Inherited neuropathies with predominant upper limb involvement: genetic heterogeneity and overlapping pathologies

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Background and purpose: In a subset of patients with inherited peripheral neuropathies the first symptom is atrophy and weakness of the intrinsic muscles of the hands, without involvement of lower limbs until later in the disease course. The exact pathomechanisms of this phenotype are currently unknown. The aim of this study was to characterize the clinical, neurophysiological and genetic features of a group of patients with a clinical diagnosis of upper limb predominant Charcot-Marie-Tooth disease (CMT).

Methods: The clinical, electrophysiology and genetic data of 11 patients with upper limb predominant peripheral neuropathy selected from a single-centre cohort of 461 patients diagnosed with inherited neuropathy were analysed and the clinical, electrophysiological and genetic characteristics of these patients reported.

Results: An overlapping phenotype of neuropathy and myopathy was detected in two patients. Four patients carry autosomal dominant mutations in GARS and a single patient had a homozygous mutation in SH3TC2. However, the underlying genetic diagnosis could not be confirmed in six patients by gene panel sequencing.

Conclusions: Upper limb-onset inherited neuropathies are genetically heterogeneous and, in some cases, there is an overlapping myopathy. Autosomal dominant GARS mutations are the most common genetic cause; however, mutations in other CMT genes may also result in this phenotype in individual patients. The majority of these patients cannot be genetically diagnosed by gene panel testing of known CMT and myopathy genes, suggesting further genetic heterogeneity and highlighting the importance of further genetic investigations in these patients and families.

Introduction

Inherited motor neuropathies comprise a genetically diverse group of disorders and often pose a diagnostic challenge. Similar molecular themes may underlie clinically differing neuropathies, and many neuropathy-associated genes are implicated in several allelic disorders. Although they are clinically heterogeneous, by far the most common Charcot-Marie-Tooth disease (CMT) phenotype is of length-dependent weakness, wasting and sensory loss, which begins in the intrinsic muscles of the feet and slowly progresses to involve proximal leg muscles and intrinsic hand muscles to a varying extent. In a subset of patients, however, CMT may first present with atrophy and weakness of the intrinsic muscles of the hands, without involvement of lower limbs until later in the disease course. This
pattern of weakness is classified as distal hereditary motor neuropathy type V (dHMNV) when it occurs without sensory involvement, or as Silver syndrome, when lower limb spasticity is present. These allelic disorders are attributed to mutations in three known genes: **BSCL2**, **GARS** and **REEP1** [1–3]. Many patients presenting with CMT with predominant hand involvement do not harbour mutations in these genes, suggesting further genetic heterogeneity [4].

The aim of the present study was to characterize the clinical, neurophysiological and genetic features of a group of patients with a clinical diagnosis of upper limb predominant CMT. From a single-centre cohort, we identified cases harbouring mutations in genes associated with diverse pathomechanisms, and highlight the overlap in distal myopathies and upper limb predominant neuropathies.

**Methods**

**Patients**

Cases were selected from a cohort of 461 patients diagnosed with inherited neuropathy at the genetic neuropathy clinic, Newcastle upon Tyne NHS Foundation Trust, between 2010 and 2018. We included all patients who lacked any clinical sign of neuropathy of lower limbs at symptom onset, but who had clinical and neurophysiological evidence of a neuropathy affecting upper limbs (Fig. 1). Clinical history and examination was used to determine the presence of upper limb weakness. Clinical assessment of hand muscle strength was by manual muscle testing, and Medical Research Council (MRC) scores for abductor pollicis brevis (APB), first dorsal interosseous (FDIO), flexor digitorum profundus (FDP), abductor digiti minimi (ADM) wrist flexion and extension were recorded. Clinically, distal weakness can be difficult to classify as being myopathic or neuropathic, but was considered to be neuropathic in patients with early loss of deep tendon reflexes, prominent weakness of ankle dorsiflexion more than plantar flexion, and involvement of extensor digitorum brevis muscle (in those cases with lower limb involvement). Sensory involvement, when present, also pointed towards neuropathy rather than myopathy. In addition, all genetically undiagnosed patients had magnetic resonance imaging of whole spine and extensive biochemical investigation.

**Neurophysiology**

Electrophysiological examination was performed in all patients according to standard techniques and interpreted by the same expert neurophysiologists. Motor and sensory nerve conduction studies were performed and qualitative and quantitative analysis of motor unit potentials and spontaneous activity were assessed on electromyography (EMG). Additional assessment of neuromuscular junction integrity using repetitive nerve stimulation and single-fibre EMG was carried out in eight patients. EMG was performed using Natus Neurology disposable 30-G concentric needles (Pleasanton, CA, USA) with a bandpass range of 10–10 000 Hz. For single-fibre EMG studies, the low pass filter was increased to 2000 Hz and the percentage of fibre pairs showing increased jitter or blocking was calculated.

**Molecular genetic analysis**

Acquired causes were excluded by detailed laboratory analysis and, in some cases, by a lack of response on immunosuppressive therapy. The genetic testing undertaken in each patient is detailed in Table 1. The inherited neuropathies 54-multigene panel assay using next-generation sequencing was carried out in the Bristol Genetics Laboratory according to the UK Genetic Testing Network-approved criteria in all patients. In addition, a myofibrillar myopathy multigene panel \{DES, MYOT, CRYAB, ZASP, BAG3, VCP and the most common TTN HMERF [c.95134T>C (p.Cys31712Arg) mutation]\} was carried out by the Northern Genetics Service, Newcastle upon Tyne NHS Foundation Trust in all genetically undiagnosed patients. Whole-exome sequencing was performed in four patients, as previously described [5]. Pathogenicity of any novel variants is supported by evolutionary conservation, *in silico* analyses, and the strong phenotypic similarities with previously reported cases carrying coding sequence mutations. In addition, all genetically undiagnosed patients had magnetic resonance imaging of whole spine and extensive biochemical investigation.

**Results**

**Clinical presentation**

We identified 11 patients from 10 families (Table 1). Two patients had a pure motor neuropathy and were classified as dHMNV. Nine patients were classified as CMT, by the presence of distal sensory loss, confirmed by abnormal sensory nerve conduction studies. The latency to clinical lower limb involvement was highly variable; in two patients no lower limb involvement was found on clinical examination (at 16 and 17 years after symptom onset,
respectively), and in the remaining nine patients the median (range) time to involvement of lower limbs was 5 (1–12) years. Four patients had a family history of neuropathy, but there was significant phenotypic variability within families in terms of the distribution of weakness at onset.

**Mutation spectrum**

Five of 11 patients had a genetic diagnosis (Table 1). Four patients from three families had mutations in the \(GARS\) gene; two were novel and two have been described previously [5,6]. One patient was homozygous for the previously reported c.2860C>T, p.(Arg954*) mutation in \(SH3TC2\) [7]. In a further patient (F10/P1), the previously described c.1403G>A; p.(Arg468His) missense mutation in \(MFN2\) was identified [8]. This variant was previously thought to be pathogenic; however, it has since been re-classified as likely benign (ClinVar).

**Neuropathy myopathy overlap**

Two patients showed phenotypic overlap between motor neuropathy and distal myopathy. Patient F10/P1 presented aged 21 years with an 18-month history of hand weakness. She had normal developmental milestones and no family history of a neuromuscular disorder. On initial examination, aged 21 years, the patient had severe weakness of finger extensors which was asymmetric (left more than right), as well as weakness of wrist extension. Thenar and hypothenar muscle bulk was initially preserved. Deep tendon reflexes were absent in the upper limbs and physiological in the lower limbs. This progressed over the next

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**Figure 1** Identification of upper limb-onset neuropathy cases in the inherited peripheral neuropathy cohort. NUTH, newcastle upon tyne hospitals.

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| Family/| Genetic testing | Gene | Nucleotide/ A.A. change | Inheritance | Phenotype | Age onset, years | Approximate time to lower limb involvement, years | Upper limb | Lower limb | Neurophysiology |
|-------|-----------------|------|-------------------------|-------------|-----------|----------------|-----------------------------------------------|-----------|-----------|----------------|
| patient/age at evaluation (years)/sex |                  |      |                         |             |           |                |                                               | Weakness | Wasting | Reflexes | Sensory | Weakness | Wasting | Reflexes | Deformity | Sensory | Median NCV | SNAPs | EMG |
| F1/P1/45 M | IPN panel, WES |      |                         | Isolated    | dHMN V   | 35             | 3.5                                          | +++      | +++      | –        | N       | N        | +       | –        | N       | N       | Mixed | N         |       | mild |
| F2/P1/31 M | IPN panel, MFM genes, WES |      |                         | Isolated    | dHMN V   | 14             | Not involved                                      | +++      | +++      | +        | N       | N        | N       | ++       | N       | N       | Axonal | N         |       | severe |
| F3/P1/69 M | IPN panel, MFM genes, WES |      |                         | Isolated    | CMT      | 58             | 10                                           | ++       | ++       | +/–      | Mild    | +        | +       | +        | N       | N       | Axonal | Inactive | neurogenic |
| F4/P1/79 F | IPN panel, SH3TC2 c.2860C>T, p.(Arg954*) |      |                         | AR          | CMT      | 55             | 5                                             | +++      | +       | –        | Mod     | N        | N       | –        | Small feet | Mild    | Demyel | Absent | Inactive | neurogenic |
| F5/P1/55 M | IPN panel, MFM genes |      |                         | Isolated    | CMT      | 18             | 12                                           | +++      | ++      | +++      | N       | +        | N       | +        | Pes cavus | Mod     | Axonal | Absent | Severe | inactive | neurogenic |
| F6/P1/74 M | IPN panel, MFM genes |      |                         | AD          | CMT      | 60             | 4                                            | +++      | +++      | –        | N       | ++       | +       | –        | Pes cavus, hammer toe | Axonal | Inactive | neurogenic |
| F7/P1/39 M | IPN panel, GARS c.647A>G, p.(His216Arg) |      |                         | AD          | CMT      | 14             | 10                                           | +++      | +++      | –        | Mild    | ++       | N       | –        | N       | Mild | Mixed | Inactive | neurogenic |
| F7/P2/70 F | GARS c.647A>G, p.(His216Arg) |      |                         | AD          | CMT      | 12             | 8                                            | +++      | +++      | –        | Mild    | ++       | +       | –        | N       | Mild | Mixed | Severe | inactive | neurogenic |
| F8/P1/30 F | GARS c.1528A>C, p.(Lys510Gln) |      |                         | AD          | CMT      | 12             | 3                                            | ++       | +       | –        | Mod     | +        | N       | +        | N       | Mild | Axonal | moderate | inactive | neurogenic |
| F9/P1/19 M | GARS c.979G>A, p.(Gly327Arg) |      |                         | AD          | CMT      | 15             | 1                                            | +++      | ++      | –        | N       | +        | N       | +        | Pes cavus | Mild     | Axonal | Inactive | neurogenic |
| F10/P1/37 F | IPN panel, MFM genes, WES |      |                         | AD          | CMT      | 20             | Not involved                                    | +++      | ++      | –        | Mild    | N        | N       | ++       | N       | N       | Axonal | Inactive | neurogenic |

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5 years to involve proximal upper limbs, wasting of the thenar eminence and mild pinprick sensory loss in her fingertips, but lower limbs remained unaffected. Scapular winging was noted from age 24 years. Muscle biopsy at age 25 years showed marked myopathic features (Fig. 2a). Neurophysiology suggested a chronic lower motor degenerative process. The patient was found to be heterozygous for a missense mutation in MFN2 c.1403G>A, p.(Arg468His), which has been previously reported in dominant CMT2A patients but has since been re-classified in ClinVar to be likely benign [8]. No genetic diagnosis has been made in this case.

Patient F2/P1 presented aged 14 years with an inability to move his fifth fingers. This progressed over the next 12 months to global and symmetrical weakness of both hands. Examination at presentation showed weakness of ADM and FDP, and wasting of the hypothenar eminence, with preservation of APB and no detectable lower limb weakness. Initial investigations showed mildly raised serum creatine kinase levels (between 300 and 1000 U/L) and a muscle biopsy from tibialis anterior at age 15 years showed mild non-specific myopathic features (Fig. 2b). Neurophysiology, however, was in keeping with a motor axonal neuropathy with neurogenic EMG in proximal and distal muscles. No myopathy-associated variants have been detected by gene panel testing and whole-exome sequencing.

**Pattern of hand muscle involvement**

All patients presented with dissociated hand muscle atrophy at disease onset. In nine patients (four GARS, one SH3TC2 and four genetically undiagnosed), preferential wasting of the thenar eminence and FDIO with relative sparing of ADM was found (Fig. 3b). In the remaining two patients (genetically undiagnosed), preferential weakness of ADM was observed at symptom onset. This dissociated pattern of muscle involvement could also be detected on nerve conduction studies (Fig. 3a).

**Discussion**

Upper limb predominant inherited neuropathy is a phenotype previously attributed to three genes: GARS, BSCL2 and REEP1. In the present study, we show that this pattern of weakness may also occur as a result of mutations in SH3TC2. The pattern of weakness in these patients suggests a different pathomechanism to the usual length-dependent axonal loss attributed to CMT2. In addition, we observed wide variability within families in terms of the clinical pattern of muscle weakness at presentation, suggesting additional factors other than the disease-causing variant leading to early upper limb involvement. Recessive mutations in SH3TC2, causing CMT4C, typically lead to an early-onset sensorimotor neuropathy with nerve

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**Figure 2** (a) Muscle biopsy (forearm) from F10/P1. Haematoxylin and eosin (H+E; left panel) shows significant variation in fibre size, with some grouping of the atrophic fibres. Internal nuclei are increased, with some fibres containing several nuclei and showing evidence of splitting. Focal and scattered infiltrates of inflammatory cells are seen with occasional necrosis. Occasional fibres contained vacuoles some of which were rimmed (inset); in other fibres there appeared to be a subsarcolemmal accumulation of basophilic material. Scattered regenerating fibres were noted. Connective tissue is increased. Scattered fibres contained material that was labelled for desmin (right panel), as well as myotilin and valosin-containing protein (VCP; not shown), some of which appeared subsarcolemmal in distribution. (b) Muscle biopsy (tibialis anterior) from case F2/P1. H+E staining demonstrated fibre size variation. On ATPase, there are some type 1 clusters but no clear fibre type grouping, in keeping with neurogenic change.
conduction velocities in the demyelinating range. Varying degrees of foot deformity and scoliosis are also typically seen. An upper-limb predominant phenotype has not previously been described. Interestingly however, the c.2860C>T, p.(Arg954*) nonsense mutation for which our patient was homozygous, has

Figure 3 (a) Neurophysiological evidence of dissociated hand muscle atrophy. Compound muscle action potential (CMAP) amplitudes recorded in abductor pollicis brevis (APB; black) and abductor digiti minimi muscles (ADM; grey) showed that, in the majority of patients, CMAP amplitudes were reduced in APB but relatively preserved in ADM, although the opposite pattern was observed in two patients. (b) Representative images of the pattern of hand muscle atrophy in patients with mutations in GARS, SH3TC2 and in two genetically undiagnosed (UnD) patients, showing preferential wasting of the thenar eminence with sparing of the hypothenar eminence.
been associated with mild mononeuropathy of the median nerve when present in a heterozygous state [9].

Interestingly, in two patients, neurophysiological and histological investigations demonstrated a clear overlapping pathology of distal myopathy and motor neuropathy. Motor neuropathy is a recognized feature of many myofibrillar myopathies, particularly those attributable to mutations in DES, MYOT, BAG3 and FLNC, in which neurogenic changes on muscle biopsy and reduced CMAP amplitudes are reported in the majority of patients [10]. Hereditary inclusion body myopathy attributable to mutations in VCP is also frequently associated with neuropathy [11]. Hereditary transthyretin amyloidosis due to mutations in TTR has also recently been shown to be associated with a phenotype of upper limb predominant neuropathy with minimal autonomic involvement [12]. We suggest that, in patients presenting with this unusual pattern of weakness, both distal myopathy- and neuropathy-associated genes may be considered. Nonetheless, six patients remained genetically undiagnosed in our cohort, suggesting that further genes causing an upper limb predominant presentation are yet to be discovered. It is also not possible to fully exclude unidentified acquired causes in these patients. Further genetic testing will be undertaken in the undiagnosed patients in this cohort, including examination of non-coding DNA regions by whole-genome sequencing, RNA sequencing, long-read sequencing or proteomics.

All patients in this cohort presented with a dissociated pattern of hand muscle involvement at disease onset, usually with isolated involvement of ABP and FDI at disease onset, although the opposite pattern was also seen. This ‘split hand’ is a common early feature in motor neuron disease (MND) [13]. It has also been described in motor neuropathies due to mutations in GARS, DMN2 and GJB1 [5,14,15]. The mechanism of the split hand in MND is a topic of controversy, with several studies arguing the site of primary pathology is at the peripheral nerve, spinal cord or cerebral cortex [15,16]. The fact that a similar pattern is seen in several motor neuropathies could indicate that in fact this is a peripherally mediated process. However, the majority of motor neuropathy genes have important functions in both central and peripheral nerves. The presence of this feature in all of these cases casts doubt over the diagnostic specificity of this feature for MND.

In summary, the upper limb-onset inherited neuropathies are a genetically heterogeneous subgroup of motor neuropathies. A non-length-dependent phenotype should raise the suspicion of overlapping pathologies in nerve and muscle, and a broad investigative approach is necessary in order to reach the diagnosis, as novel genes may need to be discovered in these patients and their families.

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Disclosure of conflicts of interest
The authors declare no financial or other conflicts of interest.

Data availability statement
The private information on patients presented here is stored in the Newcastle upon Tyne Hospitals NHS Trust. Genetic data are stored within the UK Genetic Testing Network’s database. Whole-exome sequences can be found in the RD-CONNECT database.

Supporting Information
Additional Supporting Information may be found in the online version of this article:
Table S1. Summary of neurophysiological test of upper limb neuropathy cohort.

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