Early Clinical Markers of Central Nervous System Involvement in Mucopolysaccharidosis Type II

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Objective To identify early clinical markers of neurologic involvement in mucopolysaccharidosis type II.

Study design A retrospective review of neurobehavioral standardized assessments of patients with mucopolysaccharidosis type II evaluated at the Program for Neurodevelopmental Function in Rare Disorders was completed. Patients were grouped based on the presence or absence of central nervous system (CNS) involvement at the most recent evaluation. Differences in early signs and symptoms between resulting cohorts were tested for significance, and an index severity score was developed.

Results Between December 2002 and November 2010, clinical evaluations of 49 patients and 151 patient encounters were reviewed. Thirty-seven patients exhibited neurologic deterioration. Of the 25 signs evaluated, 7 early clinical markers were strongly correlated with subsequent cognitive dysfunction: sleep disturbance, increased activity, behavior difficulties, seizure-like behavior, perseverative chewing behavior, and inability to achieve bowel training and bladder training. A new severity score index was developed, with a score ≥3 indicating a high likelihood of developing CNS disease.

Conclusion Seven early clinical markers and a severity score index of CNS involvement can be used for initial screening of children who might benefit from CNS-directed therapies. *(J Pediatr 2011;■:■-■).*

See related article, p •••

Management of patients with mucopolysaccharidosis type II (MPS II) has historically involved palliative care to reduce the associated symptoms. Various methods of replacing the iduronate-2-sulfatase enzyme have been proposed or instituted, including bone marrow transplantation,[1] human amnion membrane implantation,[2] fibroblast transplantation,[3] white blood cell infusion,[4] serum or plasma infusion,[5] gene therapy,[6-8] intraperitoneal implantation of myoblasts overexpressing iduronate-2-sulfatase,[9] and, most recently, enzyme replacement therapy (ERT),[10,11] and intrathecal (IT) ERT.[12-14] Although many of the physical symptoms can be reduced or eradicated via intravenous enzyme replacement,[11] neurologic deterioration is irreversible, presumably due to the inability of the large enzyme to cross the tight cell–cell junctions of the blood-brain barrier.

The value of IT-ERT,[13,14] adeno-associated virus vector IT gene therapy,[15] and sequence-enhanced adeno-associated virus vector IT therapy[16] to reduce the accumulation of glycosaminoglycan (GAG) in brain tissue has been well demonstrated in animal models of various mucopolysaccharidoses. Such methods of delivery have been shown to result in adequate penetration of the enzyme into tissues protected by the blood-brain barrier, with resulting improvements in general function, activity level, and ability to breed successfully. Studies investigating the safety and efficacy of IT-ERT delivery techniques in patients with mucopolysaccharidoses are underway. Early intervention has been shown to improve outcomes in other lysosomal storage diseases[17]; thus, it is reasonable to assume that it may be beneficial to treat patients with MPS II early in their neurologic disease course to achieve optimal cognitive outcome.

IT-ERT therapy and bone marrow transplantation are experimental therapies currently under evaluation that may become available in the near future. Potential dangers of treating patients with these or other future therapies may not be justified if the patients are not going to develop neurologic disease; thus, early identification of those patients who will develop neurologic impairment is critical. The purposes of the present study were to identify clinical markers of central nervous system (CNS) involvement before significant cognitive decline occurs and to develop a severity index score that can be used as a screening tool to refer patients with MPS II who are likely to benefit from CNS-directed therapy.

CNS	Central nervous system
ERT	Enzyme replacement therapy
GAG	Glycosaminoglycan
IT	Intrathecal
MPS II	Mucopolysaccharidosis type II
NP	Neural parenchyma

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Methods

Medical records of patients with MPS II referred to the Program for Neurodevelopmental Function in Rare Disorders between December 2002 and November 2010 were reviewed. The University of North Carolina at Chapel Hill's Institutional Review Board granted approval for a retrospective review of the data of all patients evaluated between December 2002 and November 2006, and patients provided informed consent for both retrospective and prospective data review thereafter.

The patients were divided into 2 groups based on the presence or absence of cognitive involvement at the most recent evaluation. Cognitive involvement was defined as a decrease in 1 SD from baseline, confirmed within a 3-month period, or an overall IQ at least 2 SDs below the mean. An exception was made with 2 older patients whose IQ scores were below the mean due to effects of severe unaided hearing loss. These patients were classified without cognitive involvement because their adaptive behavior was within the range of normal. The term "CNS disease" was used to indicate primary neuronal parenchymal (NP) involvement and excluded secondary CNS problems (eg, hydrocephalus, cervical stenosis). Children who were younger siblings of patients with CNS disease and underwent transplantation at a young age were also included in the CNS cohort. The children with CNS disease and those without CNS disease were compared in terms of 25 clinical variables associated with cognitive involvement and tested for significant differences (**Table I**; available at www.jpeds.com). The variables demonstrating significant differences were selected, and a severity index score was developed to assess the strength of the markers according to their joint distribution.

Statistical Analysis

Descriptive statistics were calculated based on the information reported in the clinical chart. The maximum of the age range was used to calculate the age at clinical manifestation. Each child was coded as never versus ever showing each specific clinical symptom. The children with CNS disease and those with only somatic disease were compared in terms of each of these symptoms using Fisher exact test. General linear mixed regression models were used to examine possible differences in the mean cognitive and gross motor developmental trajectories for each group. The models included age, CNS group, and an age-by-group interaction as predictors and cognitive or gross motor age equivalent score as outcomes. Differences in slopes were tested using the results from these mixed models. Finally, by summing the total number of markers observed in each patient, a severity index score was created representing the joint distribution of symptoms. Only patients aged >3 years were included in the analysis of the severity index score.

Results

Between December 2002 and November 2010, a total of 49 patients with MPS II were evaluated. The patients ranged in age from 2 months to 25 years, a mean age at initial visit of 71.6 ± 56.4 months. A total of 151 patient encounters were reviewed. Twenty-six patients were seen for sequential assessments. The number of evaluations per patient ranged from 1 to 7 (mean, 2.4). Patient follow-up ranged from 0 to 9.9 years (mean, 1.5 years). There was no significant difference in age or follow-up time between the CNS and non-CNS groups (all $P > .10$). Fourteen of the patients were receiving ERT at their last visit only, and 2 were receiving ERT at more than one visit. None of the patients was receiving ERT when early signs of CNS disease developed. Eight patients underwent transplantation, but only the evaluations performed before transplantation were included in the analysis. The developmental trajectories of the patients in the CNS and non-CNS groups show clear differences in cognitive function over time (**Figure 1**, A). In the CNS group, 37 patients demonstrated neurologic and cognitive deterioration; 12 patients had no cognitive decline. The patients with and without CNS disease had severe gross motor impairment. Patients with CNS disease had significantly more deterioration than those without CNS disease ($P = .005$) (**Figure 1**, B).

Demographic data for the 2 groups are displayed in **Table II**. Two of the patients were diagnosed because they were younger siblings of patients with known MPS II and neurologic involvement. Two sibling pairs were diagnosed simultaneously when the younger sibling began manifesting similar physical characteristics, prompting the clinician to consider genetic testing. Six patients died during the study period; 1 patient died after bone marrow transplantation. The median age of death was 9.6 years (range, 1.9 to 21 years).

Parents in both cohorts became concerned about their children at similar ages (age 6.8 ± 6.4 months [range, 3 to 21 months] in the non-CNS group 7.3 ± 7.3 months [range, 3 to 27 months] in the CNS group). Parents of 7 of the 12 patients without CNS disease were concerned about their child's physical abnormalities (eg, macrocephaly, inguinal hernia, coarse facies, abnormal joint function). Interestingly, parents of patients with CNS disease more frequently reported concerns about recurrent infection (13/37; 35%). In both groups, the presence of physical abnormalities prompted the physician to evaluate for MPS II. The diagnosis of MPS II was made at a mean age of 45.9 ± 28.0 months in the non-CNS group and 36.8 ± 23.2 months in the CNS group. No significant between-group differences were found in the incidence of prenatal or neonatal complications or in head circumference at any age (**Figure 2**). Unfortunately, information regarding the genotype–phenotype correlation was not available for the 2 groups.

Among the 25 signs evaluated, 7 early clinical markers were identified that correlated strongly with CNS disease (**Figure 3**; available at www.jpeds.com).

Bowel and bladder training was assessed in the 42 children who were evaluated after age 36 months. All of the 12 children in the non-CNS group were able to bladder train

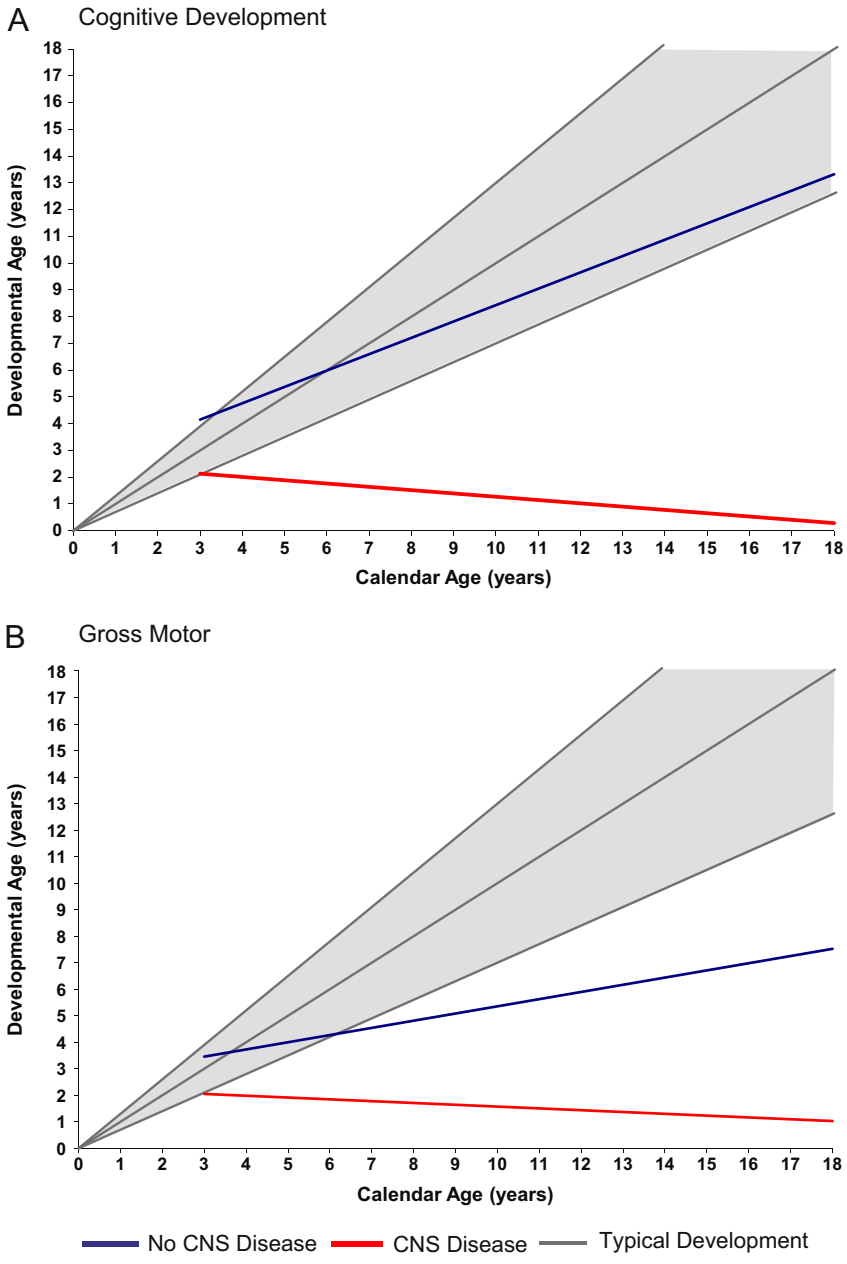

Figure 1. Longitudinal evaluation of neurodevelopmental performance in children with MPS II with and without CNS disease. Age-equivalent scores were used for comparisons and to monitor developmental progress. **A,** Cognitive and **B,** gross motor trajectories were created by plotting developmental age against chronologic age for each group over time. The *shaded area* indicates the variability (± 2 standard deviations) in typical cognitive development.

appropriately, and 11 demonstrated proper bowel training. Conversely, only 5 of 31 children in the CNS group were able to appropriately bladder train, and 4 were able to bowel train. The differences in bowel and bladder training between the groups was significant (both $P < .001$). Of the 26 patients unable to bladder train, 2 were trained for a brief period but then regressed between 60 and 72 months. One patient with CNS disease was able to bowel train appropriately but regressed before his 8th birthday.

When early markers are examined in tandem, the possible score on our index of severity ranged from 0 to 7. The scores for patients in the non-CNS group ranged from 0 to 2 (mean, 0.6), and those for patients in the CNS group ranged from 1 to 7 (mean, 4.62) (**Figure 4**; available at www.jpeds.com).

The mean age at manifestation of symptoms was determined for each of the significant clinical markers of CNS involvement (**Figure 5**). The earliest markers were increased

Table II. Patient demographic data

	No CNS involvement (n =12)	CNS involvement (n = 37)	Total (n = 49)
Age at first symptoms, months, mean (SD)	6.8 (6.4)	7.3 (7.3)	7 (6.9)
Age at diagnosis, months, mean (SD)	45.9 (28)	36.8 (23.2)	37.6 (24.6)
Age at initial visit, months, mean (SD)	103.2 (78.8)	65.3 (39.8)	75 (54.2)
Years followed, mean (SD)	1.1 (1.2)	1.6 (2.5)	1.5 (2.2)
Number of visits, mean (SD)	2.3 (1.8)	2.4 (1.7)	2.4 (1.7)
Race, n (%)			
Caucasian	9 (75)	31 (84)	40 (82)
African American	0 (0)	3 (8)	2 (4)
Asian	0 (0)	1 (3)	1 (2)
Other/unknown	3 (25)	2 (5)	6 (12)

activity (mean age, 40 months) and difficult behavior (mean age, 42 months).

Discussion

We have identified 7 early markers of neurologic disease in patients with MPS II. A combination of these markers was present in all of our patients who developed neurologic decline in this study. Only a small percentage of these patients were diagnosed with MPS II before age 30 months. Thus, early diagnosis is rare but nonetheless is necessary to prompt the clinician to assess for signs of neurologic involvement and counsel families regarding screening for CNS-directed therapy. This review of data effectively differentiates clinical markers of primary CNS disease from those of somatic deposition of GAGs.

CNS disease was defined as primary NP involvement. Secondary obstructive processes, such as hydrocephalus, carpal tunnel, or stenosis of the cervical spine, with accumulation of GAG in the choroid plexus, neural vasculature, or other locations affecting neural signal transmission, do not result from primary NP disease. This is supported by the fact that ERT is able to treat hydrocephalus and other secondary neurologic symptoms by effectively clearing accumulated GAG deposits from the choroid plexus and improving the previously disturbed cerebrospinal fluid circulation.[18] Cognitive dysfunction, however, is thought to result only from primary neural involvement and manifests as a progressive decline in cognitive abilities. These cognitive effects are the result of GAG accumulation in neural tissues. It also has been proposed that secondary effects (eg, inflammation) may be responsible for the cognitive deterioration observed.[19-21] Although such secondary processes may contribute to the CNS pathology resulting in cognitive impairment, it is believed that IT-ERT may prevent the accumulation of GAGs in neural tissue, thereby also preventing such secondary

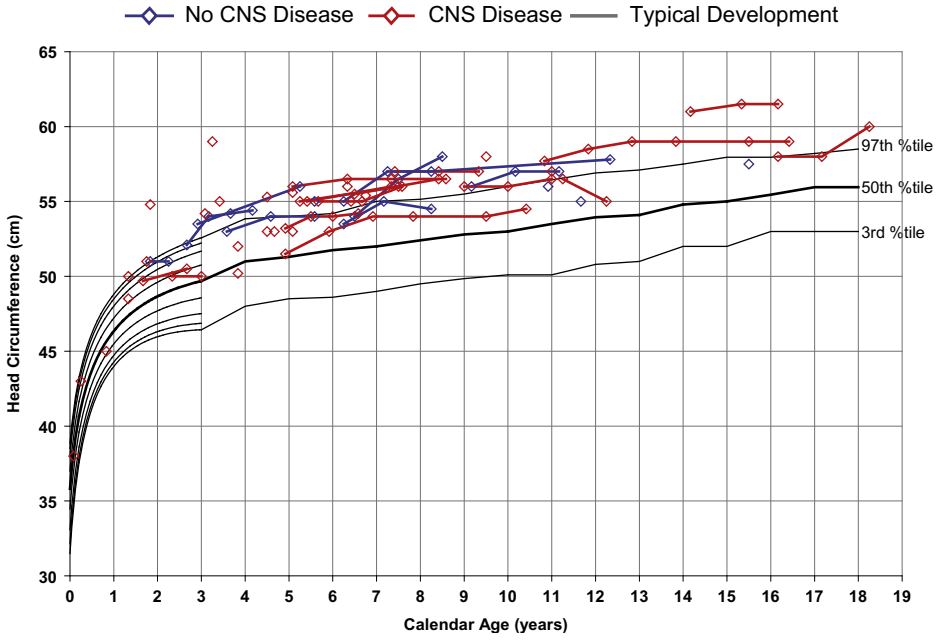

Figure 2. Head circumference of children with MPS II, plotted using Centers for Disease Control and Prevention norms, showing no difference in head growth between the CNS and non-CNS groups.

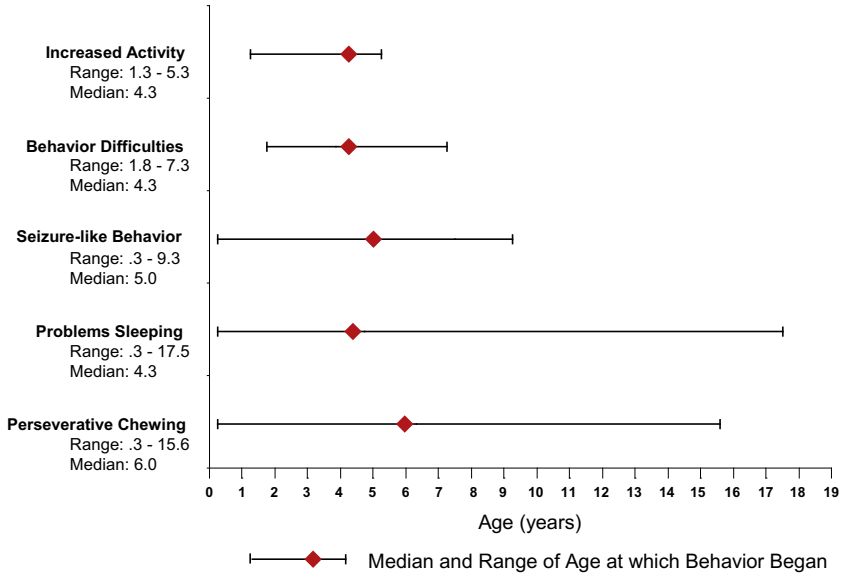

Figure 5. Age at presentation of early markers, including the median age and age range at which the behavior was first reported.

processes from developing. Our group has proposed designating neuronopathic MPS II disease with associated cognitive decline as primary NP disease (MPS II-NP). In our cohort, secondary processes, such as hydrocephalus and cervical cord compression, were seen only in patients with CNS disease, and the frequency of carpal tunnel syndrome was the same in the CNS group and the non-CNS group (31% vs 30%). Patients who required surgical interventions (eg, ventriculoperitoneal shunt, cervical decompression) demonstrated improvements in overall well being, strength, and behavior but no changes in the course of cognitive deterioration. Studies with larger samples are needed to confirm these findings.

The 7 early clinical markers that we have identified are considered direct manifestations of primary CNS disease. Sleep disturbance issues can be secondary to CNS disease, obstructive apnea, or both. The pathological mechanisms explaining how GAG accumulation leads to these sleep difficulties are even less well understood and require a more directed evaluation than was conducted in this study. The incidence of sleep disturbances was nearly 3 times greater in the CNS group than in the non-CNS group. Most of the sleep disturbances in our cohort presented as difficulty initiating or maintaining sleep, similar to those seen in patients with MPS III.[22-24] Many patients reported awaking 3 or 4 times per night and having great difficulty returning to sleep, often going without sleep several nights per week. Other sleep difficulties in the CNS group included decreased rapid-eye-movement sleep, atypical sleep stage distribution, sleep-onset insomnia, and frequent leg movements the last hour before falling asleep. Many of the patients with CNS disease had severe oxygen desaturation requiring bilevel positive airway pressure breathing support. Although 4 of 12 patients in the CNS group reported sleep disturbance at some point during the evaluation period, 2 of these patients' sleep impairment improved after tonsillectomy/adenoidectomy, and the other 2 patients had diagnosed sleep apnea with contributing oral motor hypofunction and persistent tongue protrusion. In this study, 79% of patients in the CNS group had sleep difficulties. The similar incidence of sleep disturbance in patients with MPS II and those with MPS III and the absence of nonobstructive sleep difficulty in the non-CNS group suggest that sleep problems are the result of primary CNS disease, and that the distribution of enzyme into the CNS is necessary for effective prevention.

As children develop increasingly complex and controlled cognitive abilities, they concurrently experience increased attention span and the ability to remain focused on a given task. When this process is interrupted by brain disease, the alteration manifests behaviorally as shortened attention span and increased activity level. The strong association between the age at cognitive decline and the simultaneous presence of increased activity and behavior difficulties suggests that the behavior issues are the direct result of neurocognitive decline. The increased activity is manifested as atypical, high-energy behavior. Similar behavior manifestations have been reported as "aggressive behaviors" in patients with MPS II. Careful examination of these behaviors revealed that they were merely manifestations of the frustration associated with declining cognitive abilities. Here 56% of the patients displayed behavior difficulties as their cognitive function stopped developing or declined. Their behaviors were age-appropriate for their level of cognitive function (eg, the physical actions of a 3-year-old equivalent mind in the body of a 9-year-old child).

The incidence of seizures was 44% (16/37) in the CNS group and 34% in the total cohort. An incidence of 13% was previously reported for all patients with MPS II in a large cohort study.[25] The higher incidence in the present

study is likely due to our less-stringent classification of seizures. Many of the "seizure-like behaviors" experienced by patients in our cohort were not confirmed by electroencephalography. Three main categories of seizure-like behaviors were experienced by patients in the CNS group. Absence seizures, manifesting as 3 to 20 episodes of prolonged staring per day, were observed in 5 children. Six children had tonic-clonic seizures, 3 had myoclonic seizures, and 3 had a combination of seizure-like behaviors. Many children exhibited more than one seizure-like behavior at various times. Increased incidence of seizures with progressive neurologic deterioration has been reported in other MPS disorders.[26,27]

Chronic perseverative chewing behaviors were observed only in patients in the CNS group. Although only 18 of 37 children (49%) with CNS disease manifested such behavior, it is an important clinical marker because of its complete absence in the children without CNS disease. This finding is distinct from abnormal open-mouth posture, difficulty swallowing, enlarged tongue, and gingival hypertrophy, a common constellation of symptoms caused by the accumulation of GAGs in somatic tissue. The perseverative chewing behaviors reported here are hypothesized to result from the deposition of GAGs in NP. Similar chronic chewing behaviors have been reported in patients with MPS III.

For many children with CNS disease, the cognitive decline occurs during the latter stages of normal bowel and bladder training.[28] This suggests that primary cognitive dysfunction may result in the child's poor understanding of bodily functions. Further studies are needed to confirm this. It also is feasible that neurologic control over urinary and defecatory function may be impaired and that autonomic dysfunction results from GAG accumulation in peripheral tissues. In the few children who achieved proper bowel/bladder control and declined thereafter, the loss of bowel/bladder function occurred over a period of weeks to months and was associated with a general decline in cognitive ability.

An incidental finding in our analysis was the presence of upper airway congestion at a significantly higher rate in the children with CNS disease compared with those without CNS disease. The pathological mechanism by which CNS disease imparts increased report of congestion is unclear, but might be related to increased immune reactivity due to increased GAG deposition affecting ciliary function or changes in endogenous flora. Additional investigation is needed before the presence of congestion can be considered a marker of primary NP involvement.

Many of the common somatic features of MPS II were manifested more frequently in our relatively small cohort of patients without CNS involvement compared with those with CNS disease. Umbilical and inguinal hernia, cardiac/valvular dysfunction, and macroglossia were all more common in the non-CNS group. It may be that children with primary NP involvement have less somatic involvement than those with only somatic disease. This is in contrast to previously reported data suggesting that severe MPS II is often characterized by multiple skeletal deformities (known collectively as dysostosis multiplex) and extensive organ and soft tissue involvement. In addition to the neuropsychologic impairments. Previous data suggests that non-NP MPS II is generally characterized by somatic changes of varying degrees.[29] The present study cannot provide conclusive evidence because of the relatively small number of patients in our non-CNS group. This could be an interesting variable to investigate further through large registries, such as the Shire's Hunters Outcomes Survey world registry.

Despite the limited specificity of the individual markers, the severity index score developed in this study can help providers recognize CNS involvement. A severity index score of >3 is associated with significantly increased likelihood of developing CNS disease. However, the early markers and the severity index have not yet been tested prospectively as predictors of CNS disease. Given the limited number of patients with MPS II and the sample size of the present study, a prospective study of a large sample of patients is needed to validate and further define the severity index. Once the severity index is validated, it has the potential to be used as a first-line screening measure to refer patients for future CNS therapy. This tool may become instrumental in the follow-up of infants diagnosed through newborn screening, where the diagnosis is based on enzyme testing and there is no family history. Asymptomatic newborns need frequent follow-up to determine the need for referral for CNS therapy, and such follow-up programs can become very costly without a simple screening tool, such as our severity index. ■

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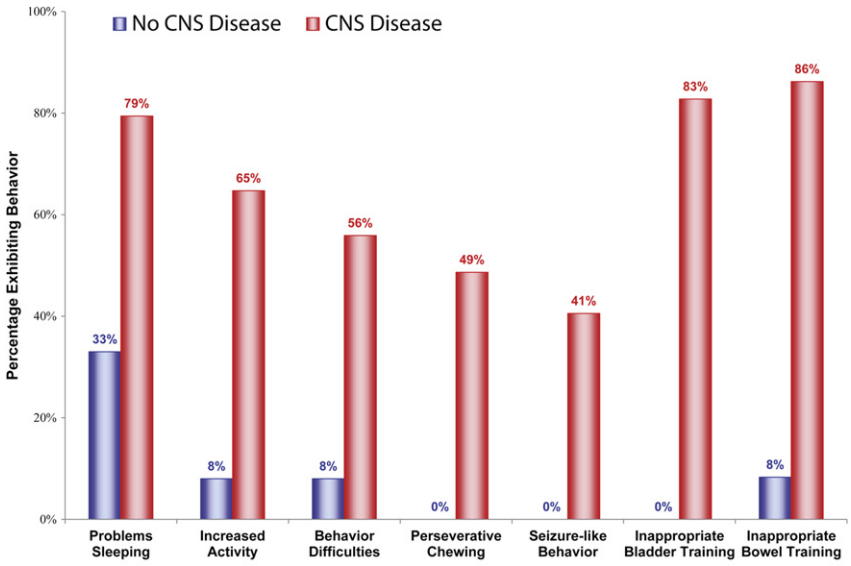
Table I. Selected signs and symptoms

Increased activity	Otitis media
Behavior difficulties	Myoclonus
Hypoactivity	Brisk deep tendon reflexes
Seizure-like behaviors	Carpal tunnel
Bladder training	Hearing deficits
Bowel training	Hearing aids
Overstuffing mouth	Sleep problems
Textural preference	Heart murmur
Perseverative chewing	Esotropia/exotropia
Head circumference	Adenoidectomy
Decreased deep tendon reflexes	Tonsillectomy
Difficulty swallowing liquids	Ventriculoperitoneal shunt
Difficulty swallowing solids	



Clinical Marker	*Description of Behavior*
Increased Activity	*Hyperactivity, difficulty redirecting attention and inability to focus on tasks. The behavior occurs with greater intensity and frequency than typical for children of the same developmental age.*
Behavior Difficulties	*Described as "aggressive" behavior, throwing objects, pushing/hitting others.*
Seizure-like Behavior	*EEG readings characteristic of seizure, observed, or reported staring episodes, episodes of myoclonus, tonic-clonic seizures.*
Sleeping Problems	*Abnormal sleep cycles, reduced REM sleep, difficulty falling asleep, inability to remain asleep.*
Chewing Behavior	*Constant motion of mastication, chewing on non edible objects, perseverative in most settings.*
Bladder Training	*Conscious control over bladder functions, not requiring diapers, at or before age 3.*
Bowel Training	*Conscious control over bowel functions, not requiring diapers, at or before age 3.*

Figure 3. Early markers of disease progression. The proportion of patients exhibiting early clinical markers of CNS disease is shown. The between-group difference is significant for all behaviors ($P < .05$). The description of behaviors provides an example of how each behavior presented during clinical evaluation.

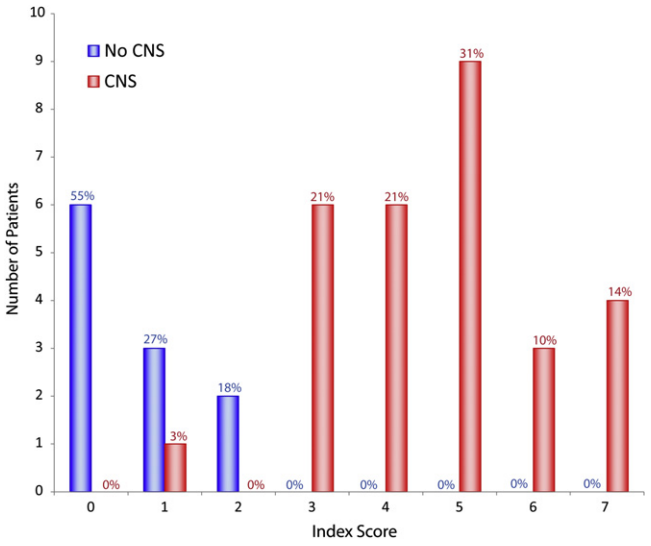


Figure 4. Histogram of early markers, depicting the joint distribution of early markers from which the index of disease severity was developed. The *y-axis* shows the number of patients, and the *x-axis* shows the index score (1 point for each CNS marker).