SENSORY NEUROPATHY IN X-LINKED RECESSIVE BULBOSPINAL NEURONOPATHY

Chun-Che Chu, Chin-Chang Huang, Hung-Chou Kuo, Chin-San Liu, and Ching-Shan Tsai

Abstract: X-linked recessive bulbospinal neuronopathy (X-BSN) is an adult-onset spinal and bulbar amyotrophy. Neurophysiologic studies demonstrate subclinical involvement of sensory nerves with diminished or absent sensory nerve action potentials and denervation changes, indicating the involvement of sensory neurons. We report the clinical features, findings of electrophysiologic study, and results of morphometric analysis of sural nerve pathology in a patient with X-BSN. Molecular genetic studies were also performed in the patient and his three daughters. Electrophysiologic studies revealed decreased amplitude sensory nerve action potentials and the presence of high amplitude motor unit potentials in all muscles tested. Sural nerve biopsy demonstrated axonal degeneration with a predominant loss of large myelinated fibers. Molecular genetic studies confirmed elongation of the CAG triplet repeats in exon 1 of the androgen receptor gene. Sequence analysis of the androgen receptor gene revealed that the number of CAG triplet repeats was 45 in the patient and was 45 to 48 in the mutant allele but only 19 to 30 in the normal allele in his three daughters. These findings suggest that both motor and sensory neurons are involved in X-BSN. Sural nerve biopsy and molecular genetic analysis are helpful in differentiation between X-BSN and other motor neuron diseases.

X-linked recessive bulbospinal neuronopathy (X-BSN), also known as Kennedy-Alter-Sung disease, is an adult-onset spinal and bulbar amyotrophy characterized by slowly progressive muscle weakness and atrophy, fasciculations in bulbar and proximal limb muscles, and hyporeflexia or areflexia [1–4]. Endocrine features such as gynecomastia, testicular atrophy, abnormal lipid metabolism, and essential tremor may occasionally occur [5–7]. Neurophysiologic studies often demonstrate subclinical involvement of sensory nerves with diminished or absent sensory nerve action potentials (SNAPs) and denervation changes, suggesting the involvement of sensory neurons in addition to bulbar and spinal motor neurons. The results of sural nerve biopsy are consistent with axonal atrophy and degeneration, with secondary demyelination [8–10]. An increased number of CAG repeats within the first exon of the androgen receptor (AR) gene have been identified as the putative cause of X-BSN [11]. Therefore, sural nerve biopsy and molecular genetic analysis have become important in distinguishing X-BSN from other forms of motor neuron diseases, and even in early detection of heterozygous female carriers [12, 13]. This report describes morphologic changes in the sural nerve and the results of molecular genetic studies in a family with X-BSN and correlates these findings with clinical manifestations and the results of electrophysiologic study.

Case Report

Clinical and family history

A 50-year-old man developed progressive limb weakness in all limbs over a 5-year period. He found that he could not lift...
dumbbells and dance as usual beginning 5 years prior to this hospital visit in 1998. He had become thinner in the last 3 years and noted progressive body weight loss, generalized weakness, and muscle wasting, especially in the proximal limbs. He had difficulty in raising his arms and rising from squatting. Neurologic examinations disclosed mild muscle weakness and wasting in all limbs with full muscle strength in distal limbs and grade 4/5 in proximal limbs according to the Scale of the Medical Research Council of Great Britain. Tendon reflexes were all absent. Sensory modalities including pin-pricks, temperature, touch, vibration, and position sensations were intact. Neither Romberg's sign nor sensory ataxia was noted. Fasciculations were noted in the perioral area, tongue, and extremities as well as mild postural hand tremor. There was neither obvious muscle cramping on exercise, dysarthria, nor dysphagia. Gynecomastia and testicular atrophy were also noted. Review of family history disclosed similar symptoms in his uncle, but no symptoms were found in his three daughters.

Biochemical data
Biochemical studies including serum concentrations of cholesterol, triglyceride, glucose, and glycohemoglobin, protein electrophoresis, and lipoprotein electrophoresis were all within normal ranges. Serum creatinine phosphokinase was elevated to 845 U/L (normal, 15–130 U/L) with 98.4% MM form and 1.6% MB form. Serum concentrations of prolactin, luteinizing hormone, follicular stimulating hormone, 17-estradiol, progesterone, testosterone, cortisol, triiodothyronine, tetraiodothyronine, thyroid stimulating hormone, and growth hormone were normal.

Electro-neurophysiologic findings
Nerve conduction studies demonstrated normal distal latencies, amplitudes of compound motor action potentials, and nerve conduction velocities in median, ulnar, peroneal, and tibial nerves, but decreased SNAP amplitudes in all sensory nerves tested [sural nerve, 7.5 µV (normal, > 10 µV); median nerve, 7.0 µV (normal, > 10 µV); ulnar nerve, 4.8 µV (normal, > 8 µV)]. Electromyographic studies showed increased amplitudes in motor unit potentials in the left flexor digitorum indicis, biceps, anterior tibialis, and vastus medialis. There were neither fibrillations nor positive waves. Somatic sensory evoked potential study revealed absence of waves in bilateral L2 responses and absence of P40 following right tibial nerve stimulation. These data indicated a peripheral sensory conduction defect in both legs and normal peripheral and central conduction from both hands.

Sural nerve biopsy
Sural nerve biopsy specimens were obtained from the left ankle in July 1998. Approximately 1.5 cm of the nerve fascicles was fixed in a 3% glutaraldehyde solution and divided into two segments, one for epoxy sections and the other for teased fiber preparation. Semi-thin sections of 0.6 μm were stained with toluidine blue and examined under a light microscope. The fiber sizes of each myelinated nerve fiber in these fascicles were measured in a morphometric analyzer (Leica Q500MC image processing and analysis system; Median Cybernetics, Silver Spring, MD, USA). Ultra-thin sections were stained with uranyl acetate and lead citrate and studied using a jeol JEM-200EXII electron microscope. About 100 nerve fibers were teased under a dissecting microscope and observed under a light microscope.

Sural nerve biopsy specimens consisted of five fasciculi. On light microscopic examination, transverse sections of the sural nerve revealed a loss of myelinated nerve fiber (MF), affecting predominantly the large MF population (Fig. 1). Some fibers had thin myelin suggesting remyelination. A few clusters of regenerating fibers were scattered around MFs. Neither interstitial changes nor thickened perineurium was seen.

Electron microscopic examination confirmed axonal degeneration with abnormal accumulation of neurofilaments and microtubules (Fig. 2). Remyelination features characterized by a relatively thin myelin sheath to the diameter of the axons were observed. Disruption of the myelin sheath with cytoplasmic debris was also observed. Unmyelinated fibers appeared relatively normal.

Morphometric study of the sural nerve showed a moderately reduced density in nerve fiber density (2,150–4,623/mm²; control, 6,000–10,000/mm²). A histogram of MFs showed a unimodal distribution with a left shift and a decreased amplitude of the peak of large MFs (Fig. 3). Preferential involvement of large MFs was also demonstrated by comparison of the density of small MFs (diameter < 7 µm) with the density of large MFs (diameter > 7 µm). Only 21.5% of MFs in the patient were large, while 43% of MFs in a normal sample taken from a 45-year-old man were large MFs. Teased fiber examination showed few fibers with segmental demyelination or remyelination.

Molecular genetic analysis
Genomic DNA was extracted from blood cells of the patient and his three daughters. Polymerase chain reaction (PCR) was performed as previously described [13] in a 100-µL reaction mixture containing 500 ng genomic DNA, 30 pmol of the forward primer 5'-TCCAGAATCTGTTCCAGAGCGTGC-3' and reverse primer 5'-GAGTTTTGCTCGTTAGGCTG-3' mixture containing 500 ng genomic DNA, 30 pmol of the forward primer 5'-TCCAGAATCTGTTCCAGAGCGTGC-3' and reverse primer 5'-GAGTTTTGCTCGTTAGGCTG-3'.

Fig. 1. Transverse semi-thin section of sural nerve biopsy showing a moderately reduced density of myelinated fibers and axonal degeneration. (Toluidine blue stain, x 200 before reduction)
and the reverse primer 5'-GCTGTGAAGGTTGCTGCCCTCAT-3', 1.5 units of FastStart Taq DNA polymerase (Roche Diagnostics, Mannheim, Germany), 20 nmol of each deoxyribonucleotide triphosphate (dNTP), 2 µL of 10x PCR buffer (pH 8.3), and 4 µL 5x GC-rich solution. The reaction mixture was subjected to PCR in a PTC-0200 DNA Engine PCR machine (MJ Research, Inc., Boston, MA, USA) consisting of denaturation at 94°C for 1 minute, annealing at 64°C for 30 seconds, and primer extension at 72°C for 2 minutes for 36 cycles. Half of the amplified DNA from each sample was analyzed by electrophoresis at 100 v for 2.5 hours on a 4% SeaKem LE agarose gel with a 100-bp DNA ladder as a size marker. The remaining PCR product was purified by ethanol precipitation to eliminate primers and dNTPs and was then ligated into a pGEM-t vector and the recombinant DNA was transfected into E. coli DH 5α cells. White colonies were isolated and nucleotide sequencing was then performed on each of the positive clones using the SequiTherm™ cycle sequencing kit (Epicentre Technologies Co., Madison, WI, USA) with the sequencing primer 5'-TCCAGAATCTGTTCCAGAGCGGTGC-3'. More than three clones were sequenced to observe the genetic instability of exon 1 of the AR gene for each of the individuals examined.

PCR-amplified DNA fragments encompassing the CAG triplet repeat in the first exon of the AR gene were obtained from the proband and other members of his family (Fig. 4). An elongated 360-bp PCR product was amplified from exon 1 of the AR gene of the X-BSN patient and three carriers, but a 290-bp DNA fragment was obtained from the normal allele in a healthy unrelated male as a normal control. The number of CAG repeats in exon 1 of the AR gene determined by DNA sequencing was 45 in the patient with X-BSN. His three daughters had 45/29, 45/30 and 48/19 CAG triplet repeats in the two alleles in exon 1 of the AR gene. However, in the unrelated healthy male control, only 22 CAG triplet repeats were found in exon 1 of the AR gene (Fig. 4).

Discussion

This study describes the clinical manifestations, results of electrophysiologic study, and pathologic changes in the sural nerve in a patient with X-BSN confirmed by molecular analysis. Although he did not have sensory symptoms, sensory neuropathy was documented from abnormal nerve conduction velocity studies with decreased amplitude SNAPs in sensory nerves. Furthermore, sural nerve biopsy revealed axonal degeneration with loss of large MFs. These data indicate that subclinical involvement of sensory nerves can occur in patients with X-BSN.

X-BSN may be confused with progressive bulbar palsy variants of motor neuron disease. Many of these cases at first appear to be sporadic, with proximal...
muscles affected first, followed by bulbar muscles [1–4]. Dysarthria and dysphagia are associated with atrophy and weakness of facial, jaw, and tongue muscles. However, contraction fasciculations of the perioral muscles, gynecomastia, and other endocrine abnormalities are characteristic [6, 7, 11]. In addition, hyporeflexia or areflexia and essential tremor may occur. Laboratory test results are usually normal except for a modest elevation in creatine kinase [9, 14]. A high level of creatine kinase (mean, 1078 ± 87 U/L; range, 274–3457 U/L) in 26 of 28 patients with X-BSN has been described [9]. Similar results may also be found in spinal muscular atrophies or other motor neuron diseases [15]. Correct diagnosis of this disorder is important for both prognosis and genetic counseling [12, 13, 16, 17]. However, electrophysiologic studies and nerve pathology data have not been emphasized. Kennedy et al found abnormal SNAPs in three cases and mild loss of nerve fibers in the peripheral nerves at autopsy in one [1]. In 1982, Harding et al found most patients had small or unrecordable SNAPs with absence of clinical sensory impairments [2]. A detailed clinicopathologic study with sural nerve biopsy and morphologic observations found that lower motor neurons were markedly depleted through all spinal segments and brainstem motor nuclei [4]. Primary sensory neurons were less severely affected. A quantitative study of primary sensory axons suggested that a distally accentuated axonopathy was the salient pathologic process [4]. Sensory nerve involvement was found not only in X-BSN patients but also in carriers in a large family and sural nerve biopsies confirmed a decrease in MFs and the presence of atrophic axons with demyelinating and remyelinating changes [8]. Some carriers may present signs of chronic denervation and low-amplitude SNAPs [9]. Our study showed similar findings of sensory nerve involvement when compared with previous studies [4, 5, 8, 9]. Therefore, the identification of sensory neuropathy by electrophysiologic study and sural nerve pathology is helpful in differentiation of this disease from other motor neuron diseases. Finally, X-BSN should be diagnosed by molecular genetic analysis.

The pathogenesis of sensory nerve involvement in X-BSN is still unclear. The highly selective involvement of fibers in the fasciculus gracilis and predominant lower leg involvement in clinical sensory nerve conduction velocity studies suggest that the involvement in primary sensory neurons is caudally dominant in terms of rostrocaudal segmental distributions [4]. Recently, it has been suggested that this disease may share nerve degeneration mechanisms with X-linked Charcot-Marie-Tooth (CMTX) disease [8, 12], as the CMTX locus seems to be close to the AR gene [18–20]. However, the clinical and neuropathologic features of peripheral nerve involvement are quite different in these two diseases. Further investigation of function of the AR gene is warranted.

References