Relative contribution of mutations in genes for autosomal dominant distal hereditary motor neuropathies: a genotype – phenotype correlation study

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Distal hereditary motor neuropathy (HMN) is a clinically and genetically heterogeneous group of disorders affecting spinal α-motor neurons. Since 2001, mutations in six different genes have been identified for autosomal dominant distal HMN; *glycyl-tRNA synthetase (GARS), dynactin 1 (DCTN1), small heat shock 27 kDa protein 1 (HSPB1), small heat shock 22 kDa protein 8 (HSPB8), Berardinelli-Seip congenital lipodystrophy (BSCL2)* and *senataxin (SETX).* In addition a mutation in the *(VAMP)-associated protein B and C (VAPB)* was found in several Brazilian families with complex and atypical forms of autosomal dominantly inherited motor neuron disease. We have investigated the distribution of mutations in these seven genes in a cohort of 112 familial and isolated patients with a diagnosis of distal motor neuropathy and found nine different disease-causing mutations in *HSPB8, HSPB1, BSCL2* and *SETX* in 17 patients of whom 10 have been previously reported. No mutations were found in *GARS, DCTN1* and *VAPB.* The phenotypic features of patients with mutations in *HSPB8, HSPB1, BSCL2* and *SETX* fit within the distal HMN classification, with only one exception; a C-terminal *HSPB1*-mutation was associated with upper motor neuron signs. Furthermore, we provide evidence for a genetic mosaicism in transmitting an *HSPB1* mutation. This study, performed in a large cohort of familial and isolated distal HMN patients, clearly confirms the genetic and phenotypic heterogeneity of distal HMN and provides a basis for the development of algorithms for diagnostic mutation screening in this group of disorders.

Introduction

Distal hereditary motor neuropathy (HMN), also known as distal spinal muscular atrophy (SMA) or the spinal form of Charcot–Marie–Tooth disease (CMT), covers a spectrum of clinically and genetically heterogeneous diseases characterized by the selective involvement of motor neurons in the peripheral nervous system. In contrast to proximal SMA, distal HMN initially and predominantly affects the distal limb muscles suggesting a length-dependent disease mechanism affecting the longest motor axons first. The vast majority of patients present a classical distal muscular atrophy syndrome in the legs without clinical sensory loss. The disease usually begins in childhood or adolescence and starts with weakness and wasting of distal muscles of the anterior tibial and peroneal compartments of the legs. Later on, weakness and atrophy may expand to the proximal muscles of the lower limbs and/or to the distal upper limbs. However, in some patients and families the disease starts or even predominates in the hands. Foot deformities (e.g. *pes cavus*) are sometimes present. Based on clinical and electrophysiological evidence, Dyck and Lambert (1968*a*, *b*) postulated that distal HMN was a separate clinical and genetic entity. Subsequently, a classification has been proposed subdividing distal HMN in seven subtypes based on age at onset, mode of inheritance, distribution of muscle weakness and additional clinical features (Harding, 1993). These subtypes were based on the description of a limited number of patients in small families. Currently, with detailed reports of a larger number of distal HMN families including several large pedigrees and the identification of gene defects, the phenotypes initially defined in this classification could be confirmed at the molecular genetic level. However, some clinical features were not included in the original classification (e.g. pyramidal signs, congenital onset and X-linked inheritance) and have resulted into further diversification of the clinical spectrum of distal HMN (Irobi *et al.*, 2006).

Since 2001, eight genes have been identified for distal HMN. Six genes have been associated with autosomal dominant (AD) distal HMN: mutations in *heat shock 27 kDa protein 1* (*HSPB1*) (Evgrafov *et al.*, 2004) and *heat shock 22 kDa protein 8* (*HSPB8*) (Irobi *et al.*, 2004*b*) are found in families with distal HMN type II, mutations in *glycyl-tRNA synthetase* (*GARS*) (Antonellis *et al.*, 2003) are associated with distal HMN type V, mutations in *Bernardinelli-Seip congenital lipodystrophy 2* (*BSCL2*)

(Windpassinger *et al.*, 2004) cause distal HMN type V and Silver syndrome, a single *dynactin 1* (*DCTN1*) (Puls *et al.*, 2003) mutation was found in a family with adult-onset motor neuron disease with breathing difficulties due to vocal cord paralysis, and mutations in *senataxin* (*SETX*) (Chen *et al.*, 2004) are reported in families with an early-onset distal HMN with pyramidal tract signs (juvenile amyotrophic lateral sclerosis; ALS4). Furthermore, a mutation was found in the *VAMP-associated protein B and C* (*VAPB*) gene in Brazilian families with clinically diverse motor neuron disorders, including atypical ALS with a slow progression, typical ALS and SMA affecting mainly proximal muscles (Nishimura *et al.*, 2004). Currently, no mutations in *VAPB* were reported in distal HMN patients and families. Mutations causing autosomal recessive (AR) distal SMA with respiratory distress (SMARD1; distal HMN type VI) were described in the *immunoglobulin μ-binding protein 2a* (*IGHMBP2*) (Grohmann *et al.*, 2001) gene. In addition, a mutation was recently found in the *pleckstrin homology domain-containing, family G member 5* gene (*PLEKHG5*) in an autosomal recessive African family with childhood onset and severe evolution (Maystadt *et al.*, 2007). The mutations in genes for distal HMN are listed in the Inherited Peripheral Neuropathy Mutation Database (www. molgen.ua.ac.be/-CMTMutations). Interestingly, most of these genes encode for ubiquitously expressed proteins with diverse cellular functions: protein translation and synthesis (GARS, BSCL2), RNA/DNA metabolism (SETX, IGHMBP2), axonal guidance and trafficking (HSP27, DCTN1, PLEKHG5), cellular protection (HSP22, HSP27) and apoptosis (HSP27) (Van Den Bosch and Timmerman, 2006). More insights into the role of these proteins in motor neuron function will increase our knowledge of the pathophysiological mechanisms underlying distal HMN.

With the identification of mutations in these genes it also became apparent that mutations in the same gene are associated with clinically separate disease entities, as mutations in *GARS* (Sivakumar *et al.*, 2005), *HSPB1* (Evgrafov *et al.*, 2004; Tang *et al.*, 2005*a*) and *HSPB8* (Irobi *et al.*, 2004*b*; Tang *et al.*, 2005*b*) have been found both in familial and isolated patients with distal HMN and hereditary motor and sensory neuropathy (HMSN; CMT) phenotypes. However, there is scarce information about the definite contribution of mutations in these genes in distal HMN patients and about possible genotype–phenotype correlations.

In this study, we investigated a cohort of 112 familial and isolated patients with a diagnosis of distal motor neuropathy and determined the distribution of mutations in the known genes associated with AD distal HMN. The cohort included 10 index patients of families that have previously been reported in manuscripts describing novel genes and related phenotypes (Timmerman *et al.*, 1992; De Jonghe *et al.*, 2002; Evgrafov *et al.*, 2004; Irobi *et al.*, 2004a, b). In addition, we included the analysis of *VAPB* to investigate whether the phenotype associated with mutations in *VAPB* could be expanded to distal HMN.

Patients and Methods
Selection criteria
We selected in our patient database all distal HMN patients using a pre-defined set of criteria. The clinical criteria for distal HMN were previously designed by the European CMT Consortium (De Jonghe *et al.*, 1998) and include: slowly progressive muscle wasting and weakness of predominantly the distal parts of the extremities with variable severity. In contrast to the published criteria, we included patients with pyramidal tract signs such as spastic gait, brisk reflexes and Babinski signs since in the mean time it has been demonstrated that these signs are common in distal HMN patients with *SETX* and *BSCL2* mutations. We excluded patients presenting with predominant proximal muscle weakness, sensory symptoms and signs, and significant central nervous system involvement other than pyramidal tract signs. The clinical diagnosis of distal HMN was confirmed by nerve conduction studies and concentric needle electromyography (EMG) i.e. normal sensory nerve conduction velocities (NCVs), normal or slightly reduced motor NCVs and neurogenic alterations on needle EMG examination.

Patient cohort
The cohort encompassed 112 index patients who were initially referred for molecular genetic testing. Genomic DNA samples were provided through Neurology Departments and Neuromuscular Centres worldwide, with the majority from European origin. For 57 out of 112 index patients, a dominant inheritance pattern, based on a parent to child transmission, could be determined. Twenty-one patients were referred as 'isolated' since they did not have a familial history of neuropathy in first and second degree relatives. No family history was available for the remaining 34 patients. Of 74 patients, we had information on age at onset of the disease; in six patients the first symptoms presented before the first year, in 19 patients the disease started in the first decade of life, in 23 index patients the onset of disease was within the second decade of life and in 26 patients the first symptoms presented after the second decade of life. In 21 index patients, pyramidal tract signs were present. Eight patients presented bulbar symptoms including facial weakness, dysarthria, dysphagia, vocal cord paralysis or breathing difficulties. We obtained detailed electrophysiological information on 63 index patients. The remaining patients had an electrophysiological evaluation by their referring neurologist confirming a diagnosis of distal HMN but no detailed data were available. All referring neurologists were active in neuromuscular centres and were familiar with the clinical and electrophysiological phenotype of

distal HMN. From all patients included in this cohort informed consent was obtained by the referring physician.

Molecular genetic analysis
The coding regions and exon–intron boundaries of *HSPB1*, *HSPB8*, *BSCL2*, *GARS*, *SETX*, *DCTN1* and *VAPB* were PCR-amplified using primer oligonucleotides designed with the Primer3 and SNPbox software tools (Supplementary Table 1) (Rozen and Skaletsky, 2000; Weckx *et al.*, 2004). PCR conditions are available upon request. PCR products were cleaned up using the Exonuclease I–Shrimp Alkaline Phosphatase enzyme (USB, Cleveland, Ohio). Mutation screening was performed by direct sequencing of the purified PCR fragments using the BigDye Terminator v3.1 Cycle sequencing kit (Applied Biosystems, Foster city, CA). Fragments were separated on an ABI 3730 automated capillary DNA sequencer (Applied Biosystems). The resulting sequences were aligned and analyzed with the SeqManII (DNAstar Inc., Madison, WI) and the novoSNP program (Weckx *et al.*, 2005). The nucleotide numbering of the genes is relative to the ATG translation initiation site with A as +1 of the corresponding cDNA sequences (*HSPB8*: NM_014365.2; *HSPB1*: NM_001540.2; *BSCL2*: NM_032667.7; *GARS*: NM_002047.2; *SETX*: NM_015046.5; *DCTN1*: NM_004082.2; *VAPB*: NM_004738.3), according to the nomenclature of DNA sequence variants (http://www.hgvs.org/mutnomen). Sequence variants were confirmed by repeated PCR and bidirectional sequencing. Where possible, segregation of the mutation with the disease phenotype in the family was analysed.

Genotyping and paternity testing
Pyrosequencing with the PSQ^{TM}HS 96A System (Biotage AB, Uppsala, Sweden) was performed to genotype the Pro182Leu *HSPB1*-mutation. Primers for pyrosequencing were designed using the SNP Primer Design software version 1.0.1 (Biotage AB available upon request). Six informative short tandem repeats (STRs), D7S2435, D7S2490 or D7S2518, D7S2470, D7S2421, D7S669 and D7S2204, were used for segregation analysis at the 7q11–q21 locus (*HSPB1*). Genotype analysis of the 11q12–q14 locus (*BSCL2*) was performed with 13 STR markers: D11S1765, D11S4076, D11S480, D11S4205, D11S1883, D11S987, CA9, CA10 (Magre *et al.*, 2001), WP1, WP3, WP7, WP8 and WP9 (Windpassinger *et al.*, 2004). Paternity was tested using 15 highly informative STRs distributed throughout the genome (ATA38A05, D1S1646, D1S1653, D1S1360, D2S2256, D3S3037, D4S2382, D4S3240, D7S509, D8S1759, D9S1118, D12S1056, D12S2082, D16S2619 and GATA152H04). STRs were PCR-amplified and PCR fragments were resolved on an ABI 3730 automated DNA sequencer. Genotypes were analysed using the ABI Prism Genescan software (Applied Biosystems) and Trace Inspector, an in-house developed software program (http://www.vibgeneticservicefacility.be/).

Results
In 17 out of 112 index patients, we found sequence variations causing pathogenic missense mutations in four distal HMN genes: *BSCL2*, *HSPB8*, *HSPB1* and *SETX* (Table 1). These mutations were not detected in a panel of 400 control chromosomes. No mutations were found in

Table I Clinical features of distal HMN patients with proven mutation

Patient	Gene	AA change	Origin	Inheritance	AAO	SAO	ALE	Walking	Weakness dist LL	Weakness prox LL	Atrophy dist LL	Pes cavus	Reflexes ank/knee	Weakness dist UL	Atrophy dist UL	Reflexes UL	Additional Features	References
CMT-206:IV.I	BSCL2	Asn88Ser	Italy	AD penetrance↓	inf	Gait difficulties, foot deformities	42	Impaired, improvement after foot surgery	P (0)	A	P	P	A/++	P (0)	P	—	plantar responses neutral	(Irobi et al., 2004a)
CMT-644:IV.3	BSCL2	Asn88Ser	Serbia	AD	45	Weakness of right hand	55	Without any support	P (3,4)	A	P (feet)	P	A/++	P (3/4)	P (thenar, interosseus I)	++		Novel family
CMT-745:III.I	BSCL2	Asn88Ser	Poland	AD	12	Weakness of hands	15	Moderately impaired	P	A	A	A	A/++	P	P (thenar)	—		Novel family
CMT-I:III.I4	BSCL2	Ser90Leu	Belgium	AD	30	Gait difficulties, weakness of hands	42	Spastic gait	P (3)	–	P	P	+++/+++	P(3)	P	+++	plantar responses extension	(Irobi et al., 2004a)
CMT-539:III.2	BSCL2	Ser90Leu	The Netherlands	AD	11	Weakness of right hand	12	Spastic gait	P (4)	A	A	P	++/+++	A	A	++	muscle tone LL ↑	Novel family
CMT-716: I.2	BSCL2	Ser90Leu	India	AD	8	Walking difficulties, atrophy hand muscles	47	Spastic gait	P (0,4)	A	P	P	A/+++	P (0,4)	P (thenar, hypothenar, interosseus)	+++	spasticity LL, knee clonus	Novel family
CMT-705:II.I	BSCL2	Ser90Leu	Belgium	AD	13	Gait difficulties, weakness of hands	15	Steppage	P (4−)	P (5−)	A	P	A/+++	P (2/4−)	P (hands, R>L)	A	muscle tone LL ↑, plantar responses extension	Novel family
CMT-734:II.I	BSCL2	Ser90Leu	Switzerland	de novo	5	Gait difficulties, weakness of hands	35	Spastic gait, bilateral foot drop	P	A	P	P	+++/+++	P (I/3/4)	P (thenar, interosseus I)	+++	muscle tone LL ↑	Novel family
CMT-278:III.I	HSPB8	Lys141Glu	Bulgaria	AD	23	Distal weakness of legs	52	Impaired, walks with canes	P (0)	P (3)	P	P	A/++	P (4)	P (hands)	++		(Irobi et al., 2004b)
CMT-M:IV.I2	HSPB8	Lys141Asn	Belgium	AD	17	Gait difficulties	70	Steppage, bilateral foot drop, orthopaedic shoes, walks with cane	P	P	P	–	A/A	P	P	A		(Irobi et al., 2004b)
CMT-196:VI.9	HSPB8	Lys141Asn	Czech Republic	AD	15	Distal weakness of legs	40	Impaired, walks with canes	P (0)	P (3)	P	A	A/+	P (3)	P (hands)	++		(Irobi et al., 2004b)
PN-474:II.I	HSPBI	Arg127Trp	Belgium	AD	18	Cramps, fasciculations	42	Moderately impaired, no aids	P (3,4)	A	P	A	A/++	A	A	++		(Evgrafov et al., 2004)
CMT-751:III.I	HSPBI	Arg127Trp	France	AD	35	Gait difficulties	–	Steppage, bilateral foot drop	P	A	P	P	A/A	P	P	—		Novel family
CMT-263:IV.I	HSPBI	Thr15Ile	Croatia	AD	25	Fatigue, weakness of legs	51	Steppage, no aids	P (0)	P (2/3)	P	P	A/A	P (2/3)	P (hands)	++		(Evgrafov et al., 2004)
CMT-391:II.I	HSPBI	Pro182Leu	Austria	Parental mosaicism	5	Gait difficulties	16	–	P	A	P	P	A/+++	P	P	—		(Evgrafov et al., 2004)
CMT-106:III.4	SETX	Thr3Ile	Austria	AD	6	Gait difficulties	27	Impaired, orthopaedic shoes	P	A	P	P	+++/+++	P	P	+++	muscle tone LL+UL ↑, plantar responses extension	(Chen et al., 2004)
CMT-61:III.4	SETX	Arg2I36His	Belgium	AD	inf	Gait difficulties	52	Steppage, bilateral foot drop	P (0,I,4)	A	P	A	++/++	P (2)	P	+++		(Chen et al., 2004)

AA = amino acid; AD = autosomal dominant; AAO = age at onset (years); SAO = symptoms at onset; ALE = age at last exam (y); LL = lower limb; UL = upper limb; P = present; A = absent; deep tendon reflexes: + = hypoactive; ++ = normal; +++ = brisk; ↑ = increased; ↓ = decreased; − = unknown; reflexes ank/knee = ankle and knee deep tendon reflexes; dist = distal; prox = proximal; inf = infancy; (MRC) = Medical Research Council scale for muscle strength, numbers between brackets indicate range of muscle weakness; R = right; L = left.

DCTN1, *GARS* and *VAPB*. Clinical and electrophysiological data of the 17 index patients are summarized in Tables 1 and 2. The 17 index patients have an HMN phenotype with distal weakness and atrophy. In seven patients with a *BSCL2* mutation, the weakness was more pronounced in the hands than legs. Age at onset was variable and upper motor neuron signs were observed in eight patients. In most patients, normal or slightly reduced motor NCVs were found with reduced compound muscle action potential (CMAP) amplitudes. Sensory NCVs were in the normal range or at the lower limit and sensory nerve action potentials (SNAP) had normal amplitudes. Two patients; CMT-705:II.1 and CMT-M:IV.12 had a slightly decreased sensory median NCV of 39.7 and 40.0 m/s respectively, due to a concomitant Carpal Tunnel Syndrome. Recent re-evaluation showed a reduced Sural nerve NCV of 36.2 m/s in CMT-263:IV.1, suggesting a transition of distal HMN to HMSN-II, although the sensory NCVs in the upper limbs remained normal (Table 2). The absence of sensory response in the Sural nerve in patient CMT-206:IV.1 may suggest a shift in diagnosis to HMSN-II as well, which was recently described in the context of *BSCL2* mutations (Table 2) (Bienfait *et al.*, 2007). Although we do not have evidence for a superimposed acquired sensory-motor neuropathy, this observation, based on a single patient, should be interpreted with caution. Of interest is that several secondary patients in this family CMT-206 had a pure motor neuropathy phenotype (Irobi *et al.*, 2004*a*).

Mutations in *BSCL2*

The previously reported c.263A>G (Asn88Ser) and c.269C>T (Ser90Leu) mutations were found in probands of three and five distal HMN families, respectively (Table 1). The segregation of the Asn88Ser mutation in the family from Italy could be confirmed (Supplementary Fig. 1) (Irobi *et al.*, 2004*a*). In the family of Serbia, however, the segregation of the pathogenic Asn88Ser mutation could not be verified since the son CMT-644:IV.1 was clinically and electrophysiologically normal. This is possibly related to his relatively young age (27 years). In family CMT-745, no additional DNA samples of the relatives were available to confirm the segregation of the mutation. Comparison of the chromosome 11q haplotypes, defining the *BSCL2* locus of families with the Asn88Ser mutation, i.e. CMT-206:IV.1 and CMT-644:IV.3, and of families CMT-I:III.14, CMT-705:II.1 and CMT-539:III.2 with the Ser90Leu mutation suggested that these patients are unlikely to be related (Fig. 1A). The Ser90Leu mutation was absent in the healthy parents and sister of patient CMT-734:II.1 (Supplementary Fig. 1). The paternity of this patient was confirmed (data not shown), pointing that the *BSCL2*-Ser90Leu mutation occurred *de novo*.

Mutations in *HSPB8*

Two mutations targeting the same lysine residue in HSPB8 were identified in three index patients (Table 1). The same c.423G>C (Lys141Asn) mutation segregated in the unrelated Belgian and Czech families of patients CMT-M:IV.12 and CMT-196:VI.9, and the c.421A>G (Lys141Glu) mutation was found in the Bulgarian patient CMT-278:III.1 (Irobi *et al.*, 2004*b*). Segregation of this latter mutation was confirmed in the affected son of the index patient (Supplementary Fig. 1). The phenotype of family CMT-M has previously been described in detail (Timmerman *et al.*, 1992, 1999).

Mutations in *HSPB1*

In this cohort, three different missense mutations in *HSPB1* were found in four index patients (Table 1) (Evgrafov *et al.*, 2004). The unrelated index patients PN-474:II.1 and CMT-751:III.1 belong to a Belgian and French family and share the same missense mutation, c.379C>T (Arg127Trp), targeting the highly conserved α-crystallin domain of HSPB1 (Figs. 1B and 2). Segregation of the c.452C>T (Thr151Ile) mutation targeting the same α-crystallin domain in the Croatian family CMT-263 could be confirmed in the index patient's mother, an uncle and a cousin (Supplementary Fig. 1). In the previously reported patient CMT-391:II.1, belonging to an Austrian family, a missense mutation (c.545C>T; Pro182Leu) was found in the C-terminal IXI/V domain (Fig. 2). The younger affected sib (CMT-391:II.3) showed a very similar disease course. Remarkably, none of the asymptomatic parents of CMT-391:II.1 did show the heterozygous *HSPB1*-mutation. False paternity in this family was excluded (data not shown). Interestingly, we detected very low levels of the mutant allele in the electropherogram of one of the parents (CMT-391:I.2) (Fig. 3A). The same low amount of mutant allele was also observed using pyrosequencing, suggesting that a fraction of this parent's lymphocytes was heterozygous for the mutation (Fig. 3B). By genotyping the five family members with six STRs at the *HSPB1* locus, we could confirm the segregation of the mutant allele from the parent to the affected children (Fig. 3C). These results demonstrated that the parent CMT-391:I.2, who is clinically unaffected, is most likely mosaic for the Pro182Leu-mutation.

Mutations in *SETX*

In two index patients, we identified a heterozygous missense mutation in *SETX*: in the Austrian patient CMT-106:III.4 we detected an N-terminal mutation, c.8C>T (Thr3Ile), and in the Belgian patient CMT-61:III.4 a missense mutation, c.6407G>A (Arg2136His), was found in the DNA/RNA helicase domain of the protein (Table 1, Fig. 2). The families to which the patients belong are described in De Jonghe *et al.* (2002) and in Chen *et al.* (2004).

Discussion

Over the last 6 years, eight genes have been identified in which mutations are associated with distal HMN.

Table 2 Electrophysiological data of distal HMN patients with proven mutation

Patient	Mutation	Age	R/L	Median motor		Ulnar motor		Peroneal motor		Tibial motor		Median sensory		Ulnar sensory		Sural sensory	
				Amp	CV	Amp	CV	Amp	CV	Amp	CV	Amp	CV	Amp	CV	Amp	CV
Normal values →				4.0	49.0	4.0	49.0	3.0	41.0	3.0	41.0	7.0	46.0	2.0	46.0	1.0	44.0
CMT-206:IV.1	*BSCL2* Asn88Ser	42	R	–	–	**0.9**	59.0	–	**30.0**	–	–	–	–	–	–	**A**	**A**
CMT-644:IV.3	*BSCL2* Asn88Ser	55	R	**1.0**	57.0	12.0	64.5	4.0	**40.5**	–	–	30.0	59.0	24.0	66.5	10.0	54.0
CMT-745:III.1	*BSCL2* Asn88Ser	15	R	–	–	–	–	–	**30.3**	–	–	–	–	–	–	–	–
			L	–	–	–	–	–	**38.9**	–	–	N	N	N	N	N	N
CMT-1:III.14	*BSCL2* Ser90Leu	42	R	–	–	6.1	62.0	1.6	65.0	–	–	19.0	61.0	11.0	71.0	9.0	–
CMT-539:III.2	*BSCL2* Ser90Leu	12	R	**0.2**	–	**0.3**	–	–	–	6.7	42	20.8	54.0	20.6	50.0	–	–
			L	–	–	–	–	–	–	–	–	–	–	–	–	12.0	45.0
CMT-716:I.2	*BSCL2* Ser90Leu	46	R	**0.3**	**29.9**	1.3	53.3	1.4	46.7	–	–	26.6	60.0	–	–	–	–
			L	–	–	**0.3**	48.8	0.8	34.6	–	–	–	–	4.2	67.7	–	–
CMT-705:II.1	*BSCL2* Ser90Leu	15	R	**1.0**	**41.8**	–	–	1.5	38.1	4.3	40.5	28.0	**39.7**	–	–	–	–
CMT-734:II.1	*BSCL2* Ser90Leu	35	R	–	–	8.0	66.7	–	–	**2.0**	**33.3**	–	–	–	–	7.1	64.3
			L	–	–	**2.9**	52.6	–	–	–	–	7.0	53.0	12.9	61.5	6.5	54.9
CMT-278:III.1	*HSPB8* Lys141Glu	40	R	–	54.5	–	58.9	**A**	**A**	–	–	–	–	–	–	–	–
			L	–	–	31.6	56.0	**A**	**A**	–	–	40.0	57.5	34.0	57.5	–	–
CMT-M:IV.12	*HSPB8* Lys141Asn	NM	R	–	**33.0**	–	**43.0**	–	–	–	–	14.0	**40.0**	10.0	46.0	–	–
			L	–	**38.0**	–	**38.0**	–	–	–	–	–	**42.0**	–	–	–	–
CMT-196:VI.9	*HSPB8* Lys141Asn	NM	R	–	–	–	–	–	–	–	–	–	–	8.0	46.0	10.0	46.0
			L	**2.2**	52.0	**1.5**	61.0	**A**	**A**	**A**	**A**	16.0	52.0	–	–	8.0	46.0
PN-474:II.1	*HSPB1* Arg127Trp	44	R	–	–	–	–	–	–	**0.3**	**26.0**	–	–	–	–	–	–
			L	12.0	52.0	–	–	**0.7**	**33.0**	**A**	**A**	–	–	–	–	20.0	**43.0**
CMT-751:III.1	*HSPB1* Arg127Trp	NM	R	N	N	–	–	**A**	**A**	**A**	**A**	–	–	–	–	20.0	N
CMT-263:IV.1	*HSPB1* Thr15Ile	51	R	4.0	55.8	**2.0**	49.4	–	–	–	–	**6.2**	58.9	5.4	52.2	8.4	**39.4**
		49	L	5.0	57.8	–	–	**A**	**A**	**A**	**A**	8.0	60.6	–	–	6.5	**36.2**
CMT-391:II.1	*HSPB1* Pro182Leu	NM	NM	–	–	–	–	–	–	–	–	–	–	–	–	–	–
CMT-106:III.4	*SETX* Thr3Ile	NM	R	5.0	52.0	7.0	53.0	**0.3**	**32.0**	11.0	**31.0**	7.9	**35.0**	–	–	12.3	**34.5**
			L	10.0	49.0	8.5	50.0	**0.2**	**31.0**	10.5	**33.0**	–	–	–	–	–	–
CMT-61:III.4	*SETX* Arg2136His	52	R	**0.7**	**27.0**	**0.6**	**30.0**	**0.7**	**33.0**	–	–	11.0	49.0	–	–	11.0	48.0
			L	**0.7**	**35.0**	**1.2**	**26.0**	–	–	–	–	12.0	48.0	11.0	47.0	10.0	46.0

A = absent response; Age = age at examination; Amp = amplitude (motor: in mV; sensory: in μV); CV = conduction velocity (in m/s); − = not measured; NM = not mentioned; N = normal; R = right; L = left; Bold numbers = abnormal values.

In this study, we investigated the relative contribution of mutations in the six genes associated with AD distal HMN: *HSPB1*, *HSPB8*, *BSCL2*, *SETX*, *GARS*, *DCTN1* as well as mutations in *VAPB* in a cohort of 112 index distal HMN patients. In 17 index patients, we found disease-causing mutations in four genes, i.e. three patients had an *HSPB8* mutation, four patients showed an *HSPB1* mutation, *BSCL2* mutations were found in eight probands and two families had a *SETX* mutation. The 17 mutations represent a mutation detection rate of 15% in the total cohort. The fact that no mutations were identified in 40 patients with a family history of distal HMN suggesting a dominant or autosomal dominant inheritance pattern indicates that additional genes must be involved in the pathogenesis of the disease. However, when we only focus on patients with a dominant inheritance the mutation yield increases to 30%. Moreover, if we

exclusively consider patients with pyramidal tract signs the mutation rate rises to 38% (24% BSCL2, 9% SETX, 5% HSP27). This finding underscores the involvement of central motor tracts as a prominent aspect in several forms of distal HMN. Pyramidal tract signs were also observed in several other families with mutations in *BSCL2* (Silver Syndrome) (Auer-Grumbach *et al.*, 2005).

Interestingly, the *BSCL2* gene was found to be mutated in 7% of our distal HMN patient cohort suggesting that *BSCL2* mutations are the most common causes of distal HMN. The major contribution of *BSCL2* mutations to the aetiology of distal HMN was recently demonstrated in a cohort of 33 patients diagnosed with distal HMN, HMSN-II or Silver syndrome (Rohkamm *et al.*, 2007) in which 12% of patients had a mutation. The slightly higher diagnostic yield of *BSCL2* mutations found in that study

A

		BSCL2: Ser90Leu			BSCL2: Asn88Ser	
Origin		Belgium	Belgium	The Netherlands	Italy	Serbia
Marker	position (bp)	CMT-I:III.14	CMT-705:II.1	CMT-539:III.2	CMT-206:IV.1	CMT-644:IV.3
D11S1765	60535120	240	234	244	234	234
D11S4076	61119711	155	157	157	155	155
WP9	61483069	163	163	161	153	161
WP8	61546083	249	245	245	247	247
WP7	61712701	235	237	237	237	241
WP3	62177793	167	167	165	169	169
BSCL2 ▶ CA9	62205260	204	204	204	204	204
CA10	62220293	150	162	164	160	156
WP1	62611191	245	245	245	245	233
D11S480	62798219	189	189	197	195	197
D11S4205	62939549	193	193	193	193	193
D11S1883	63130309	272	272	288	274	268
D11S987	67649916	103	97	105	107	105

B

		HSPB1: Arg127Trp	
Origin		France	Belgium
Marker	position (bp)	CMT-751:III.1	PN-474:II.1
D7S2435	68703302	112	114
HSPB1 ▶ D7S2518	75215811	138	130
D7S2470	75876516	228	234
D7S2421	77511915	93	94
D7S669	77710450	184	184
D7S2204	77964859	232	212

Fig. 1 Haplotype/genotype analysis of chromosome 11q markers in patients with a *BSCL2*-ser90Leu and *BSCL2*-Asn88Ser mutation (**A**) and 7q markers with a *HSPB1*-Arg127Trp mutation (**B**). The disease segregating haplotypes are shown. The haplotype phase of patients CMT-716:I.2 and CMT-745:III.1 could not be determined. The alleles of the STRs are sized in base pairs (bp). The position of the markers is according to the reference assembly of NCBI.
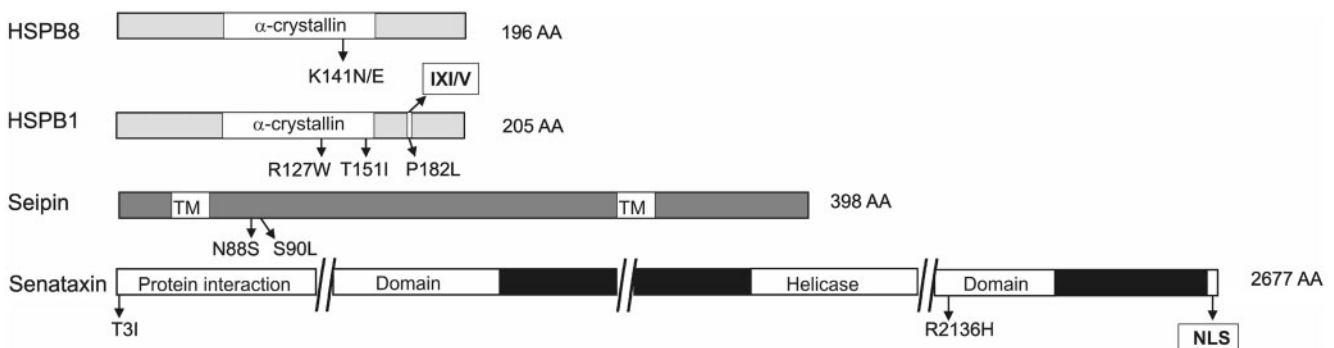
Fig. 2 Schematic presentation of protein structures of HSPB8, HSPB1, seipin and senataxin. The known and putative protein domains are shown. Mutations found in this study are indicated with an arrow. TM = transmembrane; AA = amino acids; NLS = nuclear localization signal.

may be explained by the selective inclusion of distal HMN patients with prominent hand muscle weakness. Intriguingly, only two different *BSCL2* mutations have been reported so far. It is clear from our and previous studies that both mutations represent mutational hot-spots since they can arise independently in families and can occur *de novo*. A broad spectrum of phenotypes is associated with mutations in *BSCL2* (Irobi *et al.*, 2004a; Auer-Grumbach *et al.*, 2005). The detailed clinical and electrophysiological observations within a single large Austrian family with the Asn88Ser mutation enabled Auer-Grumbach *et al.* (2005) to

delineate six endophenotypes ranging from asymptomatic individuals to Silver syndrome and spastic paraplegia. A similar broad variability was seen in the clinical presentation of our patients with *BSCL2* mutations. The disease onset varied from infancy in patients CMT-206:IV.1, CMT-716:I.2 (8 years) and CMT-734:II.1 (5 years) to late-onset in patient CMT-644:IV.3 (45 years), and the common presenting symptoms were atrophy and weakness of hand muscles and/or gait difficulties. The clinical presentation of the index patients with an Asn88Ser mutation consists of prominent distal muscle atrophy in the upper
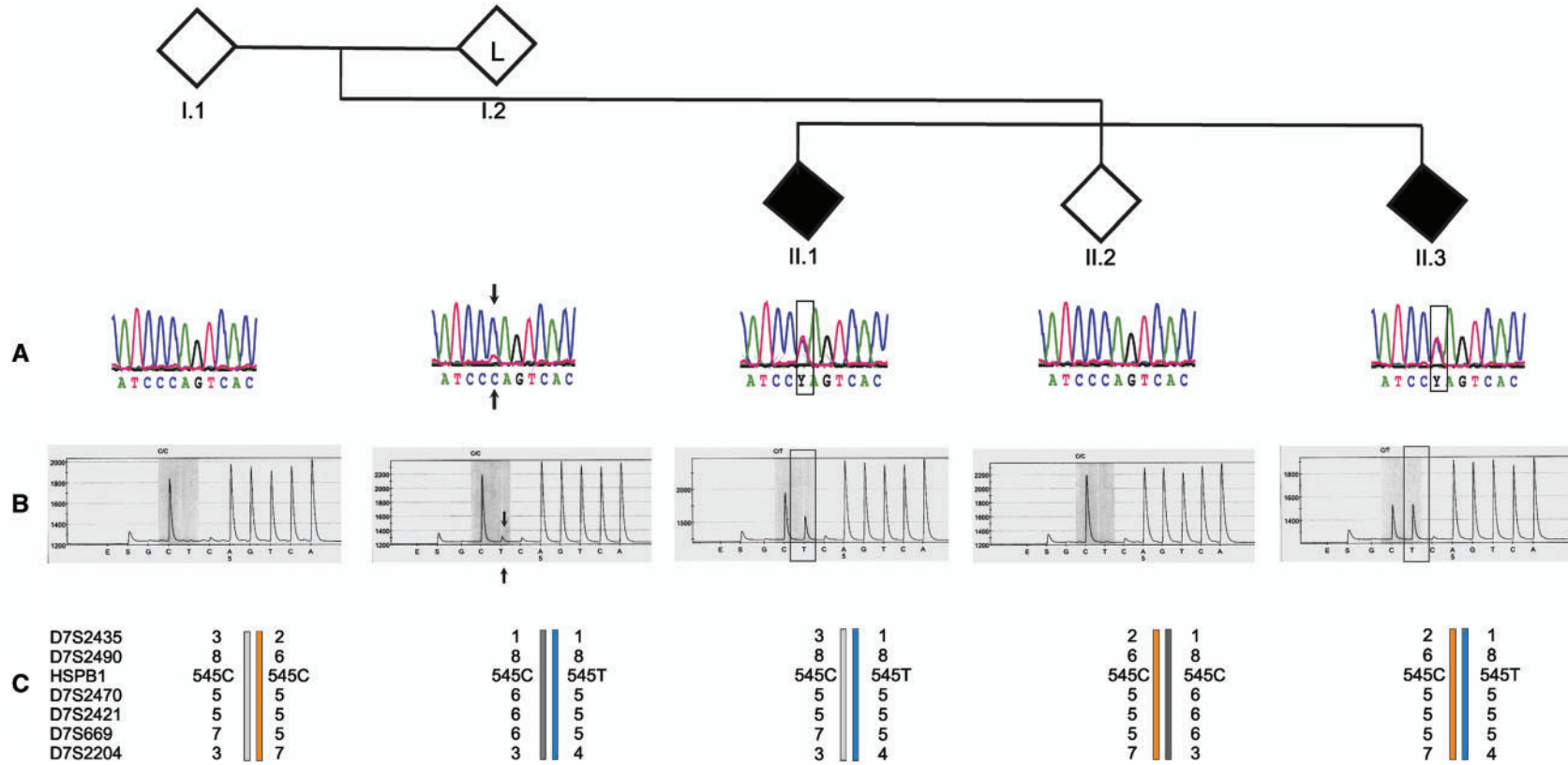
Fig. 3 Parental mosaicism for *HSPB1*-Pro182Leu in family CMT-39I. Electropherograms (**A**) and pyrosequencing profiles (**B**) show very low levels of mutant allele (arrows) in the parent (I.2). The mutant allele is boxed. (**C**) Segregation analysis of chromosome 7 STRs at the *HSPB1* locus in family CMT-39I. Symbols: diamond = gender status is masked for confidentiality reasons, black filled symbol = affected, empty symbol = unaffected, symbol filled with 'L' = parental mosaicism.

limbs and to a variable degree in the lower limbs without clear pyramidal tract signs. This is compatible with the distal HMN type V phenotype. The phenotype of four of the five index patients with a Ser90Leu mutation was dominated by prominent amyotrophy of the intrinsic hand muscles in association with marked spasticity in the lower limbs and the presence of additional pyramidal tract signs. This corresponds to the Silver syndrome phenotype. Based on these interfamilial phenotypic features, it seems that the *BSCL2*-Ser90Leu mutation is correlated with a pronounced spasticity in the lower limbs and additional upper motor neuron signs. This marked phenotypic variation between the two BSCL2 mutations has also been described in earlier reports (Silver 1966*a*, *b*; Auer-Grumbach *et al.*, 2005; van de Warrenburg *et al.*, 2006; Bienfait *et al.*, 2007). Why distinct mutations in *BSCL2* tend to result in different clinical outcomes remains unclear, moreover since both mutations target the same *N*-glycosylation motif of the seipin protein (Windpassinger *et al.*, 2004).

Pyramidal tract signs and an early disease onset were also variably present in the two index patients (CMT-61:III.1 and CMT-106:III.1) with a *SETX* mutation, which underscores the involvement of the central nervous system in this distal HMN subtype. Currently, only three heterozygous mutations in *SETX* have been reported so far to be associated with motor neuron disease (Chen *et al.*, 2004).

A similar phenotype was found in the patient with the C-terminal *HSPB1*-Pro182Leu mutation. The phenotype associated with this mutation consisted of foot deformity and gait abnormalities in early infancy (5 years) and the presence of brisk knee tendon reflexes. With disease progression the patient CMT-391:II.1 showed marked distal muscle weakness in upper and lower limbs. A mutation targeting the same C-terminal amino acid, Pro182Ser, was previously reported in a Japanese patient with distal HMN (Kijima *et al.*, 2005). Similar to our patient, the disease in this patient started in early childhood; however, no upper motor neuron signs were reported. Mutations in the C-terminal part of *HSPB1* seem to cause a more severe early-onset neuropathy than mutations in the conserved *α*-crystallin domain of the *HSPB1* protein (Arg127Trp and Thr151Ile) found in three unrelated index patients with a classic adult-onset distal motor neuropathy. The phenotype in these three patients clearly resembles distal HMN type II.

The same homogeneous juvenile to adult-onset distal HMN type II phenotype was found in the three patients with an *HSPB8*-mutation targeting an identical lysine residue. The *HSPB8*-Lys141Asn and the *HSPB1*-Arg127Trp mutations were also reported in Chinese families presenting mild sensory symptoms that were diagnosed with HMSN-II (Tang *et al.*, 2005*b*). It is likely that the disease in these HMSN-II families represents a phenotypic variant of the clinical continuum associated with *α*-crystallin mutations and that a diagnosis of HMSN-II rather than distal HMN

was prompted by the presence of more pronounced sensory symptoms. Except for an impaired vibration sense in some elderly patients, no sensory losses were noted in the studied patients with an *HSPB8* mutation. However, the presence of mild sensory deficits during a more recent re-evaluation in patient CMT-263:IV.1 (with the Thr151Ile mutation in *HSPB1*) illustrates the clinical overlap between distal HMN and HMSN-II (Table 2).

Another peculiar finding in this study was the evidence of a parental mosaicism transmitting the *HSPB1*-Pro182Leu mutation. A possible mosaicism was suspected since the affected proband's sib carried the same mutation, while the unaffected parents were negative for the mutation on standard sequencing. The absence of the disease in the mosaic parent is likely the result of the very low amount of mutant protein in the peripheral nervous system. The occurrence of gonadal mosaicism was also considered, but not examined, for a 'de novo' *BSCL2* mutation in two sibs of a Korean family with Silver syndrome and distal HMN (Cho *et al.*, 2007). These findings prove for the first time the occurrence of genetic mosaicism in distal HMN. The possibility of mosaicism even in sporadic distal HMN patients has important implications for genetic counselling.

We did not detect disease-causing mutations in *GARS*, which was somewhat unexpected since recently within a cohort of 100 patients presenting inherited or sporadic lower motor neuron degeneration, three different mutations were found in one distal HMN type V family and two families presenting distal HMN with lower limb predominance (James *et al.*, 2006).

No mutations in *DCTN1* were detected. Until now, only one mutation in *DCTN1* is described in a family with distal spinal and bulbar muscular atrophy (Puls *et al.*, 2003, 2005). No patients with a similar phenotype were included in the cohort, probably explaining the absence of *DCTN1* mutations and indicating that mutations in *DCTN1* are associated with a distinct and unique form of motor neuron disease.

In this study, we also included the analysis of *VAPB* to investigate whether the phenotype associated with the Pro56Ser mutation in *VAPB*, found in several Brazilian families with diverse forms of motor neuron disorders, could be expanded to distal HMN. We did not detect the Pro56Ser or other *VAPB* mutations in the cohort which mainly consisted of European patients, suggesting that mutations in this gene are probably not associated with distal HMN.

In summary, we examined the distribution of mutations in genes associated with AD distal HMN in a large group of familial and sporadic patients. Our data highlight the genetic and clinical heterogeneity of the disease. The mutation rate in the studied genes was relatively low in the total cohort, suggesting that other genes must be involved in the pathomechanism of motor neuropathies. We suggest that genetic testing of patients showing prominent distal muscle atrophy in the upper limbs with

a variable degree of upper motor neuron signs should start with *BSCL2*, since the yield of mutations in *BSCL2* within our distal HMN cohort is 7%. Overall, the clinical phenotype of patients with a known gene defect fit in the proposed distal HMN classification by Irobi (2004), which is based on the initial clinical distal HMN classification system of Harding (1993) (Irobi *et al.*, 2006). This study underscores the involvement of central motor tracts in distal HMN associated with mutations in *BSCL2*, *SETX* as well as *HSPB1*. In addition, we provide evidence for a genetic mosaicism in transmitting an *HSPB1* mutation. In genetic counselling, it might be important to consider parental mosaicism even in sporadic distal HMN patients. Additional descriptions of distal HMN families and patients with a known genetic defect are needed to further organize and refine the existing classification and to get more insight into the molecular basis of these disorders.

Supplementary material

Supplementary material is available at *Brain* online.

Acknowledgements

References

Antonellis A, Ellsworth RE, Sambuughin N, Puls I, Abel A, Lee-Lin SQ, et al. Glycyl tRNA synthetase mutations in Charcot-Marie-Tooth disease type 2D and distal spinal muscular atrophy type V. Am J Hum Genet 2003; 72: 1293–9.

Auer-Grumbach M, Schlotter-Weigel B, Lochmuller H, Strobl-Wildemann G, Auer-Grumbach P, Fischer R, et al. Phenotypes of the N88S Berardinelli-Seip congenital lipodystrophy 2 mutation. Ann Neurol 2005; 57: 415–24.

Bienfait HM, Baas F, Koelman JH, de Haan RJ, van Engelen BG, Gabreels-Festen AA, et al. Phenotype of Charcot-Marie-Tooth disease type 2. Neurology 2007; 68: 1658–67.

Chen Y-Z, Bennett CL, Huynh HM, Blair IP, Puls I, Irobi J, et al. DNA/RNA helicase gene mutations in a form of juvenile amyotrophic lateral sclerosis (ALS4). Am J Hum Genet 2004; 74: 1128–35.

Cho HJ, Sung DH, Ki CS. Identification of de novo BSCL2 Ser90Leu mutation in a Korean family with silver syndrome and distal hereditary motor neuropathy. Muscle Nerve 2007; 36: 384–6.

De Jonghe P, Auer-Grumbach M, Irobi J, Wagner K, Kennerson ML, Plecko B, et al. Autosomal dominant juvenile ALS and distal hereditary motor neuronopathy with pyramidal tract signs: synonyms for the same disorder? Brain 2002; 125: 1320–5.

De Jonghe P, Timmerman V, Van Broeckhoven C, workshop participants. 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. Neuromuscl Disord 1998; 8: 426–31.

Dyck PJ, Lambert EH. Lower motor and primary sensory neuron diseases with peroneal muscular atrophy. I. Neurologic, genetic and electrophysiologic findings in hereditary polyneuropathies. Arch Neurol 1968a; 18: 603–18.

Dyck PJ, Lambert EH. Lower motor and primary sensory neuron diseases with peroneal muscular atrophy. II. Neurologic, genetic, and electrophysiologic findings in various neuronal degenerations. Arch Neurol 1968b; 18: 619–25.

Evgrafov OV, Mersiyanova IV, Irobi J, Van den Bosch L, Dierick I, Schagina O, et al. Mutant small heat-shock protein 27 causes axonal Charcot-Marie-Tooth disease and distal hereditary motor neuropathy. Nat Genet 2004; 36: 602–6.

Grohmann K, Schuelke M, Diers A, Hoffmann K, Lucke B, Adams C, et al. Mutations in the gene encoding immunoglobulin μ-binding protein 2 cause spinal muscular atrophy with respiratory distress type 1. Nat Genet 2001; 29: 75–77.

Harding AE. Inherited neuronal atrophy and degeneration predominantly of lower motor neurons. In: Dyck PJ, Thomas PK, Griffin JW, Low PA, Poduslo JF, editors. Peripheral neuropathy. Philadelphia: W.B. Saunders Company; 1993. p. 1051–64.

Irobi J, Van den Bergh P, Merlini L, Verellen C, Van Maldergem L, Dierick I, et al. The phenotype of motor neuropathies associated with BSCL2 mutations is broader than Silver syndrome and distal HMN type V. Brain 2004a; 127: 2124–30.

Irobi J, Van Impe K, Seeman P, Jordanova A, Dierick I, Verpoorten N, et al. Hot-spot residue in small heat-shock protein 22 causes distal motor neuropathy. Nat Genet 2004b; 36: 597–601.

Irobi J, Dierick I, Jordanova A, Claeys K, De Jonghe P, Timmerman V. Unravelling the genetics of distal hereditary motor neuronopathies. Neuromolecular Med 2006; 8: 131–46.

James PA, Cader MZ, Muntoni F, Childs AM, Crow YJ, Talbot K. Severe childhood SMA and axonal CMT due to anticodon binding domain mutations in the GARS gene. Neurology 2006; 67: 1710–2.

Kijima K, Numakura C, Goto T, Takahashi T, Otagiri T, Umetsu K, et al. Small heat shock protein 27 mutation in a Japanese patient with distal hereditary motor neuropathy. J Hum Genet 2005; 50: 473–6.

Magre J, Delepine M, Khallouf E, Gedde-Dahl T, Jr, Van Maldergem L, Sobel E, et al. Identification of the gene altered in Berardinelli-Seip congenital lipodystrophy on chromosome 11q13. Nat Genet 2001; 28: 365–70.

Maystadt I, Rezsohazy R, Barkats M, Duque S, Vannuffel P, Remacle S, et al. The nuclear factor kappa B-activator gene PLEKHG5 is mutated in a form of autosomal recessive lower motor neuron disease with childhood onset. Am J Hum Genet 2007; 81: 67–76.

Nishimura AL, Mitne-Neto M, Silva HC, Richieri-Costa A, Middleton S, Cascio D, et al. A mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. Am J Hum Genet 2004; 75: 822–31.

Puls I, Jonnakuty C, LaMonte BH, Holzbaur EL, Tokito M, Mann E, et al. Mutant dynactin in motor neuron disease. Nat Genet 2003; 33: 455–6.

Puls I, Oh SJ, Sumner CJ, Wallace KE, Floeter MK, Mann EA, et al. Distal spinal and bulbar muscular atrophy caused by dynactin mutation. Ann Neurol 2005; 57: 687–94.

Rohkamm B, Reilly MM, Lochmuller H, Schlotter-Weigel B, Barisic N, Schols L, et al. Further evidence for genetic heterogeneity of distal HMN type V, CMT2 with predominant hand involvement and Silver syndrome. J Neurol Sci 2007; 263: 100–6.

Rozen S, Skaletsky H. Primer3 on the WWW for general users and for biologist programmers. Methods Mol Biol 2000; 132: 365–86.

Silver JR. Familial spastic paraplegia with amyotrophy of the hands. Ann Hum Genet 1966a; 30: 69–75.

Silver JR. Familial spastic paraplegia with amyotrophy of the hands. J Neurol Neurosurg Psychiatry 1966b; 29: 135–44.

Sivakumar K, Kyriakides T, Puls I, Nicholson GA, Funalot B, Antonellis A, et al. Phenotypic spectrum of disorders associated with glycyl-tRNA synthetase mutations. Brain 2005; 128: 2304–14.

Tang B, Liu X, Zhao G, Luo W, Xia K, Pan Q, et al. Mutation analysis of the small heat shock protein 27 gene in chinese patients with Charcot-Marie-Tooth disease. Arch Neurol 2005a; 62: 1201–7.

Tang BS, Zhao GH, Luo W, Xia K, Cai F, Pan Q, et al. Small heat-shock protein 22 mutated in autosomal dominant Charcot-Marie-Tooth disease type 2L. Hum Genet 2005b; 116: 222–4.

Timmerman V, Raeymaekers P, Nelis E, De Jonghe P, Muylle L, Ceuterick C, et al. Linkage analysis of distal hereditary motor neuropathy type II (distal HMN II) in a single pedigree. J Neurol Sci 1992; 109: 41–8.

Timmerman V, Beuten J, Irobi J, De Jonghe P, Martin J-J, Van Broeckhoven C. Distal hereditary motor neuropathy type II (distal HMN type II): phenotype and molecular genetics. Ann NY Acad Sci 1999; 883: 60–64.

van de Warrenburg BP, Scheffer H, van Eijk JJ, Versteeg MH, Kremer H, Zwarts MJ, et al. BSCL2 mutations in two Dutch families with overlapping Silver syndrome-distal hereditary motor neuropathy. Neuromuscul Disord 2006; 16: 122–5.

Van Den Bosch L, Timmerman V. Genetics of motor neuron disease. Curr Neurol Neurosci Rep 2006; 6: 423–31.

Weckx S, De Rijk P, Van Broeckhoven C, Del Favero J. SNPbox: web-based high-throughput primer design from gene to genome. Nucleic Acids Res 2004; 32: W170–2.

Weckx S, Del Favero J, Rademakers R, Claes L, Cruts M, De Jonghe P, et al. novoSNP, a novel computational tool for sequence variation discovery. Genome Res 2005; 15: 436–42.

Windpassinger C, Auer-Grumbach M, Irobi J, Patel H, Petek E, Hörl G, et al. Heterozygous missense mutations in the BSCL2 are associated with distal hereditary motor neuropathy and Silver syndrome. Nat Genet 2004; 36: 271–6.