ABSTRACT: Recently, we reported that mutations in the neuropathy target esterase (NTE) gene cause autosomal recessive motor neuron disease (NTE-MND). We describe clinical, neurophysiologic, and neuroimaging features of affected subjects in the index families. NTE-MND subjects exhibited progressive lower extremity spastic weakness that began in childhood and was later associated with atrophy of distal leg and intrinsic hand muscles. NTE-MND resembles Troyer syndrome, except that short stature, cognitive impairment, and dysmorphic features, which often accompany Troyer syndrome, are not features of NTE-MND. Early onset, symmetry, and slow progression distinguish NTE-MND from typical amyotrophic lateral sclerosis. NTE is implicated in organophosphorus compound–induced delayed neurotoxicity (OPIDN). NTE-MND patients have upper and lower motor neuron deficits that are similar to OPIDN. Motor neuron degeneration in subjects with NTE mutations supports the role of NTE and its biochemical cascade in the molecular pathogenesis of OPIDN and possibly other degenerative neurologic disorders.

We recently reported the discovery of neuropathy target esterase (NTE) gene mutations in a consanguineous family (family 1, Fig. 1A) and a non-consanguineous family (family 2, Fig. 1B) in which affected subjects exhibited slowly progressive spastic paraplegia and distal muscle atrophy. Herein we provide a detailed description of the clinical features and results of electrophysiological and neuroimaging studies of affected subjects in these families.

METHODS

We examined 7 members of a consanguineous South American kindred of Ashkenazi Jewish descent (Fig. 1) and 5 members of a non-consanguineous Caucasian family of German ancestry. Participation in this study was approved by the institutional review board at the University of Michigan.

Sensory and motor nerve conduction studies were performed in the dominant upper and lower extremities of subject 2 using standard percutaneous nerve stimulation and surface recordings. Sensory and motor response amplitudes and latencies were measured and conduction velocities were calculated. F-response latencies were obtained for motor nerves. Skin temperatures were maintained between 32.0°C and 34.0°C. Neuromuscular transmission was evaluated by repetitive stimulation (2 Hz) of the median and ulnar motor nerves before and after brief volitional exercise. Concentric needle electromyography (EMG) was performed on selected proximal and distal muscles of the upper and lower extremities, and insertional activity and motor unit potential recruitment and configuration were evaluated.

Brain magnetic resonance imaging (MRI) and cervical and thoracic spine MRI were performed in subject 2 without intravenous gadolinium using a 1.5-Tesla scanner. Axial T1, axial T2, and sagittal T2 sequences obtained of the brain, and sagittal T1, sagittal T2, and axial T2 sequences were obtained of the cervical and thoracic spine.

Quantitative sensation testing (QST) of the hand and foot was performed in subject 2 using CASE III or IV (WR Medical Electronics, Stillwater, Minnesota).

RESULTS

Clinical Descriptions. Clinical findings and results of diagnostic studies are summarized in Table 1. Antenatal and perinatal histories (available from the parents of subjects 2, 3, and 4) were unremarkable, and developmental milestones were attained normally. Each subject had a slowly progressive gait disturbance that began in early childhood (subject 1) around age 4 years (subjects 2 and 4) or around age 7 years (subject 3). Progressive weakness and atrophy in the hands became evident in the late teenage years (subjects 1 and 2), between age 12 and 21 years (subject 3), or around age 20 years (subject 4). Each subject...
reported urinary urgency beginning in adulthood. Subjects did not report cognitive impairment, visual disturbance, swallowing difficulties, sensory loss, or symptoms of autonomic disturbance such as postural light-headedness or impaired sweating. Subject 1 had mild dysarthria dating to early adulthood that was slowly progressive.

Neurologic examinations were performed at 82 (subject 1), 48 (subject 3), and 34 (subject 4) years of age. Subject 2 was examined six times between ages, 8 and 35 years.

Each subject exhibited marked atrophy and weakness of intrinsic muscles of the hands (most notably in the thenar group and dorsal interossei) and distal lower extremities. Although intrinsic hand muscles were markedly weak, strength was preserved in wrist extensors, wrist flexors, and proximal upper extremity muscles. Subject 4 had hamstring muscle contractures. Other subjects had moderate to marked spasticity in hamstring muscles, moderate to marked weakness of iliopsoas muscles, and marked weakness of tibialis anterior muscles. Upper extremity deep tendon reflexes were brisk (3+) in subjects 1, 2, and 3, but they were diminished in subject 4. Deep tendon reflexes at the knees were hyperactive in subjects 1, 2, and 4, but...
they were absent in subject 4. Ankle jerks were diminished (subjects 2 and 3) or absent (subjects 1 and 4).

All subjects exhibited extensor plantar responses. Vibratory sensation was diminished in the toes. Light touch and pinprick sensations were normal in subjects 3 and 4, but were diminished distally in subject 1 (who was examined at 82 years of age).

There was moderate to severe functional impairment that was generally similar between the two families. Subject 1 was unable to walk (when examined at age 82) because of severe lower extremity spasticity, weakness, and hamstring tendon contractures. His niece (subject 2, age 38 years) had prominent spastic gait and scissoring but was ambulatory without assistance. There was some evidence of intrafamily variation. Whereas subject 4 had marked spastic paraparesis and was marginally ambulatory even with a walker at 38 years of age,
his older sister (age 48) had moderate spastic paraparesis, but was fully ambulatory with a four-pronged cane.

Serial evaluations of subject 2 at age 8, 11, 12, 17, 20, and 35 years disclosed an evolving phenotype. Initial examination (age 8 years) and subsequent examinations showed a spastic gait that worsened progressively. Lower extremity hyperreflexia and extensor plantar responses were present from the initial evaluation (age 8). Tendon reflexes in the upper extremities were normal, and corticobulbar signs were absent at each evaluation. Although examinations through 17 years of age showed normal upper extremity strength, examinations at ages 20 and 35 years showed definite weakness and atrophy of thenar, hypothenar, and interosseous muscles. These muscles were markedly weak at the last examination (age 35 years). Quantitative sensation testing (performed in the hand and foot using CASE III or IV (WR Medical Electronics, Stillwater, Minnesota) was performed on each occasion and was normal until the last examination (age 35 years), when decreased vibration was detected at the great toe and index finger.

Neurophysiologic and Neuroimaging Studies and Muscle Biopsy. Motor nerve conduction studies (NCSs) and needle EMG were normal in subject 4 when they were performed at age 4 years. Motor NCSs performed in subject 3 at age 12.5 years showed decreased amplitude ulnar, median, and peroneal motor responses and a slightly increased median motor distal latency. Sensory NCSs were normal. EMG findings (subject 3, age 12.5 years) were interpreted as showing neurogenic atrophy in distal lower extremity muscles. At 20 years of age, these abnormalities included the anterior tibialis, which also showed severe motor denervation and reinnervation characterized by clinically symmetric distal involvement. The myotonic discharges recorded from a single fibrotic muscle (anterior tibialis), which also showed severe neurogenic changes. The study was interpreted as showing evidence of a moderately severe motor neuronopathy characterized by clinically symmetric distal involvement. The myotonic discharges recorded from a single fibrotic muscle were noted to be a non-specific finding associated on occasion with chronic denervation.

Serial needle EMG examinations showed evolution of this chronic denervation. Whereas examination at age 8 years showed slightly increased insertional activity, fibrillation potentials, and decreased recruitment characterized by large polyphasic motor unit potentials only in the intrinsic foot muscles, examination at age 11 showed increased insertional activity, fasciculation potentials, and alteration of motor unit potentials (increased size and duration) in distal lower extremity muscles. At 20 years of age, these abnormalities included the anterior tibial muscle. At 35 years of age, intrinsic hand muscles were similarly affected, and very mild but similar changes were found in anterior thigh muscles.

Muscle biopsy obtained from the extensor digitorum communis muscle of subject 3 at age 12 years of age was judged to be “non-diagnostic.” There was no significant histologic evidence of chronic denervation despite EMG findings from the first dorsal interosseous, tibialis anterior, and adductor digitii quinti muscles that were consistent with chronic denervation at this age.

Magnetic resonance imaging of the brain and spinal cord were performed in subject 2 (Fig. 2). No significant intracranial abnormality was identified with appropriate cerebellar and midbrain volumes, and there was no evidence of corpus callosum atrophy or agenesis. MRI of the spinal cord showed significant cord atrophy beginning at the T3–4 level and extending down to T11, where the sural sensory nerve action potential. Median and ulnar CMAP amplitudes were markedly reduced (0.3 and 2.5 mV, respectively), and peroneal and tibial CMAPs were unobtainable. The median motor distal latency was slightly prolonged, and the conduction velocity was reduced to a degree consistent with loss of large motor axons. There was no evidence of motor conduction block or abnormal temporal dispersion. The ulnar motor distal latency and conduction velocity were normal, as was the F-wave latency. There was no evidence of impaired neuromuscular transmission. Needle EMG at 30 years of age showed chronic distal partial denervation and reinnervation characterized by small-amplitude fibrillation potentials (some minute) and decreased recruitment of large-amplitude motor unit potentials. True myotonic discharges were recorded from a single fibrotic muscle (anterior tibialis), which also showed severe neurogenic changes. The study was interpreted as showing evidence of a moderately severe motor neuronopathy characterized by clinically symmetric distal involvement. The myotonic discharges recorded from a single fibrotic muscle were noted to be a non-specific finding associated on occasion with chronic denervation.
cord expanded at the conus medullaris (Fig. 3). Thoracic spinal cord cross-sectional area at T9 was reduced (25 mm²) compared with control subjects (39.3 ± 8.1 mm²). There was no evidence of intrinsic spinal cord signal aberration, compression, or significant narrowing of the central canal.

**DISCUSSION**

The salient features of subjects with NTE-MND are: (1) childhood onset of slowly progressive spastic paraplegia; (2) progressive atrophy of intrinsic muscles of the hands and distal lower extremities beginning in early through late adolescence; (3) mild diminution of distal vibration sensation; and (4) family history consistent with autosomal recessive inheritance. EMG and nerve conduction studies were consistent with progressive motor nerve impairment. Peripheral sensory nerve studies were normal. Neuroimaging identified prominent thoracic spinal cord atrophy in the one patient studied.

NTE-MND may be classified as a form of hereditary spastic paraplegia (designated SPG39). During childhood and early adolescence, NTE-MND presents as an “uncomplicated” hereditary spastic paraplegia (HSP) syndrome. The subsequent appearance (in early through late adolescence) of progressive atrophy of distal upper and lower extremity muscles signals transition to a complicated HSP syndrome involving both upper and lower motor neurons. Such lower motor neuron signs occur as a constant or variable feature in many forms of complicated HSP (SPG7, SPG10, SPG14, SPG15, SPG17, SPG20, and SPG26 HSP; see Fink for review), and in SPG3A, a common childhood-onset form of HSP that is usually uncomplicated.

Among the various forms of HSP, NTE-MND most closely resembles SPG20 HSP (Troyer syndrome), a complicated form of autosomal recessive HSP that was initially described in Old Order Amish due to SPG20/spartin gene mutation. Although both Troyer syndrome and NTE-MND have progressive spastic paraplegia and distal muscle atrophy, it is notable that many subjects with the SPG20/spartin mutation have neurologic signs that are not present in the SPG39/NTE-mutation subjects identified to date. In addition to progressive spastic paraplegia, subjects with SPG20/spartin mutation have delayed milestones (15 of 21 examined subjects), cognitive impairment (in 17 of 21 examined subjects), emotional lability (in 17 of 21 subjects), cerebellar signs (16 of 21 examined subjects), spastic dysthria (19 of 21 examined subjects), ataxia (3 of 17 examined subjects), choreoathetoid movements (3 of 21 examined subjects), and dysphagia (2 of 21 examined subjects). Moreover, 3 of 21 subjects with SPG20/spartin mutation did not have distal muscle atrophy, indicating that clinical diagnosis cannot rest on this major feature.

**FIGURE 2.** MRI of brain and spinal cord in NTE-NMD syndrome. (A) No significant intracranial abnormality is identified. The corpus callosum (not shown here) was present without evidence of atrophy. Significant spinal cord atrophy is present beginning at the T3–4 level where the cord again expands at the conus medullaris. Although atrophic, the spinal cord was not compressed, and there were no spinal cord aberrations. (B) Cord transverse area (circle) at T9 measured 25 mm² at the T9 level (compared with control subjects, not shown) that measure 39.3 ± 8.1 mm².
alone. Our observations suggest that the presence or absence of these additional signs (cognitive, bulbar, cerebellar, emotional, and extrapyramidal deficits and skeletal abnormalities) help distinguish SPG20/spartin-mutation/Troyer syndrome subjects from those with NTE mutations.

The NTE-MND subjects just described were ascertained as familial clusters suggesting autosomal recessive inheritance. Because subjects with autosomal recessive disorders often have no previous family history of the disorder, it is possible that NTE mutations contribute to motor neuron disease among subjects without a family history of the disease.

NTE is an important factor in OPIDN,9–14 a distal axonopathy that, like NTE-MND, is characterized in late-stage disease by spastic paraparesis with distal weakness and atrophy. Causes of OPIDN include occupational and accidental exposures to neuropathic organophosphorus compounds.15–20 One example of OPIDN is a disorder sometimes referred to as “jake leg palsy,” in reference to the accompanying ingestion of Jamaican ginger (“jake”) and to the characteristic gait displayed by afflicted individuals reflecting a combination of upper and lower motor neuron involvement. The disorder may have affected as many as 50,000 individuals in the Prohibition-era United States. Jake leg palsy was attributed to recreational consumption of Jamaican ginger extract, a patent medicine adulterated with tri-ortho-cresyl phosphate (TOCP).21 In addition to the clinical similarity between OPIDN and NTE-MND, neuropathologic findings in the chicken model of OPIDN (including axon degeneration of corticospinal tracts and dorsal column fibers)13 overlap the predominant localization of neurologic signs in subjects with NTE-MND mutation. These observations suggest that motor neuron syndrome associated with the organophosphorus compound–NTE interaction shares a common molecular pathogenesis with motor neuron disease attributed to homozygous (or compound heterozygous)1 NTE mutations. This observation supports the hypothesis that some apparently sporadic (or genetically occult) MNDs could be due to exposure to some neuropathic organophosphorus compounds, either alone or together with predisposing NTE gene mutations or genetic variation in factors that regulate or interact with NTE.

The molecular mechanisms by which NTE disturbance (both due to mutations in the NTE esterase domain as well as irreversible organophosphorylation of the NTE active site serine) lead to axon degeneration that particularly involves central and peripheral motor neurons are not clear. Recently, we showed that the mutations in NTE-MND subjects reduce NTE activity and alter its enzyme kinetics in vitro.22 It is unknown, however, if reduction in phospholipase activity of NTE is sufficient to cause neurodegeneration in NTE-MND. The impact of NTE-MND mutations (and irreversible organophosphorylation) on other emerging NTE functions has not been assessed. Specifically, there is recent evidence that the NTE Drosophila homolog Swiss Cheese (SWC) binds to and regulates cyclic AMP-dependent protein kinase (PKA).23 Discovering that human NTE regulates PKA in a similar manner would suggest additional mechanisms (including altered phosphorylation of target proteins and/or cAMP-regulated gene expression) by which NTE disturbance leads to neurodegeneration. It is also possible that NTE-mediated motor neuron degeneration is due to NTE protein misfolding. Abnormal protein folding is a mechanism common to many neurodegenerative disorders,24 including Parkinson disease,25 polyglutamine-expansion disorders,26 and amyotrophic lateral sclerosis.27,28 Increased recognition of NTE-MND and elucidating its molecular pathogenesis will provide insight into possible gene–environment interactions that contribute to motor neuron disease and advance our ability to treat these disorders.

This research was supported by grants from the National Institutes of Health (NINDS R01NS053917), the Department of Veterans Affairs (Merit Review Awards), the Spastic Paraplegia Foundation, and the generous support of the Paul and Lois Katzman Family Fund (to J.K.F.). We gratefully acknowledge the expert secretarial assistance of Lynette Girbach, and the participation of patients and their relatives without whom this research would not have been possible.

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