more columns of the anterior horn [45,46]. This was supported by the specific medial lumbar and cervical cord deficit seen by Oates et al. [4] in the autopsy of a SMALED patient with a *BICD2* mutation. The pathological findings in our cohort also support this idea. The lower limb pathology in family AUS1 is suggestive of lumbar anterior horn dysgenesis with incomplete involvement. However, the normal deltoid pathology in AUS1:II:2 at age 36 suggests the cervical anterior horn was spared, supporting differential segmental involvement between the cervical and lumbar motor neurons in this patient. This corresponds with her clinical pattern of weakness. Although no upper limb biopsy has been taken for TUR1:II:1, his upper limb weakness suggests he may have involvement of some cervical motor neurons as well as those in the lumbar region. The trunk weakness in AUS1:III:2 suggests that the thoracic motor neurons may be affected in addition to those of the lumbar region. This demonstrates the variable nature of *DYNC1H1* phenotypes, even in those with the same mutation, and may point to the influence of modifying factors.

The type I myofibre predominance in families AUS1, AUS2 and TUR1 suggests that the anterior horn dysgenesis primarily

affects the alpha motor neurons innervating type II myofibres, although the reason for this difference in susceptibility is unknown (see review in [47]). The reinnervation of denervated myofibres by adjacent intact motor neurons is a common phenomenon in denervating conditions [4,42], which we suspect was the case in this cohort. The normal or mildly hypertrophic myofibre size in the younger age biopsies suggests *in utero* reinnervation. The atrophic type II myofibres in AUS1:III:2 and AUS2:II:1, both aged 3, also support this theory. Additionally, in AUS1:IV:7 aged 7 and AUS1:III:2 aged 16, the presence of pure type I myofibre ‘islands’ and increased surrounding fat also supports reinnervation of type II myofibres by the adjacent surviving type I motor neurons. We suspect the ‘islands’ are a result of the type I motor neurons reaching the limit of their ability to reinnervate surrounding myofibres, and the increased fat is due to the degeneration of muscle myofibres that were not reinnervated. This is supported by the biopsy of AUS1:IV:8 showing a central area of dominant type I myofibres with a normal peripheral pattern of myofibre typing, suggesting central reinnervation by a single motor unit. These findings suggest the denervation occurs *in utero*, but then ceases or drastically slows, causing the static or slow disease progression in this patient group. The compensatory reinnervation by intact motor neurons may explain the relatively mild weakness.

Unbiased next generation sequencing is allowing rapid expansion of the known phenotypic spectrum of many neuromuscular disease genes [24]. The recent phenotypic expansions of *DYNC1H1* and *BICD2* have revealed several overlapping features, including polymicrogyria [6,14] and hereditary spastic paraplegia [10,25] in addition to SMALED. Although our finding of scapular winging and high-arched palate are novel for individuals with *DYNC1H1* mutations, scapular winging is a common feature of patients with *BICD2* mutations [11,48,49]. A high-arched palate has been described in one *BICD2* patient [49]. These findings contribute to the growing phenotypic overlap associated with mutations of these genes. Very recently, myopathy caused by *BICD2* mutations was also described [29]. The phenotype mimicking a congenital myopathy in families AUS1 and TUR1 also contributes to the phenotypic overlap.

Given the outstanding clinical heterogeneity of neuromuscular disease, genetic testing is growing in importance for providing a definitive (and sometimes unexpected) diagnosis, as was the case in our cohort. However, providers of genetic testing must be aware of the clinical overlap between SMALED, congenital myopathy and arthrogyrosis, and adjust their gene filters accordingly.

## 5. Conclusions

This cohort demonstrates that mutations in *DYNC1H1* can mimic a congenital myopathy. In addition, study of serial biopsies provides insight into the pathological progression of the disease, with hints as to its developmental origins. We highlight features that may make diagnosis of mutations in *DYNC1H1* on biopsy easier in the future, and show that lack of clinical neurogenic features in children does not preclude a *DYNC1H1* mutation. Neurogenic features may only be evident on electromyography, which can be very challenging in children and therefore are

frequently missed. The high proportion of *de novo* mutations in this gene shows *DYNC1H1* should be considered even if there is no family history. We highlight the importance of ‘diagnosis by sequencing’ in cases with unusual presentations of a known disease, and contribute to the growing phenotypic overlap between *DYNC1H1* and *BICD2*. More work is needed to fully understand the disease spectrum caused by mutations of *DYNC1H1* and other microtubule transport proteins.

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