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Phenotypic correlations in a large single center cohort of patients with BSCL2 nerve disorders: a clinical, neurophysiological and muscle MRI study

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ABSTRACT

Background

BSCL2 heterozygote mutations are a common cause of distal hereditary motor neuropathies (dHMN). We present a series of BSCL2 patients and correlate clinical, neurophysiological and muscle-MRI findings.

Methods

26 patients from 5 families carrying the p.N88S mutation were ascertained. Age of onset, clinical phenotype (dHMN, Charcot-Marie-Tooth/CMT, spastic paraplegia), physical examination, disability measured as modified Rankin score (mRS) and neurophysiological findings were collected. A whole body muscle-MRI had been performed in 18 patients. We analyzed the pattern of muscle involvement on T1-weighted and STIR sequences. Hierarchical analysis using heatmaps and a MRI Composite Score (MRI CS) were generated. Statistical analysis was carried out with STATA SE v.15.

Results

Mean age was 51.54+/-19.94 years and 14 patients were males. dHMN was the most common phenotype (50%) and 5 patients (19.23%) showed no findings on examination. Disease onset was commonly in childhood and disability was low (mRS=1.34+/-1.13) although median time since onset of disease was 32 years (range=10-47). CMT-like patients were more disabled and disability correlated with age. On muscle-MRI, *thenar eminence*, *soleus* and *tibialis anterior* were most frequently involved, irrespective of clinical phenotype. MRI CS was strongly correlated with disability.

Conclusion

Patients with the p.N88S BSCL2 gene mutation are phenotypically variable, although dHMN is most frequent and generally slowly progressive. Muscle-MRI pattern is consistent regardless of phenotype and correlates with disease severity, probably serving as a reliable outcome measure for future clinical trials.

INTRODUCTION

Distal hereditary motor neuropathies (dHMN) are a genetically heterogeneous group of neurodegenerative second motoneuron disorders. Heterozygous mutations in the Berardinelli-Seip Congenital Lypodistrophy-2 (BSCL2) gene are a common cause of dHMN if intrinsic hand muscle involvement is present (dHMN-V).[1] In 1966 Silver described the classic association of distal motor neuropathy of upper and lower limbs combined with spasticity, known as Silver Syndrome.[2] However, BSCL2 mutations cause a wide spectrum of nerve disorders, such as a pure dHMN with or without hand involvement (dHMN-V and II respectively), an axonal Charcot Marie Tooth (CMT-2) associating sensory disturbances or a pure spastic paraplegia (SPG-17).[3–5] Few families have been described[2,3,5–7] and show a broad phenotypic variability, complicating its diagnosis.

The p.N88S and p.S90L mutations account for the majority of BSCL2 patients,[3,6,8] while the p.S90W and p.R96H mutations have been exceptionally documented.[9–11] The p.N88S missense mutation is located in the exon 3 of the BSCL2 gene on chromosome 11q13. BSCL2 encodes the integral membrane protein named Seipin, with different putative functions ranging from phospholipid metabolism to fat droplet formation.[12,13] The p.N88S mutation leads to an abnormal N-glycosilation site and unfolded protein aggregation leading to endoplasmic reticulum stress and possible secondary motoneuron degeneration.[8,12]

Muscle-MRI is a powerful tool in the assessment of muscular disorders,[14–17] but studies in motorneuron disorders are scarce. In the past years, muscle atrophy on MRI has been shown to correlate with muscle testing in CMT and dHMN and to document the severity and pattern of muscle atrophy.[18] Quantitative muscle-MRI is a biomarker that can detect early changes and assess therapeutic responses in hereditary neuropathies.[19–21]

The aim of this study is to describe the full phenotypic spectrum of BSCL2 disease in a series of patients carrying the common p.N88S, with special detail to clinical and neurophysiological findings. Furthermore, we present the muscle-MRI findings of these patients in order to provide a recognizable radiological pattern and correlates with clinical data.

METHODS

Bslc2 population

A total of 26 patients from 5 families carrying the p.N88S heterozygote mutations were ascertained Clinical data were obtained cross-sectionally from electronic clinical records. The following variables were registered: gender, date of birth, age of onset (childhood: 0-14 years; youth: 15-24 years, adult onset: ≥25 years), age at diagnosis, mutation, phenotype (SPG, dHMN, CMT, asymptomatic) and limb onset. Distal muscle weakness at last visit was scored using the MRC scale for leg dorsiflexion and hand interosseous.[22] Other features like osteotendinous reflexes, Babinski sign, spasticity, sensory signs, bladder, osseous, hearing and respiratory problems were documented. Disability at last visit was measured with the modified Rankin score (mRS).[23]

Molecular analysis

- *Whole Exome Sequencing*

Both individuals from Family 2 were diagnosed in the context of a Whole-Exome Sequencing (WES) of undiagnosed cases with motor distal weakness negative for molecular screening of common causes of dominant CMT. Index singleton cases was carried out on exon targets isolated by capture using the SeqCap Exome V3.0 64 Mb kit (Nimblegen) with 100-bp paired-end read sequences, generated on a HiSeq2000 Platform (Illumina, Inc. USA) at Centre Nacional d'Anàlisi Genòmica (CNAG Barcelona, Spain). The sequencing methodology and variant analysis protocol followed the Genome Analysis Tool Kit (GATK) pipeline.[24]

- *Sanger sequencing and putative mutations assignment*

All other 4 families were diagnosed by Sanger Sequencing of exon 3 of the BSCL2 gene. For the identification of mutation carriers, the corresponding DNAs of the pools were individually amplified and sequenced with BigDye chemistry in an ABI3130 equipment (Life Technologies) to identify the mutation carrier. All the DNA sequence variants were named following the guidelines of the American College of Medical Genetics.[25]

Ancillary tests

Nerve conduction studies (NCS) from 19 patients was ascertained. Compound motor action potentials (CMAPs) and sensory nerve action potentials (SNAPs) as well as conduction velocity (CV, m/s) from the median, cubital, peroneal, sural and tibial nerves were registered. The presence of dispersed potentials was noted as well as a neurogenic trace on electromyogram. Cervical-MRI was described when available.

Whole body muscle-MRI

From June 2018, 18 patients underwent a whole body muscle-MRI (1.5T, Siemens) with axial-T1w and STIR sequences. The scan lasted 45 minutes, covering hand and feet. Fat replacement was evaluated using the Mercuri modified by Fisher score[26] and assessed by a clinically blind neurologist (RFT) and a radiologist (AG), both experienced in muscle-MRI analysis. A mean value was given bilaterally for each muscle as well as a description of muscle atrophy on axial-T1w sequences. A heatmap was generated on R software for hierarchical analysis of muscle involvement.

Data analysis

Data analysis was carried out using STATA SE v.15. Mean and standard deviation of symmetric quantitative variables, median and interquartile range of asymmetric quantitative variables, and absolute or relative frequencies of categorical variables were assessed. Statistical significance was considered at $p \leq 0.05$ and parametric test or non-parametric tests were applied when necessary (Supl material 1).

This study was approved by the Ethics Committee of the Donostia University Hospital (FER-BSC-2019-01).

RESULTS

Demographic and phenotypic presentation

All individuals were molecularly confirmed carriers of the p.N88S mutation (exon 3) and came from a small region of the coast of the Basque Country. There was a slight male predominance (53.85%). Mean age at onset of disease was 24.44+/-21.96 years, however age at diagnosis was 51.46+/19.89 years and therefore median time since onset was 32 years (range 10-47 years) (Table 1).

A pure dHMN was the clinically defined phenotype in 13 patients (50%), and although onset was frequently referred to the lower limbs (66.7%), at presentation it normaly involved lower and upper limbs distally (dHMN-V) (9 patients, 34.61%). While *pes cavus* and pyramidal features were frequent (46.15% and 50% respectively), sensory symptoms were rare (3 patients, 11.54%). 5 individuals (19.23%) remain to the last visit completely asymptomatic. Proximal weakness was anecdotal (patient 2.II.1) and only two patients had respiratory insufficiency; patient 5.I.1 (70yo) with a restrictive respiratory insufficiency and patient 1.II.1 (75yo) with a mixed respiratory insufficiency and elevated left hemidiaphragm. Both patients were males, showed a dHMN-V phenotype and had significant disability (mRS=4 and 3 respectively). The typical p.N88S BSCL2 clinical phenotypes observed have been highlighted in Table 2.

Mean disability was also low (mRS=1.34+/-1.13). Two patients walk with aid (mRS=3), and one patient required help with activities of daily living (mRS=4). No patient is to this day wheelchair-bound. the disease was slowly progressive in all 26 patients and there was a non-significant tendency for female patients to be less disabled than men

(mRS=0.92+/-0.99 vs 1.71+/-1.14; p=0.0716). No association between gender and phenotype, age or time since onset was found. Disability was higher in CMT2-like and SPG17 than dHMN-V (mRS=2.33+/-0.58, 2+/-0, 1.15+/-1.14 respectively; p=0.0000). There was a moderate correlation between mRS and age (r=0.6711, p=0.0002), while this was not clear with time since onset (r=0.2533, p=0.3106) (Figure 1).

Ancillary tests

NCS were pathological in 17 out of 19 individuals (89.47%) showing a predominantly axonal motor neuronopathy. Two patients had no electrical signs of polyneuropathy (one clinically asymptomatic patient and one with pure paraparesis) (Table S1). Dispersed CMAPs were found in the lower limbs in 7 patients (36.84%). These dispersed potentials were both in tibial and peroneal nerves and were described in one patient (1.III.16) as proximally located. However, slowing of CV was very rare and mild.

8 patients had undergone a cervical-MRI. Patient 05.II.01 (SPG phenotype) showed a protruding C4-C5 and C5-C6 discal hernia and secondary signs of myelopathy, resolving in a subsequent MRI after undergoing cervical surgery. All other patients showed no evidence of myelopathy or anterior horn degeneration.

Muscle-MRI

18 patients underwent a whole body muscle-MRI and their clinical characteristics were similar to the whole series. Mean age at scan was 50 years and 11 patients were male (61.11%). Mean duration of the disease was 26.43 years and mean disability was mRS=1.28. 10 patients showed a dHMN phenotype (55.56%), 4 SPG17, 2 CMT2, and the remaining 2 were completely asymptomatic.

15 out of 18 individuals (83.33%) had an abnormal muscle-MRI. The pattern of involvement was generally symmetric, with a proximal-to-distal gradient fat replacement within each muscle (Figure 2). Hyperintensities on STIR were seen in 6 patients (33.33%).

The most frequently affected muscles on axial-T1w were the *soleus, tibialis anterior* and *thenar eminence* (61.11%, 55.56%, and 50% respectively). Other commonly affected muscles were the *extensor digitorum longus, medial gastrocnemius, peroneus and flexor digitorum longus* (44.44%, 38.89%, 33.33% and 33.33% respectively). Axial or proximal limb muscle abnormalities were very uncommon, and this chronology and general pattern of muscle involvement was irrespective of the clinical phenotype (dHMN, SPG17 or CMT2) on hierarchical analysis (Figure 3). Even 1 asymptomatic patient (1.III.07) showed mild bilateral *soleus* fat replacement with *medial gastrocnemius* hyperintensities on STIR (Figure 2).

3 patients (16.67%), all females, had no muscle abnormalities on whole body muscle-MRI. Patient 1.II.04 (67yo) with a pure paraparesis (SPG17), patient 1.III.03 (22yo) asymptomatic with normal clinical examination, and patient

3.II.02 (41yo) asymptomatic with mild upper and lower limb amyotrophy and *tibialis anterior* weakness on examination (dHMN). The clinical vs muscle-MRI correlations can be seen in (Figure 1).

The MRI CS was positively correlated with disability measured by mRS (r=0.7119, p=0.0009). Males had a higher MRI CS than females (13.92+/-13.77 vs 2.67+/-4.27 respectively; p=0.0209). As for the phenotype, CMT were more severely affected than SPG or dHMN patients (24.33+/-15.27 vs 8.33+/-11.93 vs 8.4+/-11.06 respectively), although only reached statistical significance when CMT were confronted against dHMN+SPG (similarly affected) and asymptomatic patients (Supl material 1). MRI CS showed a moderate correlation with age (r=0.6680) and mild with time since onset (r=0.3435). Results did not vary when removing patient 03.I.01 (hands not visualized on MRI study).

DISCUSSION

We have described the clinical, neurophysiological and muscle-MRI findings of 26 patients carrying the p.N88S mutation in the BSCL2 gene. Although coming from 5 unrelated families, all were native of a small region and probably share a similar ancestry and genetical background. However, they present a wide constellation of clinical phenotypes (dHMN, SPG17, CMT2, asymptomatic) and range of age (20-99 years).

Phenotypic variability and low grade of disability in BSCL2 disease

The most common presentation was as a pure motor neuropathy. Upper limb involvement, a clinical hallmark of the disease, was not invariably present, and only 20% of patients presented with the characteristic upper and lower limb distal motor neuropathy with spasticity first described by Silver in 1966,[2] which has also been previously found to represent 15% of individuals.[3] The p.N88S mutation was identified in Family 2 through WES given patient 02.II.01 showed a paraparesis with a motor neuropathy without distal upper limb atrophy and patient 02.I.02 showed a mild (mRS 1) distal neuropathy of all 4 limbs without spasticity. However, in all other families, direct sequencing of exon 3 of the BSCL2 gene was undertaken given a more classical presentation in certain family members. Sensory symptoms are also rare (11.54%) and we show that they tend to occur in more severely affected (Table 1, Figure 1).[3,4] However, 95.83% of patients showed normal or brisk reflexes which is an uncommon finding in hereditary polyneuropathies, and therefore this could guide the diagnosis.

In our series, median time to diagnosis since onset of symptoms was 32 years. This could be partly explained by a very slow progression and overall low disability (mRS=1.34), as well as the almost 25% of non-penetrant or subclinically affected individuals carrying the p.N88S (19.23% in our series).[3] There was a tendency for female patients to be less disabled, and females showed a significant lower MRI CS than males. It is especially relevant the case of patient 1.I.02, a 99yo woman who remains unaffected from the disease. The above further emphasizes the variable penetrance of the p.N88S mutation, introducing a possible differing penetrance of the disease by gender.

Interestingly, females seem to be more severely affected in the congenital generalized lipodystrophy caused by homozygote/compound-heterozygote mutations in the BSCL2 gene[27] .Female mice lacking BSCL2 specifically in adipose tissue develop metabolic dysfunction not apparent in the same male mice model,[28] however entirely null BSCL2 female and male mice models recapitulate the metabolic disturbances observed in humans, underpinning the importance of BSCL2 in tissues other than adipose tissue and their relationship with gender.[29]

When comparing phenotypes, there was a tendency towards higher disability in "complex" BSCL2 phenotypes, either those associating sensory signs and symptoms (CMT2) or spasticity (SPG17). Muscle-MRI confirmed that CMT patients had more severe muscle fat replacement than SPG or dHMN patients, supporting the hypothesis that sensory damage appears later in the course of the disease. Only one previous case of respiratory insufficiency in a male patient with a rapidly evolving disease fulfilling El Escorial criteria for ALS, who required noninvasive ventilation, had been previously reported.[5] We present two further cases of restrictive or mixed respiratory insufficiency, although this remains uncommon and seems to present in severe cases of BSCL2 disease.

Predominance of an axonal motor neuronopathy, but occasional dispersed CMAPs on NCS

Electrophysiological studies showed a predominantly motor axonal neuropathy, with peroneal and tibial nerves similarly affected in lower limbs, and a predominant median nerve involvement in upper limbs, as originally described.[3] However, it was surprising to find the presence of dispersed CMAPs in 7 patients, mainly in tibial and sometimes peroneal nerves (Table S1). Although there is primary axonal damage, chronodispersion, CVs in the demyelination range, and partial conduction blocks have been described in BSCL2 and other motoneuron disorders suggesting a demyelinating component in the disease pathogenesis.[3,30,31] These findings are in line with sural nerve biopsies previously described,[10,31] which have revealed a predominant axonal neuropathy, with loss of large myelinated fibers and axonal regeneration clusters. Active axonal degeneration is exceptional and demyelinating features like small onion bulbs can be present, although rare.[10,31] Sural nerve CSAPs could be elicited in all cases, further supporting the later sensory involvement in the disease.

Muscle involvement in muscle-MRI is consistent across the clinical spectrum of the disease and the degree correlates with disease severity

Muscle-MRI was altered in the majority of cases (83.33%) and showed a distal symmetric pattern of muscle fat replacement in both upper and lower limbs. This proximal-to-distal gradient has been evidenced in other neuropathies and differs from the more homogeneous replacement across the muscle's length seen in distal myopathies, a differential diagnosis of BSCL2 disease.[32]

The most frequently affected muscles were *soleus*, *tibialis anterior* and *thenar eminence* muscles, followed by *extensor* and *flexor digitorum longus, medial gastrocnemius* and *peroneus*. Following the Price et al. classification of muscle involvement by nerve territory,[33] BSCL2-nerve disorders would show both an initial P-type (peroneal) and T-type (tibial) involvement, progressing to S-type (sciatic). The sparing of the deep posterior leg compartment (notably the *tibialis posterior*) seen in the present series of BSCL2 patients has also been described in CMT1A patients.[34]

The fact that distal muscles receiving the longest branches from peroneal and tibial nerves, tended to be first and more severely affected irrespective of clinical phenotype, supports the hypothesis of an associated mechanism of length-dependent motor axon degeneration in BSCL2 disease; similar to that described in CMT1A and other CMT2 and dHMN hereditary neuropathies.[34,35] However, a genotype-phenotype correlation may exist. While a prominent superficial posterior compartment (T-type) muscle involvement has been described in 21 patients with PMP22 mutations, 12 patients with HSP27 mutations and 8 patients with HSPB1 mutations, [35–37] 3 patients with DNM2 mutations showed a predominant anterior and peroneal compartment (P-type) muscle involvement.[38] We see that in BSCL2 patients both a T-type and P-type muscle involvement normally coexists.

These same muscles could present hyperintensities on STIR sequences, previous to any evidence of fat replacement in two patients, making denervation a plausible early event in the disease.[39] However, 5 patients showed STIR hyperintensities in partly fat-replaced muscles, which evidences ongoing denervation in later stages of the disease. As mentioned earlier, BSCL2 mutations may even present as an ALS-like phenotype.[5] There is no specific pattern of fat replacement in ALS, but marked STIR changes from rapid denervation have been reported.[40] Although no ALS-like phenotype was observed in our series, the predominantly distal involvement on MRI, notably *tibialis anterior* and *soleus* vs *peroneal* in ALS, and more isolated STIR hyperintensities could help differentiate ambiguous cases.

Probably the most important finding is that this T-type and P-type muscle involvement on MRI was irrespective of the clinical phenotype, even if asymptomatic (Figure 2), which could guide the genetic diagnosis of this clinically heterogeneous disease. Furthermore, there was a strong correlation of muscle fat-replacement with disability, as well as a mild-moderate correlation with time since onset of disease, supporting the role of muscle-MRI in future clinical trials developement.[41]

This study has some limitations. Although the number of patients ascertained (n=26) and total number of different families (n=5) is relatively big for a rare disease, the fact that all families share the p.N88S heterozygote variant and came from a small area makes a shared underlying genetic background probable. Despite this, it is interesting to find such a wide phenotypic spectrum, resembling the series previously reported [2,3,5–7]. Mean disability was low, however, the real burden of BSCL2 disease may be underestimated by the mRS. Third, not all patients underwent a muscle-MRI, however the distribution of age, gender, duration of disease and mean disability were similar, and all phenotypes were represented.

The p.N88S BSCL2 mutation commonly presents as a pure dHMN; nevertheless there is a variety of other presentations. Generally, the disease is slowly progressive and patients maintain autonomy until late stages. Muscle-MRI shows a consistent proximal-to-distal and symmetric fat replacement in *soleus*, *tibialis anterior* and *thenar eminence* muscles, with frequent changes on STIR sequences, regardless of the clinical phenotype. Furthermore, there is a strong correlation of muscle fat replacement and disability. Therefore, muscle-MRI can offer a recognizable pattern of muscle involvement and probably serve as a reliable outcome measure for future clinical trials.

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CONFLICTS OF INTEREST

The author's report no conflicts of interest.

DATA AVAILABILITY

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Figure 1) Clinical correlations. **(A)** Disability measured by mRS is associated with 1) age (median) and 2) phenotype (p=0.000). **(B)** MRI Composite Score (MRI CS) is positively associated with 1) mRS (p=0.0009) and 2) CMT phenotype (p=0.1473, p=0.0441 after grouping dHMN+SPG) and 3) male patients (p=0.0209). Last, 4) MRI CS shows mild correlation with time since onset of symptoms (r=0.3435).

Figure 2) Axial T1-weighted and STIR muscle-MRI sequences in the spectrum of disease. **01.III.06)** 38yo male, mRS=0, minimal TA weakness (dHMN-II), Mercuri grade 1 *soleus* fat replacement and STIR hyperintensities on *medial gastrocnemius*; **03.II.03)** 41yo female hand and shin amyotrophy ,mild TA weakness (dHMN-V), mRS=0, Mercuri 1 *thenar eminence* and *soleus* fat replacement; **05.I.02)** 69yo female with predominant spasticity and minimal TA weakness (SPG17), mRS=2, Mercuri 2-3 *soleus*, *tibialis anterior*, *extensor digitorum longus* and *peroneus*, and Mercuri 1 *semitendinosus*, *gluteus* (not shown) and axial muscles (not shown) fat replacement; **01.III.10)** 37yo male with TA and TE weakness with an axonal sensorimotor neuropathy (CMT2), mRS=2, Mercuri 4 *thenar eminence*, Mercuri 2-3 *soleus* and *tibialis anterior* and Mercuri 1 *extensor digitorum longus* and *flexor digitorum longus* muscle fat replacement, as well as hyperintensities notably on *soleus* and *thenar eminence* muscles; **01.I.08)** 58yo male with

TA and TE muscle weakness and distal sensory disturbances (CMT2), mRS=3, Mercuri 3 *thenar eminence* and *tibialis anterior*, *soleus* and *medial gastrocnemius* as well as Mercuri 2 *semimembranosus*, *extensor digitorum longus*, *flexor digitorum longus* and *peroneus* muscle fat replacement, STIR hyperintensities on *thenar eminence* and *semimembranosus* muscles. The *tibialis posterior* is characteristically spared.

TE=ThenarEminence, SM=Semimembranosus, ST=Semitendinous, TA=TibialisAnterior, P=Peroneus muscles, GAm=GastrocnemiusMedialis, So=Soleus, EDL=ExtensorDigitorumLoguns, FDL=FlexorDigitorumLongus

Neuromuscular

Figure 3) Heatmap showing muscle involvement on axial-T1w sequences. Patients and muscles are ordered according to hierarchical clustering with increasing replacement severity from the bottom to the top (patient-rows) and from the left to the right (muscles-columns). The score of a muscle in a patient is indicated by the colour of the square. Grey squares mean that data is not available. Columns on the left contain each individual's phenotype and gender, while columns in the right contain disease duration and disability measured by mRS. Legend on the bottom of the graph.

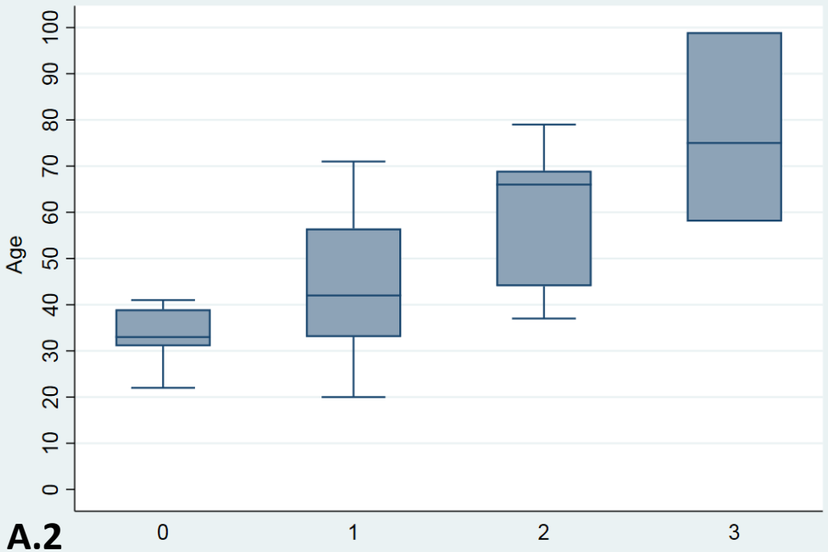
	BSCL2 cohort
Age (years)	51.54 (SD +/- 19.94)
Time since onset	32 (range 10-47)
Age onset	
- **No symptoms**	8 (30.77%)
- **Childhood (0-14)**	9 (34.62%)
- **Youth (15-24)**	3 (11.54%)
- **Adulthood (≥25)**	6 (23.08%)
Gender (Male)	14 (53.85%)
Phenotypic classification	
- **dHMN:**	13 (50%)
- **type V**	9 (34.61%)
- **type II**	4 (15.38%)
- **SPG17**	5 (19.23%)
- **CMT2**	3 (11.54%)
- **Asymptomatic**	5 (19.23%)
Limb Onset (n=18)*	
- **Lower**	12 (66.67%)
- **Upper**	4 (22.22%)
- **Both**	2 (11.11%)
Distal muscle atrophy	
- **None**	6 (23.07%)
- **Thenar eminence**	1(3.85%)
- **Tibialis anterior**	6 (23.07%)
- **Tibialis anterior and thenar eminence**	13 (50%)
Distal weakness (MRC)	
- **Hand**	3.96 (SD+/-1.49)
- **Tibialis anterior**	3.42 (SD+/-1.28)
Sensory symptoms	3 (11.54%)
Spasticity	5 (19.23%)
Reflexes (n=24)**	
- **Abolished**	1 (4.17%)
- **Normal**	10 (41.67%)
- **Brisk or Babinski sign**	13 (50%)
Osseous changes (n=21)**	
- **Pes cavus +/- hammer toes**	12 (57.14%)
- **Only hammer toes**	1 (4.76%)
- **Achilles retraction**	2 (9.52%)
- **Scoliosis**	1 (4.76%)
Neurosensorial hypoacusia	3 (11.54%)
Disability (mRS)	1.34 (SD +/- 1.23)
Neurophysiology (n=19)	
- **Motor axonal neuronopathy**	10 (52.63%)
- **Mixed polyneuropathy**	7 (36.84%)
- **Normal**	2 (10.53%)
Whole body muscle-MRI (n=18)	
- **Altered**	15 (83.33%)
- **Normal**	3 (16.67%)

Table 1) Demographic description of the cohort.
*8 patients do not report symptoms, but 3 show distal weakness on examination (dHMN), thus 5 patients have a normal are completely unaffected (asymptomatic).
**Reflexes and osseous changes missing from n=2 and n=5 patients respectively.

	dHMN-V	dHMN-II	SPG17	CMT2	Asymptomatic
Number (% of total)	9 (34.61%)	4 (15.38%)	5 (19.23%)	3 (11.54%)	5 (19.23%)
Age	47 +/-18SD	47 +/-17.15SD	63.8 +/-12.64SD	54.67 +/-16.26SD	49.2 +/-31.22SD
Gender (Male)	4	3	3	3	1
mRS	1.33 +/-1.32SD	0.75 +/-0.5SD	2 +/-0	2.33 +/-0.57SD	0
Distal muscle atrophy and weakness	Always upper and lower limb atrophy	Only lower limb atrophy	Any (lower limb, upper and lower or none)	Always upper and lower limb atrophy	
Brisk reflexes or babinski sign	5	2	5	2	0
Pes cavus/hammer toes/Achilles retraction	6	2	2	3	1
Others			+ Spasticity	+ Sensory symptoms or sensorymotor neuropathy	
Respiratory insuficiency	2	0	0	0	0

Table 2) Typical p.N88S BSCL2 clinically defined phenotypes.

A.1



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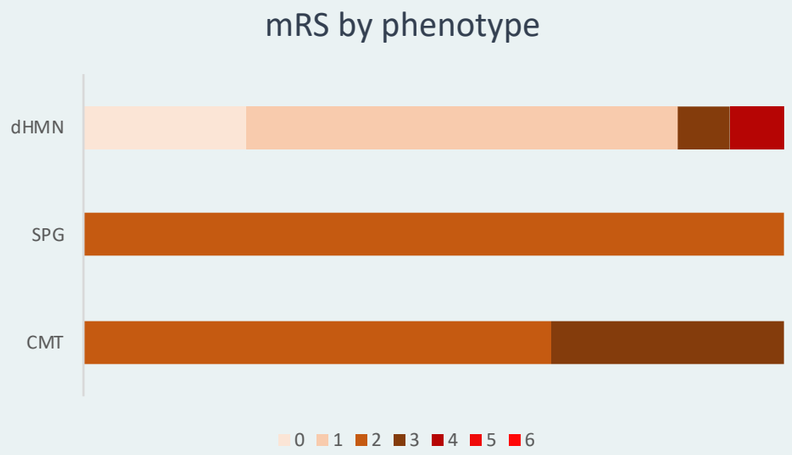

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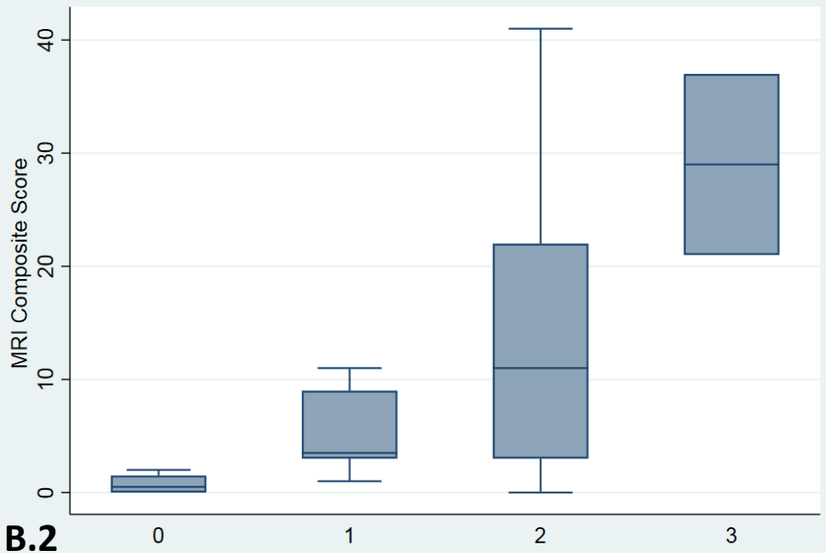

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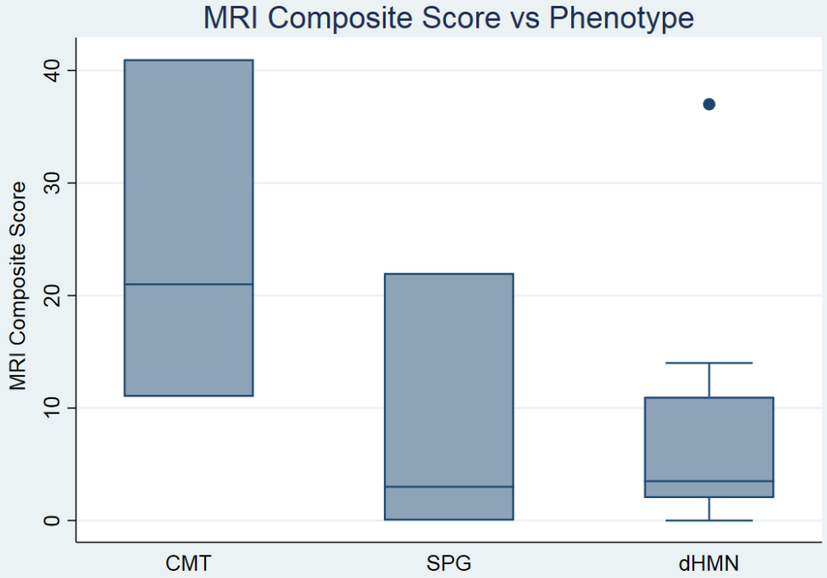

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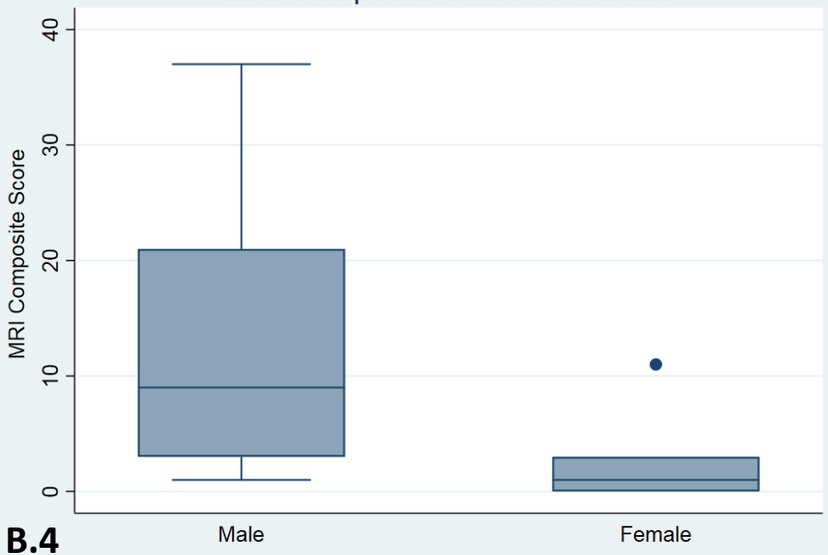

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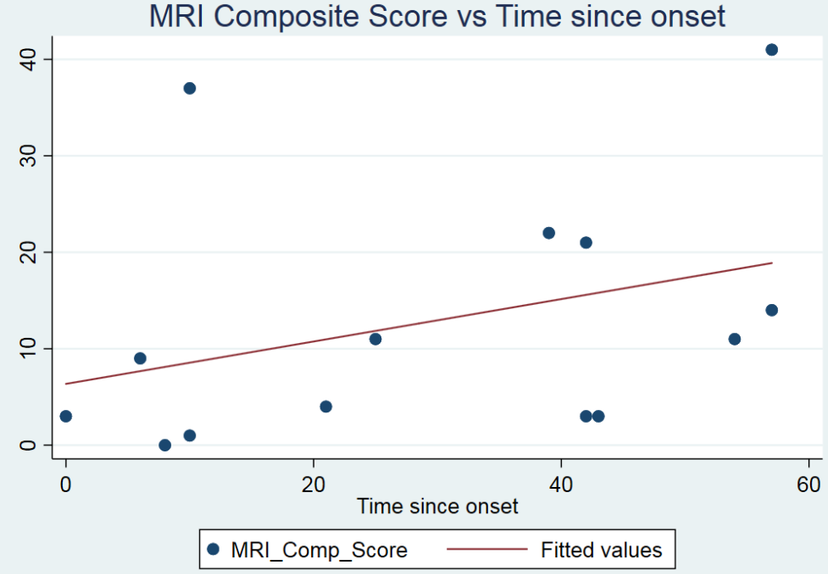

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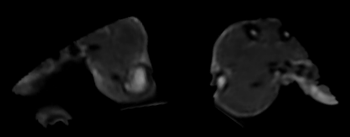
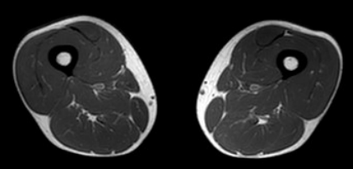
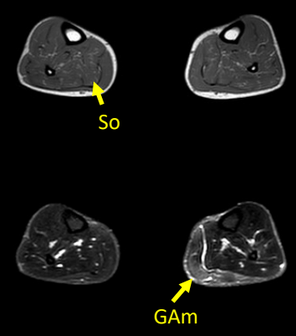
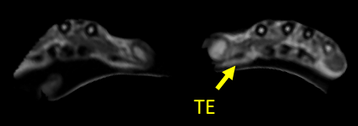
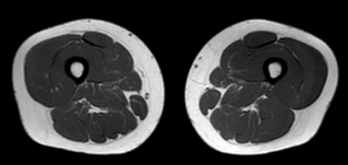
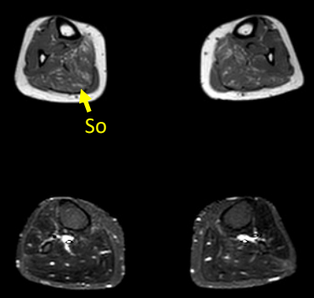
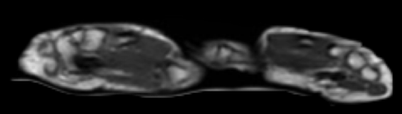
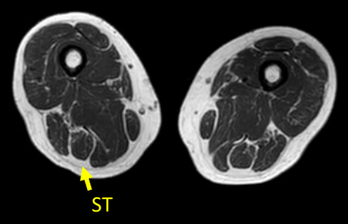
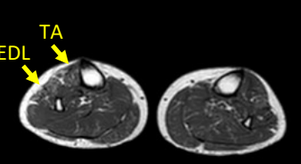
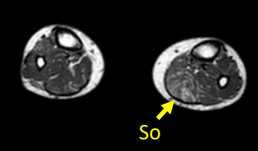
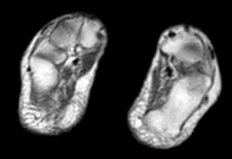
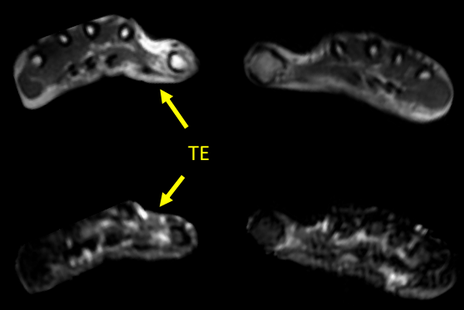
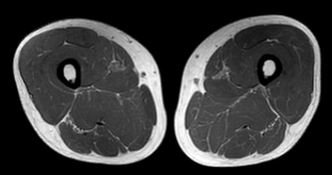
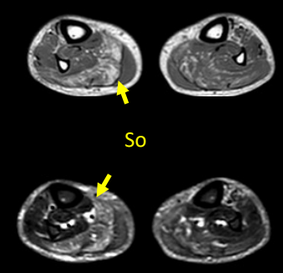
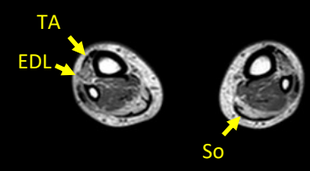
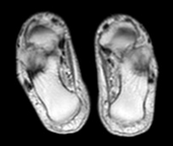
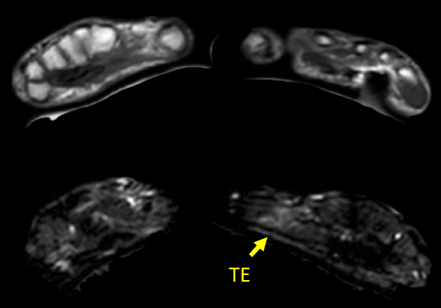
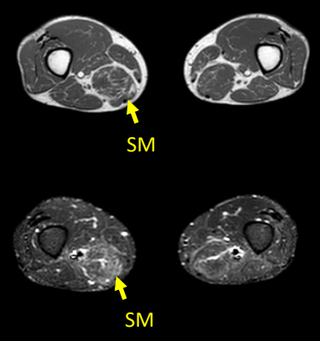
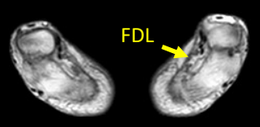
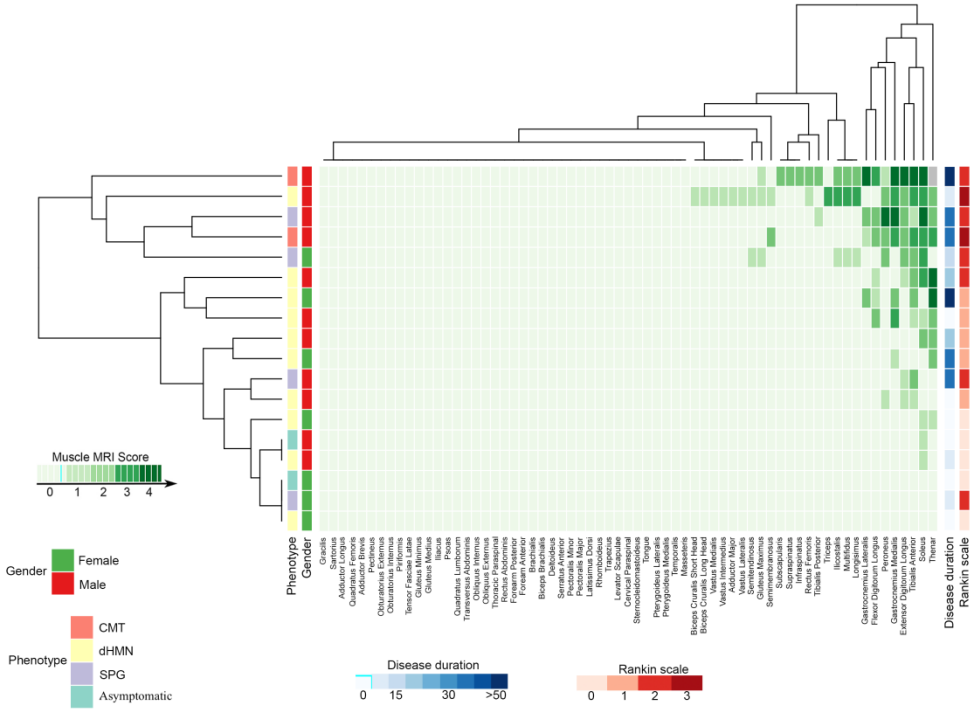
01.III.06 dHMN-II					
03.II.03 dHMN-V					
05.I.02 SPG17					
01.III.10 CMT2					
01.II.08 CMT2					



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