Phenotypes of the N88S Berardinelli–Seip Congenital Lipodystrophy 2 Mutation

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Recently, two missense mutations (N88S, S90L) in the Berardinelli–Seip congenital lipodystrophy gene have been identified in autosomal dominant distal hereditary motor neuropathy and Silver syndrome. We report the phenotypic consequences of the N88S mutation in 90 patients of 1 large Austrian family and two unrelated German families. Variation in the clinical and electrophysiological phenotype enabled us to distinguish six subtypes. In 4.4%, the disorder was not penetrant. Twenty percent of the patients were subclinically affected; some of these patients could only be detected by pathological nerve conduction studies. A distal hereditary motor neuropathy type V phenotype characterized by predominant hand muscle involvement was found in 31.1%, whereas 14.5% showed typical Silver syndrome with amyotrophy of the small hand muscles and spasticity of the lower extremities. Moreover, the phenotype present in 20% was compatible with Charcot–Marie–Tooth disease. In 10%, the clinical diagnosis of pure or complicated hereditary spastic paraparesis was made. Electrophysiological studies showed an axonal neuropathy but also chronodispersion of compound motor action potentials and conduction blocks. Sensory nerve conduction studies were rarely pathological. Our study indicates that the dominant N88S mutation in the Berardinelli–Seip congenital lipodystrophy gene 2 leads to a broad spectrum of motor neuron disorders.

Distal hereditary motor neuropathy (dHMN) is a neurological disorder caused by degeneration of motor neurons leading to distal muscle weakness and wasting.1 It is also termed distal spinal muscular atrophy (dSMA) or spinal Charcot–Marie–Tooth (CMT) disease.1,2 Depending on the age at onset, the clinical presentation, and the presence of additional features, dHMN has been divided into seven subtypes.1 dHMN type V (dHMN-V, dSMA-V) is characterized by predominant wasting and weakness of the small hand muscles that may occur unilaterally and is sometimes confined to the thenar and dorsalis interosseus I muscles only.1,3–6 Silver syndrome (SS) is a rare motor neuron disease that has been classified among the autosomal dominant group of complicated hereditary spastic paraparesis (HSP).7 It was first described in 1966 in two English families in which patients experienced from mild to severe spasticity in the lower limbs in addition to amyotrophy of the small hand and sometimes foot muscles.7

In 2000, we reported a single large Austrian family for whom the majority of affected members had a dHMN-V phenotype.8 We discussed in detail the phenotypic similarities to SS in several patients. This Austrian family was excluded for the dHMN-II locus on chromosome 12q reported by Timmerman and colleagues9 and the dSMA-V locus on chromosome 7p that Christodoulou and colleagues4 reported in 1995 in a large Bulgarian kindred.8,9 Mutations in the glycyl tRNA synthetase gene were identified as the underlying genetic defect of the disorder in this family, but mutations in the same gene were also found in families previously subclassified as having CMT disease type 2D.10–12 dSMA-V and CMT disease type 2D have several features in common, but they are different in that there are no sensory abnormalities in dSMA-V.4,13

A similar overlapping of dHMN and hereditary motor and sensory neuropathy (HMSN) phenotypes recently has also been observed in families with mutations in the small heat shock protein 22 and 27 (HSP22 and HSP27) genes, respectively.14,15

In 2001, the gene locus for SS was mapped to chromosome 11q in one of the originally reported English families, and then was classified as SPG17.16 We were able to confirm and refine this locus in four Austrian families with SS.17 Finally, in a collaborative study of 1 Belgian, 1 Italian, 1 Brazilian, 2 English, and 14 Austrian families with either a dHMN, a dHMN-V, or an SS phenotype, we demonstrated that 2 heterozygous mutations (N88S, S90L) in the Berardinelli–Seip congenital lipodystrophy 2 (BSCL2) gene encoding the protein seipin are responsible for the phenotypic expression of these disorders.18,19

In this study, we performed detailed phenotype–genotype correlation studies in 80 patients of 14 related Austrian families and 10 patients of 2 unrelated German kindreds carrying the N88S BSCL2 mutation. We demonstrate that this mutation is associated with a broad spectrum of clinical and electrophysiological features and suggest a subdivision into six main phenotypes in which screening of the BSCL2 gene should be considered.

Patients and Methods
For this study, we ascertained a total of 90 family members of 14 Austrian (Families 1–14) and 2 German (Families G1 and G2) families with proven N88S BSCL2 mutation. After information was given and written informed consent was obtained, all family members except two had a full neurological examination, which was performed by four neurologists (M.A.-G., R.F, B.S.-W., H.L). The patients were examined at the Department of Neurology of the Medical University of Graz; at the Friedrich-Baur-Institute, Department of Neurology of the Ludwig-Maximilians-University of Munich; or at their homes. Disease history was obtained from the relatives of the two patients who could not be examined. This study was approved by the local ethical committee of the medical University Graz (Graz, Austria).

Clinical Assessment
Age at onset was determined by questioning the patients about their age at the first signs and symptoms such as wasting of small hand muscles and gait disturbance.

In particular, neurological abnormalities were documented as described here. Muscle wasting was scored as absent, mild, moderate, or severe. In the upper extremities, the degree of wasting was documented separately for each side and also for the muscles affected (thenar muscles, thenar and dorsalis interosseus I muscles only, or all small hand muscles affected). Muscle weakness was determined based on the Medical Research Council (MRC) score (0–5): normal = MRC 5; mild = MRC 4, 4+; moderate = MRC 3, 4−; severe = MRC 0–2. Foot deformity was either absent, mild, moderate, or severe. Sensory modalities were regarded to be normal if there were no abnormalities except for pallesthesia and impaired when additional sensory qualities were affected. Tendon reflexes were assessed following the National Institute of Neurological Disorders and Stroke score, and patellar tendon reflexes were selected for documentation: 0 = absent; 1 = diminished; 2 = normal; 3 = brisk; 4 = very brisk or clonus. Muscle tone in the lower limbs was either normal or slightly, moderately, or severely increased. Plantar responses were documented as flexor, extensor, or Babinski sign present. Gait abnormalities were absent, mild (patients show mild gait disturbance caused by distal muscle weakness or spasticity of the lower limbs but are still fully ambulant), moderate (patients are unable to walk more than 500 meters or need walking aids), and severe (patients are partially or permanently wheelchair bound).

Electromyography and Nerve Conduction Velocity Studies
Nerve conduction velocity (NCV) studies and electromyography followed standard techniques,20 using the electromyograph MS60 (Medelec, Old Woking, United Kingdom), the electromyograph Keypoint (Dantec Medical, Skovlunde, Denmark), and a portable electromyography Myohandy (Micromed Neurodata, Mogliano Veneto, Italy). Responses for motor nerve conduction velocity (MNCV) studies were recorded from distal muscles using surface electrodes. Compound motor action potential (CMAPs) amplitudes were measured peak-to-peak, but they were measured baseline-to-peak to record conduction blocks. Sensory NCV studies were performed antidromically, using surface or ring electrodes. Median, ulnar, peroneal, and/or tibial motor nerves were measured unilaterally or bilaterally in 73 individuals, and sensory nerve conduction studies were performed on the median and sural nerve on at least one side in the majority of patients. Semiquantitative electromyography was performed with concentric needle electrodes on distal muscles of the upper and lower limbs in selected individuals.

Forty-five at-risk family members (20 males, 25 females; age range, 8–66 years; mean age, 32.4 years) who had normal results on neurological examination and did not carry the mutation were selected as a control group.

Molecular Genetic Analysis
Haplotype analysis and screening of the Berardinelli–Seip congenital lipodystrophy 2 gene.

Genotype and haplotype analysis was performed with 10 short tandem repeat markers covering the SS region of 13cM on chromosome 11q12-q14 between short tandem repeat WP8 and D11S1889.37 Direct testing for BSCL2 mutations was performed by polymerase chain reaction amplification with exon flanking primers (primer sequences are available on request) and automated sequencing of both genomic DNA strands for all translated exons. Amplification reactions were performed on a MJ Research PTC-100 thermocycler (MJ Research, Watertown, MA) using the Hot Star Tag Master Mix Kit (Qiagen GmbH, Hilden, Germany) in 25μl volumes. Cycling parameters were 15 minutes at 94°C, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 57°C, and 30 seconds at 72°C, with a final 4-minute extension at 72°C. Both sequencing and fragment analysis were
performed on a 3100 Genetic Analyser (Applera, Foster City, CA).

Genealogical Studies
Because all 14 Austrian families originated from the same geographical area and haplotype analysis suggested a common ancestor, genealogical studies were performed by conducting family history interviews with older family members to link family branches and by reviewing archived local genealogical records.

Statistics
Statistical analysis was done with the software package Statgraphics plus 5 (Manugistics, Rockville, MD). Categorical data were described by cross-tabulation. Multiple sample comparisons of electrophysiological data in the different groups were done with analysis of variance (ANOVA) and multiple range test using Fisher's least significant difference procedure to discriminate among the means.

Classification of the Patients
Clinical and electrophysiological examination apparently assessed the presence of different phenotypes also segregating within one family and suggested a subclassification of the patients carrying the N88S BSCL2 mutation. We distinguished two major groups.

GROUP I. Group I includes individuals who carry the mutation but are not aware of being affected. They may be either asymptomatic or subclinically affected. Subtype 1 (asymptomatic disease status [not penetrant]) includes individuals without any clinical abnormalities, electrophysiological changes (if performed), or both. Subtype 2 (subclinically affected disease status) includes individuals who are clinically normal or exhibit minor and nonspecific abnormalities (such as mild foot deformity, mild unilateral or bilateral thenar atrophy, or brisk tendon reflexes) and/or show distinct electrophysiological abnormalities.

GROUP II. Group II represents mutation carriers who have distinct clinical features that can be related to the disease. Depending on the phenotypic presentation, they can be divided into the following subgroups: Subtypes 3 (dHMSN-V phenotype), 4 (SS phenotype), 5 (CMT phenotype), and 6 (HSP subtype).

Subtype 3 (dHMSN-V phenotype) includes patients with exclusive or predominant symmetrical or unilateral muscle weakness and wasting in the small hand muscles, or patients in whom hand muscle involvement was the first and most disabling sign of the disease and gait disturbance occurred later in disease progression. There may be preserved or slightly brisk tendon reflexes. Depending on the presence or absence of clinical and electrophysiological sensory abnormalities, patients in this group can be further subdivided into (1) spinal CMT syndrome (no sensory abnormalities except for pallesthesia) and (2) HMSN type II (HMSN-II) if sensory abnormalities occur.

Subtype 6 (HSP subtype) includes patients without weakness and wasting of the small hand muscles but with spastic paraparesis in the lower limbs. They can be further subdivided into (1) pure and (2) complicated HSP. Pure HSP patients are afflicted with spasticity in the lower limbs but do not have additional clinical and electrophysiological features (except foot deformity). In addition to spasticity in the lower limbs, complicated HSP patients exhibit sensory abnormalities, and/or amyotrophy of the distal muscles of the legs, and/or pathological nerve conduction velocities. The latter group may also be diagnosed as having HMSN-V, as proposed in the classification by Dyck and colleagues, and CMT disease with pyramidal tract features, as described by Harding and Thomas.

Figure 1 summarizes the frequency of the six subtypes. To determine disease severity about the phenotypic presentation of each patient, we divided symptomatic mutation carriers (i.e., Subtypes 3–6) as follows: mild = mild weakness and wasting in the upper and/or lower limbs, and/or mild gait abnormalities; moderate = moderate weakness and wasting in the upper and/or lower limbs, and/or moderate gait abnormalities; severe = severe weakness and wasting in the upper and/or lower limbs, and/or prominent gait abnormalities (steppage gait, moderate or severe spasticity); very severe = unable to walk or almost wheelchair bound.

Results
Clinical Findings
Of the 90 individuals carrying the N88S BSCL2 mutation, 46 were male and 44 female. The ages at examination were 13 to 85 (mean, 43) and 10 to 84 (mean,
moderate in 19, severe in 24, and very severe in 3 patients.

Pallesthesia, which was considered age appropriate, was detected in 42 patients. In 7 patients (age range, 33–85 years; mean age, 64 years), other sensory abnormalities such as impaired surface sense or hyperesthesia were present. Positive sensory symptoms were never reported by the patients.

The tendon reflexes did not show any significant changes in the upper extremities. Patellar tendon reflexes were found to be absent in 1, depressed in 2, normal in 12, brisk in 41, and very brisk in 12 patients. The latter corresponded with increased muscle tone, which was found in 21 patients, and other pyramidal tract features, such as a positive Babinski sign present in 13 patients. Patients with spasticity in the lower limbs also reported stiffness and muscle cramps in the legs.

The disease progressed extremely slow. Most patients remained functional and active during adult life up to their 60s. Ambulation was lost in only one patient at 75 years. The disease was associated with a mild phenotype in 19, was moderate in 19, was severe in 27, and was very severe in 3 patients.

Electrophysiological Results
Seventy-three patients with the N88S BSCL2 mutation were tested. Seventeen patients belonged to Group 1 (ie, Subtypes 1 and 2), and 56 patients belonged to Group 2 (ie, Subtypes 3–6). Abnormal MNCVs were found in all but six patients (four patients in Group 1 and two in Group 2). Figure 2 shows values of MNCVs and CMAPs in the upper and lower limbs. In the lower limbs, electrophysiological abnormalities consisted of reduced CMAPs, indicating primarily axonal nerve damage. However, in addition, marked chronic-dispersion of the CMAPs was often found, and MNCVs were sometimes in the demyelinating range, pointing to additional demyelination of the peripheral nerves (Fig 3a). Moreover, partial conduction blocks were present in 16 patients (see Fig 3b). Comparing the values of MNCVs and CMAPs in the peroneal and the tibial nerve in all groups, we detected no statistically significant side differences (ANOVA, Fisher’s least significant difference procedure).

In the upper limbs, changes of the MNCVs and CMAPs were more frequently seen in the median than in the ulnar nerve in individual patients of Subtypes 3 to 5. There was no side difference between the median and the ulnar MNCVs, yet the differences of means of right median and right ulnar nerve MNCV were statistically significant (ANOVA: \( p = 0.03 \); 95% confidence interval, Fisher’s least significant difference procedure). There was no side difference in CMAPs, but again a significant difference between median and ulnar nerves on each side was found (ANOVA: \( p = \))

DESCRIPTION OF GROUP II. Age at symptomatic disease onset could be determined in 55 of 68 patients (30 female patients: age range, 10–77 years; mean age, 46 years; 38 male patients: age range, 16–85 years, mean age, 44 years) and varied between 8 and 59 (mean age, 23 years) in female patients and 6 and 66 (mean age, 19 years) in male patients. Thirty-nine (19 female, 20 male) patients initially noticed unilateral wasting of the thenar or the dorsalis interosseus I muscles, or both, with the right side more frequently affected than the left (33/39). Muscle weakness and wasting usually had a distal emphasis and affected the upper limbs, lower limbs, or both.

Gait abnormalities were mild in 33 (13 male, 20 female) patients, moderate in 25 (18 male, 7 female) patients, and severe in 3 male patients only. Gait disturbances were the result of wasting and weakness of the distal muscles of the lower limbs leading to a steppage gait in 54 (22 female, 32 male) patients and also stiffness and spasticity in 21 (8 female, 13 male) patients, or a combination of both.

Foot deformity was present in 65 members. The degree of foot deformity varied widely from mild in 19,
0.00; 95% confidence interval, Fisher’s least significant difference procedure). In the control group, there were no side differences between median and ulnar MNCVs and CMAPs, and there were the same statistically significant differences between median and ulnar MNCVs and CMAPs as in the patient groups, with the exception of left median and ulnar CMAP.

Calculations of the median and sural sensory NCVs did not indicate significant side changes in the patient or control groups, whereas there was a statistically significant difference between the median sensory nerve action potentials in the patient group and the control group. The same significance was found comparing left but not right sural sensory nerve action potentials between the patient group and the control group (Fig 4).

In summary, these results strongly suggest that the N88S BSCL2 mutation also leads to axonal damage of the sensory nerves.

Electromyographic abnormalities usually indicated chronic neurogenic disturbance with high potential amplitudes. Detailed studies of the central motor conduction times have been reported in our previous description of the disease, which confirms the presence of additional upper motor neuron involvement.

Molecular Genetic Results
In all patients, the N88S BSCL2 mutation was present as described previously. The known disease haplotype of the large Austrian family was compared with the haplotypes of the two German families (Table). As shown in the Table, Families G1 and G2 have completely different haplotypes. A common haplotype comprising three consecutive markers (WP3, CA9, CA10) can be observed in the Austrian haplotype and Family G2. These markers, however, span a small interval of 42kb, making a relationship of these two families unlikely. However, a common founder can not be formally ruled out.

Genealogical Studies
The genealogical studies enabled linkage of 13 of the 14 families to 1 large pedigree; the disease was traced to a common parent-pair, born in 1682. Thus, a large pedigree consisting of 13 generations could be constructed (Fig 5).

Discussion
This study has analyzed the range of phenotypic manifestations in 90 carriers of the N88S BSCL2 mutation. This proved to be wide ranging from asymptomatic cases to severe disease expression in patients exhibiting dHMN, CMT disease, or an HSP phenotype. The hallmark characteristic, however, was a predominant motor neuron disease affecting the upper motor neurons, lower motor neurons, or both. Most common features consisted of hand muscle weakness and wasting, gait abnormalities, stiffness in the lower limbs, and foot deformities. In a few severely affected individuals, mild sensory abnormalities also could be detected. In this article, we provide a subdivision into six clinical phenotypes that may be associated with the N88S BSCL2 mutation. Figure 1 summarizes the frequency of Subtypes 1 to 6. As shown in Figure 1, 24.4% of the mutation carriers were asymptomatic or subclinically affected, pointing to incomplete penetrance of the disease. These findings correlate with Patel and colleagues’ observations. In Subtypes 3 and 4 (45.6%), the clinical picture was dominated by symmetrical or
asymmetrical weakness and wasting of the small hand muscles; in Subtype 4, a variable degree of spasticity of the lower limbs was present, resembling the SS phenotype as originally described in 1966. Subtype 5 refers to a group of patients who were previously diagnosed as dHMN (ie, spinal CMT disease) or HMSN, respectively, when additional sensory abnormalities were found. In a smaller group (10%, Subtype 6), spasticity in the lower limbs was so prominent that the disorder was subclassified among the HSPs. Moreover, there was prominent intrafamilial variation between siblings but also between parents and children, as indicated in Figures 6 and 7, making it most difficult in some families to detect a uniform disorder. Thus, in some cases, the diagnosis of acquired motor neuropathies such as amyotrophic lateral sclerosis or multifocal motor neuropathy was suspected previously. Other patients who had no obvious gait abnormalities and were grouped under Subtype 3 were often regarded to have carpal tunnel syndrome or compression syndrome of the ulnar nerve at the wrist or elbow, occasionally leading to operative decompression without benefit. Interestingly,
when calculating the sex distribution in the six subtypes (see Fig 1), there was a statistically significant difference between male and female patients at the 95% confidence level (ANOVA: \( p < 0.05 \)). Subtypes 1 to 3 are predominantly represented by female patients, whereas subtypes 4 to 6 are more frequently represented by males.

From the variation of phenotypes observed, the question arises how to classify this disorder. In the originally reported English family linked to chromosome 11q, a subclassification among the group of the HSP was suggested and was genetically designated as SPG17.\(^{16}\) However, in the series of patients reported in this article, spasticity of the lower limbs occurred in only 24.5% of patients (Subtypes 4 and 6). The term HSP is highly misleading and confusing in the remaining subtypes. Therefore, it becomes evident from this clinical study that the broadened phenotype requires a more suitable classification, preferentially among the group of motor neuron disorders and/or based on the underlying molecular genetic background.

Broad variability was also reflected in the age at onset. Symptoms developed in most patients in the second decade of life, but some patients first noticed symptoms as late as in their 60s, and only 5 patients had signs before the age of 10 years. In some cases with mild disease severity, the age at onset could not be determined because these individuals were not aware that they were affected. A significant difference in the age at onset between male and female patients was not encountered.

Hand muscle involvement proved to be a predominant, although not regularly, feature. It was most evident that there is a preferred distribution of weakness and wasting to the thenar and dorsalis interosseus I muscles that often results in a typical adduction position of the thumb and primarily evokes problems in writing. The predilection of these two muscles still remains unclear. One could speculate that this is caused by degeneration of the anterior horn cells at a particular level of the spinal cord segments corresponding to innervation of these muscles. Magnetic resonance imaging and autopsy studies may be able to address this hypothesis. Also, the side differences that did not consequently correspond to the leading hand of the patients and that were more frequently and more severely observed in the right hand currently remain unexplainable.

Sensory examination results were usually normal, except for pallesthesia. Additional sensory abnormalities were rarely found and always occurred after a long duration of the disease and in severe cases. This observation is not surprising and has already been described in other forms of inherited neuropathies in which dominant mutations were associated with both dHMN and HMSN.\(^{10,13,14}\) However, it is diagnostically important to heed this overlapping because it is the essential clinical and electrophysiological distinguishing feature between dHMN and HMSN and HSP and HMSN-V, respectively.\(^{1,21}\) Both HMSN and HMSN-V were diagnosed in some cases described in this article.

Electrophysiological studies appeared to be a useful tool in the diagnosis of this disease. On the one hand, there is a rather uniform pattern of MNCV studies, with the median nerve more severely affected than the
ulnar nerve in patients of Subtypes 3 to 5, although there are only a few differences within the patient and control groups concerning median and ulnar MNCV and CMAP studies on the statistical basis. Axonal nerve damage becomes evident by the low CMAPs, but, in addition, many patients also show slowed MNCVs, pronounced chronodispersion of the CMAPs, and conduction blocks in the lower limbs (see Fig 3). These findings show similarities with acquired motor neuron disorders and are seen particularly in multifocal motor neuropathies. On the other hand, MNCV studies proved to be most helpful in the diagnosis of asymptomatic individuals, which is important before molecular genetic counseling and testing. Finally, the sensory nerve studies are indispensable in the diagnosis of a pure motor neuron disease in earlier stages of the disease, but clearly indicate an additional axonal sensory neuropathy with advanced disease, and then fail to distinguish dHMN and HMSN.

The genealogical studies in the large Austrian cohort enabled linkage of 13 of the 14 branches to one large pedigree consisting of 13 generations and allowed the tracing of the disease to a common parent-pair born in 1682, one of whom was most likely a carrier of the N88S BSCL2 mutation (see Fig 5). Considering the high number of offspring in each generation and the

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**Fig 5. Pedigree of the large Austrian family including 13 branches. Filled symbols indicate individuals carrying the N88S Berardinelli–Seip congenital lipodystrophy 2 mutation.**

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**Fig 6. Distribution of subtypes in 14 Austrian (1–14) and 2 German families (G1 and G2).**
50% risk for transmitting the mutated gene to a child, this disease may have an impact on prevalence and may account for a substantial fraction of the autosomal dominant inherited neuropathies and spastic parapareses in Austria. Thus, patients exhibiting one of the phenotypes described in this article should be primarily referred to genetic testing to confirm or exclude the N88S BSCL2 mutation.

So far, only two different dominant heterozygous missense mutations (N88S, S90L) in the BSCL2 gene have been identified in patients with dHMN, SS, and HSP.18,24 Eight families are reported, six of whom carry the N88S BSCL2 mutation. This frequent occurrence of an identical mutation may be because of a founder effect in the 14 Austrian and the 2 German families. However, results of haplotype analysis for short tandem repeats within and flanking the BSCL2 gene do not suggest a common ancestor, and thus point to a mutational hot spot at this position. Screening of 8 additional families and 19 single cases with a dHMN-V or a SS phenotype did not show a mutation in the BSCL2 gene indicating genetic heterogeneity within these diseases.

The phenotypes observed in the 14 branches of the large Austrian family and the 2 German families represent a distinct, although variable, clinical pattern. Also, the phenotype in the affected members of an Italian family with the N88S BSCL2 mutation, which Irobi and colleagues24 recently reported, is compatible with Subtypes 3 and 5. Interestingly, pyramidal tract features were not found in these patients. However, there is a major difference in the phenotype of the Belgium family, which Irobi and colleagues24 recently reported, carrying the S90L BSCL2 mutation that exhibits a much more severe disease, with marked weakness and wasting in the upper and lower limbs and pronounced spasticity in the lower limbs, and necessitates the use of a wheelchair in many patients even at a younger age. This variability, which is likely attributable to the type of mutation, remains unexplained and surprising, because both mutations are located in the same exon and affect a glycosylation side.18 Cellular studies on both mutations are ongoing and hopefully will serve to provide a better understanding of the pathogenesis and development of an appropriate treatment of this intriguing and challenging disease and of other inherited and acquired motor neuron disorders.

Appendix
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Fig 7. Segregation of subtypes in one branch of the Austrian family. The pedigree is slightly changed to preserve the confidentiality of the family. The sex of the individuals is not shown. Filled symbols indicate mutation carriers; numbers represent the Subtypes 1 to 6. There is nothing known about the phenotype of the members in the first generation.
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References