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RESEARCH ARTICLE

Genetic spectrum in a cohort of patients with distal hereditary motor neuropathy

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Abstract

Background: Distal hereditary motor neuropathy (dHMN) is a heterogeneous group of diseases characterized by exclusive degeneration of peripheral motor nerves, while only 20.0–47.8% of dHMN patients are genetically identified. Recently, GGC expansion in the 5'UTR of *NOTCH2NLC* has been associated with dHMN. Accordingly, short tandem repeat (STR) should be further explored in genetically unsolved patients with dHMN. **Methods**: A total of 128 patients from 90 unrelated families were clinically diagnosed as dHMN, and underwent a comprehensively genetic screening. Skin biopsies were conducted with routine protocols. **Results**: Most patients showed chronic distal weakness of lower limbs (121/128), while 20 patients initially had asymmetrical involvements, 14 had subclinical sensory abnormalities, 11 had pyramidal impairments, five had cerebellar disturbance, and four had hyperCKmia. The rate of genetic detection was achieved in 36.7% (33/90), and the rate increased to 46.7% (42/90) if patients with variants uncertain significance were included. The most common causative genes included chaperone-related genes (8/33, 24.2%), tRNA synthetase genes (4/33, 12.1%), and cytoskeleton-related genes (4/33, 12.1%). Additionally, two dominant inherited families were attributed to abnormal expansion of GGC repeats in the 5'UTR of *NOTCH2NLC*; and a patient with dHMN and cerebellar symptoms had CAG repeat expansion in the *ATXN2* gene. Skin biopsy from patients with GGC expansion in *NOTCH2NLC* revealed typical intranuclear inclusions on histological and ultrastructural examinations. **Interpretations**: This study further extends the genetic heterogeneity of dHMN. Given some dHMN patients may be associated with nucleotides repeat expansion, STR screening is necessary to perform in genetically unsolved patients.

Introduction

The distal hereditary motor neuropathy (dHMN) used to be considered as a group of pure motor neuropathies that are clinically characterized as a length-dependent muscle weakness and atrophy.[1] The majority of the cases initially shows a slowly progressive weakness of distal lower limbs; then, gradually involves proximal leg muscles, even affects the intrinsic muscles of hands.[2] However, some types of dHMN can subsequently accompany with subclinical sensory abnormalities, pyramidal signs, and other neurological symptoms.[3] In this sense, the phenotype of dHMNs can exhibit some overlapping with the axonal forms of Charcot–Marie–Tooth disease (CMT2), juvenile-onset amyotrophic lateral sclerosis (ALS), hereditary spastic paraplegia (HSP), spinocerebellar ataxia (SCA), and evenly distal myopathy.[4] Given the great heterogeneities at the ages of onset, clinical features, and accompanied neurological symptoms, it is not easy only from clinical viewpoints and electrophysiological findings to delineate the phenotype and subtypes of dHMN.[2,3] Therefore, genetic investigations are indispensable to obtain a successful diagnosis of dHMN.

With the progression of high-throughput next-generation sequence (NGS), more than 30 genes have been associated with dHMN representative of the great genetic heterogeneity.[5] Nevertheless, it still remains difficult and elusive to genetic diagnosis of dHMN, because the causative variants have been identified in only 20.0–47.8% of affected index patients with different ethnic origins.[6–10] Recently, GGC repeat expansion in the 5′ untranslated region (5'UTR) of the *NOTCH2NLC* gene has been associated with distal motor neuropathy and rimmed vacuolar myopathy.[11] Additionally, some patients with CAG repeat expansion in the *Ataxin-2* (ATXN2) gene also can affect motor neuropathy independently.[12] Therefore, it is necessary to further conduct the genetic studies on short tandem repeat (STR) in some genetically unsolved patients with dHMN.

To improve the clinical recognition and genetic diagnosis of dHMN, we collected a cohort of patients with dHMN according to the clinical and electrophysiological characteristics. Initially, targeted-panel NGS or whole exome sequencing (WES) was conducted to explore underlying genetic variants for dHMN, and then triple primed polymerase chain reaction (TP-PCR) was performed in genetic-negative patients to investigate the STR variants in the *NOTCH2NLC* gene and a panel of genes associated with SCAs.

Materials and Methods

Subjects

All the patients were recruited from January 2015 to December 2021 at the neurological department of two tertiary hospitals in China: the first affiliated hospital of Nanchang University and Peking University people hospital. All the patients were clinically evaluated by at least two experienced neurologists at the referral clinics. Inclusion of patients was based on[13] (1) clinical features of a pure motor neuropathy characterized by length-dependent motor weakness and atrophy without any sensory disturbances; (2) reduced compound motor unit action potentials or neurogenic chronic denervation with no or only subclinical sensory changes on electrophysiological studies; (3) some symptomatic family members of patients with a confirmed genetic diagnosis, even if no electrophysiological data were available. Any acquired causes of peripheral neuropathy were excluded by thorough laboratory examinations or no response on experimental immunosuppressive treatments.

The research was approved by ethics committee of the first affiliated hospital of Nanchang University. We reviewed medical records and electrophysiological studies of all patients included in this work. Based on neurological and electrophysiological findings, all patients were classified into three subgroups[6,7]: (1) dHMN that was defined as a pure motor neuropathy without sensory abnormalities; (2) motor CMT2 that was presented with clinical dHMN but accompanying with subclinical sensory electrophysiological changes; (3) dHMN-plus that was characterized as a motor neuropathy with other neurological features like cognitive impairment, pyramidal signs, cerebellar symptoms, and so on. Electrophysiological conduction study data of all patients are available on request.

Genetic analysis

DNA was obtained from peripheral blood. Twenty of 90 index patients were genetically screened by a targeted-panel NGS that commercially supported by MyGenostics Inc. (Beijing, China) before 2018. The gene panel covered 445 genes including 110 genes in CMT, 37 genes in ALS, 104 genes in HSP, 194 genes in SCA (Table S1), but other than *SORD*, *MME*, *WARS*, *SPTAN1*, *TBCK*, and *GBF1*. The remaining 70 index patients were screened using the WES commercially provided by Running Gene Inc. (Beijing, China).

Gene sequencing was performed according to the protocols of manufacturers. A mean normalized coverage was conducted to evaluate copy number variation (CNV). After variant annotation and filtering, variant classification was performed based on the American College of Medical Genetics (ACMG) guidelines. VarSome (https://varsome.com) and InterVar (https://wintervar.wglab.org) were used to adjust the pathogenicity prediction of variants. Patients with pathological or likely pathological variants were considered as genetically confirmed. Patients were considered as negative if no changes were found, or variants were classified as likely benign or benign. In order to confirm the variants, fragments containing the variants were amplified for direct Sanger sequencing in the patients and all family members available.

Subsequently, patients who were initially negative in targeted-panel NGS were directly screened for variants in the *SORD*, *MME*, *WARS*, *SPTAN1*, *TBCK*, and *GBF1* gene, because these genes were not included in the targeted panels. Sequence database from patients with negative in WES before 2021 were re-analyzed for the *SORD*, *MME* and *GBF1* gene, because the three genes were reported to be associated with dHMN before 2021.

In order to explore whether or not patients carry pathogenic expansion in the *NOTCH2NLC* gene, we conducted a TP-PCR as previously described.[14] TP-PCR was performed in all individuals who were genetically negative in the above screening. To identify abnormal nucleotide repeat of all known SCA genes, we used 22-FAM labeled primer sets for PCR, followed by capillary electrophoresis.

The primer sequences of all SCAs genes were designed using reference sequences from GenBank. The protocol and primers were described in supplemental data (Table S2).

Pathological analysis

Skin biopsies from two patients with positive expansion in *NOTCH2NLC* were performed following routine histological and histochemistry staining. Skin samples were fixed in 2.5% glutaraldehyde and postfixed in 1% osmium tetroxide. Semithin sections for orienting position were stained with toluidine blue. Ultrathin sections were examined through electron microscope. Immunohistochemical stain was conducted with anti-p62 antibody (Abcam, Cambridge, UK, ab56416).

Result

Clinical summarization

A total of 128 patients from 90 families were clinically identified as distal hereditary motor neuropathy, and were recruited into this observational study. There were 80 males and 48 females. The median age of onset was 24 years old. The disease duration ranged from 6 months to 50 years. The initial symptoms included distal weakness of lower limbs in 121 patients, hand weakness in six patients, and hoarseness in one patient. In particular, 20 patients showed asymmetric involvements at the early stage of disease. Beside the progressive weakness and wasting of distal limbs, 14 patients had minor subclinical sensory abnormalities on electrophysiological examinations (Table S3); 11 patients showed impairments of pyramidal tracts; five patients presented with signs of cerebellar involvement; and four patients had a hyperCKmia over 10 times of upper limit.

Genetic outcomes

Initially, six of 20 index patients were identified to have disease-causing variants through targeted-panel NGS, while one patient with biallelic heterozygous variants in the *MME* gene and another with pathogenic expansion in the *NOTCH2NLC* gene were found in the following genetic screening workflows. Similarly, 21 of 70 index patients were found to have disease-associated variants through WES, but two patients with homozygous variant in the *SORD* gene, one patient with pathogenic expansion in the *NOTCH2NLC* gene, and one patient with abnormal expansion in the *ATXN2* gene were identified in the additional genetic workflows (Fig. 1).

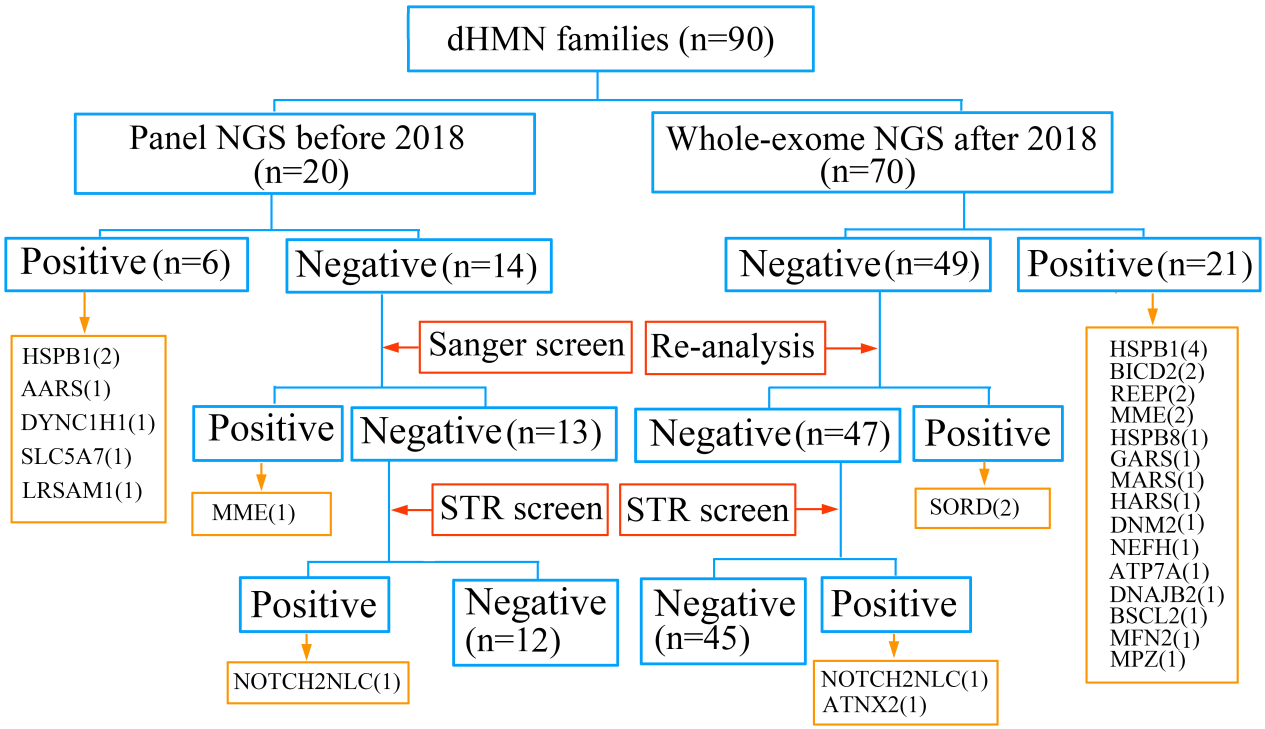

Figure 1. The workflow of genetic screening in this cohort of dHMN patients. The pathogenic genes as well as the number of positive patients were listed.

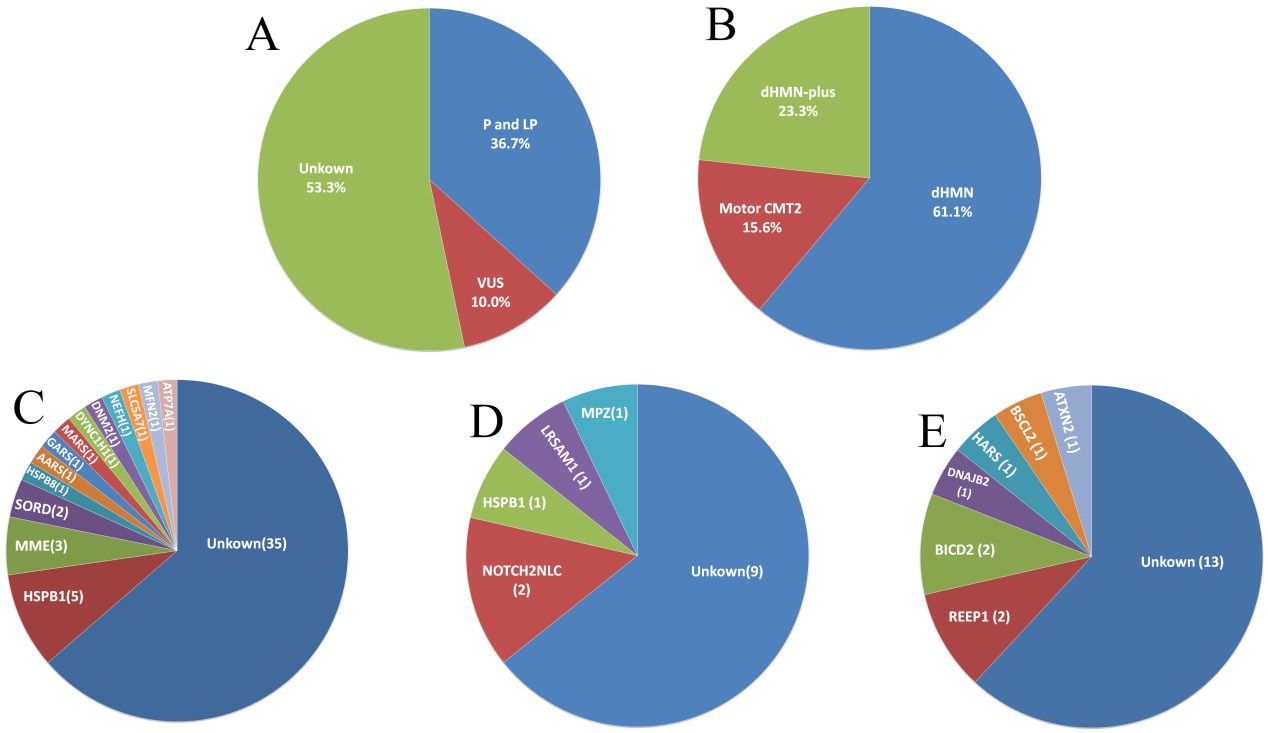

Figure 2. Spectrum and distribution of causative genes identified in this cohort. The detection rates of confirmed, VUS, and unknown genetic diagnosis, respectively (A); the clinical classification of dHMN (B); the distribution of genetic variants in patients with pure dHMN (C); the distribution of genetic variants in patients with motor CMT2 (D); the distribution of genetic variants in patients with dHMN-plus (E).

The detection rate of confirmed genetic diagnosis was 36.7% (33/90 families). If patients with variant uncertain significance (VUS) were included (Table S4), the rate would increase to 46.7% (42/90) (Fig. 2A). According to clinical features and electrophysiological changes, 61.1% (55/90) of patients were grouped to pure dHMN; 15.6% (14/90) were considered as dHMN with minor subclinical sensory abnormalities; 23.3% (21/90) were classified as dHMN-plus (Fig. 2B).

Chaperone-related genes (8/33, 24.2%) including *HSPB1*, *HSPB8*, and *DNAJB2* were the most common cause of dHMN; followed by variants in tRNA synthetase genes (4/33, 12.1%) including *AARS1*, *GARS1*, *HARS1*, and *MARS1*; and then cytoskeleton-related genes (4/33, 12.1%) covering *DYNC1H1*, *DNM2*, *BICD2*, and *NEFH*; therefore, the gene clusters of the three pathogenic pathways were responsible for 48.5% of genetically confirmed patients. Among this cohort of patients, the leading disease-causing gene of *HSPB1* accounted for 18.2% (6/33) of genetically confirmed cases; the second gene was *MME* for 9.1% (3/33); and followed by *SORD*, *NOTCH2NLC*, *BICD2*, and *REEP1* appearing in two patients, respectively. The clinical and molecular features of genetically confirmed patients were listed in Table 1 and Figure S1.

Variants of pure dHMN

Disease-causing variants were found in 20 index patients of 55 families that were classified as pure dHMN according to the clinical and electrophysiological findings (Fig. 2C). Seven patients from five families showed motor axonal neuropathy initiating from distal lower limbs, and carried with a novel (c.576_578delGGG, p.G193del) and three reported variants in the *HSPB1* gene (NM_001540).[15–17] In addition, the recurrent c.379C>T (p.R127W) in *HSPB1* confirmed it to be a hot-spot variant.[16] A novel variant of c.137C>A (p.A46D) in the *HSPB8* gene (NM_002506) was identified in a young woman presenting with typical distal weakness and wasting of lower limb.

Three families were associated with variants of tRNA synthetases including the *AARS1* (NM_001605), *GARS1* (NM_002047), and *MARS1* (NM_004990) genes. A novel variant (c.2177+1G>A) in *AARS1* occurred in a young woman whose symptoms were triggered and aggravated by recurrent pregnancies. A reported variant (c.794C>T, p.S265F) in *GARS1* was found in a juvenile-onset girl with initial symptom of the upper limb.[18] A novel variant (c.400C>T, p.P134S) in *MARS1* was identified in an affected man and his symptomatic mother.

Table 1. The clinical and mutational features of dHMN in the index patients with genetic diagnosis.

Family	Sex/AAE/AAO	Initial symptoms	Accompanied symptoms	Inheritance	Genes	Mutation	Pathogenicity	Ref
Pure dHMN								
F2	M/24/21	Right leg weakness	Hand tremor	AD	HSPB1	c.476_477delCT (p.P159Rfs*42)	P(PVS1 + PS1)	15[1]
F18-III2	M/17/12	Gait disturbance	None	AD	HSPB1	c.379C>T (p.R127W)	P(PS1 + PM1 + PM2 + PP1 + PP2)	16[2]
F25	M/40/31	Right leg weakness	None	Sporadic	HSPB1	c.379C>T (p.R127W)	P(PS1 + PS2 + PM2 + PP1 + PP2)	De novo[2]
F67	M/49/45	Leg weakness	None	Sporadic	HSPB1	c.576_578delGGG (p.G193del)	LP(PS2 + PM2 + PM4 + PP3)	De novo[2]
F88	F/55/40	Left leg weakness		AD	HSPB1	c.418C>G(p.R140G)	LP(PM1 + PM2 + PP2 + PP3 + PP4)	17[1]
F33	F/27/24	Left leg weakness	None	Sporadic	HSPB8	c.137C>A (p.A46D)	P(PS2 + PM1 + PM2 + PP3 + PP4)	De novo[2]
F7	F/36/33	Right leg weakness	None	Sporadic	AARS1	c.2177+1G>A	P(PVS + PS2 + PM2)	De novo[2]
F45	M/16/14	Hand weakness		AD	GARS1	c.794C>T (p.S265F)	LP(PM1 + PM2 + PP3 + PP4)	18[1]
F53-II1	M/51/49	Gait disturbance	None	AD	MARS1	c.400C>T(p.P134S)	LP(PM1 + PM2 + PP1 + PP2)	Novel[2]
F10	M/16/10	Gait disturbance	None	Sporadic	DYNC1H1	c.12823A>C(p.T4275P)	LP(PS2 + PM2 + PP3)	De novo[2]
F38	M/48/46	Leg weakness	Foot deformity	Sporadic	DNM2	c.2370_2372delGCC(p.P792del)	LP(PS2 + PM2 + PM4 + PP3)	De novo[2]
F48	M/22/22	leg weakness	None	Sporadic	NEFH	c.196delC(p.R66Vfs*15)	P(PVS + PS2 + PM2)	Novel[1]
F5	M/16/16	Right foot drop	None	AD	SLC5A7	c.847G>T(p.V283L)	LP(PM1 + PM2 + PM6)	Novel[1]
F58	M/7/4	Gait disturbance	None	AR	MFN2	c.163A>C (p.T55P, homo)	LP(PM1 + PM2 + PP1 + PP4)	Novel[2]
F86	F/29/4	Gait disturbance	None	AR	SORD	c.757delG(p.A253Qfs*27)	P(PVS + PM2 + PM3 + PP3)	19[2]
P90	F/19/14	Gait disturbance	None	AR	SORD	c.757delG(p.A253Qfs*27)	P(PVS + PM2 + PM3 + PP3)	19[2]
F4-II1	F/54/50	Gait disturbance	None	AR	MME	c.1342C>T(p.R448*) c.2071_2072delinsTT(p.A691L)	P(PVS + PS1 + PM2 + PM3)	20[2]
F77	F/24/16	Gait disturbance	None	AR	MME	c.1416+2T>C; c.2027C>T (p.P676L)	P(PS1 + PM2 + PM3)	20[2]
F85	M/53/43	Gait disturbance	None	AR	MME	c.2074C>T(p.Q692*) c.1342C>T(p.R448*)	P(PVS + PM2_Strong+PM3)	Novel[2]
F88	M/32/30	Hand weakness	None	XR	ATP7A	c.3854A>C(p.K1285T)	LP(PM1 + PM2 + PP1 + PP4)	Novel[2]
Motor CMT2								
F62	M/63/60	Gait disturbance	None	AD	HSPB1	c.379C>T(p.R127W)	P(PS1 + PM1 + PM2 + PP2 + PP4)	16[1]
F15	M/28/26	Left leg weakness	None	Sporadic	LRSAM1	c.1708A>C(p.M570L)	LP(PS2 + PM2 + PP3)	De novo[2]
F31	F/46/36	Leg weakness	None	AD	MPZ	c.437 T>C (p.V146A)	LP(PM1 + PM2 + PP3 + PP4)	Novel[2]
F9-III3	M/45/18	Leg fatigue	Tremor	AD	NOTCH2NLC	GGC repeat expansion	P(PS1 + PS3 + PP1)	11[2]
F72-III3	M/36/23	Gait disturbance	Tremor, dry cough	AD	NOTCH2NLC	GGC repeat expansion	P(PS1 + PS3 + PP1)	11[2]
dHMN-plus								
F27	M/29/24	Gait disturbance	HyperCKmia, bradykinesia	AR	DNAJB2	c.184C>T(homo)(p.R62W)	LP(PM2 + PM3 + PP1 + PP3)	Novel[2]
F73	F/15/2	Gait disturbance	RP, PD	AR	HARS1	c.1353G>C(homo)(p.Q451H)	LP(PM2 + PM3 + PP1 + PP3)	Novel[2]
F24	M/22/17	Gait disturbance	Pyramidal sign, arthrogryposis	AD	REEP1	c.337C>T(p.R113*)	P(PVS + PM2 + PP3)	22[1]
F52-II1	M/6/5	Gait disturbance	Pyramidal sign	AD	REEP1	c.417+1G>A	P(PVS + PM2 + PP3)	23[2]
F32-III6	F/54/51	Right leg weakness	CI	AD	BICD2	c.361C>G(p.L121V)	P(PS1 + PM1 + PM2 + PP1 + PP3)	24[2]
F82	F/21/17	Leg weakness	CI, scoliosis, FD	AD	BICD2	c.1823C>T (p.S608L)	LP(PS1 + PM1 + PP4)	21[1]
F37	F/15/11	Hand weakness	Pyramidal sign	AD	BSCL2	c.269C>T(p.S90L)	P(PS1 + PM2 + PP3 + PP4)	21[1]
F70	M/26/15	Gait disturbance	cerebellar symptom	Sporadic	ATXN2	CAG^{exp} 39 repeat	P(PS2 + PS3 + PP3)	Novel[1]

dHMN, distal hereditary motor neuropathy; AE, age at examination; AAO, age at onset; CI, cognitive impairment; FD, foot deformity; RP, retinitis pigmentosa; PD, psychomotor delay.
[1]Family co-segregation was unavailable.
[2]Family co-segregation was available.

A teenage presented with progressive dHMN involving in both upper and lower limbs, and had a c.12823A>C (p.T4275P) variant in the *DYNC1H1* (NM_001376) gene. A de novo deletion variant (c.2370_2372delGCC, p.P792del) in the *DMN2* (NM_001005360) gene was identified in a 48-year-old man exhibiting gait disturbance and foot deformity. A 22-year-old man presented with rapid-progressive distal weakness of lower limb, and carried with ac.196delC (p.R66Vfs*15) variant in the *NEFH* (NM_021076) gene.

A c.847G>T (p.V283L) variant in the *SLC5A7* (NM_001305005) gene was found in a juvenile-onset patient initiating with right foot drop, then rapid developing to distal muscle weakness of lower limbs. Intriguingly, a 7-year-old boy who showed gait disturbance and distal muscle weakness was associated with a homozygous variant (c.163A>C, p.T55P) in the *MFN2* (NM_001127660) gene, which was inherited from the asymptomatic parents, respectively. Two unrelated individuals presented with gait disturbance and length-dependent motor neuropathy, and had a loss-of-function biallelic variant (c.757delG, p.A253Qfs*27) in the *SORD* (NM_003104) gene.[19] Four dHMN patients from three families were associated with loss-of-function variants in the *MME* (NM_000902) gene, of which three patients have been described in our previous report,[20] and the last showed chronic progressive weakness and wasting of distal limbs. A novel c.3854A>C (p.K1285T) variant in the *ATP7A* (NM_000052) gene was associated with early-onset dHMN patient with initial involvement of upper limb.

Variants of dHMN with subclinical sensory abnormalities

Pathogenic variants were identified in five index patients of 14 families considered as motor CMT2 based on the electrophysiological changes (Fig. 2D). An 63-year-old man who showed a phenotype of dHMN with subclinical sensory involvement carried with a reported variant (c.379C>T, p.R127W) in the *HSPB1* gene.[16] A 28-year-old man showed motor axonal neuropathy without clinical sensory complaints, and was associated with a novel variant (c.1708A>C, p.M570L) in the *LRSAM1* (NM_001005374) gene. A dHMN family accompanied with minor subclinical sensory abnormalities was associated with a variant in the *MPZ* (NM_000530) gene (c.437T>C, p.V146A). The patients with GGC expansion in the *NOTCH2NLC* (NM_001364013) gene were described in detailed as below.

Variants of dHMN-plus

Disease-associated variants were revealed in eight index patients of 21 families diagnosed as dHMN-plus according to the clinical features (Fig. 2E). A 29-year-old man presenting with gait disturbance, bradykinesia, and hyperCKmia carried with a homozygous variant (c.184C>T, p.R62W) in the *DNAJB2* (NM_001039550) gene. A childhood-onset girl showing gait disturbance, psychomotor delay, and retinitis pigmentosa was associated with a homozygous variant (c.1353G>C, p.Q451H) in the *HARS1* (NM_002109) gene. A reported *BSCL2* (NM_032667) variant (c.269C>T, p.S90L) was found in teenage girl who initiating with axonal motor neuropathy of the upper limb and pyramidal sign.[21] Two families carried with premature variants (c.337C>T, p.R113* and c.417+1G>A, respectively) in the *REEP* (NM_022912) gene, and exhibited distal motor neuropathy plus pyramidal sign.[22,23] Two families were considered as spinal muscular atrophy with lower extremity predominance accompanied with cognitive impairment or joint deformities, and carried with variants (c.361C>G, p.L121V and c.1823C>T, p.S608L, respectively) in the *BICD2* (NM_015250) gene.[21,24] The patient with CAG expansion in the *ATXN2* (NM_001372574) gene would be described in detailed as below.

Patients with trinucleotide expansion

Patient one

The patient (F9-III3) (Fig. S1) was a 32-year-old male who had fatigue in his distal lower limbs since 18 years of age, and gradually had difficulty in walking on his tiptoes and muscle fasciculation in distal lower limbs. He noticed tremor in jaw and both hands at age 30. Physical examination on admission showed a steppage gait. His muscle strength (Medical Research Council) grade was 5 in neck flexors, 5 in the upper limbs, 5 in the proximal lower limbs, and 3–4 in the extension and flexion of ankle and toes. Interosseous muscle atrophy of feet was observed. Achilles tendon reflex was diminished. No abnormalities of sensations were found. Nerve conduction studies (NCS) revealed a remarkable reduction of motor nerve conduction velocity (NCV) predominantly affecting the lower limbs, while the sensory NCV was only mildly decreased (Table S3). Electromyogram (EMG) findings showed neurogenic changes and myotonic discharge. Serum creatine kinase (CK) was 175 IU/L (normal range: 22–269 IU/L). Cerebral MRI was normal. Abnormal expansion of GGC repeats were found in the 5'UTR of the *NOTCH2NLC* gene (Fig. 3A). In his family, other four individuals showed muscle weakness of lower limbs. In brief, his aunt (F9-II2) showed muscle weakness and wasting of distal lower limbs in her 20s, had hand tremor and recurrent headache since 35 years of age, and was bound to wheelchair at age 65. His sister (F9-III2) had

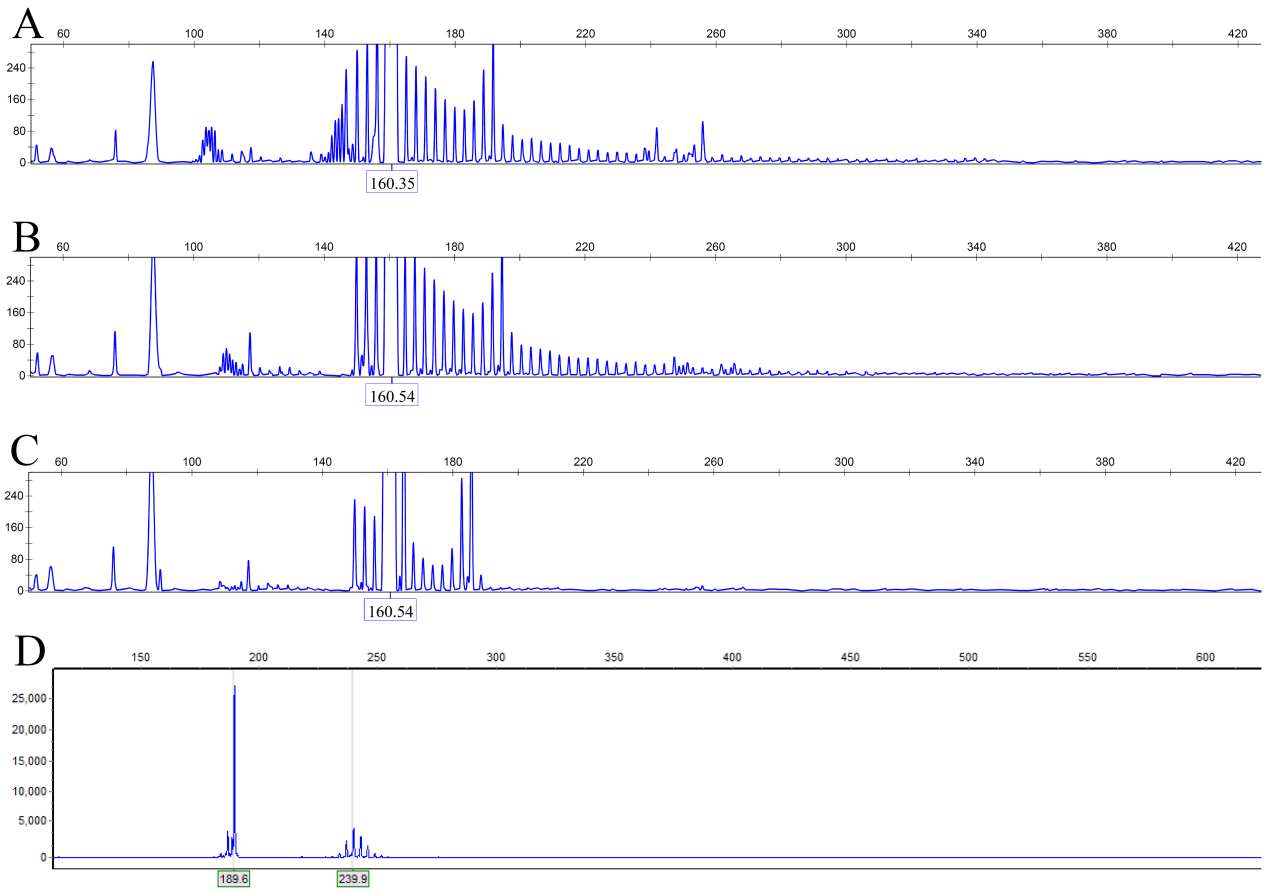

Figure 3. The chromatogram of STR screen. The long saw-tooth curves indicate that the numbers of GGC in patient F9-III3 (A) and patient F72-III3 (B) exceed a value of at least 100 repeat expansion in the *NOTCH2NLC* gene, but only single peak wave without saw-tooth pattern in a healthy control (D). The TP-PCR shows an expansion with 39 CAG repeats in the *ATXN2* gene in the patient F70 (C).

muscle weakness of distal lower limbs at age 22, and had hand tremor at age 36. His father (F9-II3) and grandmother (F9-I1) developed weakness in distal limbs in their early 20s and died in their 50s with unavailable causes.

Patient two

The patient (F72-III3) (Fig. S1) was a 36-year-old male who had dry cough with uncertain cause at age 23. At age 25, he developed distal lower limb weakness and had difficulty walking on his tiptoes. Recently, he presented resting and postural tremors in both hands. Physical examination on admission revealed steppage gait and weakness with MRC grade 4 in bilateral ankle and toe flexion. Tendon reflexes were reduced in the ankles. There were no sensory deficits. NCS revealed an obvious reduction in motor NCV and prolonged distal latency in the lower limbs, while the sensory NCV was slightly decreased (Table S3). EMG findings indicated neurogenic changes. Serum creatine kinase (CK) was 302 IU/L. Cerebral MRI was intact. Abnormal expansion of GGC repeats were identified in the 5'UTR of the *NOTCH2NLC* gene (Fig. 3B). His family's pedigree was consistent with an incomplete dominance inheritance pattern (Fig. S1). The brother (F72-III1) had similar symptoms with dry cough and weakness of the distal lower limbs. The uncle (F75-II4) developed dry cough in his early 20s, weakness in distal lower limbs in his 30s, hand tremor at age 35, and urinary incontinence at age 45. The father (F72-II2) was said to have dry cough and leg weakness, and died of pneumonia at age 66.

Patient three

The patient was a 26-year-old male from a nonconsanguineous family. He initially had difficulty walking on his tiptoes at age 15, and gradually developed slow walking and falling down due to an uneven ground. Physical examination on admission revealed that muscle strength

grade was MRC 5/5 in the proximal limbs, 3 in the foot dorsiflexion, and 4 in the foot plantarflexion. Deep tendon reflexes were diminished in both lower limbs. Pain, light touch, vibration, and joint position sensations were normal. Babinski sign was negative on both sides. Horizontal nystagmus was noticed. Finger nose test indicated a cerebellar involvement. NCS revealed a severe reduction in motor NCV in the lower limbs, while the sensory NCV was normal. EMG findings indicated neurogenic changes. Cerebral MRI showed a mild cerebellar atrophy. Genetic screening revealed 39 repeats of CAG expansion in the *ATXN2* gene (Fig. 3C). The parents were said to have no abnormalities, while the clinical and genetic investigations were unavailable.

Pathological changes

In order to identify the pathogenicity of *NOTCH2NLC* positive expansion, skin biopsies were performed in the two index patients. The ductal epithelial cells of sweat glands showed a lot of eosinophilic intranuclear inclusions that were positive to P62 antibody (Fig. 4A). Electron microscopy revealed a pile of round-halo filamentous materials in the center of the nucleus (Fig. 4B).

Discussion

In this dHMN patient cohort, the rate of genetic diagnosis was achieved in 36.7% (33/90) of families through a comprehensive genetic investigation including the recently reported *SORD* and *NOTCH2NLC* genes. These rates are similar to the other published series screened by NGS techniques from worldwide,[3,6–10] which proved once again the genetic heterogeneity of dHMN, and a large part of dHMN patients still remain uncertain in genetic diagnosis.

The reasons for the relatively low rate of molecular diagnosis might origin from many ways[5]: (1) some exonic variants in this study were classified as VUS due to lack of co-segregation analysis or function investigation; (2) some deep intronic mutations, copy number variations, and gene structural abnormalities might be omitted owing to the limitations of NGS techniques; (3) numerous STRs expansion were undetectable in classic genetic screening, though the *NOTCH2NLC* and SCA-related genes were screened in this study; (4) there were still many unknown genes responsible for the phenotype of genetically unresolved dHMN cases, though 22 genes with pathogenic or likely pathogenic significance have been found in our patients; (5) even if thorough examinations were clinically conducted in these patients, it was impossible to completely exclude other secondary causes for some patients with distal motor neuropathy.

Chaperones participate in multiple important processes in the proteostasis system, so the genetic defects of chaperones have been referred to as chaperonopathies.[25] ATP-independent chaperones including HSPB1, HSPB3, HSPB8, and DNAJB2 have become the leading causative genes of dHMN.[4] In our patient cohort, *HSPB1*, *HSPB8*, and *DNAJB2* accounted for 24.2% of patients with genetic confirmation, which were consistent with other cohort's studies. *HSPB1* and *HSPB8* mutations were inherited dominantly and most involved in missense variants, while they might be also associated with a frameshift or deletion mutations. Although *DNAJB2*-related dHMN

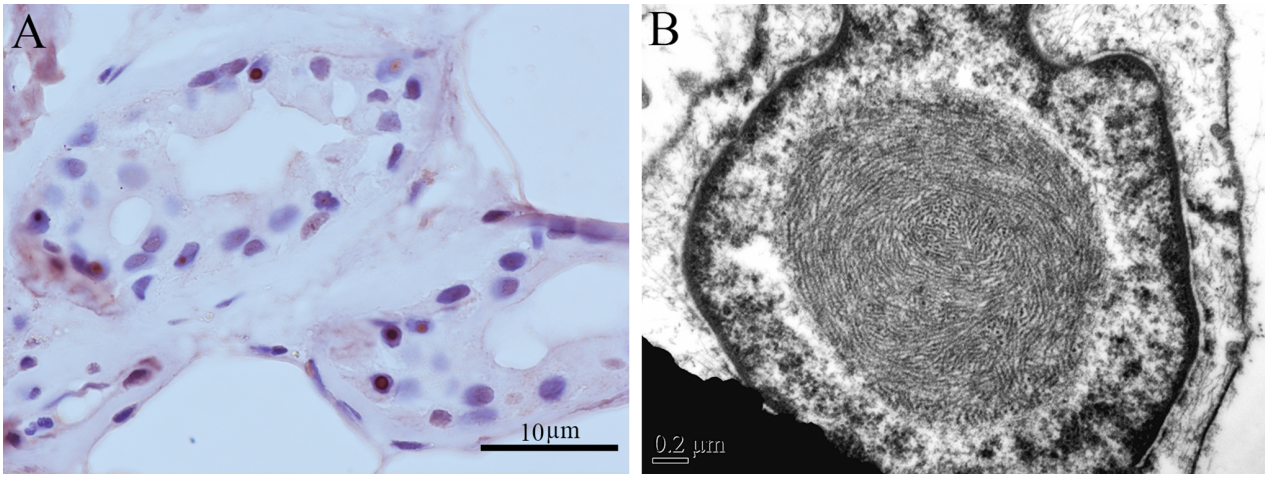

Figure 4. The pathological changes of skin in dHMN patient with *NOTCH2NLC* positive expansion. Skin biopsy of patient F9-III3 with *NOTCH2NLC* positive expansion showed lots of eosinophilic intranuclear inclusions that were positive to P62 antibody (A) and a pile of round-halo filamentous materials in the center of the nucleus on electron microscopy (B).

patients were relatively common in a Spanish cohort,[7] only one patient caused by *DNAJB2* variants in autosomal recessive inheritance was found in our cohort. Given DNAJB2 mainly expressing in brain, nerve, and muscle, it was reasonable that the patient showed a dHMN-plus phenotype including distal motor neuropathy, bradykinesia, and hyperCKmia. The chaperones have greatly involved in the growing list of dHMN subtypes; thus, more attentions should be paid to chaperone-related genes in genetically unsolved cases.

Since axonal neuropathy is closely associated with dysfunctions of cytoskeleton in axonal transport, it is unsurprised that lots of cytoskeleton-related genes have involved in the pathogenesis of dHMN.[26] In this study, four dHMN patients were identified with disease-causative variants in the *DYNC1H1*, *DNM2*, *BICD2*, and *NEFH* genes, and accounted for 12.1% of patients with genetic diagnosis. Although the cytoskeleton-related genes were hot-spot candidates for dHMN in different ethnic populations, the specific genes and mutational types had great genetic heterogeneities, as in our patient cohort. In addition, most of patients showed an early-onset motor neuropathy, while great clinical heterogeneities were observed in these patients associated with cytoskeleton-related genes. Since the cytoskeleton participates in axonal transport, as well as in the dynamics of various organelles and plasma membrane receptors, there is clear significance to explore the underlying cytoskeleton genes for genetically unsolved cases.[21]

Amino acyl-tRNA synthetases are an essential family of enzymes responsible for attaching amino acids to their cognate tRNAs in all cells and tissues.[27] The pathogenic variants of tRNA synthetases may directly disrupt protein synthesis, and cause the dysfunction of axonal transport. Traditionally, variants in the *AARS1*, *GARS1*, *HARS1, KARS1*, and *YARS1* genes have been implicated in several subtypes of CMT, meanwhile variants in the *AARS1*, *GARS1*, and *WARS1* genes have also been associated with dHMN.[28] We identified that variants in *AARS1*, *GARS1*, *HARS1*, and *MARS1* were co-segregated with dHMN patients, and as the common cause of dHMN in our patient cohort. Nevertheless, *MARS1* and *HARS1* were the first time to be reported to cause dHMN. It is well known that CMT2 and dHMN have significant overlapping in clinical and genetic aspects, and the coexistence of both phenotypes can be observed in the same patient with different disease stages or the same family whose members carrying the same pathogenic mutation but showing different phenotypes.[29] Accordingly, it was reasonable that variants in *MARS1* and *HARS1* might be associated with the phenotype of dHMN, though pathogenic function analysis was still needed.

Although the above-mentioned three genetic pathways accounted for nearly half of genetically confirmed cases, there were still many other disease-causative genes associated with dHMN. Among them, the genetic list not only included classic genes such as *MFN2, MPZ, BSCL2, SLC5A7*, and *ATP7A* but also covered the newly discovered *MME* and *SORD* genes. *SORD* variant had been reported that it might account for a majority of autosomal recessive-dHMN cases,[10,19] while we found the *MME* variants were the most common for recessive inherited cases in our patient cohort. Intriguingly, *MFN2* variant for CMT/dHMN used to be single heterozygous mutation, while a homozygous mutation of *MFN2* was found in a childhood-onset dHMN patient. In addition, a homozygous *HARS1* variant was also detected in a patient with dHMN, psychomotor delay, and retinitis pigmentosa. The phenotype of biallelic variants in *HARS1* usually presented with Usher syndrome type 3B or multisystem ataxic syndrome.[30] Our patient with homozygous p.Q451H variant in *HARS1* showed a dHMN-plus syndrome which partly overlapped with Usher syndrome type 3B or multisystem ataxic syndrome.

In this study, we screened the STR variants in dHMN patients, and identified GGC expansion in *NOTCH2NLC* in two autosomal dominant families and CAG expansion in *ATNX2* in a patient with dHMN-plus cerebellar ataxia. As we discussed in a previous study,[11] the GGC repeat expansion in the *NOTCH2NLC* has been associated with a new type of hereditary distal neuromyopathy based on a common pathological basis of widespread eosinophilic intranuclear inclusions in both nerves and muscles. Herein, we found that the GGC expansion in *NOTCH2NLC* can be responsible to a part of patients with dHMN with minor subclinical sensory abnormalities, of which the electrophysiological changes indicated both axonal and demyelinating involvements, and the clinical features showed some extra-motor neuropathy symptoms such as tremor, dry cough, urinary incontinence, or headache. The clinical and electrophysiological characteristics might be useful diagnostic indicators for distal neuropathy associated with *NOTCH2NLC* repeat expansions.

Previous studies have indicated that symptoms or signs of peripheral nerve involvement in SCA2 are not uncommon.[12] Electrophysiological evidence of the isolated involvement of motor neurons and/or axons has been detected in some presymptomatic carriers before the onset of cerebellar signs.[31] In this study, we also identified that CAG expansion in the *ATXN2* gene was associated with distal motor neuropathy of lower limb. Although the pathophysiological mechanisms responsible for the motor nerve involvement in SCA2 are not well understood, the STR screening for CAG expansion in *ATXN2* should be routinely included in the genetic diagnosis of dHMN.

Although the majority of dHMN patients showed an autosomal dominant inherited mode, autosomal recessive

dHMNs have been associated with variants in the *SORD*, *IGHMBP2, PLEKHG5, DNAJB2, SIGMAR1, SYT2, ATM, TBCE*, and *VRK1* genes.[20,32] Our study indicated that variants in the *MME* gene were associated with autosomal-recessive dHMN, and as the second disease-causing gene for genetically confirmed cases in our patient cohort. Compared with patients with autosomal-recessive *MME*-related CMT2 displaying as adult-onset progressive motor-sensory neuropathy,[33] some affected patients with *MME*-related dHMN showed a juvenile-onset and slow progressive length-dependent motor neuropathy.

In summary, this study extends the genetic heterogeneity of dHMN, which clinically exhibits an overlap syndrome ranging from motor neuropathy to SCA, HSP, ALS, SMA, CMT2, distal myopathy, and other neurologic abnormalities. Although the NGS method makes it possible to convenient genetic diagnosis, there are more than half of dHMN patients still with unknown genetic causes. Given some dHMN patients have been associated with STR variants in *NOTCH2NLC* and *ATXN2*, it is necessary to conduct STR screen in genetically unsolved patients with dHMN.

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Conflicts of Interest

The authors have declared no conflicts of interest.

Ethics Approval

The research was approved by ethics committee of the first affiliated hospital of Nanchang University.

Availability of Data and Material

All relevant data are described within the paper. Deidentified data can be requested. Data can be requested by all interested researchers, who can be contacted via the corresponding author.

Authors' Contributions

C. W. draft manuscript and analysis of data. H. X., R. C., Y. Z., S. C., and Y. Y. contributed to the acquisition and analysis of data. M. Z., Y. Y., and J. D. performed the genetic and pathological study. M. Z. and D. H. contributed to electrophysiological analysis. M. Z. and D. H. contributed the study design and drafting the manuscript.

References

1. Verschueren A. Motor neuropathies and lower motor neuron syndromes. Rev Neurol (Paris). 2017;173(5):320-325.
2. Harding AE. Inherited neuronal atrophy and degeneration predominantly of lower motor neurons. In: Dyck PJ, Thomas PK, Griffin JW, Low PA, Poduslo JF, eds. Peripheral Neuropathy. 3rd ed. Saunders; 1993:1051-1064.
3. Bansagi B, Griffin H, Whittaker RG, et al. Genetic heterogeneity of motor neuropathies. Neurology. 2017;88(13):1226-1234.
4. Rossor AM. Broadening the genetic spectrum of distal hereditary motor neuropathy. Eur J Neurol. 2021;28(4):1104-1105.
5. Pipis M, Rossor AM, Laura M, et al. Next-generation sequencing in Charcot-Marie-tooth disease: opportunities and challenges. Nat Rev Neurol. 2019;15(11):644-656.
6. Liu X, Duan X, Zhang Y, et al. Molecular analysis and clinical diversity of distal hereditary motor neuropathy. Eur J Neurol. 2020;27(7):1319-1326.
7. Frasquet M, Rojas-García R, Argente-Escrig H, et al. Distal hereditary motor neuropathies: mutation spectrum and genotype-phenotype correlation. Eur J Neurol. 2021;28(4):1334-1343.
8. Xie Y, Lin Z, Pakhrin PS, et al. Genetic and clinical features in 24 Chinese distal hereditary motor neuropathy families. Front Neurol. 2020;11:603003.
9. Argente-Escrig H, Burns J, Donlevy G, et al. Clinical, genetic, and disability profile of pediatric distal hereditary motor neuropathy. Neurology. 2021;96(3):e423-e432.
10. Liu X, He J, Yilihamu M, Duan X, et al. Clinical and genetic features of biallelic mutations in SORD in a series of Chinese patients with Charcot-Marie-tooth and distal hereditary motor neuropathy. Front Neurol. 2021;12:733926.
11. Yu J, Luan XH, Yu M, et al. GGC repeat expansions in NOTCH2NLC causing a phenotype of distal motor neuropathy and myopathy. Ann Clin Transl Neurol. 2021;8(6):1330-1342.
12. Crum BA, Josephs KA. Varied electrophysiologic patterns in spinocerebellar ataxia type 2. Eur J Neurol. 2006;13(2):194-197.
13. 2nd Workshop of the European CMT Consortium:53rd ENMC International Workshop on Classification and Diagnostic Guidelines for Charcot-Marie-Tooth Type 2 (CMT2-HMSN II) and distal hereditary motor neuropathy (distal HMN-spinal CMT) 26–28 September 1997, Naarden, The Netherlands. Neuromuscul Disord. 1998;8(6):426-431.
14. Deng J, Zhou B, Yu J, et al. Genetic origin of sporadic cases and RNA toxicity in neuronal intranuclear inclusion disease. J Med Genet. 2021. doi:10.1136/jmedgenet-2020-107649

15. Capponi S, Geroldi A, Fossa P, et al. HSPB1 and HSPB8 in inherited neuropathies: study of an Italian cohort of dHMN and CMT2 patients. J Peripher Nerv Syst. 2011;16 (4):287-294.
16. Evgrafov OV, Mersiyanova I, Irobi J, et al. Mutant small heat-shock protein 27 causes axonal Charcot-Marie-tooth disease and distal hereditary motor neuropathy. Nat Genet. 2004;36(6):602-606.
17. Houlden H, Laura M, Wavrant-De Vrièze F, et al. Mutations in the HSP27 (HSPB1) gene cause dominant, recessive, and sporadic distal HMN/CMT type 2. Neurology. 2008;71(21):1660-1668.
18. Lee HJ, Park J, Nakhro K, et al. Two novel mutations of GARS in Korean families with distal hereditary motor neuropathy type V. J Peripher Nerv Syst. 2012;17(4):418-421.
19. Cortese A, Zhu Y, Rebelo AP, et al. Biallelic mutations in SORD cause a common and potentially treatable hereditary neuropathy with implications for diabetes. Nat Genet. 2020;52(5):473-481.
20. Hong D, Fang P, Yao S, et al. Variants in MME are associated with autosomal-recessive distal hereditary motor neuropathy. Ann Clin Transl Neurol. 2019;6(9):1728-1738.
21. Novarino G, Fenstermaker AG, Zaki MS, et al. Exome sequencing links corticospinal motor neuron disease to common neurodegenerative disorders. Science. 2014;343 (6170):506-511.
22. Toft A, Birk S, Ballegaard M, et al. Peripheral neuropathy in hereditary spastic paraplegia caused by REEP1 variants. J Neurol. 2019;266(3):735-744.
23. Liu SG, Che FY, Heng XY, et al. Clinical and genetic study of a novel mutation in the REEP1 gene. Synapse. 2009;63 (3):201-205.
24. Wan C, Wang Y, Zhou Q, et al. Adult-onset SMALED2 due to a novel *BICD2* mutation presenting with asymmetrical lower limb involvement. Clin Neuropathol. 2019;38(3):109-117.
25. Lupo V, Aguado C, Knecht E, et al. Chaperonopathies: spotlight on hereditary motor neuropathies. Front Mol Biosci. 2016;3:81.
26. Du K, Meng L, Lv H, et al. Sural biopsy to detect the axonal cytoskeleton defects in KIF5A-related Charcot-Marie-tooth disease type 2. Clin Neuropathol. 2021;40(3):142-149.
27. Li JQ, Dong HL, Chen CX, Wu ZY. A novel WARS mutation causes distal hereditary motor neuropathy in a Chinese family. Brain. 2019;142(9):e49.
28. Zhang H, Zhou ZW, Sun L. Amino acyl-tRNA synthetases in Charcot-Marie-tooth disease: a gain or a loss? J Neurochem. 2021;157(3):351-369.
29. McMacken G, Whittaker RG, Charlton R, et al. Inherited neuropathies with predominant upper limb involvement: genetic heterogeneity and overlapping pathologies. Eur J Neurol. 2021;28(1):297-304.
30. Galatolo D, Kuo ME, Mullen P, et al. Bi-allelic mutations in HARS1 severely impair histidyl-tRNA synthetase expression and enzymatic activity causing a novel multisystem ataxic syndrome. Hum Mutat. 2020;41 (7):1232-1237.
31. Pelosi L, Iodice R, Antenora A, et al. Spinocerebellar ataxia type 2-neuronopathy or neuropathy? Muscle Nerve. 2019;60(3):271-278.
32. Argente-Escrig H, Frasquet M, Vázquez-Costa JF, et al. Pediatric inherited peripheral neuropathy: a prospective study at a Spanish referral center. Ann Clin Transl Neurol. 2021;8(9):1809-1816.
33. Higuchi Y, Hashiguchi A, Yuan J, et al. Mutations in MME cause an autosomal-recessive Charcot-Marie-tooth disease type 2. Ann Neurol. 2016;79(4):659-672.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. The pedigrees of dHMN patient with confirmed genetic diagnosis in Table 1.
Table S1. The gene list of target-panel NGS for dHMN before 2018.
Table S2. The primers and protocols for STR screening in SCAs.
Table S3. The electrophysiological changes in patients with dHMN and minor sensory abnormalities.
Table S4. The clinical and mutational data of dHMN patients with VUS variants.

