Motor Neuron-Related Disorders

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Abstract

Motor neuron disease (MND) is a group of neurological disorders which is characterized by selectively progressive degeneration of motor neurons in the brain and spinal cord. On the basis of the degree of upper or lower neuron involvement, MND is broadly divided into several subtypes: pure upper neuron diseases (primary lateral sclerosis), pure lower motor neuron diseases (progressive spinal muscular atrophy, progressive bulbar palsy, spinal muscular atrophy, X-linked spinal and bulbar muscular atrophy, etc.), and mixed upper and lower motor neuron diseases (amyotrophic lateral sclerosis). Generally, MND can be sporadic, or it can occur as an inherited disorder. To date, a great number of genes have been identified to be responsible for inherited MND. The distinct causative genes usually result in different clinical phenotypes. Therefore, the genetic testing is crucial for inherited MND. In this chapter, we mentioned several cases of inherited motor neuron diseases and described the way how these definitive causative genes were identified. In addition, to make clear definition for MND, several other inherited neurologic diseases, such as hereditary spasticity paraplegia and inherited peripheral neuropathy, were also presented in this chapter.

Keywords

Motor neuron disease • Amyotrophic lateral sclerosis • Hereditary spastic paraplegia • Spinal muscular atrophy • Familial amyloidotic polyneuropathy • Charcot-Marie-Tooth disease • Kennedy's disease • Molecular diagnosis

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3.1 Amyotrophic Lateral Sclerosis (ALS)

A 49-Year-Old Male with Progressive Limbs Weakness and Muscle Atrophy

Clinical Presentations

A 49-year-old man came to our department with a history of over 20 years of progressive limbs weakness associated with muscle atrophy. He was first noted mild weakness of his bilateral legs at the age of 29. He gradually experienced difficulty in walking. Five years later, his arms were affected as well, with difficulty in lifting a heavy load over his head. In the next five years, he had increased difficulty in handgrip and walking. Obvious muscle atrophy was observed in both hands and legs. The disease affected his speech muscles when he was 43 years old. He exhibited a mild dysarthria but still had a relatively unimpaired swallowing ability. His cognition and behavior ability were not impaired during the entire disease course. The patient first started to take riluzole at the age of 49, though with a limit effect. More supportive care was currently home provided to enhance the quality of life.

Physical examinations on his first visit showed obvious atrophy of his bilateral intrinsic hand muscles and diffusely brisk deep tendon reflexes. Babinski sign was present bilaterally at the moment. In his recent examinations, positive signs included mild dysarthria, muscle weakness of both upper limbs and lower extremities. Muscle weakness involved all his extremities, with a decreased strength of the upper extremities (3–4/5) and lower extremities (2–4/5, distal weaken than proximal). The gag reflex was normal. Tendon reflexes were disappeared in all four extremities. Pathogenic signs weren't observed at this moment. His sensory and cerebellar function was normal. His cognition was evaluated normal as well.

The former medical history was unremarkable, apart from a record of slightly increased level of blood sugar. In total, six persons were affected by this disease in the family. They experienced the similar problems in their 20s (Fig. 3.1) and died of respiratory failure within a couple of years after onset. His younger sister developed limbs wasting at the age of 32 and underwent dysarthria at age 45.

There are no specific clinical findings on routine laboratory examinations. The blood tests showed unremarkable findings. Serum levels of T3, T4, and TSH were in normal range. Both folic acid and vitamin B12 were within normal range. There were no significant findings on test for rheumatoid factor and assessment of immunology system. The brain and cervical spinal magnetic resonance imaging (MRI) examination revealed unspecific findings. There were no abnormal findings on electroencephalography. Electromyography test showed extensive denervation in limb muscles and thoracic paraspinal muscles.

Primary Diagnosis

In this case, the patient mainly presented with slow progressive limbs weakness and severe

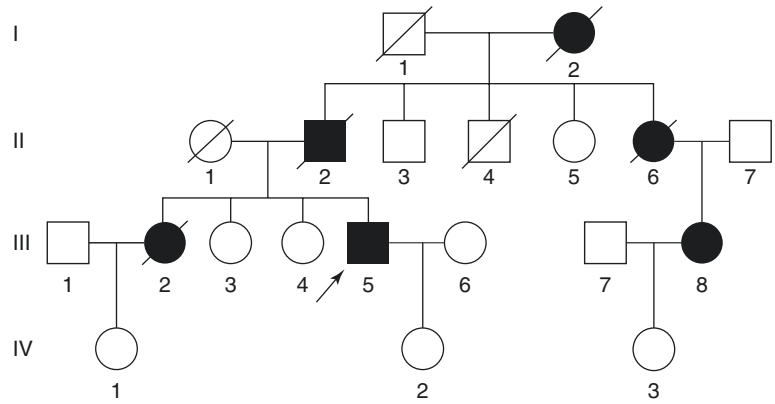

Fig. 3.1 The family pedigree of the patient. The *black field* indicates affected individuals; *circle*, female; *square*, male; *diagonal line* across symbol, deceased; *arrow*, proband

muscle atrophy, which were evidences of lower motor neuron deficits. EMG results also highly suggested the involvement of lower motor neuron. The Babinski signs observed in early stage indicated the entanglement of the upper motor neuron. Taken together, the clinical picture hinted the impairment of upper and lower motor neuron. Owing to the feature of midlife-onset and slowly progressive course in this patient, the diagnosis consideration should include the disturbance of neoplasm, metabolism, chronic inflammation, degeneration, and hereditary. Neoplasm could be excluded on the basis of the unspecific MRI findings and blood tests. Normal laboratory testing results basically ruled out the possibility of metabolism and chronic inflammation. Both upper and lower motor neuron were affected in the present case, indicated the deficits of the motor neuron processes. Therefore, the diagnosis of probable amyotrophic lateral sclerosis (ALS) could be made on the basis of revised El Escorial criteria [1]. The differential diagnosis also included lesions of cervical spine, multifocal motor neuropathy (MMN), and other forms of motor neuron diseases. Unremarkable MRI examination in the brain and spinal cord basically excluded the possibility of structural lesions of these sites. Multifocal motor neuropathy had typical electrophysiologic features of conduction block, which could be detected by nerve conduction examination. The multiple neurological examinations could provide more strong evidence of UMN impairment and help distinguish MND from other LMN disorders. Moreover, a positive family history was obviously observed in the disease with an autosomal-dominant pattern. Therefore, the genetic testing was required to unearth the genetic cause and help make the correct diagnosis.

Additional Tests or Key Results

Through targeted sequencing by combining the genes responsible for ALS in a custom panel, the patient was detected with a novel c.175G>C (p.G59R) mutation in *DCTN1* gene. The novel mutation was further certificated through Sanger sequencing in his affected younger sister (Fig. 3.2).

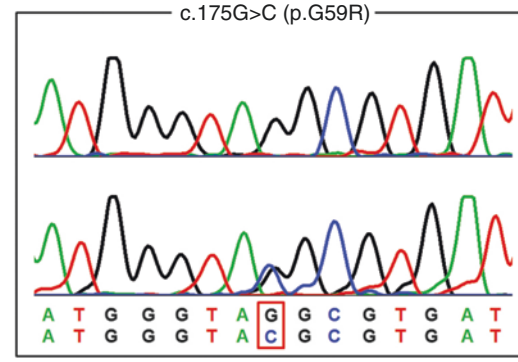

Fig. 3.2 Mutation analysis of the patient. Chromatogram of c.175G>C (p.G59R) mutation in *DCTN1*. The upper panel indicates the normal *DCTN1* sequence, whereas the lower panel shows the heterozygous mutated sequence

Discussion

ALS is manifested by progressive degeneration of both upper and lower motor neurons located in the cerebral cortex, brainstem, and spinal cord. This disease is first described by Jean-Martin Charcot in 1874 and become well recognized because of the two famous persons with ALS, Lou Gehrig and Stephen William Hawking, and increasingly common today due to the prevalence of ice bucket challenge in 2012. The typical symptoms comprise muscle weakness, muscular atrophy, pyramidal tract signs, and the absence of sensory impairment. The average age onset of ALS is 65 years [2]. Most patients die from failure of respiration within 3–5 years after the onset, but about 10% of patients may still survive as much as 10 years and even longer. Unlike classical ALS, which tends to affect people with a rapidly progression, the clinical feature of the present patient was atypical. In this case, the course of the disease was slowly progressive, with a period of 13 years from limbs onset to bulbar symptom. In his family, his affected younger sister also experienced the similar slow course of progression. During the early stage of the disease, upper motor signs were identified in our case; nevertheless, lower motor signs were the primarily symptoms in the whole disease course. This suggests a wide variability of UMN and LMN involvement in the whole process of ALS. Given the critical role of involvement of both UMN and LMN in ALS diagnosis, careful

neurological examinations should be performed at different stages of the disease.

About 5–10% of ALS patients show familial inherited pattern (autosomal-dominant or autosomal-recessive inheritance). More than 19 disease-causing genes have been described in familial ALS (FALS) [3]. Among them, *SOD1*, *TDP-43*, and *FUS* make up about 30% in all FALS cases [4]. Moreover, ALS is also a kind of disease with highly genetic heterogeneity and clinical variability. Different causative genes can still result in similar phenotypes in ALS patients. Therefore, it is a big challenge to identify underlying mutations with traditional sequencing method. Targeted sequencing, a new relatively rapid and economic molecular diagnosis strategy, makes it possible to screen culprit genes or mutations in FALS patients.

The patient was finally detected with *DCTN1* mutation through targeted sequencing. The first mutation of *DCTN1* related to MND was reported in 2003, and several *DCTN1* mutations were described in families affected with ALS or ALS-FTD. However, there was an obvious variability with regard to the associated phenotypes of *DCTN1* mutations. The severity of disease and the course of disease are varied largely among the interfamilial or intrafamilial members with *DCTN1* mutations.

The pathogenic mechanism of ALS is believed to be utmost complicate, which would involve multiple mechanisms. Although huge improvement has been made to elucidate the mechanisms of ALS during the past two decades, the specific mechanism is still unclear. To date, there is no effective medical treatment for ALS. Many drugs that have been initially developed and showed a promise in the field of cure ALS, but the majority of them failed the final clinical trial.

3.2 Hereditary Spastic Paraplegia (HSP)

A 24-Year-Old Male with 10-Years History of Abnormal Gait

Clinical Presentations

A 24-year-old man came to our neurological department with a chief complaint of abnormal gait for over 10 years. He had gait disturbance at the age of 14. The symptoms progressed slowly and became evident in the last 3 years. He felt weak in the lower limbs and had difficulty in going downstairs because of his stiff legs. The upper limbs were intact. He denied cognitive impairment, headaches, eyelid drooping, double vision, swallowing difficulty, or shortness of breath. He did not complain urinary bladder incontinence. He had no other medical problems and was not taking any medications. The patient was of Chinese descent, and his mother also developed gait disturbance at the age of 35 (Fig. 3.3a).

The neurological examination showed higher muscle tone, increased reflexes in the lower limbs, positive bilateral Babinski sign, and ankle clonus. His gait was stiff with scissoring. He had difficulties with tandem gait. His speech is fluent. No cognitive impairment, abnormal signs of cranial nerves, or extrapyramidal disturbances were observed. Strength and sensory examinations in upper extremities were normal. No muscle weakness, muscle atrophy, or sensory deficits in the lower limbs was noted. Romberg test and finger-to-nose test were negative. No scoliosis or kyphoscoliosis was observed.

The routine laboratorial screening tests, including routine blood test, blood biochemical test, vitamin B12, folate, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and thyroid

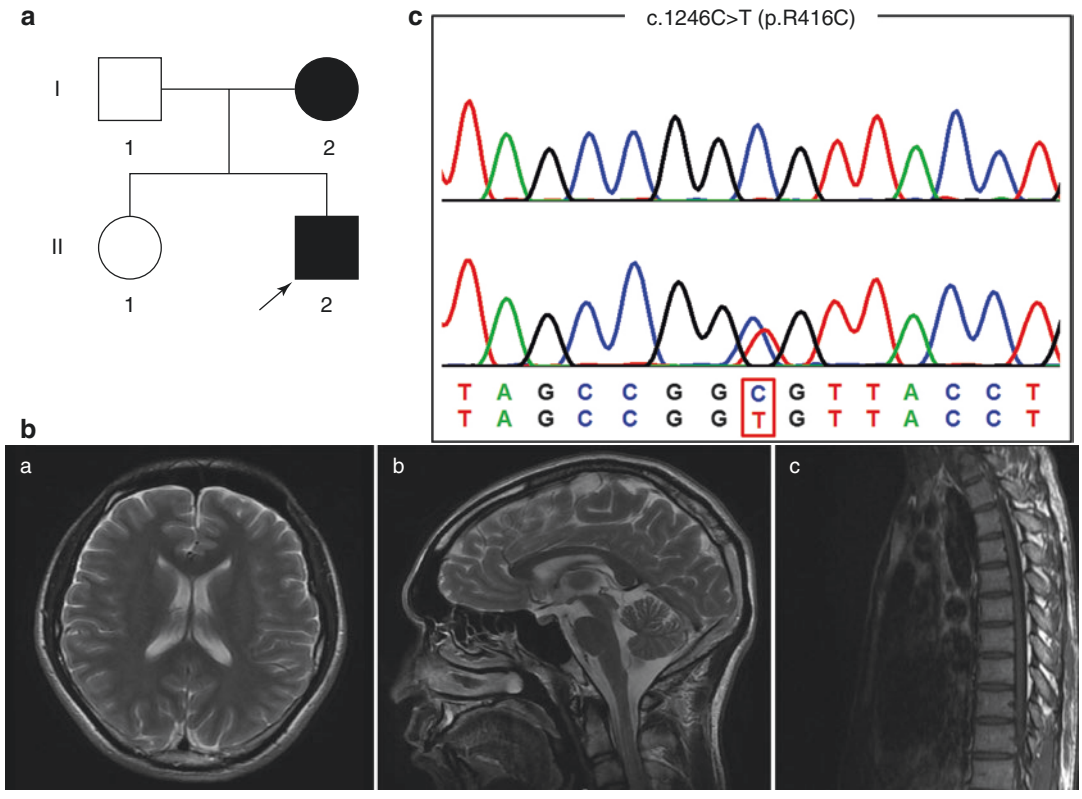

Fig. 3.3 The pedigree, MRI examination and sequencing chromatograms. (**a**) The pedigree of the patient. *Arrow* indicates the proband. The *black box* indicates the affected patient. (**b**) The brain and spinal cord MRI examination indicate normal structure. (**c**) The sequencing chromatograms reveal a heterozygous variant c.1246C>T of the *ATL1* gene in the patient

function, were within normal ranges. The examination of a group of autoantibodies, such as antinuclear antibodies, anti-double-stranded DNA antibodies, and anti-Smith antibodies, was negative. Serology for HIV, hepatitis, and syphilis were negative as well. The X-ray examination for knee joint and hip joint was normal. No abnormalities were found in brain magnetic resonance imaging (MRI) examination (Fig. 3.3b).

Primary Diagnosis

Lower extremity spasticity, high muscle tone, hyperreflexia, positive Babinski sign, and absence of sensory impairment suggested the pyramidal dysfunction. The normal brain MRI findings and intact function of upper limbs hinted the involvement of the spinal cord below the cervical enlargement. As the patient's mother also had symptom of gait disturbance indicating a clear family history, an inherited disease affecting the spinal cord should be verified. The following diseases should be taking into account: familial amyotrophic lateral sclerosis, primary lateral sclerosis, structural abnormalities of spinal cord, spinocerebellar ataxias (SCA), hereditary spastic paraplegias (HSP), inherited metabolic diseases (homocysteine remethylation defects, arginase deficiency, etc.), and subacute combined degeneration of spinal cord.

Additional Tests or Key Results

The examination of thoracic spinal cord MRI, CSF analysis, and electrophysiological study were further performed. No abnormalities were found in spinal cord MRI examination (Fig. 3.3b). The nerve conduction studies and electromyography assessments were normal as well. The lumbar puncture with cerebrospinal fluid (CSF) analysis showed normal protein, glucose, cell counts, and IgG synthesis rate.

Since the serum vitamin B12 level, CSF analysis, and MRI examination of the spinal cord were normal, the disease of myelopathy (metabolic, compression) and structural abnormalities disorders were unlikely. Owing to long-term disease course, normal neurological examination in the upper extremities, and normal neurophysiologic test, amyotrophic lateral sclerosis was excluded. For inborn disorders of metabolism, the inheritance pattern is often an autosomal-recessive trait, and the clinical picture is usually not restricted to pyramidal signs [5]. Moreover, the level of serum homocysteine was normal. Therefore, inherited metabolic diseases were not primarily under consideration. The negative result of *ATXN3* analysis, the gene responsible for SCA3, helps us to exclude the disease of SCA3. As HSP is the most common disease responsible for hereditary lower limb spasticity [6], we pay our attention to this disease.

Genomic DNA of this patient was extracted from peripheral EDTA-treated blood. A gene panel covering the causative gene associated with HSP was designed (Table 3.1). Targeted next-generation sequencing was further performed. After filtering, a heterozygous variant in *ATL1*

Table 3.1 The genes responsible for HSP were included in the gene panel

Disease	Gene	Disease	Gene	Disease	Gene	Disease	Gene
SPG1	*L1CAM*	SPG18	*ERLIN2*	SPG45	*NT5C2*	SPG59	*USPS*
SPG2	*PLP1*	SPG20	*SPG20*	SPG46	*GBA2*	SPG60	*WDR48*
SPG3A	*ATL1*	SPG21	*ACP33*	SPG47	*AP4B1*	SPG61	*ARL6IP1*
SPG4	*SPAST*	SPG22	*SLC16A2*	SPG48	*AP5Z1*	SPG62	*ERLIN1*
SPG5A	*CYP7B1*	SPG26	*B4GALNT1*	SPG49	*TECPR2*	SPG63	*AMPD2*
SPG6	*NIPA1*	SPG28	*DDHD1*	SPG50	*AP4M1*	SPG64	*ENTPD1*
SPG7	*PGN*	SPG30	*KIF1A*	SPG51	*AP4E1*	SPG65	*NT5C2*
SPG8	*KIAA0196*	SPG31	*REEP1*	SPG52	*AP4S1*	SPG66	*ARS*
SPG10	*KIF5A*	SPG33	*ZFYVE27*	SPG53	*VPS37A*	SPG67	*PGAP1*
SPG11	*SPG11*	SPG35	*FA2H*	SPG54	*DDHD2*	SPG68	*FLRT1*
SPG12	*RTN2*	SPG39	*PNPLA6*	SPG55	*C12orf65*	SPG69	*RAB3GAP2*
SPG13	*HSPD1*	SPG42	*SLC33A1*	SPG56	*CYP2U1*	SPG70	*MARS*
SPG15	*ZFYVE26*	SPG43	*C19orf12*	SPG57	*TFG*	SPG71	*ZFR*
SPG17	*BSCL2*	SPG44	*GJC2*	SPG58	*KIF1C*	SPG72	*REEP2*

c.1246C>T was identified which was confirmed by Sanger sequencing (Fig. 3.3c). This variant had been reported previously as pathogenic in HSP families [7]. Further sequencing demonstrated that the proband's mother had the same heterozygous variant in *ATL1*. Therefore, the patient was diagnosed with HSP.

Discussion

Hereditary spastic paraplegias (HSP), also termed spastic paraplegias (SPG), is a genetically and clinically heterogeneous group of neurological disorders which is commonly characteristic with progressive spasticity, extremities weakness, and some dorsal column impairment [8]. Postmortem studies of HSP patients indicated a length-dependent degeneration of the longest axons in the corticospinal tract [9]. HSP is clinically divided into two forms, pure HSP and complex HSP. Besides the corticospinal signs, other neurological signs may present in complicated HSP, such as ataxia, cognitive impairment, epilepsy, peripheral neuropathy, thin corpus callosum, and optic atrophy.

Up to now, at least 55 causative genes have been found to be associated with HSP [6, 8]. The transmission modes of HSP include autosomal-dominant (AD), autosomal-recessive (AR), X-linked, and maternal trait of inheritance [10]. Due to the genetic heterogeneity of HSP, it is difficult for the neurologist to detect all of the candidate genes to make a molecular diagnosis. Targeted next-generation sequencing, a high-throughput DNA sequencing technology that performs parallel sequencing of the genomic regions of interest, makes it possible to sequence thousands of genes [11]. Using this technology, we quickly detected the causative gene in this family.

Spastic paraplegia 3 (SPG3) is one of the most frequent autosomal-dominant type of HSP and is related to *ATL1* gene on chromosome 14q12-q2 [12]. The age of onset of SPG3 is around 4 years old and is rarely later than that age [13, 14]. Most SPG3 patients have a pure HSP phenotype [15]. Our patient also displayed a pure form HSP but with a late age of onset. The signs of vibration sensation deficits at the ankles and urinary sphincter hyperactivity were less frequently happened in SPG3. Accordingly, our patient did not show these two symptoms.

Treatment for HSP is exclusively symptomatic. Fortunately, the progression of HSP is slow, and wheelchair dependency is relatively rare. The goal of symptomatic treatment is to improve mobility and relieve the discomfort associated with spasticity. Medical therapy of spasticity may begin with oral baclofen. When the oral drugs are useless, intramuscular injections of botulinum toxin can be further under consideration. Moreover, the physical therapy concentrated on strengthening exercises should be combined with the pharmacotherapy [16, 17]. Genetic counseling for patients and their family is considerable.

3.3 Spinal Muscular Atrophy (SMA)

A 31-Year-Old Male with Progressive Muscle Weakness and Atrophy

Clinical Presentations

A 31-year-old man admitted to our hospital presented with progressive lower and upper limbs' muscular weakness for 20 years and aggravated these 5 years. He noted lower limbs' muscle weakness 20 years ago, manifested with difficulty in raising the stairs and running. This manifestation was progressive gradually, and the upper limbs also involved. During the last 5 years, these manifestations significantly aggravated, and now he is having difficulty in walking and cannot raise his arms, complicated with muscle atrophy in his upper and lower limbs. Medical history was unremarkable.

Neurological examinations revealed that cranial nerves were negative, without tongue muscle atrophy and fasciculation. Muscle atrophy was observed in his proximal limbs. Manual muscle testing (MMT, 0–5 grade) showed weakness of neck muscle (5- grade), upper proximal muscles (4 grade), and lower proximal muscles (2 grade), and the distal limb muscle strength was 5 grade. His muscular tension was decreased. All his upper and lower limbs' deep tendon reflexes were disappeared. Hoffmann, Babinski, and Romberg signs were bilaterally negative. His deep and superficial sensation examinations were symmetric and normal.

The blood routine examination was normal. Aspartate amino transferase (AST) was 55 U/L (normal range 0–50 U/L). Serum creatine kinase (CK) was 1435 U/L (normal range 55–170), and isoenzymes of creatine kinase (CK-MB) was 78 U/L (normal range <25 U/L). Serum lactate dehydrogenase (LDH) was 823 U/L (normal range 313–618 U/L). ECG test was normal without arrhythmia. Lung function test showed that FVC was 91.8%. The brain MRI disclosed unremarkable findings.

Interestingly, one of his uncles also presented with similar manifestations (Fig. 3.4a). He was 59 years old and noted lower limbs' muscle weakness at his age of 4. The muscle weakness progressed gradually as well as the upper limbs. At age of 30, he was unable to walk without assistant and needed crutch when walking. He was wheelchair dependent when he was 50 years old. Neurological examinations showed cranial nerves were negative. Muscle atrophy was observed, especially in his proximal limbs. MMT showed weakness of neck muscle (4 grade), upper proximal muscles (0–1 grade), upper distal muscles (3 grade), and lower limbs' muscles (0 grade). His muscular tension was decreased. All limbs' deep tendon reflexes were disappeared. Hoffmann and Babinski signs, as well as sensation examinations, were normal.

Primary Diagnosis

The patient showed muscle weakness and atrophy at all of the limbs, especially the proximal muscles. The muscle tension and deep tendon reflexes were disappeared, without Hoffmann and Babinski signs. The CK level was mildly increased. These symptoms, signs, and investigations hint the impairment of peripheral nervous system, and the localization of lesion should be considered from muscle, neuromuscular joint, to lower motor neurons.

The muscle weakness and atrophy was childhood onset, with a progressive course and positive family history, all of which implied that hereditary neurological diseases should be considered first for this patient, such as progressive muscular dystrophy (PMD), spinal muscular atrophy (SMA), and Kenney's disease (KD).

Additional Tests or Key Results

Electromyography (EMG) test revealed extensive neurogenic lesions, without conduction block. After informed consent, the proband also performed a muscle biopsy, the result of which further confirmed the neurogenic lesions (Fig. 3.4b). The childhood-onset progressive muscular weakness and atrophy, decrease muscle tension, and deep tendon reflexes, combined with neurogenic lesions from EMG and histopathological tests, strongly suggested the diagnosis of SMA. Thus, genetic test of survival of motor neuron 1 gene (*SMN1*) was carried out by poly-

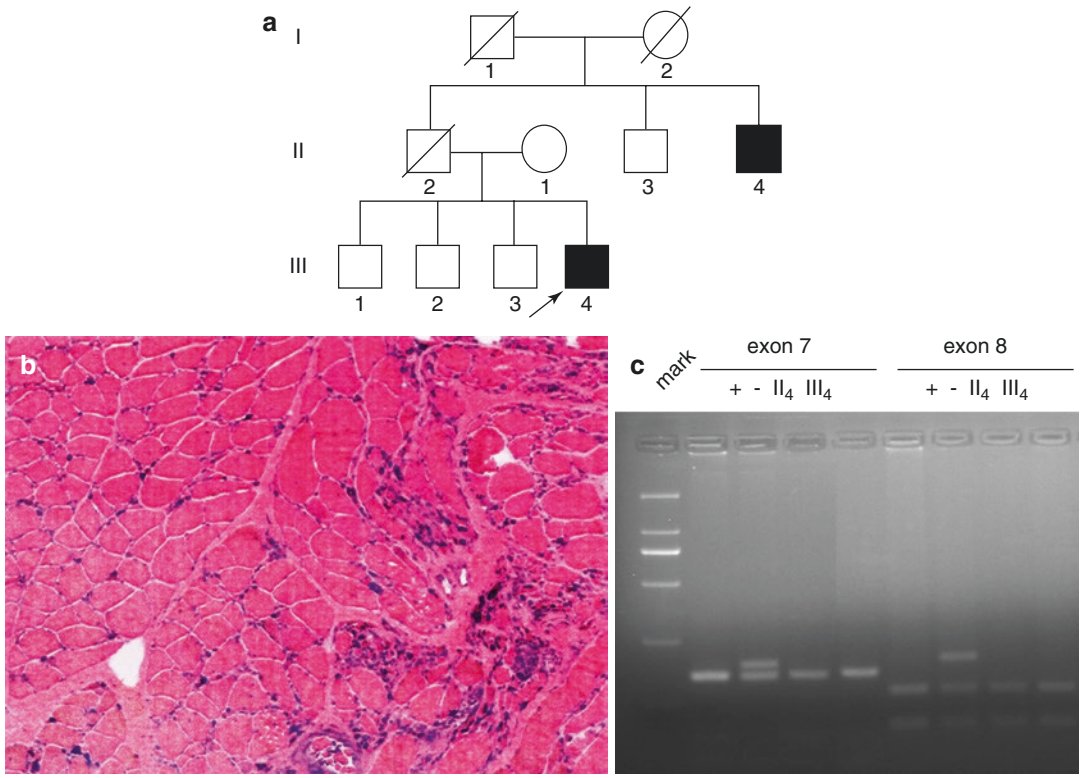

Fig. 3.4 (**a**) The patient's pedigree chart. *Arrow* indicates the proband; *square*, males; *circle*, females; *filled symbol*, affected individual; *diagonal lines* across symbol, deceased individual. (**b**) Muscle biopsy pathological examination showed neurogenic lesions by HE staining. (**c**) Detection of *SMN1* and *SMN2* genes by PCR-RFLP. Both the proband (III_4) and his uncle (II_4) possessed the homozygous deletion of exons 7 and 8 in *SMN1* gene. *Plus*, positive control. *Minus*, negative control

merase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Both the proband and his affected uncle carried the similar homozygous deletion of exon 7 and exon 8 (Fig. 3.4c).

Discussion

Childhood-onset SMA is an autosomal-recessive neurological disorder in humans with a high frequency of 1.4/10,000 and a carrier frequency of 1/42 in the mainland of China [18]. It is featured by selective degeneration of motor neurons in the spinal cord and progressive muscular weakness and atrophy. Based on onset age and motor function achieved, childhood-onset SMA can be classified into three types, SMA I–III [19]. Patients with SMA I, the most severe type, usually develop muscular weakness before 6 months and die within the first 2 years because of respiratory failure. Patients with SMA II, the intermediate type, usually show onset after 6 months. Patients can sit but not walk without help, and their life span is significantly reduced. Patients with SMA III, the mild type, show onset after 18 months. They are able to walk but not run, and they become wheelchair bound during adulthood.

The causative gene, *SMN1*, was identified in 1995 [20]. Interestingly, survival of motor neuron 2 gene (*SMN2*), a highly identical homolog to *SMN1* in 5q13, modifies the disease severity and represents a promising therapeutic target for SMA now. *SMN1* and *SMN2* are highly identical, and the main difference is a C to T substitution at the 840 position of exon 7, which alters a Dra I enzyme site. Besides, a G to A substitution at the 236 position of exon 8 also creates a Dde I enzyme site. The Dra I and Dde I enzyme sites could be used to distinguish *SMN1* and *SMN2* by PCR-RFLP technology.

In this SMA family, the proband and his affected uncle showed their first symptoms at the age of 10 and 4, respectively, indicating that they were type III SMA patients. Besides, both of them showed typically clinical features for SMA, including progressive muscle weakness and atrophy, especially the proximal limbs, disappeared deep tendon reflexes, neurogenic lesions in EMG, and histopathological tests, as well as the deletion of *SMN1* gene. However, they possessed a similar disease course. Previously, we reported two rare families with two SMA patients in two continuous generations, but their symptoms were significantly different, which correlated with the copy number of *SMN2* gene [21]. In conclusion, this is a typical SMA pedigree, and we hope it will facilitate the understanding and diagnosis of SMA for clinical neurologists.

To date, no effective treatment is available for SMA. Recently, a set of drugs rendering the inclusion of exon 7 in *SMN2* gene were under investigated, including histone deacetylase inhibitors (HDACi), hydroxyurea, ceftriaxone, antisense oligo, and new synthetic compounds [22–24]. Weihl et al. [25] reported seven type III/IV SMA patients who were treated with valproate (VPA) and showed an increase of muscle strength and subjective function. However, in the following several large clinical trials, VPA or VPA plus L-carnitine failed to improve muscle strength or motor abilities in SMA patients [26–29]. The induced pluripotent stem (iPS) cell technology and CRISPR/Cas9 technology may be the new treatments for SMA in the future to achieve the cell replacement and gene correction [30–32].

3.4 Familial Amyloidotic Polyneuropathy (FAP)

A 45-Year-Old Female with Progressive Paresthesia and Limb Muscle Atrophy

Clinical Presentations

The patient was a 45-year-old woman who had progressive paresthesia for 4 years and weakness for 2 years in her four limbs. She developed stabbing, burning pain, and numbness at her pelma at age 41. The symptoms progressed slowly and extended up to knees in the next two years. Weakness and atrophy in her bilateral lower extremities and numbness in two hands were found afterward. She needed aid to walk and suffered from orthostatic hypotension, dry skin, and skin ulcer when she was transferred to our clinic at age 45. On further questioning, she reported visual problem since the age of 43. She was diagnosed with peripheral neuropathy in local hospital. Methycobal and idebenone were administrated in the course of disease, but no effect was found in stopping the progression of the disease. Her condition continually worsened. Her developmental milestone was unremarkable. The patient had no past history of chronic diseases, such as diabetes and hypertension. She had no history of neurotoxin exposure. The patient's nephew, sister, father, uncles, aunts, and grandfather had similar symptoms (Fig. 3.5).

Physical examinations showed orthostatic hypotension (blood pressure 120/80 mmHg in the supine position and 80/60 mmHg upon standing). Neurological examinations revealed weakness, atrophy (Fig. 3.6), and generalized areflexia in the four extremities. Muscle power examination revealed normal or weakness of the following muscle group: neck flexion, Medical Research Council (MRC) grade 5/5; shoulder

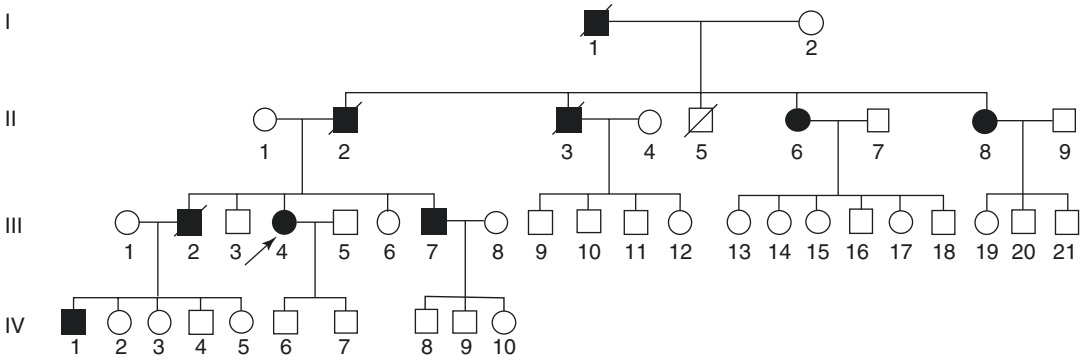

Fig. 3.5 The family pedigree of the patient. *Square* indicates male; *circle*, females; *empty symbol*, unaffected individual; *filled symbol*, affected individual; *arrow*, proband; *diagonal line* across symbol, deceased individual. I_1 died at age 65 with symptoms of FAP since age of 60. II_2 died at age 54 with symptoms of FAP since age of 45. III_2 died at age 52

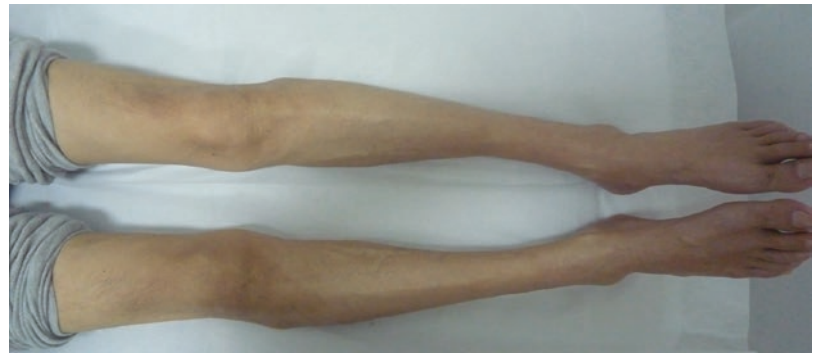

Fig. 3.6 Lower limbs muscle atrophy of the patient. The lower part of legs looks like "stork legs" or "inverted champagne bottle"

abduction, MRC grade 4/5; wrist flexion and extension, MRC grade 3/5; hip flexion, MRC grade 3/5; ankle flexion and extension, MRC grade 2/5. Sensation examination revealed pinprick and temperature sensations were absent, and vibration and proprioception sensations were decreased. Babinski sign was negative.

The patient had normal laboratory studies, including full blood count, blood biochemical tests, vitamin B12 and folate levels, thyroid function tests, parathyroid and testosterone hormone levels, and autoantibodies and paraneoplastic antibodies test. Nerve conduction studies displayed normal motor nerve conduction but reduced sensory nerve action potential (SNAP) in the upper limbs and absent SNAP and reduced compound muscle action potentials (CMAP) in the lower limbs, suggestive of a distal axonal neuropathy or length-dependent, axonal type sensorimotor peripheral neuropathy. Ophthalmoscope after pupil dilation and eye ultrasound revealed vitreous opacity in bilateral side.

Primary Diagnosis

Progressive paresthesia and weakness in four extremities as the main features of this patient, reduced muscle power, decreased superficial and deep sensation and generalized areflexia in neurological examinations, and abnormal results in nerve conduction study suggest the impairment of peripheral nervous system. Orthostatic hypotension, dry skin, and skin ulcer suggest autonomic nervous system involvement. Visual problem and vitreous opacity in ophthalmoscope and eye ultrasound examination suggest extranervous system involvement. Level diagnosis was thus located in peripheral nervous system, autonomic nervous system, and extra-nervous system. Based on the autosomal-dominant inheritance of her family, hereditary motor and sensory neuropathy should be considered. However, the rapid progress course did not support this disease. Combined with obvious autonomic dysfunction and vitreous opacity, familial amyloid polyneuropathy (FAP) should be considered. In the three main types of FAP, transthyretin-related FAP (TTR-FAP) and apolipoprotein A-1 FAP could cause a nerve length-dependent polyneuropathy. But the length-dependent polyneuropathy is not the predominant feature of apolipoprotein A-1 FAP, which induced the major organ damage preferably. Therefore, TTR-FAP could be the first diagnosis. To make it clear, nerve biopsy and genetic screening of *TTR* gene should be conducted.

Additional Tests or Key Results

Biopsy of sural nerve showed the presence of extracellular amyloid deposits in the endoneurial space, which confirmed our hypothesis (Fig. 3.7). The entire coding sequence and the exon/intron boundaries of *TTR* gene were sequencing. Mutation c.145A>G (p.T49A) were detected in the patient and his affected relatives, which had been reported as deleteriously before (Fig. 3.8). This result further confirmed the diagnosis of FAP.

Discussion

TTR-FAP is an autosomal-dominant inherited disease related to mutations within the *TTR* gene. The mutations in *TTR* result in misfolding of the protein and the formation of amyloid fibrils. Insoluble amyloid fibrils can deposit in multiple organs and tissues, which leads to sensorimotor neuropathy, autonomic neuropathy, cardiomyopa-

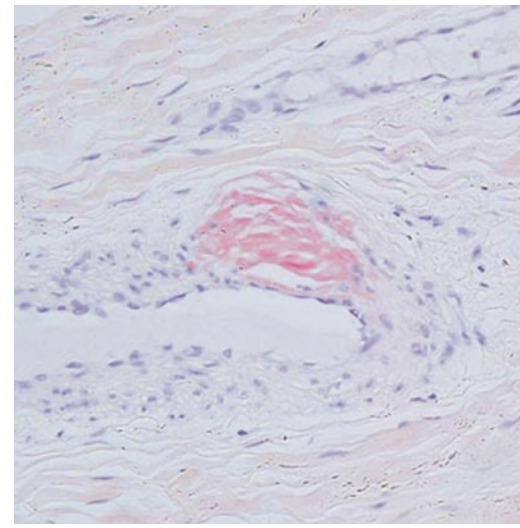

Fig. 3.7 Sural nerve biopsy from the patient. Congo red staining verifies the presence of amyloid in the endoneurium and around an endoneurial vessel

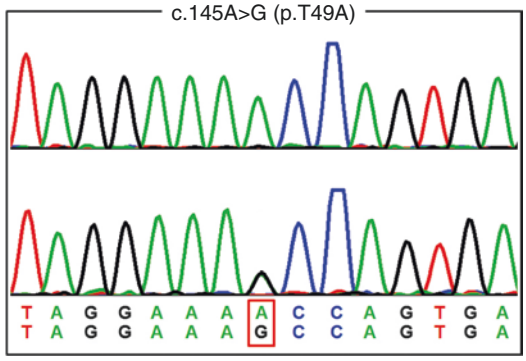

Fig. 3.8 Chromatogram of the heterozygous c.145A>G (p.T49A) mutation within *TTR* gene

thy, nephropathy, leptomeningeal amyloidosis, and vitreous opacities [33]. A wide clinical spectrum and considerable phenotypic heterogeneities make the diagnosis challenging. Therefore, many patients lose therapeutic opportunities, and the median survival time is about 10 years in endemic areas, even shorter in other places [34]. TTR-FAP is often fetal, devastating, and irreversible.

As in this patient, length-dependent sensorimotor axonal polyneuropathy is the typical peripheral nerve deficit trait of FAP. It is firstly involved in unmyelinated nerve fibers, then small myelinated nerve fibers, and large myelinated nerve fibers. Clinically, neurological deficits progress in a direction from feet to ankle, lower leg, thigh, fingers, fore arm, and anterior trunk. Generally, symptoms start with impaired pain and thermal sensation, followed by abnormality of light touch, deep sensation, and motor fiber deficits. So numbness and pain of the feet should be the first sign, which usually is neglected by the patients and their doctors. Other than that, it can't be detected by the routine conduction studies.

TTR-FAP should be considered highly, if a patient has a positive family history, length-dependent sensorimotor axonal polyneuropathy, and symptoms of amyloid deposits in extra-neurological organs. However, some patients present as sporadic TTR-FAP because of late onset and low penetrance. These patients should differentiate with CIDP because of high CSF protein level, differentiate with diabetic polyneuropathy because of length-dependent sensorimotor polyneuropathy or early small fiber deficit, and differentiate with toxic neuropathy because of axonal neuropathy. In a previous study, 18 in 90 nonfamilial cases were misdiagnosed and treated as CIDP [35]. For these intractable cases, nerve biopsy and *TTR* gene screening is mandatory to make a differential diagnosis.

For the patients with TTR-FAP, symptomatic treatments are necessary and can provide immediate relief, such as treating neuroglia with gabapentin, treating gastroparesis with domperidone, and treating vitreous opacity with vitrectomy [36]. However, the key to treatment is to stop the successive amyloid deposition. In this file, the liver transplantation is the standardized treatment strategy, which could eliminate the production of the mutant TTR and halt the polyneuropathy progression. But the liver transplantation has no effect to cardiomyopathy and ocular amyloidosis and need to be done in the early course of FAP [37]. Otherwise, TTR stabilizer tafamidis, which was approved by European medical agency, is another option for the patients in the early stage [38]. Gene therapies also show great promise [36]. But patients in the current study were complicated with severe motor deficits when they were diagnosed, and all the modifying treatments were not eligible.

3.5 Charcot-Marie-Tooth Disease (CMT)

A 39-Year-Old Male with Leg Weakness and Kyphoscoliosis

Clinical Presentations

A 39-year-old Chinese man presented with one year history of bilateral distal leg weakness. Over the previous year, it was difficult for him to climb the stairs. He always experienced slippers fall off when walking over uneven surfaces. He found his both legs were progressively becoming thinner. The symptoms did not show a fluctuating course. There was no history of joint pain, numbness, headache, cognitive problems, hearing impairment, eyelid drooping, swallowing difficulty, or shortness of breath. He said he was not a good runner and was unable to keep up with his peers during childhood. Since he was 10 years old, his parents noticed that he had kyphoscoliosis. He had no other medical problems and was not taking any medications. No family history of neurologic disease was recorded (Fig. 3.9a).

Neurological examinations showed symmetrical weakness of ankle dorsiflexion (Medical Research Council [MRC] grade 4/5) in the lower extremity. He had moderate atrophy of distal muscle of the lower limbs. Tendon reflex in all limbs were absent. He also had claw hands, pes cavus with hammer toes, and kyphoscoliosis (Fig. 3.9b). The sensation of pinprick and vibration were reduced below both knees. The hand and the foot were cold and wet. Cognition, cranial nerves, and cerebellar functions were normal. No pathological reflexes were found.

The laboratorial studies revealed that the C-reactive protein, erythrocyte sedimentation rate, creatine kinase, vitamin B12, folate, thyroid function, immunoglobulins, and electrolytes

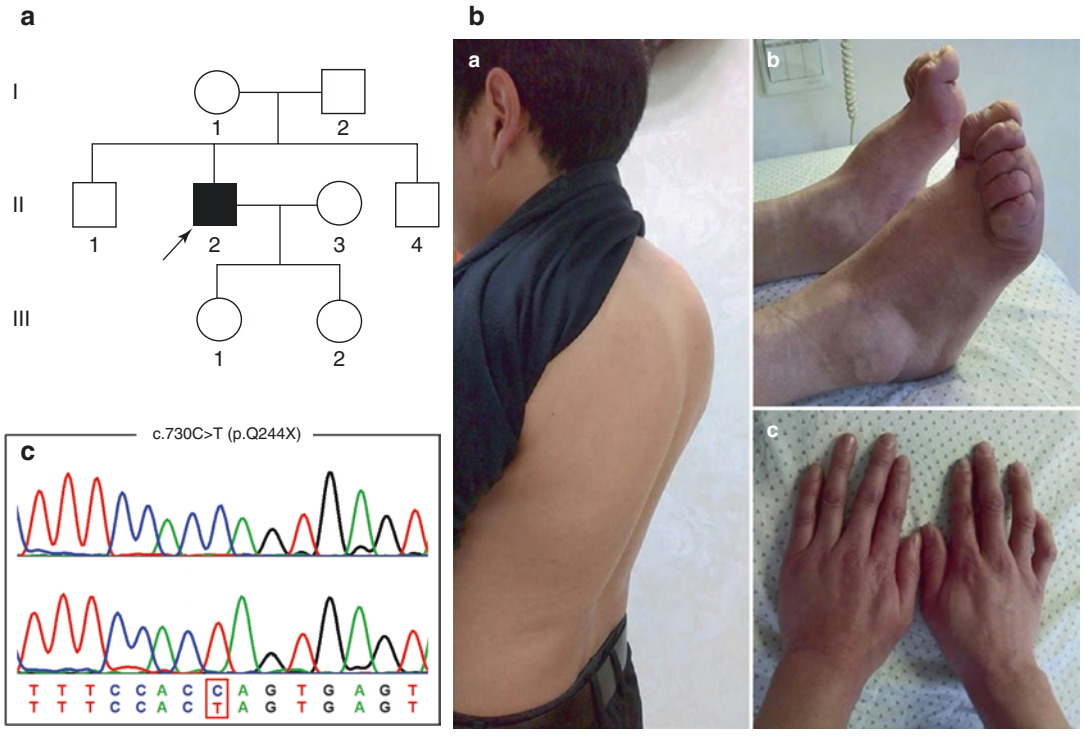

Fig. 3.9 The pedigree, clinical manifestation, and sequencing analyses of the patient. (**a**) Pedigree of the patient. The index is indicated by the *arrow*. The *black box* indicates the affected patient. (**b**) Clinical manifestation of the patient: scoliosis (*a*), foot deformity (*b*), and claw hand (*c*). (**c**) The sequencing chromatograms showing a homozygous variant in *SH3TC2*. The substitution (C > T) at base 730 results in a stop signal (TAG) at codon 244 (p.Q244X) in *SH3TC2*

were all within normal range. Serology for HIV, hepatitis, and syphilis were negative. The antineutrophil cytoplasmic and antinuclear antibodies were also negative.

Primary Diagnosis

The signs of symmetrical distal motor weakness, generalized areflexia, sensation impairment in a distal stocking pattern, and negative Babinski sign hint the involvement of peripheral nerves. Diseases that affect the peripheral nerves should be differentiated, such as chronic acquired neuropathies and inherited polyneuropathy. Diabetes is one of the most common causes of neuropathy, but our patient did not suffer from this problem. Moreover, no history of toxic contact and nutritional deficiency was reported. Systemic diseases like POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, skin changes) and systemic lupus erythematosus (SLE) exhibit symptoms of peripheral nerve involvement. Nevertheless, only a negligible number of patients display polyneuropathy before advanced stages of the disease. In our patient, the positive antinuclear antibodies, hepatomegaly, and skin changes were not observed. Hence, we did not consider these systemic diseases.

Further electrophysiological studies were necessary to confirm the deficit of peripheral nerves. Lumbar puncture with cerebrospinal fluid (CSF) analysis was required to exclude the chronic acquired neuropathies, such as chronic inflammatory demyelinating polyneuropathy (CIDP).

Additional Tests or Key Results

CSF analysis showed normal protein and cell counts. The IgG-oligoclonal bands were present in both CSF and serum. Electrophysiological examination revealed decreased motor nerve conduction velocities (MNCV) and absent sensory nerve action potentials (SNAP) in the tested nerves (Table 3.2).

According to the electrophysiological study, we can verify the impairment of peripheral nerves. The CSF examination is normal, helping to differentiate it from CIDP. The following characteristics of this patient may point to genetic neurological disorders: early age of onset, slowly progressive disease course, widespread slowing of conduction velocities, and presence of pes cavus and scoliosis. The differential diagnosis for inherited polyneuropathies includes Charcot-Marie-Tooth (CMT), familial amyloid polyneuropathy (FAP), Refsum's disease, and porphyric neuropathy.

FAP is featured by the predominant involvement of small diameter sensory and autonomic nerves and deposition of amyloid in various organs. The pattern of inheritance in all types is autosomal dominant. Sensory loss, pain, and autonomic changes are prominent in the disease. Cardiac enlargement and irregularities in cardiac rhythm have occurred in most patients. The diagnosis of Refsum's disease is based on a

Table 3.2 Electrophysiological study of the patient

Motor nerve conduction study								
	Median		Ulnar		Peroneal		Tibial	
Nerve	Right	Left	Right	Left	Right	Left	Right	Left
MNCV (m/s)	31	30	29	27	18	13	25	19
CMAP (mV)	4.484	8.323	6.270	6.849	0.336	0.083	0.550	0.771
Sensor nerve conduction study								
	Median		Ulnar		Radial		Sural	
Nerve	Right	Left	Right	Left	Right	Left	Right	Left
SNCV (m/s)	NP	NP	NP	NP	NP	NP	NP	NP
SNAP (μV)	NP	NP	NP	NP	NP	NP	NP	NP

MNCV motor nerve conduction velocity, *CMAP* compound motor action potential, *SNCV* sensory nerve conduction velocity, *SNAP* sensory nerve action potential, *NP* not potential

combination of clinical manifestations including retinitis pigmentosa, ataxia, and chronic polyneuropathy. Cardiomyopathy and neurogenic deafness are present in most patients. The most characteristics of porphyric neuropathy are the relapsing nature, acute onset, abdominal pain, psychotic symptoms, and predominant motor neuropathy. In our patient, normal color Doppler echocardiography and ECG examination were reported. Some typical symptoms, such as cardiac enlargement, ataxia, and abdominal pain, were not observed. Consequently, we did not primarily consider these inherited diseases.

Since CMT is the most common disease responsible for the inherited polyneuropathy, we pay our attention to this disease. The MNCV of the examined nerve was significantly decreased indicated the demyelinating type of CMT. As *PMP22* duplication/deletion was the major genetic cause for demyelinating CMT, we first carried out multiplex ligation-dependent probe amplification (MLPA) analysis to detect the copy number of *PMP22*. However, the result was negative. To make sure the causative gene responsible for this patient, we designed a gene panel covering 70 genes associated with CMT (Table 3.3). Then the targeted next-generation sequencing (NGS) was performed. After filtering, a homozygous variant (c.730C>T) caused a premature SH3TC2 protein (p.Q244X) was observed in the *SH3TC2* gene. This variant was verified by Sanger sequencing (Fig. 3.9c) and was not present in our 500 controls. On the basis of the American College of Medical Genetic and Genomics (ACMG) standard and guideline, this novel variant in *SH3TC2* was classified as pathogenicity [39]. Thus, the patient was finally diagnosed with CMT. Pathological examination was not tested as the patient refused nerve biopsy.

Discussion

CMT, also known as hereditary motor and sensory neuropathy, is the most common hereditary neuromuscular disorder. It is clinically characterized by progressive motor weakness and sensory abnormalities. The incidence of CMT was evaluated up to 1 in 2500 people. In light of the nerve conduction studies, CMT could be subdivided into three main groups: a demyelinating form (MNCV <38 m/s; CMT1 if autosomal dominant), an axonal form, and an intermediate form (MNCV lies between 25 and 45 m/s). Further subdivision of these CMT types is based mainly on causative genes [40]. All Mendelian inheritance modes are

Table 3.3 List of genes responsible for CMT and other hereditary peripheral neuropathy

No.	Gene	No.	Gene	No.	Gene	No.	Gene
1	*AARS*	19	*INF2*	37	*RAB7B*	55	*DST*
2	*BSCL2*	20	*KARS*	38	*SBF1*	56	*FAM134B*
3	*CCT5*	21	*KIF1B*	39	*SBF2*	57	*HSN1B*
4	*CTDP1*	22	*LITAF*	40	*SEPT9*	58	*IKBKAP*
5	*DHTKD1*	23	*LMNA*	41	*SH3TC2*	59	*KIF1A*
6	*DNM2*	24	*LRSAM1*	42	*SOX10*	60	*NGF*
7	*DYNC1H1*	25	*MED25*	43	*SURF1*	61	*NTRK1*
8	*EGR2*	26	*MFN2*	44	*TRPV4*	62	*SCN11A*
9	*FGD4*	27	*MPZ*	45	*YARS*	63	*SPTLC1*
10	*FIG4*	28	*MTMR2*	46	*DCTN1*	64	*SPTLC2*
11	*GARS*	29	*NDRG1*	47	*FBXO38*	65	*WNK1*
12	*GDAP1*	30	*NEFL*	48	*HSPB3*	66	*ALAD*
13	*GJB1*	31	*PDK3*	49	*IGHMBP2*	67	*CPOX*
14	*GNB4*	32	*PLEKHG5*	50	*REEP1*	68	*HMBS*
15	*HK1*	33	*PMP22*	51	*SLC5A7*	69	*PPOX*
16	*HOXD10*	34	*PRPS1*	52	*ATL1*	70	*TTR*
17	*HSPB1*	35	*PRX*	53	*ATL3*		
18	*HSPB8*	36	*RAB7A*	54	*DNMT1*		

described for CMT, and over 50 causative genes have been described to be related with CMT (http://www.molgen.ua.ac.be/CMTMutations/; http://neuromuscular.wustl.edu/).

The genetic diagnosis of CMT patient was performed according to the inheritance pattern, clinical phenotype, and neurophysiologic results. Our patient displayed a demyelinating form of CMT without positive family history. As *PMP22* is the most causative gene [41], the *PMP22* duplication/deletion analysis should be investigated first in patient with autosomal-dominant or sporadic demyelinating form of CMT.

Using targeted NGS, we identified a homozygous variant in *SH3TC2* in our patient. *SH3TC2* is the causative gene responsible for CMT type 4C (CMT4C) [42]. Both missense and nonsense mutations in the gene have been reported [43]. CMT4C is an autosomal-recessive demyelinating form of CMT. It is clinically manifested by early-onset demyelinating peripheral neuropathy frequently associated with spinal deformities and cranial nerves involvement. In the first decade of life, the CMT4C patient usually has severe spine deformities, such as scoliosis or kyphoscoliosis [44–46]. The progress of spine deformities is usually faster than motor deficits. Our patient showed slowly progressive motor impairments but had early onset of spinal deformities. In addition, the symptoms of cranial nerves deficits, such as hearing loss, dysphagia, diplopia, and vocal cord paresis, are found in most CMT4C cases [45]. However, our patient did not show these symptoms. Nerve biopsy is an effective approach to observe the myelin abnormalities in CMT4C patients.

Currently, there is no effective pharmacologic therapy for CMT. Fortunately, CMT4C patient shows a slowly progressive course. The treatment objectives for CMT are to enhance the quality of life and reduce deformities. Patients with CMT should be avoiding taking drugs that cause peripheral nerve toxicity. A series of trials using ascorbic acid for CMT1A has been carried out; however, these trails failed to show any benefit to CMT1A patients [47]. Many different approaches have been used to treat feet and spine deformities, including rehabilitative therapy and surgical treatment [40]. Surgery was required when the scoliosis cause respiratory difficulties. Genetic counseling for patients and their families is important.

3.6 Charcot-Marie-Tooth Disease Plus Acute Inflammatory Demyelinating Polyradiculoneuropathy (CMT + AIDP)

A 29-Year-Old Male with a More Than 20-Years History of Unsteadiness and Aggravated for One Month

Clinical Presentations

Mr. Xu is a 29-year-old shopkeeper who presented to us with a more than 20-years history of unsteady on his feet. He had noticed "heaviness" in his feet since he was a toddler and was easy to stumble while running. His symptoms progressed gradually very slow that did not affect his daily life until last month. His walking has deteriorated rapidly since last month, and he has to put a lot of effort to lift his feet, as if he is "walking with concrete blocks on," particularly while walking upstairs.

On examination, Mr. Xu's general systemic examinations, including pulse and blood pressure, were unremarkable. The cranial nerves were normal. Symmetrical distal atrophy was found in the hands and in the legs to knees level. Muscle tone was normal. Power examination revealed symmetrical weakness in the feet (MRC grade: 3/5) and throughout the hands (MRC grade: 4/5). Deep sensation including joint position sense and vibration sense were decreased in the legs to knee level, while light touch sensation and pain sensation were not impaired. Ankle and knee reflexes were absent, and other reflexes were decreased.

Primary Diagnosis

The history and examination findings suggested that there was a combination of sensory and motor symptoms, affecting the distal of all four limbs. Nerve conduction study revealed widespread slowing of conduction velocity in sensory and motor nerves, as shown in Table 3.4. This could be compatible with a length-dependent peripheral nerve disorder. Because of the early onset and very long history, hereditary peripheral neuropathy would be the first consideration, of which Charcot-Marie-Tooth disease (CMT) is most frequent. On further query, Mr. Xu reported that his father and uncle's legs seem to be thinner than normal, but there was no muscle weakness. This family history also argued in favor of the diagnosis of CMT.

Additional Tests or Key Results

However, the CSF analysis indicated an elevated protein level of 1.02 g/L (0.1–0.45 g/L). This increased protein level would not be produced by CMT, which always has a normal or slightly elevated protein level. At this point, we had to go back to our history collection. Did

Table 3.4 Nerve conduction study

Nerve	Stimulate point	Latency (ms)	Amplitude (mV)	Velocity (m/s)
Motor nerve conduction velocity (MNCV)				
Ulnar (R)	Wrist	9.5	2.4	
	Elbow	24.1	1.6	14.3
Median (R)	Wrist	14.7	3.0	
	Elbow	29.9	2.0	13.5
Peroneus (R)	Ankle	23.3	0.5	
	Capitula fibula	41.3	0.5	13.9
Tibial (R)	Medial malleolus	21.0	0.4	
	Popliteal fossa	51.2	0.3	11.6
Sensory nerve conduction velocity (SNCV)				
Superficial ulnar (R)	Pinkie	3.0	2.7	34.5
Superficial median (R)	Middle finger	3.7	1.4	35.1
Sural (R)	14 cm above heel	2.8	0.9	24.7
Superficial peroneus (R)	One third of leg	2.8	0.2	35.7

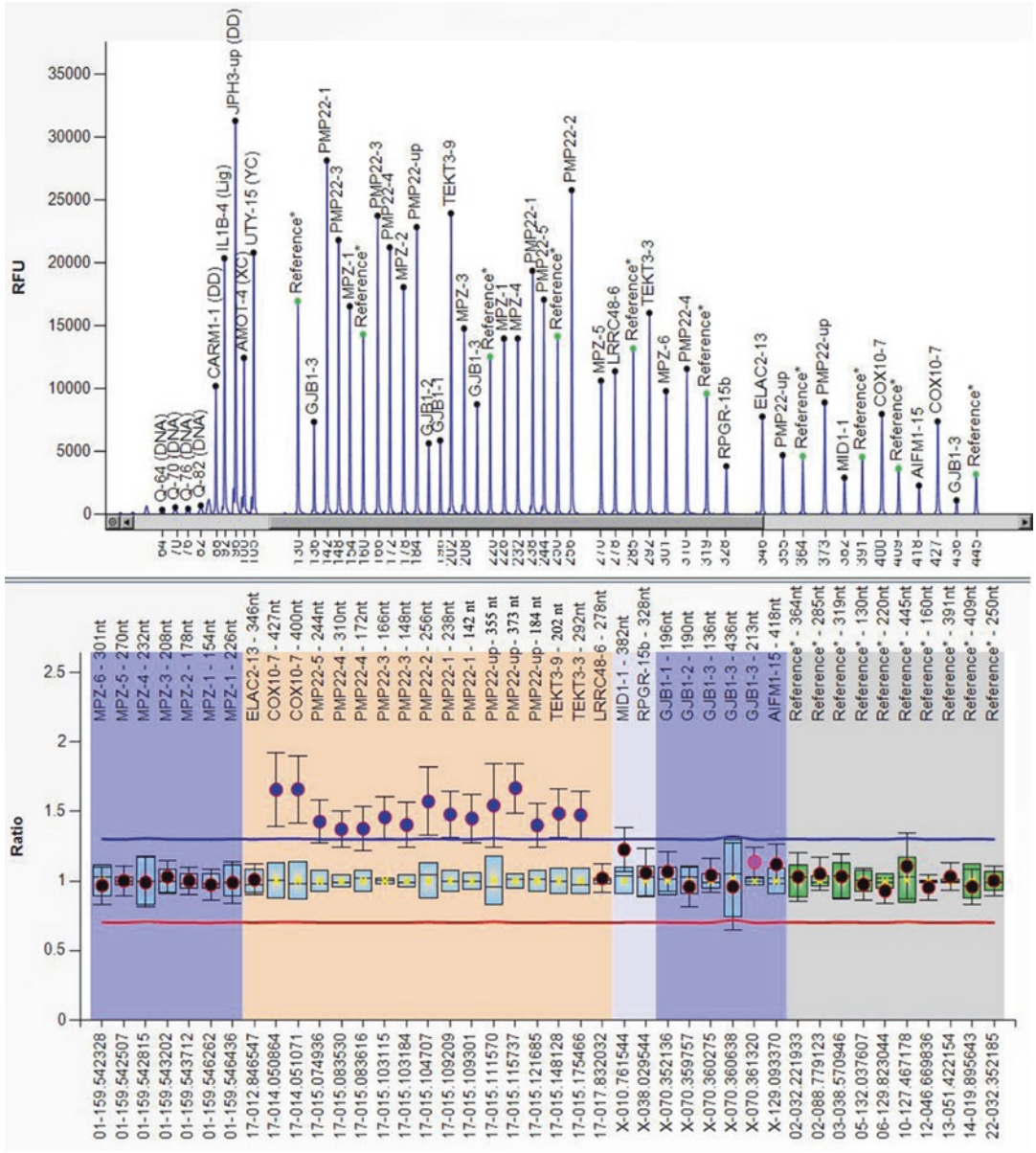

Fig. 3.10 Multiplex ligation-dependent probe amplification (MLPA) revealed the abnormal duplication of *PMP22* gene

Mr. Xu really have a 20-year disease history? Was the family history reliable? Was it possible that his unsteadiness only developed for the latest month and the long disease duration as well as the family history was our misleading? If there was only one month's history, the diagnosis of acute inflammatory demyelinating polyradiculoneuropathy (AIDP) should be in the first order. However, the symmetrical distal atrophy of the limbs could not be explained by a one-month history.

PMP22 genotyping was requested and was positive (Fig. 3.10), which confirmed the diagnosis of CMT1A. However, did this mean that the disorder of CMT could cause an elevated CSF protein level? We revealed the relevant papers

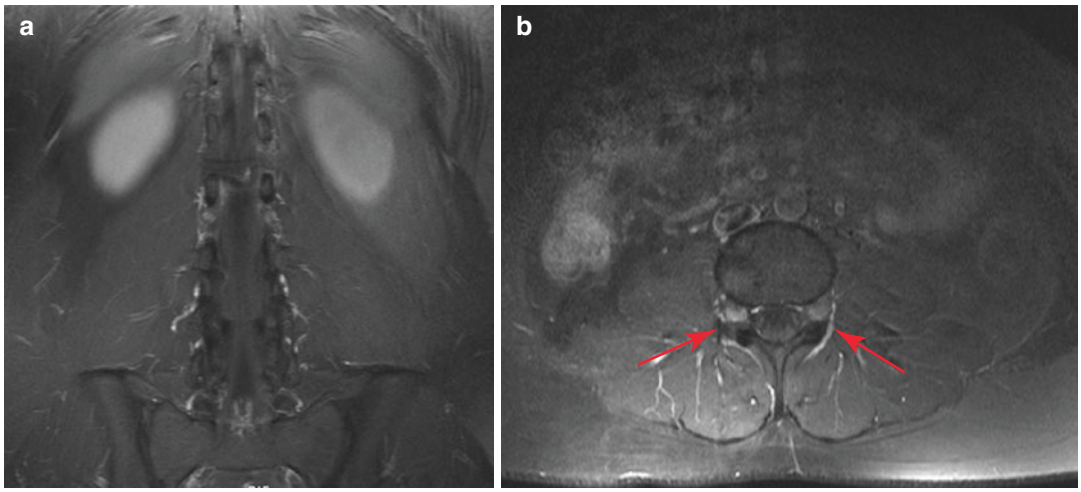

Fig. 3.11 MRI of the lumbosacral spine, coronal (**a**) and axial (**b**). T2-weighted images show thickened nerve roots that completely fill the dural sac. In (**b**), the *red arrow* heads indicate the hypertrophic ganglia

and found a few case reports of this phenomenon [48–51]. Most of these patients presented with hypertrophy of spinal roots, which was also seen in our patient (Fig. 3.11). Some researchers did a biopsy of the peripheral nerve, and a pathology that was compatible with AIDP/CIDP was found. Although we failed to persuade Mr. Xu to receive a nerve biopsy, his symptoms did alleviate a lot after the administration of steroids for several weeks. As a result, we hypothesize that there was an AIDP/CIDP superimposed on CMT1A, for the combination of a high CSF protein level with the recent worsening. However, we were unable to differentiate AIDP from CIDP because there was no confirmatory evidence in laboratory or morphologic findings.

Discussion

This patient showed chronic motor-sensory polyneuropathy since childhood and acute symptoms worsening for one month. His history and laboratory findings suggested AIDP/CIDP overlapping CMT1A. Both AIDP/CIDP and CMT have the distal muscle weakness and areflexia. However, in this case, the patient's early age of onset, the presence of muscle atrophy in lower limbs, and deformities were explained by CMT. Although the patient's inheritance mode was not clear, it suggested an autosomal dominance trait.

The occurrence of CMT1A overlapping with AIDP/CIDP has been reported in several cases. Most of them were genetically confirmed and all had an increased protein level in CSF. Besides, hypertrophy of spinal roots was also an important character.

Although CMT is a neural hereditary disease that with no effective therapy at present, AIDP/CIDP is a gained disorder that respond well to IVIG or glucocorticoid. As a result, we suggest that the phenomenon seen in our case should be recognized, and targeted therapy should be started up as early as possible to prevent the development of the disease.

3.7 Kennedy's Disease

A 51-Year-Old Male with Weakness of Limbs and Atrophy of Tongue

Clinical Presentations

A 51-year-old man visited our clinic for one-year difficulty of climbing stairs and weakness of holding stuffs. He also reported a progressively developing swallowing problem and a slurred speech within 3 months. He denied any seizures, vertigo, blurred vision, memory loss, and sensory abnormalities. Besides, he reported no recent illness, sick contacts, or travel abroad and family history of neuromuscular disorders.

On neurological examinations, his vital signs were notable for an obvious atrophy of tongue muscle, weakness of neck muscle, and mild to moderate atrophy and weakness of limb muscles. His strength of both proximal upper limbs was 4/5 per Medical Research Council (MRC) scale; strength of both distal upper limbs was 5−/5 per MRC scale. His strength of both proximal lower limbs was 3/5, and strength of distal lower limbs was 4/5. The patient's bilateral tendon reflexes were decreased. Sensation was grossly intact. The rest neurological examinations were normal apart from his breast development (gynecomastia).

His cervical MRI revealed slightly bulging of disk C3/4 and C6/7. Blood tests revealed a significantly elevated creatine kinase (CK) up to 672 U/L (reference range 38–174 U/L) and a slightly elevated lactate dehydrogenase (LDH). The rest imaging and lab tests including cerebrospinal fluid (CSF) tests were all negative.

Primary Diagnosis

The patient's weakness and atrophy of multiple muscles might hint the involvement of upper motor neuron, lower motor neuron, muscles, or peripheral nerves. The decreased tendon reflex and absence of pyramidal signs further narrowed the localization to lower motor neuron, muscles, and peripheral nerves. The atrophy of tongue muscle is more likely to be found in motor neuron disease (MND). However, the patient was a middle-aged male while displaying unusual breast development. Thus, a diagnosis of X-linked recessive spinal and bulbar muscular atrophy (SBMA) was strongly hinted. Meanwhile, other pure lower motor neuron disorders like spinal muscular atrophy (SMA) and peripheral nerve disorders especially acquired diseases including diabetic peripheral neuropathy (DPN) and chronic inflammatory demyelinating polyneuropathies (CIDP) might not be excluded. Additionally, considering patient's mild elevated serum CK, late-onset recessive inherited muscle disorders including some types of limb girdle muscular dystrophy (LGMD) might not be excluded either. Besides, in some stages of amyotrophic lateral sclerosis (ALS), patient's sign of upper motor neuron involvement might be absent. More precise diagnosis should rely on neurophysiological studies.

Additional Tests or Key Results

Electromyography (EMG) examination and nerve conduction study (NCS) were then carried out in this patient. The results are listed below (Table 3.5). The neurophysiological tests revealed a sporadic anterior spinal cord neuron involvement. However, the amplitude of sensory nerve action potential (SNAP) in sural nerve was also obviously decreased, hinted an involvement of sensory neuron or sensory nerve axon. The neurophysiological studies ruled out the possibilities of muscle and peripheral nerve diseases and narrowed down the diagnosis to motor neuron diseases. The involvement of sensory nerve is much more common in SBMA rather than ALS. Additional hormone tests and gene test for *AR* gene were further performed. The hormone tests demonstrated a significantly elevated serum estradiol (196.9 pmol/L, reference range in male: 28–156 pmol/L). The Sanger sequencing of the first exon of *AR* gene revealed a prolonged CAG expansion (52 repeats, Fig. 3.12).

Discussion

SBMA, also known as Kennedy's disease, is an X-linked recessive neurodegenerative disease. It's clinically characterized by slowly progressive weakness and atrophy of bulbar and proximal limbs muscles [52]. It is related to a prolonged

Table 3.5 Electromyography (EMG) and nerve conduction study (NCS) tests

Electromyography (EMG)						
		Spontaneous potentials				
Muscles (right)	Insertion potential	Fibrillation	Positive sharp wave	Fasciculation	MUP	Recruitment order
Tibialis anterior	N	1+	1+	N	>5 mV	Simple
Gastrocnemius caput medialis	N	N	1+	N	>5 mV	Simple
Vastus medialis	N	N	N	N	>5 mV	Simple
Interosseus dorsal I	N	N	1+	N	>5 mV	Simple
Flexor carpi radialis	N	N	N	N	>5 mV	Simple
Biceps	N	N	1+	N	>5 mV	Simple
Rectus abdominis	N	N	N	N	>5 mV	Simple
Trapezius	N	N	1+	N	>5 mV	Simple
Sternocleidomastoid	N	N	N	N	>5 mV	Simple
Glossus	N	N	N	N	Slightly abnormal	Simple-mixed
Masseter	N	N	N	N	Slightly abnormal	Interference
Nerve conduction study (NCS)						
Motor (right)	Latency (ms)	Amplitude (mV)	Distance (mm)	Velocity (m/s)	F-wave latency (ms)	
Median						
Wrist-APB	3.0	5.3	48		25.3	
Elbow-wrist	7.1	4.6	235	57.3		
Ulnar						
Wrist-ADM	3.3	8.6	53		27.9	
Below elbow-wrist	6.7	7.8	204	60.0		
Above elbow-below elbow	9.0	6.4	100	58.8		
Peroneal						
Ankle-EDB	4.4	0.8	60		NP	
Below knee-ankle	10.9	0.7	312	48.0		
Above knee-below knee	13.2	0.6	95	41.3		
Sensory (right)		Latency (ms)	Amplitude (mV)	Distance (mm)	Velocity (m/s)	
Median	Digitus III-wrist	2.4	30	128	68.1	
Ulnar	Digitus V-wrist	2.2	13	112	69.1	
Sural	Middle lower leg- lateral malleolus	2.9	2.2	120	52.2	

ADM abductor digiti minimi, *APB* abductor pollicis brevis, *EDB* extensor digitorum brevis, *NP* no potential

expansion of CAG repeats within exon 1 of androgen receptor (*AR*) gene on the X chromosome [53]. The European Federation of the Neurological Societies (EFNS) guideline indicated that an expansion beyond 38 repeats is pathogenic [54].

Even though SBMA is an inherited disorder, the report of family history remains uncommon in China [55]. In this case, the patient didn't recall any family history as well, which made the diagnosis of SBMA more difficult. The involvement of anterior spinal cord is a shared feature both in ALS and SBMA, which might easily lead to a misdiagnosis.

However, SBMA displays its identical features. Firstly, the progression of SBMA tends to

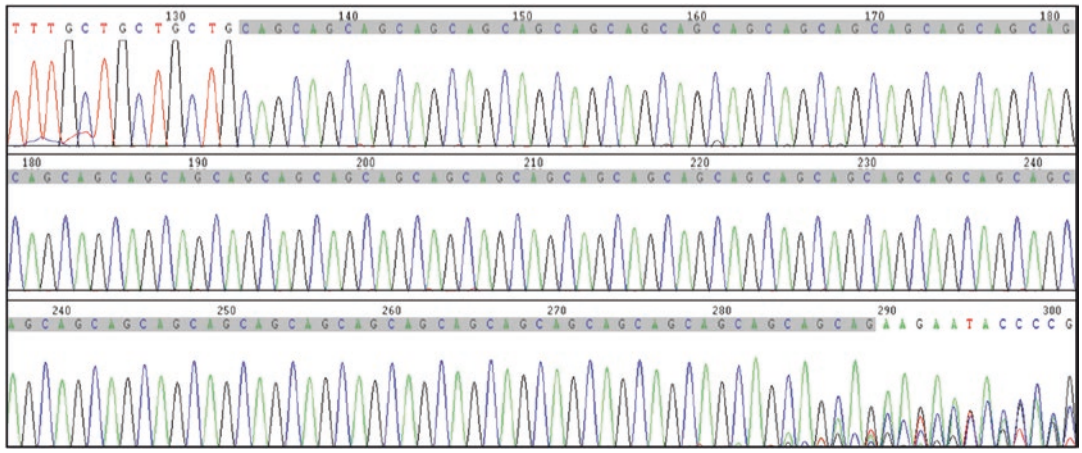

Fig. 3.12 Sequencing chromatogram of the expanded CAG repeats within *AR* in the index patient. The sequence highlighted with *gray* indicates expanded allele with 52 CAG repeats

be much slower. Patients suffered from SBMA usually live relatively long life before disabled. Moreover, it is not rare to identify muscles involvement in SBMA patients, since the expanded and misfolded polyglutamines can be seen everywhere in human body including muscles. Thus, it has been reported frequently that the serum CK in SBMA patients usually elevated [55–58]. The most important feature of SBMA is the reduction of SNAP amplitude in NCS tests. As was reported before, the reduction of SNAP amplitude can be recognized in most SBMA patients, especially in lower limbs [55, 57], which differ from ALS [59]. In this case, the patient's neurophysiological tests revealed a significantly decreased sural SNAP, which lead to further hormone and gene tests for SBMA.

Although no effective treatments have been established in SBMA so far, a few clinical trials have been accomplished. For instance, leuprorelin is a potent luteinizing hormone-releasing hormone (LHRH) analog which suppresses the release of gonadotrophins and reduces the level of testosterone generated by the testes. In 2003, Kasuno et al. [60] reported its improvement of motor function in SBMA transgenic mice. Later in 2010, a randomized and multicenter trial about leuprorelin treatment in SBMA patients was performed. Though the primary endpoint outcomes of this clinical trial failed to show efficacy, it did show improved swallowing function in patients with disease duration less than 10 years, suggesting that the disease duration might have influenced the results [61]. Additionally, the dutasteride trial conducted by Fernández-Rhodes and colleagues failed to prove an efficacy in the treatment of SBMA [62]. The clenbuterol trial found a significant improvement of mean 6-min walk test and forced vital capacity values, but the changes of outcome measures were not significantly different (e.g., ALSFRS-R scale) [63]. Indisputably, the understanding of the pathogenesis in SBMA is remarkably strong; the translation of mechanism into clinical application remains unsatisfactory. There's still a long way to go.

References

1. Brooks BR, Miller RG, Swash M, Munsat TL; World Federation of Neurology Research Group on Motor Neuron Diseases. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord. 2000;1(5):293–299.
2. Logroscino G, Traynor BJ, Hardiman O, Chiò A, Mitchell D, Swingler RJ, Millul A, Benn E, Beghi E; EURALS. Incidence of amyotrophic lateral sclerosis in Europe. J Neurol Neurosurg Psychiatry. 2010;81(4):385–390.
3. Chen S, Sayana P, Zhang X, Le W. Genetics of amyotrophic lateral sclerosis: an update. Mol Neurodegener. 2013;8:28.
4. Chesi A, Staahl BT, Jovičić A, Couthouis J, Fasolino M, Raphael AR, Yamazaki T, Elias L, Polak M, Kelly

C, Williams KL, Fifita JA, Maragakis NJ, Nicholson GA, King OD, Reed R, Crabtree GR, Blair IP, Glass JD, Gitler AD. Exome sequencing to identify de novo mutations in sporadic ALS trios. Nat Neurosci. 2013;16(7):851–855.
5. Wortmann SB, Espeel M, Almeida L, Reimer A, Bosboom D, Roels F, de Brouwer AP, Wevers RA. Inborn errors of metabolism in the biosynthesis and remodelling of phospholipids. J Inherit Metab Dis. 2015;38(1):99–110.
6. Lo Giudice T, Lombardi F, Santorelli FM, Kawarai T, Orlacchio A. Hereditary spastic paraplegia: clinical-genetic characteristics and evolving molecular mechanisms. Exp Neurol. 2014;261:518–539.
7. Magariello A, Tortorella C, Citrigno L, Patitucci A, Tortelli R, Mazzei R, Conforti FL, Ungaro C, Sproviero W, Gambardella A, Muglia M. The p.Arg416Cys mutation in SPG3a gene associated with a pure form of spastic paraplegia. Muscle Nerve. 2012;45(6):919–920.
8. de Souza PV, de Rezende Pinto WB, de Rezende Batistella GN, Bortholin T, Oliveira AS. Hereditary spastic paraplegia: clinical and genetic hallmarks. The Cerebellum. 2016;16:525–551.
9. Deluca GC, Ebers GC, Esiri MM. The extent of axonal loss in the long tracts in hereditary spastic paraplegia. Neuropathol Appl Neurobiol. 2004;30(6):576–584.
10. Finsterer J, Loscher W, Quasthoff S, Wanschitz J, Auer-Grumbach M, Stevanin G. Hereditary spastic paraplegias with autosomal dominant, recessive, X-linked, or maternal trait of inheritance. J Neurol Sci. 2012;318(1–2):1–18.
11. Li LX, Zhao SY, Liu ZJ, Ni W, Li HF, Xiao BG, Wu ZY. Improving molecular diagnosis of Chinese patients with Charcot-Marie-Tooth by targeted next-generation sequencing and functional analysis. Oncotarget. 2016; 7(19):27655–27664.
12. Zhao X, Alvarado D, Rainier S, Lemons R, Hedera P, Weber CH, Tukel T, Apak M, Heiman-Patterson T, Ming L, Bui M, Fink JK. Mutations in a newly identified GTPase gene cause autosomal dominant hereditary spastic paraplegia. Nat Genet. 2001;29(3):326–331.
13. Namekawa M, Ribai P, Nelson I, Forlani S, Fellmann F, Goizet C, Depienne C, Stevanin G, Ruberg M, Durr A, Brice A. SPG3A is the most frequent cause of hereditary spastic paraplegia with onset before age 10 years. Neurology. 2006;66(1):112–114.
14. Orlacchio A, Montieri P, Babalini C, Gaudiello F, Bernardi G, Kawarai T. Late-onset hereditary spastic paraplegia with thin corpus callosum caused by a new SPG3A mutation. J Neurol. 2011;258(7):1361–1363.
15. Durr A, Camuzat A, Colin E, Tallaksen C, Hannequin D, Coutinho P, Fontaine B, Rossi A, Gil R, Rousselle C, Ruberg M, Stevanin G, Brice A. Atlastin 1 mutations are frequent in young-onset autosomal dominant spastic paraplegia. Arch Neurol. 2004;61(12):1867–1872.
16. Ribeiro AM, Ferreira CH, Mateus-Vasconcelos ECL, Moroni RM, Brito LM, Brito LG. Physical therapy in the management of pelvic floor muscles hypertonia in a woman with hereditary spastic paraplegia. Case Rep Obstet Gynecol. 2014;2014:306028.
17. Fink JK. Hereditary spastic paraplegia: clinico-pathologic features and emerging molecular mechanisms. Acta Neuropathol. 2013;126(3):307–328.
18. Sheng-Yuan Z, Xiong F, Chen YJ, Yan TZ, Zeng J, Li L, Zhang YN, Chen WQ, Bao XH, Zhang C, Xu XM. Molecular characterization of SMN copy number derived from carrier screening and from core families with SMA in a Chinese population. Eur J Hum Genet. 2010;18(9):978–984.
19. Kolb SJ, Kissel JT. Spinal muscular atrophy: a timely review. Arch Neurol. 2011;68(8):979–984.
20. Lefebvre S, Bürglen L, Reboullet S, Clermont O, Burlet P, Viollet L, Benichou B, Cruaud C, Millasseau P, Zeviani M, Le D. Identification and characterization of a spinal muscular atrophy-determining gene. Cell. 1995;80(1):155–165.
21. Chen WJ, He J, Zhang QJ, Lin QF, Chen YF, Lin XZ, Lin MT, Murong SX, Wang N. Modification of phenotype by SMN2 copy numbers in two Chinese families with SMN1 deletion in two continuous generations. Clin Chim Acta. 2012;413(23-24):1855–1860.
22. Xu C, Chen X, Grzeschik SM, Ganta M, Wang CH. Hydroxyurea enhances SMN2 gene expression through nitric oxide release. Neurogenetics. 2011;12(1):19–24.
23. Nizzardo M, Nardini M, Ronchi D, Salani S, Donadoni C, Fortunato F, Colciago G, Falcone M, Simone C, Riboldi G, Govoni A, Bresolin N, Comi GP, Corti S. Beta-lactam antibiotic offers neuroprotection in a spinal muscular atrophy model by multiple mechanisms. Exp Neurol. 2011;229(2):214–225.
24. Naryshkin NA, Weetall M, Dakka A, Narasimhan J, Zhao X, Feng Z, Ling KK, Karp GM, Qi H, Woll MG, Chen G1, Zhang N, Gabbeta V, Vazirani P, Bhattacharyya A, Furia B, Risher N, Sheedy J, Kong R, Ma J, Turpoff A, Lee CS, Zhang X, Moon YC, Trifillis P, Welch EM, Colacino JM, Babiak J, Almstead NG, Peltz SW, Eng LA, Chen KS, Mull JL, Lynes MS, Rubin LL, Fontoura P, Santarelli L, Haehnke D, McCarthy KD, Schmucki R, Ebeling M, Sivaramakrishnan M, Ko CP, Paushkin SV, Ratni H, Gerlach I, Ghosh A, Metzger F. SMN2 splicing modifiers improve motor function and longevity in mice with spinal muscular atrophy. Science. 2014;345(6197):688–693.
25. Weihl CC, Connolly AM, Pestronk A. Valproate may improve strength and function in patients with type III/IV spinal muscle atrophy. Neurology. 2006;67(3):500–501.
26. Swoboda KJ, Scott CB, Crawford TO, Simard LR, Reyna SP, Krosschell KJ, Acsadi G, Elsheik B, Schroth MK, D'Anjou G, LaSalle B, Prior TW, Sorenson SL, Maczulski JA, Bromberg MB, Chan GM, Kissel JT; Project Cure Spinal Muscular Atrophy Investigators Network. SMA CARNI-VAL trial part I: double-blind, randomized, placebo-controlled trial of L-carnitine and valproic acid in spinal muscular atrophy. PLoS One. 2010;5(8):e12140.

27. Kissel JT, Scott CB, Reyna SP, Crawford TO, Simard LR, Krosschell KJ, Acsadi G, Elsheik B, Schroth MK, D'Anjou G, LaSalle B, Prior TW, Sorenson S, Maczulski JA, Bromberg MB, Chan GM, Swoboda KJ; Project Cure Spinal Muscular Atrophy Investigators' Network. SMA CARNIVAL TRIAL PART II: a prospective, single-armed trial of L-carnitine and valproic acid in ambulatory children with spinal muscular atrophy. PLoS One. 2011;6(7):e21296.
28. Darbar IA, Plaggert PG, Resende MB, Zanoteli E, Reed UC. Evaluation of muscle strength and motor abilities in children with type II and III spinal muscle atrophy treated with valproic acid. BMC Neurol. 2011;11:36.
29. Kissel JT, Elsheikh B, King WM, Freimer M, Scott CB, Kolb SJ, Reyna SP, Crawford TO, Simard LR, Krosschell KJ, Acsadi G, Schroth MK, D'Anjou G, LaSalle B, Prior TW, Sorenson S, Maczulski JA, Swoboda KJ; Project Cure Spinal Muscular Atrophy Investigators Network. SMA valiant trial: a prospective, double-blind, placebo-controlled trial of valproic acid in ambulatory adults with spinal muscular atrophy. Muscel Nerve. 2014;49(2):187–192.
30. Ebert AD, Yu J, Rose Jr FF, Mattis VB, Lorson CL, Thomson JA, Svendsen CN. Induced pluripotent stem cells from a spinal muscular atrophy patient. Nature. 2009;457(7227):277–280.
31. Marchetto MC, Winner B, Gage FH. Pluripotent stem cells in neurodegenerative and neurodevelopmental diseases. Hum Mol Genet. 2010;19(R1):R71–76.
32. Jang YY, Ye Z. Gene correction in patient-specific iPSCs for therapy development and disease modeling. Hum Genet. 2016;135(9):1041–1058.
33. Planté-Bordeneuve V, Said G. Familial amyloid polyneuropathy. Lancet Neurol. 2011;10(12):1086–1097.
34. Coelho T, Maurer MS, Suhr OB. THAOS-the transthyretin amyloidosis outcomes survey: initial report on clinical manifestations in patients with hereditary and wild-type transthyretin amyloidosis. Curr Med Res Opin. 2013;29(1):63–76.
35. Ikeda K, Kano O, Ito H, Kawase Y, Iwamoto K, Sato R, Sekine T, Nagata R, Nakamura Y, Hirayama T, Iwasaki Y. Diagnostic pitfalls in sporadic transthyretin familial amyloid polyneuropathy (TTR-FAP). Neurology. 2008;70(17):1576–1577.
36. Plante-Bordeneuve V. Update in the diagnosis and management of transthyretin familial amyloid polyneuropathy. J Neurol. 2014;261(6):1227–1233.
37. Yamashita T, Ando Y, Okamoto S, Misumi Y, Hirahara T, Ueda M, Obayashi K, Nakamura M, Jono H, Shono M, Asonuma K, Inomata Y, Uchino M. Long-term survival after liver transplantation in patients with familial amyloid polyneuropathy. Neurology. 2012;78(9):637–643.
38. Merkies IS. Tafamidis for transthyretin familial amyloid polyneuropathy: a randomized, controlled trial. Neurology. 2013;80(15):1444–1445.
39. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL, Committee ALQA. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405–424.
40. Pareyson D, Marchesi C. Diagnosis, natural history, and management of Charcot-Marie-Tooth disease. Lancet Neurol. 2009;8(7):654–667.
41. Watila MM, Balarabe SA. Molecular and clinical features of inherited neuropathies due to PMP22 duplication. J Neurol Sci. 2015;355(1–2):18–24.
42. Senderek J, Bergmann C, Stendel C, Kirfel J, Verpoorten N, De Jonghe P, Timmerman V, Chrast R, Verheijen MH, Lemke G, Battaloglu E, Parman Y, Erdem S, Tan E, Topaloglu H, Hahn A, Muller-Felber W, Rizzuto N, Fabrizi GM, Stuhrmann M, Rudnik-Schoneborn S, Zuchner S, Michael Schroder J, Buchheim E, Straub V, Klepper J, Huehne K, Rautenstrauss B, Buttner R, Nelis E, Zerres K. Mutations in a gene encoding a novel SH3/TPR domain protein cause autosomal recessive Charcot-Marie-Tooth type 4C neuropathy. Am J Hum Genet. 2003;73(5):1106–1119.
43. Lupo V, Galindo MI, Martinez-Rubio D, Sevilla T, Vilchez JJ, Palau F, Espinos C. Missense mutations in the SH3TC2 protein causing Charcot-Marie-Tooth disease type 4C affect its localization in the plasma membrane and endocytic pathway. Hum Mol Genet. 2009;18(23):4603–4614.
44. Lassuthova P, Mazanec R, Vondracek P, Siskova D, Haberlova J, Sabova J, Seeman P. High frequency of SH3TC2 mutations in Czech HMSN I patients. Clin Genet. 2011;80(4):334–345.
45. Piscosquito G, Saveri P, Magri S, Ciano C, Gandioli C, Morbin M, Di Bella D, Moroni I, Taroni FF, Pareyson D. Screening for SH3TC2 gene mutations in a series of demyelinating recessive Charcot-Marie-Tooth disease (CMT4). J Peripher Nerv Syst. 2016;21(3):142–149.
46. Azzedine H, Ravise N, Verny C, Gabreels-Festen A, Lammens M, Grid D, Vallat JM, Durosier G, Senderek J, Nouioua S, Hamadouche T, Bouhouche A, Guilbot A, Stendel C, Ruberg M, Brice A, Birouk N, Dubourg O, Tazir M, LeGuern E. Spine deformities in Charcot-Marie-Tooth 4C caused by SH3TC2 gene mutations. Neurology. 2006;67(4):602–606.
47. Micallef J, Attarian S, Dubourg O, Gonnaud PM, Hogrel JY, Stojkovic T, Bernard R, Jouve E, Pitel S, Vacherot F, Remec JF, Jomir L, Azabou E, Al-Moussawi M, Lefebvre MN, Attolini L, Yaici S, Tanesse D, Fontes M, Pouget J, Blin O. Effect of ascorbic acid in patients with Charcot-Marie-Tooth disease type 1A: a multicentre, randomised, double-blind, placebo-controlled trial. Lancet Neurol. 2009;8(12):1103–1110.
48. Liao JP, Waclawik AJ. Nerve root hypertrophy in CMT type 1A. Neurology. 2004;62(5):783.
49. Odaka M, Yuki N, Kokubun N, Hirata K, Kuwabara S. Axonal Guillain-Barre syndrome associated with axonal Charcot-Marie-Tooth disease. J Neurol Sci. 2003;211(1–2):93–97.
50. Pareyson D, Testa D, Morbin M, Erbetta A, Ciano C, Lauria G, Milani M, Taroni F. Does CMT1A

homozygosity cause more severe disease with root hypertrophy and higher CSF proteins? Neurology. 2003;60(10):1721–1722.
51. Vital A, Vital C, Lagueny A, Ferrer X, Ribiere-Bachelier C, Latour P, Petry KG. Inflammatory demyelination in a patient with CMT1A. Muscle Nerve. 2003;28(3):373–376.
52. Kennedy WR, Alter M, Sung JH. Progressive proximal spinal and bulbar muscular atrophy of late onset. A sex-linked recessive trait. Neurology. 1968;18(7): 671–680.
53. La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature. 1991;352(6330):77–79.
54. Burgunder JM, Schols L, Baets J, Andersen P, Gasser T, Szolnoki Z, Fontaine B, Van Broeckhoven C, Di Donato S, De Jonghe P, Lynch T, Mariotti C, Spinazzola A, Tabrizi SJ, Tallaksen C, Zeviani M, Harbo HF, Finsterer J. Efns. EFNS guidelines for the molecular diagnosis of neurogenetic disorders: motoneuron, peripheral nerve and muscle disorders. Eur J Neurol. 2011;18(2):207–217.
55. Ni W, Chen S, Qiao K, Wang N, Wu ZY. Genotype-phenotype correlation in Chinese patients with spinal and bulbar muscular atrophy. PLoS One. 2015;10(3): e0122279.
56. Atsuta N, Watanabe H, Ito M, Banno H, Suzuki K, Katsuno M, Tanaka F, Tamakoshi A, Sobue G. Natural history of spinal and bulbar muscular atrophy (SBMA): a study of 223 Japanese patients. Brain. 2006;129(Pt 6):1446–1455.
57. Rhodes LE, Freeman BK, Auh S, Kokkinis AD, La Pean A, Chen C, Lehky TJ, Shrader JA, Levy EW, Harris-Love M, Di Prospero NA, Fischbeck KH. Clinical features of spinal and bulbar muscular atrophy. Brain. 2009;132(Pt 12):3242–3251.
58. Querin G, Bertolin C, Da Re E, Volpe M, Zara G, Pegoraro E, Caretta N, Foresta C, Silvano M, Corrado D, Iafrate M, Angelini L, Sartori L, Pennuto M, Gaiani A, Bello L, Semplicini C, Pareyson D, Silani V, Ermani M, Ferlin A, Soraru G, Italian Study Group on Kennedy's Disease. Non-neural phenotype of spinal and bulbar muscular atrophy: results from a large cohort of Italian patients. J Neurol Neurosurg Psychiatry. 2015;87(8):810–816.
59. Hama T, Hirayama M, Hara T, Nakamura T, Atsuta N, Banno H, Suzuki K, Katsuno M, Tanaka F, Sobue G. Discrimination of spinal and bulbar muscular atrophy from amyotrophic lateral sclerosis using sensory nerve action potentials. Muscle Nerve. 2012;45(2):169–174.
60. Katsuno M, Adachi H, Doyu M, Minamiyama M, Sang C, Kobayashi Y, Inukai A, Sobue G. Leuprorelin rescues polyglutamine-dependent phenotypes in a transgenic mouse model of spinal and bulbar muscular atrophy. Nat Med. 2003;9(6):768–773.
61. Katsuno M, Banno H, Suzuki K, Takeuchi Y, Kawashima M, Yabe I, Sasaki H, Aoki M, Morita M, Nakano I, Kanai K, Ito S, Ishikawa K, Mizusawa H, Yamamoto T, Tsuji S, Hasegawa K, Shimohata T, Nishizawa M, Miyajima H, Kanda F, Watanabe Y, Nakashima K, Tsujino A, Yamashita T, Uchino M, Fujimoto Y, Tanaka F, Sobue G. Efficacy and safety of leuprorelin in patients with spinal and bulbar muscular atrophy (JASMITT study): a multicentre, randomised, double-blind, placebo-controlled trial. Lancet Neurol. 2010;9(9):875–884.
62. Fernandez-Rhodes LE, Kokkinis AD, White MJ, Watts CA, Auh S, Jeffries NO, Shrader JA, Lehky TJ, Li L, Ryder JE, Levy EW, Solomon BI, Harris-Love MO, La Pean A, Schindler AB, Chen C, Di Prospero NA, Fischbeck KH. Efficacy and safety of dutasteride in patients with spinal and bulbar muscular atrophy: a randomised placebo-controlled trial. Lancet Neurol. 2011;10(2):140–147.
63. Querin G, D'Ascenzo C, Peterle E, Ermani M, Bello L, Melacini P, Morandi L, Mazzini L, Silani V, Raimondi M, Mandrioli J, Romito S, Angelini C, Pegoraro E, Soraru G. Pilot trial of clenbuterol in spinal and bulbar muscular atrophy. Neurology. 2013;80(23): 2095–2098.