Myoclonus From Selective Dentate Nucleus Degeneration in Type 3 Gaucher Disease

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Objective: To describe a case with a new genetic variant of type 3 Gaucher disease presenting with stimulus-sensitive and action myoclonus in the presence of selective dentate abnormalities.

Design: Clinical, pathologic, and molecular genetic studies.

Setting: Medical school departments.

Patient: A 6-year-old girl with type 3 Gaucher disease experienced progressively crippling generalized stimulus-sensitive and action myoclonus. Repeated electroencephalographic examination did not show cortical activity associated with the myoclonus, suggesting its subcortical origin. Neuropathological examination revealed selective degeneration of the cerebellar dentate nucleus and dentatorubrothalamic pathway in the face of essentially complete lack of storage in the brain. Mutation analysis identified the following 2 mutant alleles: one with a V394L mutation and the other with the lesion RecTL (D409H + L444P + A456P + V460V), which resulted from a recombination event, with the pseudogene located 16 kilobases downstream from the structural gene.

Conclusion: Given the restricted abnormalities, this genetically unique case provides insight into the pathogenesis of myoclonus and suggests a prominent role for the cerebellar dentate nucleus in its genesis.

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G AUCHER disease (GD) is a lysosomal storage disease resulting from the deficient activity of acid β-glucosidase and the accumulation of its substrate, glucosylceramide, primarily in the cells of the macrophage-monocyte system. The storage results in a multisystem disease with progressive visceromegaly and gradual replacement of the bone marrow with distinctive lipid-laden macrophages (Gaucher cells).1-3 There are 3 subtypes that are distinguished by the absence (type 1) or presence and severity of neurologic involvement (types 2 and 3), although clinical presentations may vary. Type 2 disease is a rapidly progressive neurodegenerative disorder resulting in death by 2 to 3 years of age. Type 3 GD has been subclassified into types 3a and 3b.4 Patients with type 3a GD exhibit mild-to-moderate hepatosplenomegaly and slowly progressive neurologic deterioration. Recurrent myoclonic seizures are common. Patients with type 3b GD exhibit splenomegaly along with extensive hepato megaly that frequently is accompanied by esophageal varices. Early development of horizontal supranuclear gaze paresis is the major neurologic sign.4,5 In most patients, the reduction of glucocerebrosidase activity is caused by mutations in the gene that codes for glucocerebrosidase.4,6 The mechanism by which acid β-glucosidase deficiency leads to such specific neurologic signs in types 2 and 3 disease is unclear, as there is no neuronal storage and, typically, perivascular cells with accumulated substrate are diffusely distributed throughout the brain.1,3 Investigators have proposed that the accumulation of psychosine (glucosylsphingosine), an abnormal neuronotoxic degradation product, is responsible for the neurologic symptoms.7-10 Recent studies have suggested that psychosine inhibits the electron transport chain (possibly acting via cardiolipin at sites I and III) resulting in a change in the lipid environment of the membrane that is responsible for the mitochondrial dysfunction.11,12 Development of animal models of GD has lead to new insights to the genetics of the disease and paved the way for new therapies.13 The development of enzyme replacement treatment for
type 1 GD has dramatically changed life expectancy in these patients. Enzyme replacement therapy has been tried in a small number of patients with type 3 GD with promising results in the improvement of visceral function and stabilization of the neurologic deterioration.

There are several types of myoclonus. Hallett and coworkers coined the term “reticular-reflex myoclonus” for the variant that arises from the magnocellular bulbar reticular formation in the vicinity of the spinal nucleus of the accessory nerve. In cortical myoclonus, neuronal hyperexcitability originates in cortical neurons and is propagated downward. In reticular-reflex myoclonus, cortical spikes, if present, follow rather than precede the reticular discharges that are propagated upward and downward. Reticular- and cortical-reflex myoclonus can coexist in the same patient. Unlike reticular-reflex myoclonus, which is generalized, cortical-reflex myoclonus may be confined to the area of the body being stimulated. Both types of myoclonus are often associated with action myoclonus, which is an abnormal response to proprioceptive feedback during movement. Action myoclonus occurs most frequently in diffuse neuronal diseases such as postanoxic encephalopathy, uremia, and various progressive myoclonic epilepsies, as well as type 3 GD.

We herein describe a 6-year-old girl with type 3 GD. Progressively crippling, generalized action and stimulus-sensitive myoclonus dominated her clinical picture. Results of neuropa thological examination suggest that the source of the myoclonus was selective degeneration of the cerebellar dentate nucleus and of the dentatorubrothalamic pathway.

CLINICAL FINDINGS

The patient was born at 37 weeks' gestation with a birth weight of 3.5 kg to an Ashkenazi Jewish mother and a non-Jewish Irish father. There was no family history of GD, although the maternal grandmother had anemia and bone problems requiring a hip replacement. The patient’s early development was normal; she walked at 1 year of age and spoke on time. She was toilet trained at 2 years and learned to dress herself.

At 1 year of age, stridor developed that was worse during sleep. Splenomegaly was noted at 18 months. At 2 years of age, her parents reported frequent falls and a wide-based gait. The diagnosis of GD was made at 28 months when typical Gaucher cells were observed in the bone marrow. It was confirmed by the demonstration of markedly reduced acid β-glucosidase activity (<10% of normal) toward the natural substrate, glucosylceramide, in cultured skin fibroblasts (53 vs 580 nmol/h per milligram for normal cells). She was small and stocky for age, ie, height of 81 cm (less than the third percentile for age) and weight of 11.5 kg (15th percentile for age). She had mild anemia (hemoglobin level, 113 g/L) and thrombocytopenia (76 000 × 10^9/L). A skeletal survey revealed an Erlenmeyer flask deformity of the femur. An electroencephalogram (EEG) and computed tomographic scan of the head revealed no abnormalities. Electrophysiologic study revealed a mild distal axonopathy.

Myoclonic jerks, which at first were not stimulus sensitive, appeared at 3 years of age and initially were helped by clonazepam. Most were frequent, sudden jerks of a limb or facial muscles, exacerbated by movement. Some were sudden extension jerks that made her fall backward without loss of consciousness or awoke her from sleep. A repeat EEG was normal, without spike correlation with the myoclonus. Her parents noted that when they spun her around, her eyes lagged in 1 direction. She would thrust her head sideways to track a target, and there was a delay in her eyes returning to their central position. Hearing and vision were normal. She was a bright and alert social child who was well liked by her many friends.

At about 4 years of age, psychological testing yielded an IQ of 110. She was short for her age (103 cm at 6 years; less than the third percentile for age), had a normal head circumference (49 cm), and had a slight, lower dorsal kyphosis and a scoliosis convex to the right. Splenomegaly was massive; hepatomegaly was minimal. She spoke in well-formed, sophisticated sentences, but was dysarthric. The prominent oculomotor apraxia was confirmed, but the range of her extraocular movements was full, without nystagmus. There was no facial asymmetry, and she could stick out her tongue. As a result of the myoclonus, her gait deteriorated to the point of her being nonambulatory by 4 years of age, by which time she had lost her equilibration reflexes and tended to fall like a log. She could only stand with support and titubated when sitting. Results of sensory examination were normal. She was diffusely hyperreflexic with bilateral extensor plantar responses.

She had her first generalized myoclonic seizure at 4 years of age, described as a sudden extension of her limbs that lasted about 30 seconds, without loss of consciousness. By 5 years of age, the myoclonus had increased dramatically, with voluntary movement and in response to tapping or touching, but not to light or sound. It was so severe that she was unable to sit or keep her head up and could barely use her hands, and it interrupted her speech so frequently as to jeopardize intelligibility. The myoclonus did not respond to primidone, valproic acid, phenytoin sodium, ethosuximide, diazepam, or lorazepam. She had difficulty feeding and lost weight, and her worsening stridor occurred during the day as well as at night. She received prophylaxis with a combination of sulfamethoxazole and trimethoprim (Bactrim) and occasional blood transfusions for anemia. By 6 years of age, she had had 11 hospital admissions for virtually continuous, intractable myoclonus, dehydration, or pneumonia. Inability to move voluntarily, respiratory distress, and difficulty swallowing became so disabling that she was aphantic and totally incapacitated, yet remained alert. Cyanosis and hemoptysis developed, and she died a few days after her sixth birthday.

GENETIC STUDIES

Genomic DNA was isolated from cultured skin fibroblasts and screened for the 4 common acid β-glucosidase mutations (N370S, L444P, 84GG, and IVS2+1) by means of polymerase chain reaction (PCR) amplifica-

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The patient had 1 allele carrying the L444P mutation. The initial screening method could not differentiate between simple, singly mutated L444P alleles and several complex L444P-bearing alleles found in approximately 1.5% of Jewish and 3.5% of non-Jewish patients. To determine if the L444P mutation was present alone or as 1 of 3 known L444P-containing complex alleles, the complete acid β-glucosidase coding region and adjacent intron-exon boundaries were amplified by PCR and sequenced. Sequencing revealed a C→T base substitution at complementary DNA (cDNA) position 1448 (genomic position 7319) encoding the leucine→proline substitution at position 444 (L444P) and 4 additional nucleotide substitutions producing the following 3 missense mutations and a polymorphism: (1) a G→T transversion at cDNA position 1297 (genomic position 6799) encoding a valine→leucine substitution at amino acid 394 (V394L); (2) a G→A transition at cDNA position 1342 (genomic position 6844) encoding the substitution of an aspartate by histidine at residue 409 (D409H); (3) a G→C transversion at cDNA position 1483 (genomic position 7354) encoding the substitution of an alanine by proline at residue 456 (A456P); and (4) a polymorphic G→C transversion at cDNA position 1497 (genomic position 7368) in codon 460 (V460V). These results were consistent with earlier studies indicating that, using dot blot analysis of genomic DNA from the patient and both parents, demonstrated that the V394L mutation came from the mother and that the 4 other mutations derived from the paternal allele. Thus, the mutation analysis identified 2 mutant alleles, one with a V394L mutation and the other with the lesion RecTL (D409H + L444P + A456P + V460V), which resulted from a recombination event with the pseudogene located 16 kilobases downstream from the structural gene. The V394L missense allele, as well as the 3 individual missense mutations (D409H, L444P, and A456P) of the complex RecTL allele, were expressed in the baculovirus system and characterized. The mutant proteins expressed from the D409H, L444P, and A456P alleles were severely compromised, with turnover rates of less than 0.2%, 5.0%, and greater than 0.03% of the healthy enzyme, respectively. In addition, immunoblotting studies (using polyclonal anti–acid β-glucosidase antibodies) of these 3 expressed mutant proteins consistently produced lighter signals than that of the expressed healthy enzyme demonstrating reduced stability. These results indicated that essentially no residual activity was derived from the multiply mutated RecTL allele. The V394L allele expressed a stable protein product with a catalytic efficiency that was approximately 8.5-fold lower than that of the healthy enzyme. In addition to the reduced turnover rate, the V394L mutant protein had been shown to have reduced stability at 0°C (half-life $t_{1/2}$ = 3.9 minutes; normal = 11 minutes) and pH of 3.5 ($t_{1/2}$ = 13 minutes; normal = 30 minutes), suggesting that the intracellular lysosomal activity expressed from this allele would be very compromised. Thus, the combination of the inactive RecTL allele with the pH-sensitive V394L allele produced very little, but detectable, functional enzyme, consistent with the observed type 3 phenotype.

**NEUROPATHOLOGICAL FINDINGS**

The external appearance of the brain was unremarkable. The weight of the left hemibrain was 595 g. The neuronal populations of the cerebral and cerebellar cortices, basal ganglia, thalamus, and major brainstem nuclei, including the red nucleus, oculomotor nucleus, dorsal vagal motor nucleus, nucleus ambiguus, and inferior olivary nucleus, were well preserved. There was no evidence of anoxic-ischemic neuronal necrosis in vulnerable neuronal populations. Alzheimer type II astrocytes were found in the basal ganglia, substantia nigra, and inferior olivary nucleus.

There was a marked neuronal loss in the cerebellar dentate nucleus with many of the residual neurons showing pyknosis and nuclear condensation. Some of the dentate neurons also had coarse granular staining around apical dendrites, consistent with grumose degeneration, and most evident with synaptophysin immunostaining (Figure 1). There were clusters of dystrophic synaptic termini adjacent to the dentate neuronal cell bodies and processes. The dentate hilus appeared pale and attenuated (Figure 2), and the number of fibers emanating from the dentate nucleus was markedly diminished, with loss of myelin and axonal profiles (Figure 2 and Figure 3). The fiber loss was selective to the dentatorubrothalamic pathway, with loss of fibers in the superior cerebellar peduncle, but not in the cerebellar white matter or the inferior or middle cerebellar peduncles (Figures 2 and 3).
The corticospinal tract had minimal fiber loss and a few swollen axons. Sections of cerebral white matter were unremarkable except for a small focus of ependymal injury in the occipital lobe, where there was a cluster of macrophages with the cytoplasmic vacuolation characteristic of Gaucher cells (Figure 4). Although electron microscopy was not possible on these sparse cells, fine structural studies of macrophages from the spleen revealed the diagnostic cytomembranous inclusions of GD, including membrane-bound tubular inclusions. No Gaucher cells were detected in any of the other sections of brain.

Sections of the spleen and liver had abundant Gaucher cells, but none was detected in sections from the larynx, including the vocal cords. The laryngeal nerves were

Figure 2. The hilus of the dentate nucleus is attenuated (A), and myelin stains (Luxol fast blue) demonstrate profound loss of myelin in this region (B) (original magnification ×40).

Figure 3. The outflow pathway of the cerebellar dentate nucleus shows selective loss of fibers. A myelin stain (Luxol fast blue) shows marked loss of fibers in the superior cerebellar peduncle (A), whereas the middle cerebellar peduncle within the same section has preserved myelin (B). A silver stain for axonal filaments (Bodian copper) shows marked loss of fibers within the hilus of the cerebellar dentate nucleus (C), whereas fibers in the surrounding cerebellar white matter are preserved (D) (original magnification ×160).
not identified, but there was no Gaucher cell accumulation in peripheral nerves in the vicinity of the larynx or in other peripheral nerves. There was no evidence of neurogenic atrophy in the laryngeal muscles.

**COMMENT**

This case was characterized by devastating myoclonus in the presence of restricted dentate abnormalities. The myoclonus was multifocal and generalized, without an obvious precipitating cause. It evolved into reticular-reflex myoclonus, which was selectively exacerbated by proprioceptive input. The myoclonus did not have an EEG correlate, supporting its subcortical source. It became so severe in this child that she became unable to feed herself and could barely speak. Mutism with cerebellar lesions is thought to reflect disruption of pathways linking the dentate nucleus to the red nucleus and thalamus, with projections to the supplementary motor areas. In this patient, it appeared to be a consequence of constant overwhelming myoclonus. We did not identify a nuclear, infranuclear, or local pathologic lesion to account for the progressively disabling stridor. This suggests that the stridor was due to disruption of supranuclear influences from the cerebellar nuclei or, perhaps, a consequence of myoclonus that did not entirely subside in sleep. The very restricted abnormalities, with sparing of other parts of the brain, explain this patient’s normal psychosocial and cognitive development.

Oculomotor apraxia was an early sign of the illness in this patient. Following the original description of congenital oculomotor apraxia in children by Cogan, acquired oculomotor apraxia has been reported in various diseases, including types 2 and 3 GD, suggesting a brainstem or cerebellar origin for this finding. This case indicates that it may arise from an abnormality restricted to the dentate nucleus and its outflow.

The dentate nucleus is known to play an important role in the genesis of myoclonus. The major outflow of the dentate nucleus is via the superior cerebellar peduncle to the contralateral ventrolateral thalamic nucleus, but it also projects to the intralaminar nuclei of the thalamus, the red nucleus, the reticulotegmental nucleus, the brainstem reticular formation, and the inferior olivary nucleus. The pathophysiological basis of this girl’s myoclonus was presumably disruption of pathways from the dentate to the lower brainstem reticular formation. Loss of dentate neurons was probably responsible for her ataxia, as there was no loss of Purkinje cells.

The most consistent pathologic change in patients with action myoclonus has been degeneration of the Purkinje cells or dentate nucleus neurons. Loss of Purkinje cells or dentate neurons increases the excitability of the reticular formation to sensory input. Although Lance and Adams suggested that degeneration of the dentate nucleus and Purkinje cells is not likely to be the primary cause of myoclonus, as the cerebellum is not consistently degenerated in autopsy studies of patients with myoclonus, the selective neuronal injury of dentate neurons in this case belies this suggestion.

Most neurodegenerative diseases with progressive myoclonic epilepsy are associated with changes in both neocortical and subcortical structures, although some have no demonstrable cerebral abnormality. Moss and coworkers described selective and symmetrical degeneration of the dentate and second-order somatosensory nuclei in 4 patients from 2 separate pedigrees who had myoclonus, ataxia, and epilepsy that were later shown to be variants of dentatorubropallidolysian atrophy. Dentate nucleus degeneration and Purkinje cell loss in asso-
Brain. In Krabbe (globoid cell leukodystrophy) disease, patient were due solely to selective neuronal destruction and the systemic type 3 phenotype. These clinical reports are confirmed by animal experiments in which unilateral injections of chlorophenolthione into the deep cerebellar nuclei, inferior olivary nucleus, and red nucleus produced generalized stimulus-sensitive myoclonus.50

Pathological studies in neuronopathic types 2 and 3 GD have disclosed 1 or more patterns of neuronal alteration: (1) mild and nonselective, (2) cerebellodentate, (3) bulbar, and (4) thalamocortical.49 The bulbar-thalamocortical pattern is typical of infantile type 2 GD, whereas the first pattern characterizes the Norrbottnian type 3 GD in patients who are homoallelic for the L444P mutation.51,52 In young adults with type 3 disease, dentate degeneration is striking, whereas the cerebral cortex is normal.39 There are few published autopsy studies of patients with type 3 GD with progressive myoclonus. Conradi and coworkers60 described a young child with type 3 disease who had oculomotor apraxia, progressive myoclonus, and bulbar signs and thus resembled our patient clinically. However, unlike our patient, in whom abnormalities were limited to dentate nucleus devastation, Gaucher cells were present diffusely in the perivascular spaces of the white and central gray matter, surrounded by astrogliosis. There was a slight loss of Purkinje cells and severe loss of neurons with astrogliosis in the dentate nucleus. Two siblings with type 3 disease studied by Winkelman and coworkers39 resembled our patient clinically in that stimulus-sensitive myoclonus, generalized seizures, supranuclear gaze palsies, and cerebellar ataxia developed. They differed pathologically, however, in that Gaucher cells were found diffusely in the brain and subarachnoid space. The neocortex was normal, and changes in the cerebellar cortex were limited to mild astrogliosis of the molecular layer. There was severe neuronal depletion and gliosis in the dentate, emboliform, and fastigial nuclei. In the spinal cord, there was diffuse astrogliosis in the posterior horns without discernible neuronal loss.

The genotype, severity, and course of type 3 GD vary.1,4,39,40 A number of mutations have been identified in patients with type 3 disease, with the L444P lesion accounting for approximately 70% of the mutant alleles, whereas less common (eg, RecNciI) and family-specific lesions account for the remainder.20 Expression studies revealed that our patient’s mutant RecTL allele produced little, if any, active enzyme, whereas the V394L allele produced a sufficient amount of active, albeit unstable, enzyme to preclude a type 2 phenotype. Together the V394L and RecTL alleles did not produce enough activity to prevent the development of neurologic involvement and the systemic type 3 phenotype.

The devastating neurologic symptoms in this patient were due solely to selective neuronal destruction in the dentate nucleus without evidence of storage in the brain. In Krabbe (globoid cell leukodystrophy) disease, Miyatake and Suzuki6 and Suzuki’s7 showed that the inactivity of galactocerebrosidase results in accumulation of galactosylsphingosine (psychosine), which is neurotoxic and is formed because galactocerebrosidase degradation takes place through an alternate pathway. The psychosine hypothesis was extended to explain changes in neuronopathic type 2 and 3 GD.6 Nilsson and Svennerholm8 reported that psychosine, which is not present in normal brains, was present in all brains with types 2 and 3 GD that they examined. The levels were higher in type 2 disease, corresponding to a more fulminating course and severe neuronal loss. The psychosine concentrations were raised to lesser levels in brains with type 3 disease. The highest concentrations of accumulated glucosylceramide in type 3 were found in the cerebellum of patients who had survived splenectomy for several years. The ceramide composition of the accumulated glucosylceramide suggested that brain gangliosides were the major precursors of the glucosylceramide in brains with type 2 disease, but accumulation in cerebellar cortex in type 3 GD was partly of extracerebral origin. Kaye et al55 reported that neuropathological findings in the brains with type 2 disease are correlated with the glucocerebroside accumulation. Despite the similar pattern of glucocerebroside accumulation in the brains with type 3 disease, no neuropathological abnormalities were seen. Psychosine may cause selected neurons to undergo apoptosis, but the basis for the selective vulnerability of neurons in the dentate nucleus in type 3 disease is not known.4,7,8

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