DIFFERENTIAL DIAGNOSIS IN CHILDREN WITH MULTISYSTEM INVOLVEMENT: MITOCHONDRIAL AND OTHER COMPLEX METABOLIC PATHOLOGIES

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DEDICATION

This work was a labor of love for so many families and medical professionals who gave of their time and efforts to insure the cases were collected and processed appropriately for the study. I dedicate this work to them. I would also like to use this work to remember those undiagnosed children who did not benefit from its intended clinical utility, to the ones we lost during the journey.

Finally, I would like to dedicate this work to my family, three members in particular. Dorothy Blume, another health care provider, who inspired me from a young age and made it possible for me to pursue higher education. My parents, Kendall and Brenda Parker, always supported my dreams and service to children.

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ABSTRACT

MARY ELIZABETH PARKER

DIFFERENTIAL DIAGNOSIS IN CHILDREN WITH MULTISYSTEM INVOLVEMENT: MITOCHONDRIAL AND OTHER COMPLEX METABOLIC PATHOLOGIES

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The purpose of this work was to develop and test a tool of clinical indicators to assist in diagnosis and referral of undiagnosed children with complex multisystem involvement. Three studies were conducted.

In the first study a chart of primary clinical indicators was created from literature on cerebral palsy (CP), Rett syndrome (RTT), metachromatic leukodystrophy (MLD), Krabbe disease, and mitochondrial disorders and cases of undiagnosed children. CP and RTT are established diagnostic entities while MLD, Krabbe, and mitochondrial disorders are less known. Primary clinical indicators were selected with 60% or more prevalence. The primary clinical indicators for CP are hypertonicity; quadriplegia, hemiplegia, or diplegia; and dyskinesia; for RTT are ataxia, apraxia, hand stereotypies, and regression; for MLD are hypertonia, hypotonia, initial gait disturbance, and regression; for Krabbe are hypertonia, regression, irritability, and primary feeding issues; for mitochondrial disorders are hypotonia, regression, three or more organ systems affected, primary feeding issues, and dysmorphism; for the 10

undiagnosedchildren are hypotonia and primary feeding issues. A category of "other" was added to the tool for the latter category.

In the second study the tool was assessed for validity and refined. Thirty-three subjects with known diagnoses were included. Primary clinical indicators were compiled and compared to the tool. CP and RTT were accurately identified, and with modifications to the tool, both leukodystrophies (changed from MLD) and mitochondrial disorders (regression and dysmorphism removed) also demonstrated good clinical utility.

The purpose of the third study was to assess the efficacy of the revised clinical indicator tool in guiding clinical diagnoses of complex multisystem disorders. Twenty-one subjects with diagnoses blinded to the primary researchers were included in a medical record review. CP demonstrated the greatest prevalence, and no subjects had RTT, MLD, or Krabbe. The tool was again revised by adding a category of "complex."

In conclusion the tool demonstrated the ability to differentiate CP and RTT with additional validation needed for complex multisystem disorders in future studies with greater subject number and geographical scope.

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CHAPTER I

INTRODUCTION

Health care professionals need guidance in evaluating and treating patients with multisystem involvement who do not have definitive diagnoses. In the case of children with special needs, 30 to 40% do not have a specific diagnosis.[1,2] While The Human Genome Project greatly enhanced diagnostics with the mapping of disease specific sites on all 46 chromosomes, the full implications for the undiagnosed have yet to be realized.[3, 4]

A tool to guide healthcare professionals in accurately diagnosing or making appropriate referrals of clients with undiagnosed disorders, particularly children, would be beneficial. In this work the operational definition of *undiagnosed* is a phenotype (presentation) that does not correlate with any known disease entity clinically via laboratory or other diagnostic means. While various algorithms, descriptions, and clinical pathways are available for known diagnoses such as cerebral palsy, few tools are available and relevant to children with complex metabolic pathologies such as mitochondrial disorders. The inability to assess and serve the undiagnosed population leads to *dumpdiagnoses*, operationally defined here as the diagnosis that most closely fits and provides for services and reimbursement by third party payers. Moreover, patients with multisystem system diagnoses are referred to many health care

providers for treatment, but the responsibility for centralized data collection from all providers is undefined. Clinical indicators (cluster of signs and symptoms) that may be exhibited are currently not compiled in any national database or referenced in any literature. The possibility of grouping according to these indicators is plausible as a mechanism of organization, but is not yet in place for undiagnosed children with multisystem disorders.

BRIEF LITERATURE REVIEW

The National Organization for Rare Disorders, Inc. (NORD) published a study in 2003 by Krammer[2] stating the following:

- 36% of 138 respondents remained undiagnosed for over one year.
- 14% remained undiagnosed for 6 years or more.
- 28% could not attend school because of their rare disorder.
- 21% were under 16 years of age.

The data in this study were compiled from patients across the lifespan, thus reflecting the magnitude of the undiagnosed population of all ages within the health care system. This study supported NORD's mission to unite health care entities together even though a study of diagnostic practice of health care professionals was not included. In an attempt to serve this population the National Institutes of Health (NIH) in May of 2008 launched a multiple unit project on undiagnosed patients.[5] These initiatives reflect the need to develop a process for better classification approaches, but there is essentially no literature

describing any current clinical strategies to aid in a diagnosis and plan of care for this undiagnosed population.

Cerebral palsy (CP) and Rett syndrome (RTT) are well known disorders affecting children that have been better classified over time and can provide a structure for newer classification schema.[6-15]Other diseases that have developing classification models include Krabbe disease, metachromatic leukodystrophy, Leigh syndrome, and mitochondrial disorders. Classifications of these disorders can also serve as models for better defining and grouping undiagnosed pediatric populations, and therefore all of these classified disorders are reviewed briefly.

In 2005 a consensus definition of cerebral palsy was developed and published:

"Cerebral palsy (CP) describes a group of disorders of the development of movement and posture, causing activity limitation, that are attributed to non-progressive disturbances that occurred in the developing fetal or infant brain. The motor disorders of cerebral palsy are often accompanied by disturbances of sensation, cognition, communication, perception, and/or behavioral; and/or by a seizure disorder."[6(p.572)]

This definition incorporated classification components from previous clinical observations and laboratory studies including motor abnormalities such as hypertonia, associated impairments such as seizures, anatomic and radiological findings, and causation and timing. The definition of hypertonia was based on

previous work and was operationally configured as three subsets: "spasticity," "dystonia," and "rigidity," based on degree of muscle activation and co-activation, joint movement, and response to external force.[7]

Paneth[8] suggested that CP was an unclear diagnosis due to the following: it is a clinical diagnosis with room for error across diagnosticians; clinical presentations are variable due to neurological differences in each individual; delineation of CP versus other or concomitant neurological dysfunction is slight; and motor impairments early in life may be transient and not indicate lifelong pathology. Paneth referred back to the consensus definition of CP, affirming that the four important terms are *group*, *development*, *activity limitation* and *nonprogressive*, which suggests that clinicians should include the diagnostic processes and operational definitions in any diagnosis of CP.Accardo and Hoon[9] reiterated that sound diagnostics are needed when determining whether a child has cerebral palsy. They referred back to the Sanger et al.[7] work on hypertonia and expanded the scope of diagnostics to include the ELGAN (Extremely Low Gestational Age Newborns) study.[10] They purported that an accurate diagnosis of CP (versus benign cerebral hypotonia, for example) is pivotal in management and prognosis determination. As part of the ELGAN Study in 2008 an algorithm was developed to assist with the classification system of children with cerebral palsy.[10] Utilizing the Bayley Scales of Infant Development II (BSID-II), the Gross Motor Function Classification System (GMFCS), the Vineland Scales, and the Modified

Checklist for Autism in Toddlers the authors composed a flow sheet and tables illustrating co-morbidities and classifications of 1056 children at 2 years of age who were born before 28 weeks gestation. Of the sample, 11.4% (120) met the diagnostic criteria of CP based on their work. Two significant limitations of this study are that there were no diagnostic validations of the diagnosis, and the authors did not suggest its use clinically.

A decision tree developed in Europe for the inclusion or exclusion of cases of cerebral palsy includes: 1) whether or not the condition is progressive, and 2) whether or not the condition is due to a syndrome/brain anomaly or chromosome abnormality.[11] Functional definitions of cerebral palsy are also employed as clinical indicators for children with cerebral palsy. The Gross Motor Function Classification System- Expanded and Revised (GMFCS- E & R)[12]expands the age range through 18, by including an age band of 12 to 18. Levels I through V reflect stages of mobility.

Utilizing combinations of these decision tools and functional measures to arrive at a diagnosis of CP prevents its expanded use as a *dump diagnosis*. With this as a prototype other disorders can be assessed via modifications of given tools. A holistic approach in diagnostics can benefit not only those with suspected CP, but those with more complex metabolic disorders.

Classical Rett syndrome (RTT) is an example of a disorder of regression. RTT diagnostic criteria include: female; normal pre- and perinatal periods;

normal head circumference at birth with deceleration of head/brain growth; early behavioral, social, and psychomotor regression/loss of previously acquired developmental milestones, leading to dementia and communication difficulties; loss of purposeful hand skills; hand wringing or other stereotypies; and gait apraxia with truncal apraxia/ataxia.[16,17] Further classification was derived with the staging of RTT.

An initial staging of classical RTT was published in 1986 with clarification and modifications by the original authors in the following decade.[13-15] Percy et al.[18]developed the *Rett Syndrome Motor-Behavioral Analysis Evaluation Instrument* to distinguish RTT from infantile autism and evaluate therapeutic interventions for RTT. The tool includes gathering information in the following categories: social interaction, respiratory pattern, speech, self-abuse/aggression and movement. Within the stages of RTT each of the aforementioned categories can qualify the status of those affected.

Another disorder of regression is Krabbe disease, which is defined by typical development in first few months of life followed by the development of irritability, extensor posturing, difficulty feeding, vomiting, somnolence, nystagmus or other visual impairments, and a distinctive pattern of yawning.[19] A staging classification and algorithm for Krabbe disease has been developed to determine outcomes of treatment following umbilical cord blood

transplantation.[20]This tool looks at clinical indicators such as tone abnormalities and seizures.

The late-infantile form of metachromatic leukodystrophy (MLD) appears between birth and 4 years of age and should be considered in pediatric differential diagnosis when regression in development is noted.[21-23] Initial presentation is typically a gait disturbance. Progressively the motor skills of the child regress into ataxia and spastic quadriplegia, resulting in the loss of independent mobility. Seizures and visual disturbances are frequent and disabling.

Leigh syndrome is similar to CP in that it can have multi-factorial etiology.[6,24,25] Rahman et al.[24]analyzed 67 patients, 35 with confirmed Leigh syndrome and 32 with Leigh-like presentations. The latter did not meet the criteria established by the authors for true Leigh syndrome: progressive neurological disease with motor and intellectual developmental delays; signs and symptoms of brainstem and/or basal ganglia disease; elevated lactate in blood and/or cerebrospinal fluid; documented neuropathology by imaging; post-mortem studies; or similar presentation in a sibling. Common clinical indicators in both Leigh and Leigh-like subjects were developmental delay, elevated deep tendon reflexes, and respiratory disturbances.

Mitochondrial disorders are a complex group of metabolic diseases that often elude diagnosis.[26] Wolf and Smeitink[27] formulated the *Mitochondrial*

Disease Criteria (MDC) based on clinical, metabolic, imaging and histopathological data, the majority of which require invasive testing. Filiano et al.[28]created a classification system based on phenotypical descriptors of 12 subjects (six males, six females) who had not previously been diagnosed with mitochondrial encephalomyopathy, but had signs of mitochondrial dysfunction. Hypotonia, epilepsy, autism and developmental delay were defined as HEADD syndrome and indicate a cluster of descriptors to lead physicians and investigators to order muscle biopsies for individuals with this presentation to determine if they indeed have a mitochondrial disease. A later study summarized the main neurological manifestations (from most frequent to less frequent) of 31 patients with mitochondrial disease as: global developmental delay, spasticity, hypotonia, convulsions, sensory neural hearing loss, dystonia, optic atrophy, proximal muscle weakness, ataxia, and retinitis pigmentosa.[29]

The complexity of the presentation of mitochondrial disorders in contrast to CP, RTT, metachromatic leukodystrophy, Krabbe disease, and Leigh syndrome justifies more comprehensive clinical assessment tools for these disorders as well as for the undiagnosed populations. The potential benefits of research involving undiagnosed pediatric clients are the following: with the ability to differentially diagnose and refer, all health care professionals can assist in the process of decreasing the number of undiagnosed cases; earlier diagnosis may positively affect client outcomes and the potential for a disorder to be treated in the earlier

stages of the disease process; and earlier diagnosis may ultimately save or prolong lives. The purpose of this work was to develop a tool to assist in establishing a working physical therapy diagnosis, prognosis and plan of care based on the clusters of clinical indicators. Secondarily, the tool can assist with potential referrals by identifying clinical indicators which point to the need for more extensive testing to clarify the underlying diagnosis.

Methodology

A tool to assist in the differential diagnosis and treatment of undiagnosed pediatric clients with rare disorders was developed based on the combined results of three distinct studies. The studies are based on literature review, retrospective case reviews, and history of disease progression derived from caregivers of children with undiagnosed conditions and placed in medical records. All medical records were reviewed via a protocol established and approved by the regulating Institutional Review Boards of the authors' affiliated universities.

Study number one entailed collection and charting of clinical indicators for conditions such as CP, RTT, MLD, Krabbe, and mitochondrial disorders via review of published pediatric case studies. In order to capture the scope of the many clinical indicators that describe complex disorders, additional support for the clinical indicators were gleaned from review of clinical records of 10 children with undiagnosed multisystem disorders. Record review included the compilation of descriptors in clinical assessments and diagnostic reports that

contributed to the pool of clinical indicators. Clinical indicators were operationally defined for clarity and consistency. The goal of this first study was to group clusters of primary clinical indicators for each disorder's category. Descriptive analyses of these clinical indicators were performed. For a given clinical indicator to be considered a primary clinical indicator for that diagnostic category, its presence was required in at least 60% of the cases.

In study number 2 groupings of clinical indicators developed in the first study were tested in a non-blinded review of 33 cases of children with known diagnoses by calculating frequencies of cases with the cited primary clinical indicators for their retrospective diagnoses. The record review procedure was the same as that used in study one. The goal of study two was to investigate the validity of the original primary clusters of clinical indicators for each category of disorder by confirming their presence in a group of diagnosed children. The clusters of primary clinical indicators developed from this sample were compared to the clusters in the chart from the previous literature review, using Fisher's Test or Kappa analysis where indicated.

Study number 3 evaluated the updated clinical indicator tool which was developed and refined in the first two studies. Clinical indicators described in the charts of another 21 subjects with known diagnoses were collected. The primary researcher was blinded to the diagnoses and utilized the clinical indicator tool to arrive at a working diagnosis for each case, which was then compared to the

known diagnosis. The goal of Study 3 was to test the ability of the tool to assist in an accurate clinical differential diagnosis.

CHAPTER II

REVIEW OF LITERATURE

Differential diagnosis of a pediatric patient with multisystem involvement and a complex presentation is a challenge in health care. Patient phenotypes do not always follow familiar patterns. The disease process may be dynamic causing different manifestations at different times. In order to recognize a disorder clinically signs and symptoms (clinical indicators), need to cluster in a manner that consolidates a definition, some in the context of the lifespan. If a disorder is undefined based on clinical presentation, further diagnostic testing is indicated. To address differential diagnosis in patients with complex multisystem and metabolic disorders that have not been well-defined to date, it is important to review the established methods of differential diagnosis for other pediatric disorders in order to develop a decision-making tool. Based on their presentation in pediatric clients, five conditions were chosen for review: cerebral palsy (CP),Rett syndrome (RTT), metachromatic leukodystrophy (MLD), Krabbe disease, and mitochondrial disorders with emphasis on Leigh syndrome. The first two disorders, CP and RTT, were chosen secondary to having well established definitions and diagnostic tools, in contrast to the other disorders that are less well known.

CEREBRAL PALSY

Cerebral palsy is a well known condition in pediatrics, occurring in 2-3:1000 live births; it is the most common cause of motor deficits in young children.[1] First mentioned in non-English peer reviewed journals, it was later identified by the English physician William Little[2] and consequently referred to as Little's disease.[3] Further documentation by William Osler in the 1880's led to the name "cerebral palsy." A well detailed history of the early identification of CP can be found in *Early Diagnosis and Therapy in Cerebral Palsy: A Primer on Infant Developmental Problems*[3] including descriptors provided by Sigmund Freud. More recently the following diagnosis was established by a consortium of researchers:

'Cerebral palsy (CP) describes a group of disorders of the development of movement and posture, causing activity limitation, that are attributed to non-progressive disturbances that occurred in the developing fetal or infant brain. The motor disorders of cerebral palsy are often accompanied by disturbances of sensation, cognition, communication, perception, and/or behavioral; and/or by a seizure disorder.'[4(p.572)]

Components of this classification include motor abnormalities (e.g., hypertonia), associated impairments (e.g., seizures), anatomic and radiological findings, and causation and timing.

The consortium reviewed over 100 years of various diagnostic criteria and definitions, including information for the *Surveillance of cerebral palsy in Europe (SCPE)*[1] which provides the categories adopted by the consortium: spastic (including elevated tone and hyperreflexia); ataxic (including poor motor coordination); and dyskinetic (including dystonic or choreo-athetoid presentations). Bax et al.,[4] however, caution that CP should be defined by the predominant tone pattern or abnormality of movement, e.g., spasticity, dystonia, choreoathetosis or ataxia, and that there should be careful use of the word 'mixed' as it must be operationally defined.

The SCPE also proposed two helpful decision trees that lend themselves for further consideration when analyzing clinical indicators of CP.[1] The first details criteria the SCPE used for inclusion into their CP register. They initiate the decision tree by asking if a child's disorder is of central pathology and continue with a series of questions, including the presence of hypotonia, that could lead to a known genetic or syndromic causality and from there derive the CP classification. This method is helpful in preventing an incorrect diagnosis of the static disorder of CP, in contrast to a possible progressive metabolic disorder. The second decision tree begins with the consideration of increased muscle tone and continues through a set of "yes" or "no" questions until one of the SCPE classifications is derived, e.g., dystonic CP. (See Appendix A). A study similar to the SCPE was performed in the United States in 2002 and defined 20 different

CP subtypes by including the classical descriptors of limb involvement, such as spastic diplegia, and a category for CP not otherwise specified.[5] Consistent application of these paradigms could assist with increased diagnostic precision and less misdiagnosis of CP.

Finally, The SCPE discussed the inconsistencies in the diagnosis of CP.[1] The group stated while many definitions existed, the key points all definitions should encompass were:

- CP is an umbrella term of a group of disorders.
- CP is permanent, but not unchanging.
- CP involves a disorder of movement/posture/motor function
- CP is due to a non-progressive insult in the developing brain.

Other areas of incongruity were age of registration in a database; including or not including CP from postnatal insult; including or not including children with known syndromes or chromosomal abnormality; and how to classify children with severe hypotonia. All of these issues were addressed in the decision making trees in hopes of clarification across diagnostic registries.

An example of where tools like these may be helpful is found in the work of Rajab et al.[6] 10 subjects of consanguineous origin were diagnosed with cerebral palsy despite consistent traits of microcephaly and mental retardation which were attributed to a genetic abnormality, not an insult to the developing brain. Utilization of the definition of cerebral palsy and applying the decision making

trees would indicate the need for more genetic testing. In turn a more conclusive etiology, such as an autosomal recessive patterning of inheritance, which the authors suggest, may be found. Moreover, genetic testing may assist with addressing other impairments not related to cerebral palsy, e.g., tremors and visual loss noted in these subjects. Ultimately, more appropriate interventions could be rendered.

CP is well established as a diagnosis, but the criteria and tools aforementioned are not always applied, leading to documented misdiagnoses of CP in the literature.[7-10]These sources give a range of misdiagnoses including congenital myotonic dystrophy, dystonia, ataxia-telangiectasia, and metabolic disorders. Russman[11] in an extensive review points out that many brain malformations lead to a diagnosis of CP, when in fact they differ from acquired lesions such as the aforementioned, e.g., PVL. Disorders such as polymicrogyria, schizencephaly, agenesis of the corpus callosum, hydrocephalus, and holoprosencephaly are non-progressive in nature, but nonetheless, the reason for the disability. CP in these cases is just a descriptor, and may even be considered a *dump diagnosis* in these cases. In the larger picture many of these malformations have underlying genetic bases, thus requiring more extensive workups and genetic counseling.

Another resource for clinicians is the work of Gupta and Appleton[12] who state CP is an 'umbrella' term that does not define a cause of the disorder in and

of itself. These authors specifically state that the diagnosis of CP should necessitate an attempt to find the cause of the disorder such as RTT and MLD. They conclude that children with a suspicion of the CP diagnosis should be evaluated for etiology especially when one or more of the following are found: no definitive perinatal insult to the brain; family history of "cerebral palsy;" regression; sensory loss, ataxia, muscle atrophy, oculomotor dysfunction, and non-volitional movement disorders.

Functional definitions of cerebral palsy have also evolved with the publication of the Gross Motor Function Classification System- Expanded and Revised (GMFCS-E & R):[13]

- Level I indicates that the child walks without limitations.
- Level II indicates that the child walks with limitations.
- Level III indicates that the child walks using a hand-held mobility device.
- Level IV indicates that the child has limitations in self-mobility and may use power mobility.
- Level V indicates those children who are transported in a manual wheelchair.

The GMFCS-ER elaborates on the actual patient presentation described in the SCPE paradigms and is useful for functional mobility classifications.

Cerebral palsy, however, has multimodal causation and the diagnosis maybe given on clinical presentation or based on a lack of genetic mutations that can explain the neuromotor impairments.[14] Unlike the days of Little and Osler, current diagnostics can be performed using a variety of imaging techniques.[15, 16] Cranial ultrasound (CUS), computed axial tomography (CT), and magnetic resonance imaging (MRI) are in order as progressively detailed means used to diagnose brain pathology that may lead to CP.[17] Shimony et al.[15] suggest the following synopsis for linking pathology to presentation:

1. In the premature infant, white matter involvement or periventricular leukomalacia (PVL)→ spastic diplegia with vision issues.
2. In the premature or term infant, white with possible deep nuclear gray matter→spastic quadriplegia with learning issues.
3. In the term infant, cortical arterial vascular interruption, focal cerebrovascular accident→hemiplegia with learning disorders and epilepsy.
4. In the term infant, deep gray matter involvement of the thalamus and basal ganglia→athetoid with feeding/oral motor difficulties.

MRI is purported in this study to be the most sensitive tool for identifying impairments. MRI techniques range from traditional imaging to diffusion, spectroscopy, and functional (fMRI).

In terms of differential diagnosis via imaging Ashwal et al.[18] proposed an algorithm for evaluation of a child with possible CP. The process begins with ruling out a progressive, neurodegenerative disease. This algorithm mirrors the SCPE paradigms as it reviews whether imaging diagnostics were performed in the neonatal period and, if not, recommends the use of MRI over CT.[1] Finally, it defines CP by the MRI findings in conjunction with history and examination. This study demonstrates the balance of clinical decision making with the use of diagnostic imaging to validate the diagnosis of CP. While some researchers suggest that CP need not be validated with imaging, others suggest that MRI is the gold standard to confirm the diagnosis.[15, 17-19] However, the authors of the proposed definitions of cerebral palsy caution that as of 2005 imaging was just emerging in technical ability to be a valid diagnostic tool.[4] They corroborated the suggestion made by Aswal et al.,[18] that when possible imaging should be performed on all children with CP. Given the possibility of misdiagnosis without definitive etiology it may be optimal to corroborate clinical indicators with imaging in suspected cases of CP.

RETT SYNDROME

Imaging is not the gold standard for some complex multisystem disorders. For example, a definitive diagnosis of Rett syndrome (RTT) is determined by analyzing the MECP2 gene.[20] This process is relatively common when physicians are first attempting to diagnose a child with developmental issues per review of

medical records (personal observation). Mutations in the MECP2 gene residing on the X chromosome cause most cases of Rett syndrome (RTT).[21] Characteristics of females with Rett include normal pre- and perinatal periods; normal head circumference at birth with deceleration of head/brain growth; early behavioral, social, and psychomotor regression/loss of previously acquired developmental milestones/evolving dementia and communication difficulties; loss of purposeful hand skills; hand wringing or other stereotypies; and gait apraxia with truncal apraxia/ataxia.[22, 23]

The stages of classical RTT were published in 1986 with clarification and modifications by the original authors in the following decade.[24-26]

- Stage I is defined by stagnation of development beginning as early as 6 months of age.
- Stage II begins between 1-3 years of age and is the time of rapid developmental regression.
- Stage III is the pseudostationary phase when seizures worsen but developmental regression has ceased.
- Stage IV is the final stage where loss of ambulation and the need to use a wheelchair is noted (Stage IV A denotes previous ambulators and Stage IV B denotes non-ambulators throughout life).

In 1988 The Rett Syndrome Diagnostic Criteria Work Group[23] expanded the staging criteria by including possible differential diagnoses. Throughout the

staging/lifespan criteria they proposed RTT could be considered a contrasting diagnosis to disorders such as benign congenital hypotonia or cerebral palsy at Stage I; infantile spasms[27] or autism[28-30] at Stage II; and spastic ataxic cerebral palsy, leukodystrophy or another storage disorder at Stage III. This expansion speaks to the evolving nature of RTT and the importance of defining clinical indicators throughout the lifespan.

Percy et al.[28] developed the *Rett Syndrome Motor-Behavioral Analysis Evaluation Instrument* to distinguish RTT from infantile autism. Subjects were scored on social interaction, respiratory pattern, speech, self-abuse/aggression and movement on a scale ranging from '0' (never) to '4' (constant/near constant). Results indicated that RTT was distinguishable from infantile spasms, but the tool has not had any further published applications. This instrument may be a valuable means to quantify functional status in RTT.

Caveats in the diagnosis of RTT include 20% of females that present with phenotypical RTT but do not test positive for the RTT gene. Some can be explained by the presence of mutations in the cyclin-dependent kinase-like 5 (CDKL5) and Forkhead box G1 (FOXG1) genes causing atypical RTT.[31-35]These exceptions demonstrate the importance of processing clinical indicators within the perspective of genotype versus phenotype.

METACHROMATIC LEUKODYSTROHPY

Another multisystem disorder with a complex presentation and known genetic cause is metachromatic leukodystrophy (MLD) which was first defined by Joseph Godwin Greenfield in 1933 as "a form of progressive cerebral sclerosis."[36] MLD is an autosomal recessive lysosomal storage disorder that is caused by a mutation of the arylsulfatase A gene on chromosome 22 resulting in a decrease in the enzyme arylsulfatase A that is necessary to break down sulfatide.[37-39] Sulfatide storage negatively affects the myelin-producing cells, oligodendrocytes, causing demyelination of the white matter of the brain (leukodystrophy). It can also accumulate in organs, blood, and bone marrow. Studies report the incidence from 1:40,000 to 1:100,000.[40,41]

The late-infantile form of MLD (the most common form) appears somewhere between birth and 4 years of age.[37, 38, 42] Initial presentation is typically a gait disturbance. The motor skills of the child progressively deteriorate resulting in ataxia, or spastic quadriplegia, and a loss of independent mobility. Seizures and visual disturbances are frequent and disabling. Like RTT, MLD is a disorder noted by initial regression of acquired skills followed by progressivedeterioration of function. The following sampling of cases gives a more comprehensive look at the presentation of MLD.

MacFaul et al.[43] described 24 cases of late-infantile MLD. Mean age of onset was 17 months with 23 out of 24 cases initially showing gait disturbance as

the first sign. In half of the sample gait was impaired by balance deficits, toe walking, and abnormal foot positioning. These children also lost social and language abilities as the disease progressed. Spasticity, elevated or diminished deep tendon reflexes, ataxia, nystagmus, dysarthria, intention tremor, and opisthotonus were noted in the group in varying degrees.

Lugowska et al.[44] analyzed the genotype/phenotype correlation in a sample of patients with MLD. Motor deterioration including gait disturbances; ataxia; spasticity; dystonic, myotonic and opsoclonic movements; nystagmus; hypotonia; and abnormal reflexes was noted in the late-infantile form. In a study of peripheral neuropathy in patients with MLD, similar clinical indicators were noted. The most common were delay in attainment of developmental milestones, frequent falls, ataxia, spasticity, and seizures.[45] Of note in these two studies is mention of the differential diagnoses of both Guillain-Barré syndrome (GBS) and Krabbe disease that confounded some of the data. In the study on peripheral neuropathy it was suggested that sural nerve biopsy was the key to differential diagnosis of MLD over GBS. In the previous study it was reported that MRI imaging indicated demyelination of a leukodystrophy, but in one case the pattern of myelin loss was closer to that of Krabbe disease; thus, MRI may not be definitively diagnostic in this disorder. In sum, MLD is a progressive disorder causing demyelination (leukodystrophy) that manifests with visual and motor deficits most notably in conjunction with seizures.

KRABBE DISEASE

Krabbe disease (globoid cell leukodystrophy) is another autosomal recessive lysosomal storage disorder with devastating impact.[46] Krabbe disease was first described in 1916 by Knud Krabbe in *A new familial infantile form of diffuse brain atrophy*.[47] Five cases of infants were detailed by Krabbe who noted these commonalities in presentation: typical development in the first few months of life and then development of irritability, extensor posturing, difficulty feeding/vomiting, somnolence, nystagmus and other visual impairments, and a distinctive pattern of yawning. The majority of cases are of this early infantile form.[46] Krabbe is due to a deficiency of a lysosomal storage enzyme, galactocerebroside β-galactosidase (GALC), caused by a mutation on chromosome 14.[46, 48]The multinucleated globoid cells that are a hallmark of the disease are macrophages that hold the undigested glycolipid. Other noted changes in the nervous system of individuals with Krabbe are loss of myelin and the myelin-producing oligodendrocytes as well as Schwann cells in the peripheral nervous system.[49] The incidence of Krabbe varies widely, from 1:100,000 to 6:1,000 in the Muslim Druze kindred in Israel secondary to consanguinity.[46,50]

Zafeiriou et al.[51] describe a male of nonconsanguineous origin with late-infantile Krabbe who began demonstrating signs and symptoms at 13 months of age and was given a diagnosis of spastic diplegia. He demonstrated irritability, increased muscle tone, increased deep tendon reflexes, clonus, Babinski sign, and

Rossolimo reflex, but his MRI was negative for any abnormality. His condition deteriorated and at 2 years of age he had developmental delay, feeding problems, apnea, and visual impairment caused by optic atrophy. He was given the diagnosis of spastic tetraplegia. MRI showed involvement of the deep white matter, internal capsule, basal ganglia, and thalamus.

Zafeiriou et al.[52] in another case study report the findings of a female born to nonconsanguineous parents. Signs of neurological dysfunction were irritability, seizures including myoclonic jerks, decreased muscle tone, increased deep tendon reflexes, bilateral Babinski sign, developmental delay, sensitivity to sound, peripheral neuropathy, macrocephaly, and feeding problems. It was not until 12 months of age that an MRI indicated white matter involvement of deep white matter and the thalamus.

Nagar et al.[53] detail the findings of another female with Krabbe. At 7 months of age the infant demonstrated excessive crying/irritability, developmental delay, and refusal to feed. MRI indicated cerebral cortical atrophy, ventricular enlargement, and deep white matter abnormalities in the corpus callosum, internal capsule, and pyramidal tracts. The cerebellum, thalamus, optic nerves, and cervical spinal cord also showed aberrations.

Sahai et al.[54] postulated a connection with Krabbe and multiple sclerosis (MS) due to a kinship connection in the immediate family of the subject, and the fact that both disorders affect myelin. This case details the history of a female

infant with Krabbe born to nonconsanguineous parents. She demonstrated irritability, episodic vomiting, feeding difficulties, weak palmar grasp, lower extremity twitching, decorticate positioning, head lag in pull to sit, irregular eye movements, increased deep tendon reflexes, fluctuations in tone, right lower extremity clonus, and seizures. She died at 10 weeks of age. CT and MRI indicated involvement of the lateral thalami, putamen, corona radiata, and dentate nuclei of the cerebellum. Her paternal side of the family had three members with MS. The authors hypothesize that the mutation in the GALC gene may have an interaction with other genes implicated in MS, and suggested clinical indicators may be missed in early infancy secondary to a low level of concern, thus stressing the importance of a detailed family history. They also cited a case of a 21 week gestation fetus with a definitive diagnosis as well as a 7 week old infant with only a peripheral neuropathy that may have gone unnoticed without familial cause for investigation.

The following staging system was proposed for Krabbe disease by Hagberg.[55] In Stage I an infant will demonstrate excessive irritability, hyperesthesia, periodic fevers without a known etiology, and elevated tone in the extremities. While apparently normal at birth the infant becomes hypersensitive to sensory stimuli and will cry frequently, without a known provocation. Delay or regression in motor skills may be noted as well as vomiting and seizures. Stage II is a period of rapid regression with opisthotonic and decorticate positioning as

well as hyperreflexia. Seizures continue during Stage II. The terminal Stage III is marked by decerebrate rigidity and blindness; the infant has little interaction with the environment.

Other models of clinical indicators for diagnosis are imbedded in the literature but are often presented for other purposes. For example, another staging classification for Krabbe disease has been developed to determine outcomes of treatment following cord blood transplantation.[56] These authors provided a valuable resource of operational definitions of clinical indicators of Krabbe, including spasticity in extremities, jerky eye movements, mild thumb clasp, seizures, and abnormal reflexes. The staging criteria ranged from Stage I, child appears normal with subtle neurological signs, to Stage IV, child demonstrates advanced disease. These stages do not correlate with those of Hagberg[55] and the staging criteria have not been applied outside the original domain of cord blood transplantation.

Peripheral neuropathy is a lesser known clinical indicator for Krabbe disease, but is described in two interesting studies by Siddiqui et al.[57, 58] These early nerve conduction studies (NCS) correlated closely with clinical severity and the effect of stem cell transplantation. With the ability to test for Krabbe in a newborn screen and then treat with stem cell transplantation, this clinical evidence has dual value.[59]

Differential diagnosis of Krabbe includes consideration of other disorders such as MLD, Alexander disease, Pelizaeus-Merzbacher disease, and Niemann-Pick disease.[46] Krabbe can be definitively diagnosed by clinical testing via blood lymphocytes and/or fibroblasts to rule out these confounding entities. MRI findings may be limited at early stages of the disease and not as definitive as the genetic testing, and thus they are not sound for differential diagnosis or expedient if stem cell transplantation is an option.[52]

A tool which consolidates clinical indicators that can be observed by medical professionals may prove to be more useful in differential diagnosis. Not only would it expedite clinical testing, but it may allow for earlier referral for experimental treatments. In a recent pilot case study (unpublished), delay in diagnosis of an infant with Krabbe details the diagnostic hurdles, and retrospectively suggests that the clinical indicators (including irritability, abnormal eye movements, and distinctive tone and posturing) may have led to an earlier diagnosis. In the case of this infant many tests were performed to rule out Smith-Lemli-Opitz syndrome and mitochondrial disease. Based on imaging studies a tentative diagnosis of Leigh syndrome was given along with failure to thrive. Ultimately lysosomal storage enzyme studies indicated Krabbe disease but the diagnosis came 2 weeks post-mortem.

MLD and Krabbe (leukodystrophies) are just two examples of over 40 lysosomal storage disorders (LSDs) which affect 1:5000 persons.[60-62] While

prenatal and newborn screening for all LSDs in one test is not available at this point, it is possible that a tool for assessing early clinical indicators may be of use and more cost effective. Analyses of LSDs with attention to medical and therapeutic interventions are included in two articles by Meikle et al.[60, 61] and Haley et al.[62] The latter study provides a functional measurement tool for mucopolysaccharidosis I (MPS I) and Pompe disease that could be extrapolated for use in the identification of clinical indicators for these two LSDs. The MPS I physical performance measure (MPS-PPM) was developed to gauge treatment outcomes following enzyme replacement or stem cell transplantation; however the identification of decreased endurance and functional motor tasks in the MPS I population may provide for expanded clinical indicators of this disease. Likewise the Pompe-Pediatric Disability Inventory (Pompe-PEDI) could provide evidence of early mobility issues that could assist with early observational diagnostics. Tools like the MPS-PPM and the Pompe-PEDI may be helpful guides in settings where many children with suspected metabolic dysfunction are referred and treated.

In sum both LSDs and leukodystrophies need to be considered in differential diagnosis of patients with complex multisystem disorders. Since LSDs and leukodystrophies are not synonymous nor mutually exclusive categories, other considerations need to be made. In addition to MLD and Krabbe, Leigh

syndrome and Alexander disease are leukodystrophies with differing (non-lysosomal storage) etiologies that may initially present in a similar fashion.[36,47,63-66] MRI imaging is valuable for these leukodystrophies but often not definitively diagnostic, so skilled clinical evaluations are also required. Cheon et al.'s[63] review of imaging for differential diagnosis of leukodystrophies suggested the following:

- White matter abnormalities can be identified, localized, and characterized by MRI.
- Response to treatment can be confirmed by MRI.
- MRI may be non-specific to type of leukodystrophy, relying on the clinician to discern more information through additional testing (clinical and metabolic) as well as a review of patient history.
- MLD has a characteristic tigroid pattern in the periventricular white matter, but this is only noted in T2-weighted imaging. Biochemical diagnosis, however, is the gold standard.
- Krabbe has characteristic high signal attenuation in parts of the thalami, basal ganglia, and internal capsule by CT scan. Biochemical diagnosis, however, is the gold standard.
- Leigh syndrome may manifest as symmetrical involvement in the thalamus with infrequent involvement of the cerebral white matter by MRI.

- Alexander disease has characteristic Rosenthal fibers and macrocephaly.[65] Biopsy or autopsy used to be the only way to definitively diagnose but in 2001 a genetic marker was found to expedite diagnosis.[66] Both CT and T2-weighted MRI are useful, but not definitive.

Molecular genetics that discern the biochemical markers are now providing more conclusive diagnostics for leukodystrophies.[67]

MITOCHONDRIAL DISORDERS

Another complex metabolic disorder that warrants consideration is the family of mitochondrial disorders, given their variable presentations. Recent estimates state that a child with a mitochondrial disorder is born every 15 minutes, and the exact number affected is unclear secondary to misdiagnosis.[68] The literature indicates that mitochondrial disorders should be suspected when a typical disease has atypical features; when three or more organ systems are involved; and when recurrent setbacks or exacerbations result from infection.[68,69] According to Cohen,[70] based on his years of clinical practice and research, mitochondrial disorders should be considered when any of these presentations are seen in combination: encephalopathy including seizures, developmental delay or regression, myoclonus, neuropathy, cardiac problems, hearing problems, short stature, extraocular muscle problems, diabetes, renal tubular dysfunction, visual loss, and lactic acidosis. Other presentations that are

suspect according to Cohen include atypical cerebral palsy, failure to gain weight, and respiratory problems. Incidence of mitochondrial disorders is rising with greater recognition and diagnostic capabilities and may allow for some undiagnosed cases to be categorized. Moreover, the medical community may see a shift from diagnoses like CP or probable Leigh syndrome to more definitive genotypes via molecular genetics.[68]

Mitochondrial disorders are complex genetic and metabolic diseases that can cause an array of symptoms from hypotonia to autism.[70-75] In mitochondrial disorders either the mitochondrial deoxyribonucleic acid (mtDNA) or the nuclear DNA (nDNA) mutates and causes the respiratory chain (electron transport chain) to malfunction, creating a defect in the mitochondrial oxidative phosphorylation (OXPHOS) which disturbs the creation of adenosine triphosphate (ATP) . The respiratory chain consists of five multisubunit complexes in the inner membrane of the mitochondria: Complex I, NADH dehydrogenase ; Complex II, succinate hydrogenase; Complex III, cytochrome BC1; Complex IV, cytochrome c oxidase (COX); and Complex V, ATP synthase. These subunits with the assistance of other mediators perform oxidative phosphorylation to create ATP that all cells need for energy. This energy provides the means for glucose dependent structures, e.g., brain and muscle, to work, so the malfunction of the mitochondria has serious and pervasive implications.

Important distinctions need to be made between mtDNA and nDNA defects.[75-77]The mtDNA is maternally inherited from the oocyte (egg). If a woman has a mtDNA mutation all of her children will inherit the same mutation, but only the daughters will propagate it. A mutation in the mtDNA will disperse randomly during cell division; thus some cells will be affected with some tissues being more involved than others. This random effect is called heteroplasmy and is the reason mitochondrial disorders presentations vary when caused by a mtDNA mutations. Moreover, a critical amount of disrupted mtDNA is required for cell and tissue malfunction to occur, which is known as the threshold effect. The threshold effect is further complicated by the bottleneck effect, when only a certain amount of mitochondrial material can be transferred to each cell. Because of continual mitotic cell division during an individual's life, one person can have one clinical manifestation as an infant, and another disorder as an adult, thus resulting in mitotic segregation.

When the mtDNA is affected the following disorders can occur:[71, 73-75]

1. Mutations in the genes that synthesize protein can result from point mutations in transfer ribonucleic acid (RNA) or ribosomal RNA genes as well as single deletions.
    a. Kearns-Sayre syndrome is a multisystem disorder with onset before 20 years of age. It manifests with progressive opthalmoplegia,

pigmentary retinopathy, and heart block. It may also cause ataxia, dementia, diabetes, short stature, and hypoparathyroidism.

b. Mitochondrial encephalomyopathy lactic acidosis and stroke-like episodes (MELAS) is a disorder that causes seizures, cortical blindness, and hemiparesis.

c. Myoclonic epilepsy with ragged-red fibers (MERFF) causes generalized myoclonic seizures, myoclonus, mitochondrial myopathy, and cerebellar ataxia.

2. Mutations in protein-coding genes also interfere with development as evidenced by these examples.

   a. Leber hereditary optic neuropathy is more often found in males and causes early adulthood blindness.[77]

   b. Leigh syndrome is a disorder that causes neuropathy, ataxia, and retinitis pigmentosa. Leigh syndrome, however, is a term used to describe an array of mitochondrial disorders affecting the nervous system, and the actual mutation determines the variety of presentations.

As stated previously, mtDNA presentations can vary. Graf et al.[78] describe a family that had members with a myriad of presentations from autism to Leigh syndrome. A mitochondrial DNA G8363A transfer RNA^{Lys} mutation was detected in multiple family members of a male child diagnosed with autism. He was the

product of a full term pregnancy that was significant for gestational diabetes. He had transient neonatal hypoglycemia. Gross motor milestones were within normal limits for the first 18 months. He lost speech and play skills by 2 years of age, and his symptoms progressed to hyperactivity, lack of emotional control, self-injurious behavior, and seizures by 3 ½ years of age. MRI of the brain was normal. G8363A transfer RNA^{Lys} mutation was detected. His sister's gestation was unremarkable and she developed normally until 15 months of age. At that time she began walking unsteadily, falling, demonstrating myoclonus, and having difficulty swallowing. She was diagnosed with Leigh syndrome. An MRI of her brain at 5 years of age showed abnormality in the putamen. At 7 years of age this abnormality apparently resolved but abnormality in the posterior medulla was noted. As with her brother, a G8363A transfer RNA^{Lys} mutation was found as well as elevated lactate levels and an absence of COX (Complex IV). A maternal half sister also had the mutation but had no abnormal findings and was phenotypically normal. Another maternal half sister, however, had the mutation and presented with seizure disorder, oppositional behavior, and tremor with mild motor dyspraxia. Her brain MRI was normal. Heteroplasmy was demonstrated in this family.

Shah et al.[79] indicate a possible link between infantile spasms, a type of seizure disorder, and the A3243G mtDNA mutation. While the authors do not describe in detail the phenotypes of the 56 subjects, in a few cases they state the

relationship between the mutation and developmental delay, hypotonia, failure to thrive, and gastroesophageal reflux (GERD).

Another example of a mtDNA mutation is the T9176C location on the ATPase 6 gene, causing Leigh syndrome with leukodystrophy.[80] The authors reported at the time that this was a novel mutation causing Leigh syndrome with leukodystrophy in two sons born to nonconsanguineous parents. At 4 months of age the first son began showing signs of irritability, hypotonia, decreased sucking, loss of social smile, and head lag in pull to sit. By 5 months of age he had elevated deep tendon reflexes, the Babinski sign, decreased hearing, no eye contact, and feeding difficulties due to sucking and swallowing deficits. MRI indicated white matter involvement in the bilateral frontoparietooccipital subcortices, periventricular areas, posterior limb of the internal capsule, and cerebellum. The infant died at 7 months of age. His brother developed normally until 5 months of age. At 6 months he demonstrated hypotonia, nystagmus, no eye contact, decreased hearing, and feeding difficulties similar to his brother. He died at 10 months of age. Unlike his brother he had bilateral optic atrophy, but their MRI findings were identical. According to the authors, the fact that neither brother showed basal ganglia or brainstem involvement that is typical of Leigh syndrome, demonstrates that this is another form of the disorder.

A single case study by McPherson and Zabel[81] describes the case of a 15 year old female who as an infant demonstrated distal arthrogryposis, and as an

adolescent developed progressively worsening migraines. MRI indicated a left posterior infarct with resulting right homonymous hemianopsia. Other signs included hearing loss, seizures, and muscle weakness. The female was found to have a mitochondrial mutation of T3271C indicating mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS) which explained her clinical indicators.

The mtDNA case studies presented indicate commonalities despite the different overall phenotypes. Hypotonia, ataxia, feeding difficulties, as well as visual impairments are common clinical indicators. What is significant is the range of phenotypes without all being terminal; in contrast, the nDNA derived mitochondrial disorders present a different picture.

Mutations in nDNA result in Complex I-V disorders, with II being completely nuclear DNA derived.[73,82] These nDNA mutations are subject to Mendelian genetics and either parent can pass on the mutation.[82,83] Defined nDNA defects that cause mitochondrial disorders include Complex I-V deficiencies as well as disorders related to coding of coenzyme Q10 and cytochrome c. The nDNA mutations can cause more serious consequences than mtDNA mutations because each cell is affected; there is no heteroplasmy. It is important to note that these nDNA mutations are frequently autosomal recessive and cause Leigh syndrome in most cases. Of all the mitochondrial disorders, 10-

20% are from mitochondrial DNA mutations, with the majority resulting from nDNA dysfunction.

Bourgeron et al.[82] reported the first definitive link of nDNA mutation to mitochondrial disease in 1995. Two sisters with mutations in Complex II that codes for succinate dehydrogenase were described as presenting with Leigh syndrome. Their parents were of consanguineous origin. In contrast Van den Heuvel et al.[84] described an infant with a Complex I defect who was born to nonconsanguineous parents. This infant began showing signs of the disease at 8 months when he presented with severe vomiting, failure to thrive, and hypotonia. By 13 months he had a demonstrated developmental delay, seizures, bradypnea, cyanosis, hypotonia, and diminished deep tendon reflexes. He died at the age of 16 months. In the same year Loeffen et al.[85] published findings of the first reported nDNA mutation causing a Complex I defect and Leigh syndrome. This infant was of nonconsanguineous origin and presented similarly to the previous case at 5 weeks of age. He died at 11 weeks. Recently an interesting case of Complex II defect with Leigh syndrome resulting from a nuclear DNA mutation was reported in a male with mild symptoms who actually improved over time.[86]

Not all studies of interest on mitochondrial disorders report whether the origin of defect is mtDNA or nDNA mutation; however, many of these studies give excellent clinical indicators and warrant review. Gire et al.[87] described six neonates with mitochondrial disorders but did not categorize them as mtDNA- or

nDNA-derived. Commonalities included consanguinity, polyhydraminos, hydrops fetalis, hypotonia, dysmorphism, and multisystem involvement. Both Complex I and/or IV deficiencies were found in the muscle biopsies. Only one subject was alive at publication of the primary article in 2002. In this study no mtDNA deletions were found, but no other specific correlation to mitochondrial or nDNA mutations were noted. This lack of molecular marker does not provide an etiology for the mitochondrial malfunction in the complexes in the muscle tissue, but does definitively prove a mitochondrial disorder is present. Whether the mitochondrial disorder is primary (genetic mutation) or secondary (another disease causing the mitochondrial dysfunction) is unknown.

Similar findings in eight infants by Moroni et al.[88] corroborate the evidence that both mitochondrial and nuclear encoded mutations can cause white matter changes in the brain. This study indicated deficiencies in Complex I, II, III, IV and V as well as pyruvate dehydrogenase (an assistant in oxidative phosphorylation); thus, both forms of derivation of mitochondrial disease were present. Three of the patients demonstrated early developmental delay, failure to thrive, recurrent vomiting, truncal hypotonia, elevated tone in the extremities, and cognitive impairments. Another four patients had more of a regression pattern than delay, losing already acquired developmental milestones in the first years of life. All subjects had white matter involvement despite their differences in biochemical (complex/enzyme) results and varying clinical presentations.

Clinical indicators were consistent with those seen in other studies despite the seeming variability within these subjects.

Certain diagnostic criteria and phenotype commonalities start to emerge when multiple cases of mitochondrial disorders are compiled. However, many of the categorical systems include diagnostic testing out of the scope of practice for non-physicians.[89-93] The 'gold standard' classification for mitochondrial disorders is the Modified Walker criteria which are predominantly derived from invasive biochemical and molecular testing.[92, 93] Wolf and Smeitink[89] formulated the *Mitochondrial Disease Criteria (MDC)* or *Nijmegan criteria* based on clinical, metabolic, imaging, and histopathological data. This system does include clinical indicators such as ptosis, exercise intolerance, and muscle weakness which can be quantified without invasive testing. On the other hand, Filiano et al.[90] created a classification system based on phenotypical descriptors of 12 subjects (six males, six females) who had not previously been diagnosed with mitochondrial encephalomyopathy but had signs of mitochondrial dysfunction. The subjects ranged from 2-20 years of age and were tested negative for other similar presenting disorders including RTT and MLD. Based on this small sample size hypotonia, epilepsy, autism, and developmental delay were defined as HEADD syndrome and comprised a cluster of descriptors indicating that muscle biopsies were necessary to determine if the subjects indeed have a mitochondrial disease.

A later study summarized the main neurological manifestations of 31 patients with mitochondrial disease (from most frequent to less frequent) as: global developmental delay, spasticity, hypotonia, convulsions, sensory neural hearing loss, dystonia, optic atrophy, proximal muscle weakness, ataxia and retinitis pigmentosa.[91] The most common finding in seven of the patients was Leigh syndrome, showing diffuse demyelination of the brain on MRI and loss of previously acquired motor milestones. Unlike the demyelination seen in Krabbe and MLD, the process in mitochondrial disorders can be varied and not linked to a single autosomal recessive gene.[94] This fact makes imaging diagnostics challenging and again not conclusive.

One particular manifestation of mitochondrial disorders is Leigh syndrome which warrants further description as a diagnostic entity. Leigh syndrome was first recognized in the literature in 1951 by Denis Leigh as a "subacute necrotizing encephalomyelopathy."[64] Leigh syndrome is a metabolic disorder caused by disruption to the processing of pyruvic and lactic acid.[95,96] It has multifactorial etiology caused by mutations affecting the mitochondrial respiratory chain (oxidative phosphorylation or OHPHOS) or deficiencies in related enzymes that assist in energy metabolism. Inheritance may be X-linked, autosomal recessive, maternally derived, or due to a spontaneous mutation. Because Leigh syndrome is a subset of mitochondrial disorders it also has varying presentations. The literature and interpretations of Leigh are diverse,

complicated, and at times disparate; thus, a comprehensive look at this descriptor of mitochondrial disorders warrants further analysis separate from the discussion of mitochondrial diseases.

Leigh[64] first described a single case with 'focal, bilaterally symmetrical subacute necrotic lesions or softenings' in the brain which may include the brainstem, thalamus, basal ganglia, cerebellum, and spinal cord. Further descriptions emphasize primary involvement of the basal ganglia and brainstem.[95,97] The first case detailed by Leigh concerned a male infant who was blind and deaf with hypertonic upper and lower extremities. The infant was somnolent at hospital admission and died at 7 months of age.[64]

Rahman et al.[95] described 67 patients, 35 with confirmed Leigh syndrome and 32 with Leigh-like presentations. The latter did not meet the criteria established for true Leigh syndrome because they did not have a neurological disease with developmental delay of a progressive form; brainstem and/or basal ganglia involvement; elevated lactate in blood and/or cerebrospinal fluid; or one of more of the following: neuropathology by radioimaging/post-mortem studies or a sibling with similar phenotype. Common clinical indicators in both Leigh and Leigh-like subjects were developmental delay, elevated deep tendon reflexes, and respiratory disturbances. Besides a predominance of males in both groups, the other clinical indicators were not strongly correlated with genetic causes.

Yüksel et al.[98] detailed two specific cases of Leigh syndrome caused by a mutation in a nDNA coding gene called surfeit locus protein 1 (SURF1) which affects COX, a facilitator of the OXPHOS process (Complex IV). The authors' goal was to discuss the relationships among clinical and imaging results and biochemical analysis/genetics. The two subjects were males whose initial presentations appeared to be indicative of Cornelia de Lange syndrome, e.g., depressed nasal bridge, but both lacked the hallmark signs, e.g., confluent eyebrows. The first subject demonstrated developmental delay, hypotonia, absent deep tendon reflexes, and respiratory problems in the first 3 years of life. His facial dysmorphisms included frontal bossing, brachycephaly, hypertrichosis (forehead), esotropia, and low-set, large ears. MRI indicated involvement of the brainstem and subthalamic nuclei. The second subject had a similar presentation but was of known consanguineous origin. In addition his MRI showed involvement of the brainstem and subthalamic nuclei, but also showed mild cerebellar and significant cerebral atrophy, as well as substantia nigra and central tegmental tract involvement. Both were homozygous for a mutation in SURF1. Their findings support that Leigh syndrome due to SURF1 with COX deficiency presents in this manner; they suggest that secondary to brainstem involvement there is a serious and rapid deterioration by 2 years of age with hypotonia, truncal ataxia, respiratory problems of neurologic origin, and progressive encephalopathy. Of probable diagnostic importance is the bilateral involvement

of the subthalamic nuclei not seen in other similar diseases. While MRI findings cannot be used as outward clinical indicators, the facial dysmorphisms and neurological manifestations in these two cases with known etiology may assist with differential diagnostics by leading to testing of SURF1.

Tay et al.[99] detailed another four cases with novel SURF1 mutations that not only demonstrate the need to discern etiological proof of Leigh, but also the fact that clinical indicators may be valuable in the diagnostic process. The first male subject began to deteriorate at 6 months of age. He was vomiting regularly and found to have low bicarbonate and distal renal tubular acidosis. By 11 months he required mechanical ventilation and demonstrated multiple cardiac issues. He eventually also had a tracheostomy and gastrostomy tube placement. While initial MRI at 6 months was normal, by 18 months he showed symmetric brainstem, cerebellum, and diencephalon abnormalities. The second male was of consanguineous origin and began to regress at 10 months of age. By 14 months he was diagnosed with hypotonia, microcephaly, occasional nystagmus, optic atrophy, and respiratory anomalies. He had proximal renal tubular acidosis as well as elevated lactate and pyruvate. He died in his sleep at 2 years of age secondary to apnea. Autopsy indicated encephalomalacia of the putamen and globus pallidus. His sister showed a similar course and clinical indicators and also died at 2 years of age. The final male subject survived due to less severe presentation, but with persistent hypotonia, developmental delay, and feeding

difficulties. What was unusual is that he also had ragged red fibers in his muscle tissue which is not a consistent finding in SURF1 mutations; rather this is seen in the mitochondrial disease, MERFF, previously described.

Another two-subject study details the course of females with Leigh syndrome and leukodystrophy with a known Complex II deficiency (nDNA).[100] The sisters were of consanguineous origin. The older sibling was developing typically until 10 months of age when she began to deteriorate rapidly (developmental regression), to an eventual vegetative existence until her death at 19 months. With an elevated lactate level she was given the diagnosis of Leigh syndrome. CT indicated leukodystrophy of the frontal lobes and occipital horns of the ventricles. Her sister had a similar early presentation and course. Her CT showed frontal and occipital lobe white matter involvement as well as caudate and thalamic involvement. MRI indicated caudate, thalamus, and substantia nigra aberrations. Both MLD and Krabbe were excluded biochemically. While there were some differences in testing (only the second subject had a muscle biopsy), the confirmed diagnosis in both subjects was a Complex II mitochondrial disorder secondary to a deficiency in succinate cytochrome c reductase which assists in OXPHOS. Of note is that due to parental consanguinity this Complex II disorder appears to be an autosomal recessive inheritance of the nuclear genome. These cases reiterate that Leigh syndrome can be related to leukodystrophy.

A larger study conducted in Korea between 2001-2006 suggested that Complex I may be the most common cause of Leigh syndrome in that country.[101] The researchers began with 46 patients suspected of having a mitochondrial disorder. Sixteen demonstrated the criteria established in this study for Leigh syndrome including MRI confirmation of symmetric basal ganglia, thalamus, and brainstem involvement. Of those, 15 underwent biochemical analysis and nine demonstrated Complex I deficiencies, with a third being confirmed as mtDNA in origin. This has a major challenge, however. Important pearls from this study are that the individuals confirmed to have Leigh syndrome did not have the characteristic elevated plasma lactate or muscle histology of classic mitochondrial disease, and with one third suspected to have nDNA mutations that are yet to be defined, the etiology of Leigh syndrome continues to be difficult to discern in all cases. This study points to the early belief that Leigh was simply a gray matter disorder, and that a leukodystrophy was a novel finding, but as the literature on various related disorders is reviewed, the commonality of gray and white matter in Leigh is revealed. Perhaps Leigh should be subdivided based on biochemical/genetic markers, to make more clear diagnostic entities.

Staley et al.[102] suggest in a case review that the diagnosis of Leigh can be made with clinical observation, imaging, and muscle biopsy results without definitive molecular evidence. They support this by stating that not all nDNA mutations have been located; so, if no mtDNA mutation is found as in 20-50% of

all cases of Leigh, then the diagnosis is assumed to be due to a yet undefined nDNA mutation. Leigh could be a *dump diagnosis* of sorts. This again points to the importance of clinical indicators to lead patients for further diagnostics and definitive testing.

DISCUSSION OF DIFFERENTIAL DIAGNOSIS

In the six diagnoses briefly outlined above there is definite overlap. For example, patients with mitochondrial disorders may or may not have Leigh syndrome.[91] Hypotonia, feeding issues, developmental regression, and seizures can indicate a plethora of disorders. Literature that assists with differential diagnoses in multisystem disorders is not always disseminated in the terms that lend itself to that purpose. Kang et al.[103] is an exception; this study details three cases of children with inborn errors of metabolism that were thought initially to have a mitochondrial disorder. The first infant was found to have white matter abnormalities and elevated lactate in his basal ganglia; however the definitive test was for lysosomal enzymes, indicating Krabbe disease. The second infant had seizures and opisthotonic posturing with a hypotonic core and with elevated tone in her extremities. Initial imaging of her brain appeared normal but subsequently she demonstrated brain atrophy with white matter involvement. Urinalysis found the metabolic disorder of molybdenum cofactor deficiency, and the mitochondrial testing was negative. The third infant also had a hypotonic core with elevated deep tendon reflexes. EEG demonstrated subclinical multi-focal

seizures and the MRI demonstrated abnormal white matter involvement. This infant also had elevated lactate in the basal ganglia but consequently was diagnosed with Alexander disease, a leukodystrophy, via cerebral biopsy in which the characteristic Rosenthal fibers were noted. The authors suggest that the abnormal metabolic processes in Krabbe disease, molybdenum cofactor deficiency, and Alexander disease cause a secondary adverse effect on mitochondria since all three demonstrate effect on lactate; thus their clinical indicators must be rigorously reviewed and validated with appropriate diagnostic testing. In fact this relationship points to the additional conundrum of whether a mitochondrial disorder is primary or secondary.

Hypotonia is one clinical indicator in many of the disorders discussed thus far. Does 'hypotonia' mean the same thing in each study? Recent literature concerning the operational definition of hypotonia attempted to solidify and specify what the medical community considers to be low tone. Sender and Jayawant[104] discuss the "floppy" infant and operationally differentiate among hypotonia (decreased tone), weakness (decreased muscle force), and ligamentous laxity with increased joint mobility. A study of physical and occupational therapists reported consensus that hypotonia meant that an infant displayed impaired strength, decreased activity tolerance, and delayed motor skill development.[105] Unlike the previous report, however, they did not operationally define hypotonia separate from weakness, as they interpreted the literature to not

be in agreement. In contrast a comprehensive evaluation of hypotonia by Peredo and Hannibal[106] serves as a useful tool in operationally defining hypotonia. Again, the term "floppy" infant is used as an overlying descriptor. The following differentiations are helpful in looking at hypotonia as a clinical indicator:

- Central hypotonia is seen more often in infants who do not track visually, do not imitate facial expressions, and who appear lethargic.
- Central hypotonia is more often related to decreased consciousness, core weakness, and dysmorphic features, as well as other characteristics.
- Peripheral hypotonia is seen more often in infants with typical consciousness and attention to the environment.
- Peripheral hypotonia is more often related to the motor neurons in the anterior horn.

In review, hypotonia is a common clinical indicator in infants with undiagnosed multisystem disorders, and correctly differentiating etiology as soon as possible with the least invasive means is paramount in determining a diagnosis.[107]

CLINICAL INDICATORS

What about clustering signs and symptoms? Can clinicians determine, based on a certain number of clinical indicators, the likelihood and necessity of a referral for further diagnostic inquiry based on clusters? To date there are no

clinical prediction rules that are applicable. One systematic review elucidates the importance of critical evaluation of these tools. Beneciuk et al.[108] concluded that studies with good research designs may be valuable tools in the physical therapy setting given the lack of validation studies of specific treatments for given diagnoses. In the context of the undiagnosed population the operational definition of clinical prediction rules as defined by Childs and Cleland[109] includes making a diagnosis, estimating a prognosis, and beginning treatment.

CONCLUSION

A tool to assist health care professionals with identifying clusters of clinical indicators could benefit many individuals with complex metabolic disorders. Expanding the scope of identification beyond the physician realm may ultimately serve all aspects of patient care, and make the task of diagnosis more expeditious with appropriate referrals. Ultimately, both providers and patients would benefit with a honed diagnostic paradigm.

CHAPTER III

STUDY 1

Health care professionals need guidance in evaluating and treating patients with multisystem involvement who do not have definitive diagnoses. In the case of children with special needs, 30 to 40% do not have a specific diagnosis.[1,2] While The Human Genome Project greatly enhanced diagnostics with the mapping of disease specific sites on all 46 chromosomes, the full implications for the undiagnosed have yet to be realized.[3,4]

Clinical indicators (cluster of signs and symptoms) that may be exhibited in children with undiagnosed multisystem disorders are currently not compiled in any national database or referenced in any literature. The possibility of grouping according to these indicators is plausible as a mechanism of organization but is not yet in place. With expert scientists summarizing the diagnostic indicators and clinical researchers describing the patient presentations, themes emerge that assist with developing a differential diagnostic methodology. There are inconsistencies and different opinions among experts as to what is, and what is not, a clinical indicator; therefore, consensus definitions and congruity among resources must be examined.

A tool to guide healthcare professionals in accurately diagnosing or making appropriate referrals of clients with undiagnosed disorders, particularly

children, would be beneficial. The purpose of this study was to develop a chart of clinical indicators based on consensus information derived from the literature and from case studies of undiagnosed children.

METHODS

Clinical Indicators from the Literature

In order to develop an initial chart of diagnosis-specific clinical indicators, five conditions were chosen for review based on diagnostic criteria, tools for diagnosis, and variation of complex presentations: cerebral palsy (CP), Rett syndrome (RTT), metachromatic leukodystrophy (MLD), Krabbe disease, and mitochondrial disorders including Leigh syndrome. CP and RTT were chosen for their established diagnostic criteria as a basis for clinical indicators. Moreover, CP was chosen to elucidate the problems with its use as a misdiagnosis. MLD, Krabbe, and mitochondrial disorders were added as examples of complex multisystem disorders that may go undiagnosed without further investigation.

Each condition was investigated via literature search through primarily PubMed with a few contributions from MEDLINE (Ovid) and CINAHL. Locating publications of initial diagnostic recognition was attempted in all cases. For CP and RTT the criteria are well established and easily validated across sources. For MLD, Krabbe, and mitochondrial disorders, including Leigh syndrome, a wider search was conducted to insure that cases from multiple different sources

representing subjects from more than one geographic area and ethnicity were included.

Clinical indicators were retrieved from the literature specific to these diseases, and operational definitions of these clinical indicators were developed based on that literature as well as supporting references from medical resources to corroborate the descriptors in the literature. (See Appendix B). The cases chosen consisted of data from peer-reviewed journals that provided descriptors throughout the disease course. In some instances the authors were globally descriptive of their subjects, and for many these cases were new areas of application of diagnostic paradigms. For each disorder cases were reviewed to compare and contrast clinical indicators. If the majority of cases reviewed for each disorder (over 60%) demonstrated the same clinical indicators, those indicators were inserted in the chart as primary clinical indicators for the disorder. (See Appendix C).

To improve utility of the initial chart of clinical indicators for health care providers the list of primary clinical indicators was further honed via the following criteria: 1) the clinical indicators must be able to be discerned through observation or non-invasive testing, e.g., hypertonia via passive range of motion; and 2) the clinical indicators must be discriminatory; in other words 'developmental delay' was noted for many cases but it was secondary to varying etiologies and not operationally defined uniquely. "Seizures," "failure to thrive,"

and "autism" were also excluded by these criteria. The following diagnosis-specific indicators were derived from the literature as cited.

Cerebral Palsy Indicators

Cerebral palsy is an established diagnosis in pediatrics with many definitions and tools available to be used for both diagnostic and treatment assessments.[5-7] The currently accepted definition of CP states that it is a non-progressive disorder caused by an insult to the fetal or infant brain.[5] *Surveillance of Cerebral Palsy in Europe (SCPE)*[6] provides a good example of the role of clinical indicators in determining the diagnosis of cerebral palsy. Disorders of movement are the main characteristics, e.g., spasticity (hypertonia). (See Appendix A). The classical descriptors of limb involvement, such as spastic diplegia are also included. Additional validation of these clinical indicators is found in the literature.[7-9]

Rett Syndrome Indicators

Rett syndrome (RTT) is ultimately determined by analyzing the MECP2 gene[10,11], but it is clinically a regressive disorder found primarily in females with autism similarities. Regression, loss of purposeful hand skills with hand wringing or other stereotypies, apraxia/ataxia, dementia, and bruxism are signs and symptoms of RTT.[12-14]

Metachromatic Leukodystrophy Indicators

Metachromatic leukodystrophy (MLD) is a disorder noted by initial regression of acquired skills followed by progressive deterioration of function.[15] MLD is an autosomal recessive lysosomal storage disorder.[16-18] MLD causes demyelination of the white matter of the brain (leukodystrophy).[19,20]
The late-infantile form of MLD (the most common form) appears somewhere between birth and 4 years of age.[16,17,21] Initial presentation is typically a gait disturbance, e.g. toe walking with abnormal foot positioning.[22] The children progressively deteriorate with an eventual loss of independent mobility. Hypertonia; hypotonia; elevated or diminished deep tendon reflexes; peripheral neuropathy; nystagmus; dysarthria; intention tremor; opisthotonus; dystonic, myotonic and opsoclonic movements; developmental delay; and seizures, as well as lost social and language abilities, are also noted in the presentation.[22,23,24]

Krabbe Disease Indicators

Krabbe disease (globoid cell leukodystrophy) is another autosomal recessive lysosomal storage disorder with most cases occurring in early infancy.[25] Commonalities first documented were infants with early typical development, but who later developed irritability; extensor posturing; difficulty feeding/vomiting; somnolence; nystagmus and other visual impairments; and a distinctive pattern of yawning.[26] Other clinical indicators in the literature are: clonus, decreased muscle tone, fluctuating tone, abnormal reflexes (e.g. palmar grasp, Babinski),

apnea, visual impairment caused by optic atrophy but also including blindness, seizures including myoclonic jerks; peripheral neuropathy; macrocephaly; episodic vomiting/feeding problems; lower extremity twitching; head lag in pull to sit; hyperesthesia; periodic fevers without a known etiology; hypersensitive to sensory stimuli (e.g. sound) causing frequent crying; developmental delay or regression of motor skills; and opisthotonic and decorticate/decerebrate rigidity.[27-32]

Mitochondrial Disorders Clinical Indicators

Mitochondrial disorders are a complex genetic and metabolic disease group that can cause an array of symptoms from hypotonia to autism.[33-38] The literature suggests that mitochondrial disorders should be suspected when a typical disease has atypical features; when three or more organ systems are involved; and when recurrent setbacks or exacerbations result from infection.[39] Autism, developmental delay, hypotonia, seizures, failure to thrive, gastroesophageal reflux (GERD), regression, irritability, feeding difficulties, microcephaly, abnormal reflexes, ataxia, muscle weakness, vision and hearing disturbances, vomiting , developmental delay, seizures, bradypnea and other respiratory issues, muscle weakness, abnormal deep tendon reflexes, dysmorphism, spasticity, dystonia, muscle weakness, cognitive deficits, dystonia/dyskinesia, and ataxia are examples of the myriad of presentations.[40-55]

Cases for Review

Ten case studies of children with complex multisystem disorders were also compiled to determine the potentially unique clinical indicators that could lead to a diagnosis. Birth history including gestation, Apgars,[56] and delivery were reported as available in the medical records. Initial presentation and course of the disease process were reviewed to discern primary clinical indicators in each case. The clinical indicators in the cases were then compared to the clinical indicators in the tool.

RESULTS

Clinical Indicators from the Literature

CP and RTT have established clinical indicators which are described below. MLD, Krabbe, and mitochondrial disorders including Leigh syndrome lack established diagnostic models, and thus all clinical indicators for these diagnoses were collected, with final inclusion of only those indicators present in 60% of the cases cited. For MLD 10 studies were reviewed; for Krabbe, eight were reviewed; for mitochondrial disease, 19 were reviewed followed by another five that were exclusively Leigh syndrome cases.

CP is characterized primarily by motor impairments in the disorders of movement category. Spasticity (hypertonia), ataxia (with hypotonia), and dyskinesias are primary clinical indicators as well as classical descriptors of limb involvement. Regression is not noted, as CP is a static disorder. There is no

predominance of other categorical clinical indicators, e.g., body systems. RTT diagnosis includes a combination of ataxia and apraxia affecting gait in the disorders of movement category. Regression is also a significant early clinical indicator. RTT has the distinction of loss of hand function due to stereotypies which is unique to this disorder. MLD is characterized with disorders of movement (hypertonia and hyptonia) with regression and initial gait disturbance as early significant clinical indicators. Krabbe is similar in presentation to MLD which is a logical as they are both leukodystrophies. Krabbe has the unique clinical indicator of irritability as a primary early sign. In addition primary feeding issues are a hallmark clinical indicator of Krabbe. Mitochondrial disorders including Leigh syndrome are suspected when multiple systems are involved due to the vast range of organs primarily affected. Hypotonia, primary feeding issues, regression, and dysmorphism are also primary indicators of mitochondrial disease.

Clinical Indicators from Case Review

Of the 10 cases the gender split was even, five males, and five females. Four of the children are deceased, the youngest died at 7 months of age, and the oldest died at 5 years of age. In the 10 case reviews two clinical indicators were present in 60% of the cases: hypotonia and primary feeding issues. Combinations of presentations did emerge as well, e.g., hypotonia and primary feeding issues were present together in 4:10 cases. Based on the clinical indicator tool, all of the

cases demonstrated clinical indicators most closely aligned to mitochondrial disorders, indicating complex multisystem disorders in these subjects. None of the subjects had primary motor disorders of CP, nor exclusively distinguishing characteristics like stereotypic hand movements. Regression was present in three of the children, two of whom are deceased. (See Appendix D for the case histories).

DISCUSSION

The purpose of this study was to develop a chart of clinical indicators based on the literature and from case studies of undiagnosed children to assist in identification of patients in need of referral for further testing to confirm a specific diagnosis. Some of the disorders discussed had individual indicators that discriminated, e.g. loss of hand function because of stereotypies in RTT. Clusters of clinical indicators in other cases pointed to given diagnoses, e.g. irritability and regression in Krabbe disease.

Based on the literature presented describing cerebral palsy (CP), Rett syndrome (RTT), metachromatic leukodystrophy (MLD), Krabbe disease, and mitochondrial disorders including Leigh syndrome, as well as the 10 undiagnosed case studies presented, threads of commonalities and differences emerge. Hypotonia and primary feeding issues were the most prevalent in the 10 undiagnosed case studies indicating that these subjects most likely have or had a complex multisystem disorder typical of mitochondrial disorders, as their

symptomology did not point to a definitive neuromuscular disorder such as CP, or a disorder with classic motor manifestations such as RTT with its characteristic hand stereotypies.

Limitations of this tool include the following; secondary impairments may be clinical indicators, e.g., scoliosis, but not initial signs or symptoms; variant forms of a disorder, e.g., CP of genetic origin presenting with hypotonia without ataxia and microcephaly may not fit this model of clinical indicators. Lastly, just a small number of individuals with undiagnosed complex disorders were analyzed, limiting generalizability of the selected clinical indicators to a larger population of undiagnosed children.

Future studies could assess secondary indicators to determine their usefulness in discriminating between disorders. Also, this instrument should be further validated by comparing the cited disease-specific indicators with primary indicators from other diagnosed children. With greater referrals for diagnostic work up of suspected mitochondrial disorders comes the benefit of potential discovery of new biochemical markers for these disorders.

CONCLUSION

While this tool cannot be used to provide a definitive diagnosis, it is a means of identification of clinical indicators that will facilitate referrals of children with complex multisystem disorders.

CHAPTER IV

STUDY 2

Based on a thorough literature review and an analysis of 10 case studies of children with undiagnosed disorders, a preliminary list of primary clinical indicators was developed for individuals with multisystem involvement. (See Appendix C). These indicators were defined as primary if they were present in 60% or more of reviewed cases. The next step was to assess the validity of these indicators and further refine it as a tool having good clinical utility. The research question is: Do the primary clinical indicator groups correctly identify children with given diagnoses? The research hypothesis is: Each complete groups of clinical indicators for a diagnosis will accurately describe a single diagnosis and no other diagnoses.

METHODS

IRB approval was secured from the primary researcher's two affiliated universities. Subjects were then recruited from the primary researcher's clinical affiliations with therapy providers and community outreach/service entities. Consent was secured for review of the cases of children with known diagnoses. These case reviews of the medical records were compiled, including all clinical indicators. Only clinical indicators found to be primary in the original study were included and analyzed using descriptive and non-parametric statistics when

indicated. Brief case synopses were compiled to document progression, regression, or disease plateau for use in future analysis.

Subjects

33 subjects' medical records were collected between 2007 and 2011. Of the 33 subjects, 16 were female and 17 were male. Their dates of birth ranged from 1996-2010, and three are deceased. Medical records varied from complete history including hospitalizations, laboratory results, imaging reports, etc. to more limited scope of medical history via parental report and rehabilitation evaluations. No restrictions were given during the request for medical records, and therefore a variety of diagnoses resulted, including CP, RTT, leukodystrophies, and a variety of other genetic disorders.

Data Analysis

Once clinical indicators were compiled for each of the cases, they were compared with the primary clinical indicators in the tool. Results were dichotomized based on the presence of all primary clinical indicators listed in the tool being found in the reviewed cases. If all primary clinical indicators were present in the case for a specific diagnosis, there would be agreement. Contingency tables (2 by 2) were developed for CP and RTT. (See Appendix E). Utilizing PASW Statistic 18 for non-parametric analysis, the primary clinical indicator group counts were processed for CP and RTT using the Fisher's Exact Test. Due to a limited sample of individuals with MLD and Krabbe (one in each

diagnostic group) and the variation in presentation of clinical indicators for mitochondrial disease, those disorders were not analyzed statistically based on the current clinical indicator tool.

RESULTS

Regarding medical diagnoses of the 33 cases, six were diagnosed with CP, five were diagnosed with RTT, one was diagnosed with MLD, one was diagnosed with Krabbe, seven were diagnosed with a mitochondrial disorder, and 13 had other complex disorders. (See Appendix F.)

All six CP cases had all three clinical indicators. In the case of RTT, four out of five subjects had all of the four clinical indicators, and the remaining RTT subject had three of the four clinical indicators; she did not demonstrate regression. The presence of all three clinical indicators in the six individuals with CP demonstrated perfect agreement, and a good agreement was found for the RTT cases. The Fisher's Exact Test demonstrated statistical significance with $p<0.01$ in each instance. For the remaining disorders contingency tables were not constructed at this time due to small cell counts.

The individual subjects in both MLD and Krabbe demonstrated all the primary clinical indicators of their given diagnosis. Two additional subjects scored similarly to the subject with MLD, Case 5 with Alexander disease (another leukodystrophy), and Case 33 with a leukodystrophy (of unknown genetic etiology).

Mitochondrial disorders demonstrated their heterogeneous nature by showing a difference in presentation; at the time of reporting none of the seven subjects had demonstrated an active regression; six demonstrated hypotonia; three demonstrated primary feeding disorders; five demonstrated involvement of three or more organ systems; and two had documentation of dysmorphism. Case 7 and Case 32 both demonstrated the combination of hypotonia, three or more organ systems involvement, primary feeding issues, and dysmorphism.

For disorders allocated to the "complex" group, check marks have been added to the clinical indicator tool to show the frequencies of indicators present in these 11 cases. The most common primary clinical indicator in these cases is hypotonia, present in 5 cases (45%). The most clinical indicators demonstrated in one subject was Case 11 who has Costello syndrome with hypertonia, irritability, three or more organ systems affected, and dysmorphism. (See Appendix G for case histories).

DISCUSSION

Well-studied disorders such as CP and RTT were readily differentiated from other diagnoses utilizing the pilot groups of primary clinical indicators, a finding that supports the literature as well as these sections of the clinical indicator tool. The one outlier in the RTT group that did not demonstrate a regression may indicate a milder phenotype of the genetic mutation.

A limitation of this study is the small number of subjects with MLD and Krabbe which did not allow for meaningful statistical analysis. However, the fact that each of those single cases demonstrated all of the primary clinical indicators suggested for those disorders gives support to those sections of the tool. Because two additional cases of leukodystrophy also exhibited the same group of primary clinical indicators, it is plausible that other general leukodystrophies will present with the same primary clinical indicators. Therefore the specific MLD delineation in the tool will be replaced by the category of leukodystophy. A new contingency table (2 by 2) based in this change is found in Table 3a in Appendix H. A Fisher's Exact Test was conducted to analyze the discrete data in this contingency table for leukodystrophies, with the finding of significance at a level of $p<0.01$.

Mitochondrial disorders proved troublesome to describe with this small sample size. Hypotonia and the involvement of three or organ systems were most common. Although regression is a documented primary clinical indicator of mitochondrial disorders in the literature, it was not demonstrated in the seven subjects in this study with a diagnosis of mitochondrial disorders. Dysmorphism also had a limited presence. Therefore, a new contingency table (2 by 2) was developed on the three remaining clinical indicators: hypotonia, three or more organ systems involved, and primary feeding issues. (See Table 3b in Appendix H.) The Fisher's Exact Test demonstrated significance ($p=0.001$) with the

modified group of three primary clinical indicators of mitochondrial disorders for the seven subjects with that diagnosis.

With a variety of approaches to diagnosis of mitochondrial disorders, and the infancy of this line of medical testing, the validity of the medical diagnosis may have affected the results. Moreover, the medical records that were provided may have excluded additional clinical information that would have corroborated the diagnosis of mitochondrial disease.

In expanding the diagnostic category of MLD to all leukodystrophies and honing the clinical indicators for mitochondrial disorders it appears that the diagnostic utility of this tool improved. With a wider application for all leukodystrophies and a narrower focus for mitochondrial disorders, the next step is to test the revised clinical indicator tool by applying it to a group of cases with diagnoses to which the primary researcher is blinded in order to see if the revised tool has the ability to direct the practitioner to a diagnostic category. Table 4 in Appendix I reflects the primary clinical indicators found in the 11 complex cases as well as the leukodystrophy and mitochondrial disorder modifications and is a revision of the tool from the previous study.

CONCLUSION

The clinical indicator tool piloted in this study demonstrated the ability to differentiate among diagnostic groupings of certain disorders and the revised tool has the potential to be further validated in studies with larger sample sizes. The

greatest area of challenge will be the establishment of a valid clinical picture of mitochondrial disorders given their heterogeneous nature.

CHAPTER V

STUDY 3

In the previous two chapters, a clinical indicator tool was developed and modified in order to assist in providing clinical diagnosis and referral for undiagnosed children. First, based on the literature and a series of case reviews, the tool was developed and refined. It was then further tested by comparing its primary clinical indicators of selected disorders with clinical indicators present in 33 case studies of diagnosed children. The tool was adjusted according to the results of this second study. (See Appendix I). The purpose of this third study was to assess the efficacy of this current version of the clinical indicator tool in guiding clinical diagnoses of complex multisystem disorders by comparing the diagnosis discerned by the clinical indicator tool with the known diagnosis in an additional group of case studies. The research question is: Does the clinical indicator tool have the ability to identify the correct diagnosis in children with complex multisystem disorders? The research hypothesis is: A potential clinical diagnosis can be suggested by using the primary clinical indicators in this tool.

METHODS

Subjects

IRB approval was secured from the primary researcher's two affiliated universities. Subjects were then recruited from the primary researcher's clinical

affiliations with therapy providers and community outreach/service entities. Consent was secured and each case was reviewed and chronicled. The cases of 21 subjects, 14 males and seven females, were reviewed. All children described in these case studies are still living at this time. The range of dates of birth is 1994-2008 with records spanning from 2005-2010.

Procedure

Case reviews of the medical records were developed, including all clinical indicators, by research assistants so that the primary researcher would be blinded to the diagnoses. Only clinical indicators found to be primary in the prior two studies were included for comparison. Brief case synopses were compiled to document progression, regression, or disease plateau for use in future analysis. (See Appendix J). The primary researcher continued to be blinded to the diagnoses while applying the tool to select a clinical diagnosis for each of the 21 cases.

Data Analysis

Based on the tool, diagnoses were determined for each of the cases that demonstrated all of the primary clinical indicators of a given disorder. If a case did not demonstrate the primary clinical indicators for one of the given diagnoses, it was labeled as "other." Due to the diagnostic expertise of the researcher, diagnoses for those cases in the "other" category were also hypothesized and added parenthetically but were not analyzed. For CP, Kappa

analysis was performed to see the agreement between the diagnosis derived by the primary researcher utilizing the clinical indicator tool and the medical diagnosis. No other statistical analysis was undertaken due to small sample size, but descriptive results are included.

RESULTS

Of the five diagnoses included in the clinical indicator tool, CP demonstrated the greatest prevalence in the medical record sample, with seven cases (33%). Kappa analysis of the CP diagnosis rendered by the primary researcher utilizing the revised clinical indicator tool and the medical diagnosis indicated a substantial agreement at 0.80 with $p<0.001$, correctly diagnosing five of the seven cases of CP. None of the 21 cases had a medical diagnosis of RTT or Krabbe, nor did any case include the primary clinical indicator group for each of these disorders, suggesting specificity of their indicators. The researcher also correctly diagnosed four cases as complex and indicated those clinical indicators in the revised version of the clinical indicator tool by check marks to denote frequency. The one child (Case 4) correctly diagnosed with a mitochondrial disorder displayed all three of the primary clinical indicators for mitochondrial disorders found in the revised tool: hypotonia, primary feeding issues, and three or more organ systems involved. Case 1 was also diagnosed correctly with a leukodystrophy, based on the tool, but Case 9 was not determined to have a leukodystrophy, yet that was his diagnosis.

When the primary researcher could not determine a clear diagnosis based on clinical indicators, the case was labeled "complex." These were cases that demonstrated groupings of clinical indicators not found in the tool, but indicative of a complex disorder not otherwise specified. Of the 21 cases four (19%) were deemed complex and those medical diagnoses included Case 5, microcephaly; Case 6, hydrocephalus; Case 12, lack of motor coordination and congenital diaphragmatic hernia; and Case 20, Vater syndrome. To indicate frequencies of the primary clinical indicators in the group check marks were made in the "complex" column of the clinical indicator tool.

Other cases 2, 11, and 14 were accurately determined by the primary researcher as "other," including Down syndrome, arthrogryposis, and torticollis; these diagnoses were not one of the six diagnoses listed in the tool. The remaining four cases were characterized by the primary researcher as follows: Case 3 was given the diagnosis of CP, but the medical diagnosis was epilepsy; Case 10 was suspected to have a sensory processing or autism spectrum disorder, but the medical record gave a diagnosis of lack of motor coordination; Case 17 was determined to be a systemic disorder, and the medical diagnosis was similar, juvenile rheumatoid arthritis; and, Case 19 had all of the clinical indicators of CP, but was diagnosed with Angelman syndrome. (See Appendix J for case histories).

A final version of the tool was created with a column of "complex" that can be utilized to document clinical indicators, and then investigate a diagnosis based on the clinical phenotype. (See Appendix K).

DISCUSSION

The results of this study support the primary clinical indicators chosen for the diagnosis of CP. The other findings are less conclusive. The sample of case studies was a convenience sample of children currently being treated in local facilities, and recruitment of specific diagnostic groups was not conducted due to the lack of prevalence of such cases in one geographic area. However, we surmised that specificity of the primary clinical indicator groups could also be supported by inclusion of other diagnostic categories. The absence of primary clinical indicator groups for the five targeted disorders in the remaining sample suggests that those indicators are able to differentiate diagnoses.

Although the Kappa analysis demonstrated substantial agreement, the investigator misdiagnosed two cases of CP. Case 3 had a medical diagnosis of epilepsy but presented with hypertonicity in his lower extremities; however, this could have been due to an injury he sustained, rather than CP; Case 19 was incorrectly given the diagnosis of CP when the patient had Angelman syndrome, with autistic like tendencies in addition to the clinical indicators of CP. The use of additional clinical indicators to reject a working diagnosis should be examined in

future studies. With the application of an "other complex" category to the clinical indicator tool, the primary researcher had a 90% correct diagnostic rate.

One limitation to this study is that variability in the medical records led to imprecise diagnoses, such as "lack of motor coordination." Another limitation is the small sample size for selected diagnoses, yet these cases are infrequently found in the defined geographic areas of this study. The clinical indicator of regression proved to be problematic and could be affected by the point in time the records were gleaned; by the varying longitudinal expanse of time covered in the records; and the morbidity and mortality of individuals with these varying disorders. Future studies are planned to enhance clinical indicator data collection on other case studies of undiagnosed children to further refine and test this tool.

Caution is needed in providing the additional category of "complex" disorders to absorb the undiagnosed and other presentations, but the compilation of clinical indicators for this group over time may result in other diagnostic primary and secondary clinical indicators for additional disorders not yet recognized. These patients should be referred for further diagnostic testing if they do not fit well into any of the current diagnostic paradigms.

CONCLUSION

The clinical indicator tool piloted in this study demonstrated ability to discriminate CP from other pediatric disorders based on specific groups of primary clinical indicators. Ability of these groups of primary clinical indicators

to differentiate cases was suggested by other diagnostic cases that didn't include these sets of indicators. However, the validity of the primary clinical indicator groups for disorders including RTT, Krabbe, leukodystrophies, and mitochondrial disorders cannot be supported completely, given the lack of reviewed cases with those diagnose.

CHAPTER VI

CONCLUSION

The purpose of this work was to develop a tool to guide health care professionals working with individuals with complex disorders and to assist with referrals for additional testing if the clinical indicators in the tool suggest a new direction of diagnostics. First, a literature search was conducted to establish clinical indicators for the given diagnoses. Cerebral palsy (CP) and Rett syndrome (RTT) were used as the "gold standards" as these disorders are well documented in the literature and have established clinical indicators. In contrast, metachromatic leukodystrophy (MLD), Krabbe disease, and mitochondrial disorders including Leigh syndrome were included as complex multisystem/metabolic disorders that may confound health care professionals early in the diagnostic process. From the literature search and a review of 10 undiagnosed cases, clinical indicators for each disorder were compiled in a tool. Next, the tool underwent validation by application to a group of pediatric cases with known diagnoses. Finally, the tool underwent further testing in a group of pediatric cases where the diagnoses were blinded to the primary researcher.

Clinical indicators established in the literature review (based on 60% of cases demonstrating them) were compiled in a chart. CP was indicated by hypertonia, a form of –plegia, and a form of dyskinesia. RTT was indicated by

ataxia, apraxia, hand stereotypies, and regression. MLD was indicated by hypertonia, hypotonia, initial gait disturbance, and regression. Krabbe disease was indicated by hypertonia, regression, irritability, and primary feeding issues. Mitochondrial disorders were indicated by hypotonia, regression, three or more organ systems primarily affected, primary feeding issues, and dysmorphism. An "Other" category was added to address the clinical indicators found in the 10 undiagnosed subjects. (See Appendix C).

Descriptive and non-parametric analyses were utilized as warranted to process the data found in all three studies. The clinical indicator tool was able to discriminate among the diagnostic groups of CP and RTT in 33 children. There were limitations to the application for Krabbe and MLD due to smaller numbers of children affected; however, two subjects with another form of leukodystrophy scored similarly to the one subject with MLD, suggesting the tool may be able to discriminate leukodystrophies generally. Mitochondrial disorders, due to their heterogeneous nature, proved to be problematic to quantify; however, they did prove to be complex and warrant additional studies. The clinical indicator tool was also able to discriminate between cases with and without CP in a group of 21 children but had limited application to the other disorders due to composition of the group. The clinical indicator tool went under further revisions, with the addition of a "complex" disorder designation, a change from MLD to

leukodystrophies, and omitting regression and dysmorphism from the primary clinical indicators in the final tool. (See Appendices I and K).

FUTURE RESEARCH

The clinical indicator tool developed in this study to discern relevant clinical should enhance clinical practice. When a clinician suspects a complex multisystem disorder this tool can be used to assist in directing a diagnostic course of action. Of course, this initial work needs replication in additional studies with a variety of diagnoses represented in the subject pool to further validate its use.

One avenue of future application of the clinical indicator tool could be in the differential diagnosis of individuals diagnosed with CP but whose clinical indicators point to a more complex disorder secondary to dysmorphism or multisystem involvement. This application of the tool could be corroborated with data from the *Surveillance of Cerebral Palsy in Europe (SCPE).*[1] In fact one study testing the validity and reliability for the SCPE made the following observations and recommendations: 1) there is a need for collaboration to solidify the use of the SCPE and be consistent in terminology; 2) there is a need to expand training and application of the SCPE.[2]

Another expansion of use of this tool would be to isolate a larger group of individuals with the complex disorders and validate statistically the clinical

indicators for MLD, Krabbe, and mitochondrial disorders if discerned in the group. In turn the case study series could provide additional literature to be disseminated to treating clinicians to offer a clearer picture of the variance in presentation and utility of the clinical indicators. Clinicians may then expedite referrals of those without diagnoses to research entities, one of which is the National Institutes of Health Undiagnosed Disease Program (NIH UDP). The NIH UDP was established in 2008 to address the paucity of options for diagnostic testing for complex, multisystem disorders.[3] An essential point made as the program reflects on progress to date is the necessity of having precise descriptions or phenotypes developed based on extensive medical record collections on each subject.[4] The clinical indicator tool and process of compilation of medical records can assist with this phenotyping.

Finally, the current draft of the clinical indicator tool needs to be used for multisite data collection by other front line health care providers for the purpose of additional data collection. The ultimate goal is to use this tool to assist in diagnosing and referring individuals with complex multisystem involvement.

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Chapter VI

1. Cans C. Surveillance of cerebral palsy in Europe: a collaboration of cerebral palsy surveys and registers. *Dev Med Child Neurol.* 2000;42(12):816-824.
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APPENDIX A

Figures 1 & 2

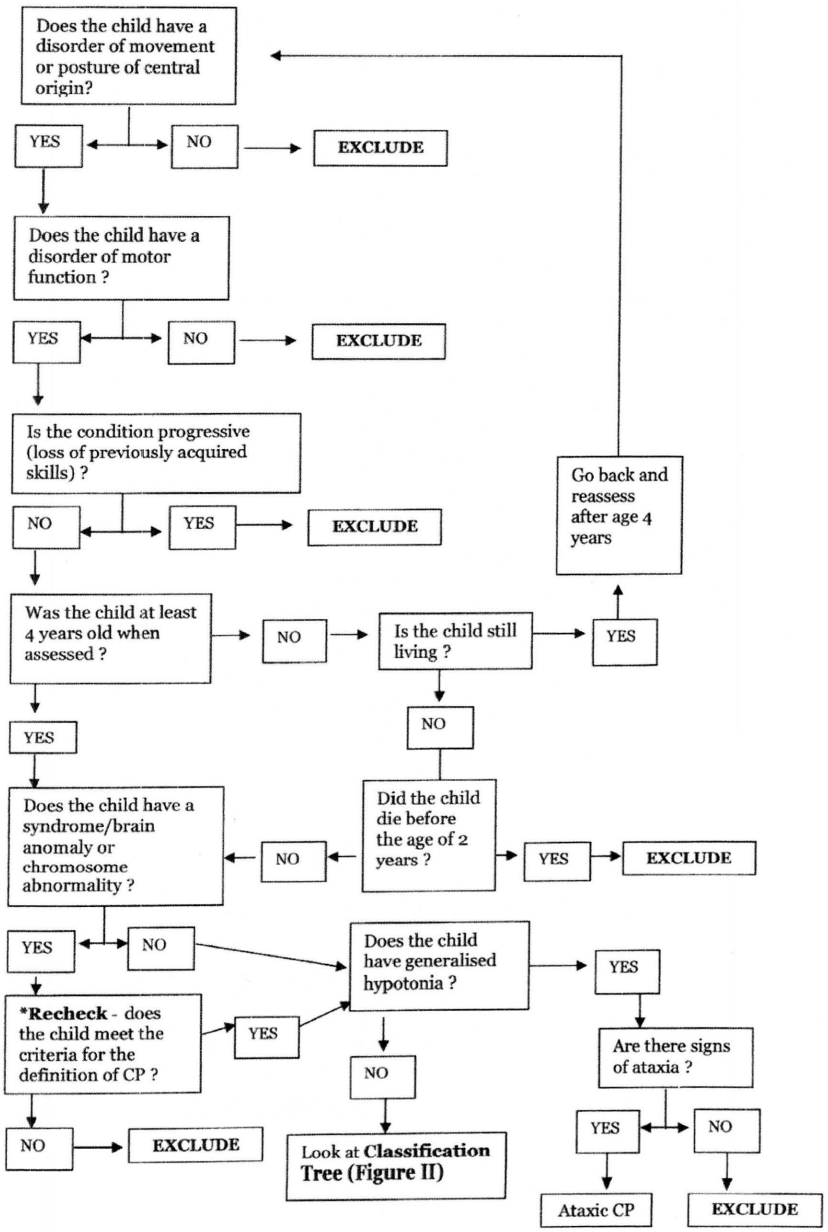

Figure 1: SCPE decision tree for Cerebral Palsy

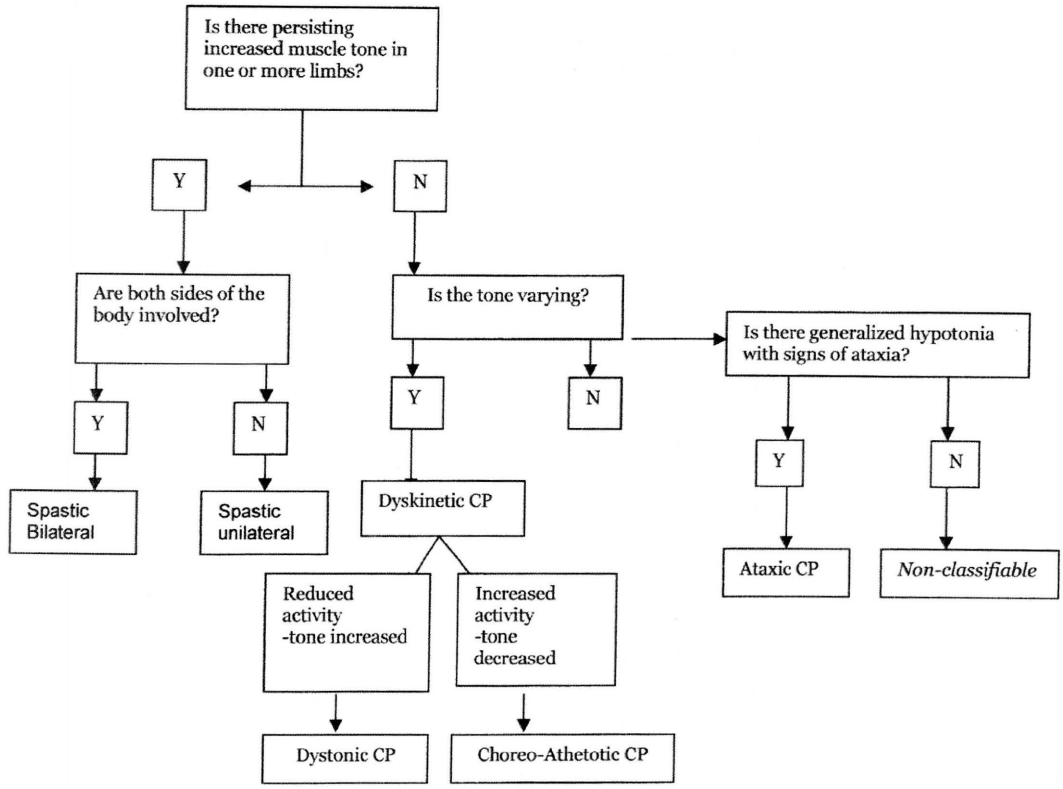

Figure 2: SCPE Classification tree for sub-types of Cerebral Palsy

Figures 1 and 2 reprinted with permission:

SCPE Collaborative Group, Surveillance of Cerebral Palsy in Europe: A collaboration of cerebral palsy surveys and registers. *Dev Med Child Neurol* 2000, 42:816-24

APPENDIX B

Operational Definitions of Clinical Indicators*

Operational Definitions of Clinical Indicators*

Disorders of Movement

Hypertonia- increased tension in muscles; elevated tone.

Quadriplegia- loss of motor function in all extremities with changes in tone.

Hemiplegia- unilateral loss of motor function with changes in tone.

Diplegia- loss of motor function with changes in tone in the lower extremities most commonly.

Dyskinesia- impaired functioning of muscles during voluntary movements.

Spasticity- increased muscle tone which is velocity related.

Dystonia- impaired activation of muscle secondary to tone abnormalites.

Choreo-athetosis- fluctuating muscle tone with jerky movements more proximal than distal.

Hypotonia- decreased resting tension in muscles; decreased tone.

Hypotonia with ataxia- decreased tone in combination with impaired coordination of muscles during voluntary movements.

Ataxia- impaired coordination of muscles during voluntary movements.

Apraxia- disorder of motor planning and execution that causes an inability to perform purposeful movements; not due to loss of sensory or motor ability.

Initial gait disturbance- impairment of alignment and function of gait, e.g. toe walking.

Hand stereotypies- repeated movements of the hands in a non-purposeful, volitional manner.

Development

Regression- a returning to a previous less developed state; loss of previously acquired skills.

Irritability- response to change in environment; sensitivity to stimulate resulting in decreased sensory regulation.

Body Systems

Three or more organ systems primarily affected- cardiac, respiratory, integumentary, musculoskeletal, nervous, endocrine, etc.

Primary feeding issues- noted from birth or at onset of disease process; includes vomiting, dysphagia, structural malformations, etc. that prevent oral feeding. Feeding issues that develop as the disease progresses or due to growth are not included.

Dysmorphism- not in typical form; including arthrogryposis, macrocephaly, and microcephaly.

*Adapted from reference list and Taber's Cyclopedic Medical Dictionary, 21st ed., 2005

APPENDIX C

TABLE 1

Table 1

Primary Clinical Indicator Tool

Clinical Indicators	Cerebral Palsy	Rett Syndrome	Metachromatic Leukodystrophy	Krabbe Disease	Mitochondrial Disorders	Other
Disorders of Movement						
Hypertonia (↑DTRs)						
One of the following -plegias						
-Quadriplegia						
-Hemiplegia						
-Diplegia						
One of the following dyskinesias						
-Spastic						
-Dystonia						
-Choreo-athetosis						
-Hypotonia with ataxia*						
Hypotonia (↓DTRs)						
Ataxia						
Apraxia						
Initial gait disturbance						
Hand stereotypies						
Development						
Regression						
Irritability						
Body Systems						
3+ organ systems 1° affected						
Feeding issues 1°						
Dysmorphism**						

*Hypotonia in combination with ataxia in CP

**Including arthrogyposis, macrocephaly, and microcephaly

APPENDIX D

Case Studies of Children with Undiagnosed Disorders

Case 1

Case 1 is a female born at 39 weeks via induction secondary to polyhydraminos. She weighed 8 pounds 3 ounces and her Apgars were 5, 8, and 8. She demonstrated microcephaly with decreased head growth secondary to multiple brain anomalies, Lennox-Gastaut seizures, metabolic disorder, respiratory issues, developmental delay, feeding issues/dysphagia/GERD, visual disturbances including cataracts, hypotonia, scoliosis, and hip dislocation. Images of this subject indicate some facial dysmorphism that were related to her high palate and microcephaly. She died at 3 years of age in her sleep. While undiagnosed at her death physicians and family suspect a neurodegenerative disorder of metabolic, possibly of mitochondrial etiology.

Case 2

Case 2 is a female born at 38 weeks gestation weighing 8 pounds 8.5 ounces. She had mild jaundice which resolved in the first week of life. She stopped eating at 2 months and began uncontrollable screaming attributed to colic. She demonstrated respiratory issues, feeding problems/weak suck/GERD, FTT, a port wine birthmark, lower extremity abnormal posturing with gait issues. By 2 years of age there was noted improvement in GI and respiratory issues. She is alive at publication without a definitive diagnosis.

Case 3

Case 3 is a male born full term via Caesarian section (C-section) due to breech position. He weighed 8 pounds 2 ounces with Apgars of 8, 9. He developed typically until 3 months of age when his eyes started 'rolling around in circles' per his mother's report. A possible diagnosis of opsoclonus myoclonus was speculated, but never confirmed. He continued to deteriorate over the next few months with decreased oral motor skills, feeding issues, abnormal movement patterns, stridor, sleeping issues, respiratory issues, decreased head control, decreased self-consoling, regression, myoclonic seizures, and cataracts. He demonstrated pervasive hypotonia. By 11 months of age he needed significant life support. His MRI showed atrophy of cortex, cerebellum, and basal ganglia. He was also diagnosed with ventricular hypertrophy, Complex I dysfunction via muscle biopsy, but no genetic marker. This led physicians to speculate that his mitochondrial dysfunction may have been secondary to another still undefined disease process. He died at 13 months of age.

Case 4

Case 4 is a male born via planned C-section at 37 weeks weighing 7 pounds 8 ounces. He had repeated respiratory infections the first 3 months of life with respiratory and gastrointestinal illnesses (including bouts of vomiting) pervasive throughout his life. He demonstrated developmental delay, hypotonia, extrapyramidal cerebral palsy, frequent falls with one fall causing a femur

(indicative of osteopenia) at 4 years of age. He also demonstrated failure to thrive, regression, and seizures. He died at 5 years of age.

Case 5

Case 5 is a male who was born at 40 weeks gestation after an uncomplicated gestation and birth. He weighed 6 pounds 3 ounces. Shortly after delivery he was transferred to the neonatal intensive care unit (NICU) secondary to respiratory distress. He developed feeding issues with failure to thrive, thrush, bilateral conjunctivitis, hypercalciuria, and hypercalcemia. By 14 ½ months he only weighed 13 pounds and was noted to have oral aversion, oral leukoplakia, photophobia, corneal clouding, continuous red, inflamed eyes, and hearing loss. Biotinidase deficiency and chronic granulomatous disease had been ruled out. Hereditary benign intraepithelial dyskeratosis had been discussed, but not validated. At 5 months of age a G tube was placed. By 26 months of age his oral intake of pureed foods had improved thus routine tube feedings were not necessary. He has bilateral hearing aids but it is his eyes that continue to be a constant area of concern. Overall clinical indicators continue to be postnatal failure to thrive, bilateral hearing loss, oral aversion, progressive ocular abnormalities, and oral/esophageal abnormalities.

Case 6

Case 6 is a male born at 41 weeks gestation weighing 8 pounds 4 ounces with Apgars of 8, 9. Early concerns arose about the difference in functioning in

the right and left sides of his body. At one year of age an MRI revealed an area of concern in the deep parietal white matter, left more involved than right, which could be indicative of periventricular leukomalacia. Further EEG, mitochondrial and metabolic work ups showed slight aberrations but no significant pattern of pathology. Working diagnoses include gross and fine motor delays, mild myopathy, and motor apraxia. However, additional diagnoses of Asperger disorder, attention deficit disorder, seizure disorder, mild right hemiparesis, and verbal apraxia have been reported as well.

Case 7

Case 7 is the sister of Case 6, and is 2 years 8 months younger than her brother. She was born full term weighing 8 pounds 8 ounces with Apgars of 9, 9. She was notably hypotonic at birth and was a poor feeder. Since birth her parents have been concerned about her respiratory status, specifically shallow breathing. NCV/EMG testing at 18 months did not show significant pathology, however upon a repeat of these tests with muscle biopsy a year later there was a mild distal myopathy. She tested negative for Rett syndrome. Over the next 2 years concerns about GI issues, specifically constipation arose. By 4 years 8 months she was given the diagnoses of hypotonia with hypermobility, with some suspicion of a connective tissue disorder such as Ehlers-Danlos syndrome. She wears bilateral ankle foot orthotics (AFOs) for stability.

Case 8

Case 8 is a female born weighing 6 pounds 11 ounces with Apgars of 9, 9. Feeding difficulties were immediate, and she began to projectile vomit at 5 weeks of age. By 8 months she was diagnosed with failure to thrive (FTT) and GERD. She received an NG tube and eventually a G tube. She was diagnosed with hypotonia, developmental delay, and abnormal movement patterns including persistent primitive reflexes. Recurrent respiratory and gastrointestinal illnesses plagued her first years of life. Gagging on food became a major issue and pica developed. Behavior was also an issue with physical refusals and behavioral outbursts In addition self-stimulation behaviors developed such as spinning in sitting, arm flapping, and hand movements. Due to these movements and perseverative play autism is considered as a clinical indicator despite her social nature.

Muscle biopsy results indicated some abnormalities suggesting a fatty acid or mitochondrial disorder. It was noted that with every increase in mobility came with a concomitant GI exacerbation. Her dysmorphisms include frontal bossing, and a depressed nasal bridge.

Case 9

Case 9 is a male born via C-section at 37 weeks 2 days gestation weighing 4 pounds 8 ounces. Apgars were 2, 7, and 8. He required positive pressure ventilation and supplemental oxygen at birth. He was diagnosed with

intrauterine growth retardation secondary to marginal insertion of a small placenta. He was diagnosed with laryngomalacia, feeding difficulties, seizures, hypotonia, developmental delay, and irritability. He died at 7 months of age.

Case 10

Case 10 is a female born at 40 weeks gestation weighing with 6 pounds 4 ounces with Apgars of 8, 8 and no significant complications. She has two older typically developing siblings. Early concerns about cardiac status included a patent ductus areteriosus (PDA) which resolved spontaneously. She was breastfed but did not grow at a typical rate. At 2 months her parents noted abnormal eye movements and she was diagnosed with congenital nystagmus. Eventual diagnoses of FTT and developmental delay were made, and for a while a diagnosis of Pelizaeus-Merzbacher was considered. This disorder was ruled out as she survived her first year, and so other metabolic diseases including proprioinic academia were considered. Choanal atresia was diagnosed and surgically repaired. A G tube was placed, but the feedings were not tolerated well, so it was removed.

As Case 10 developed her motor skills were delayed, but her verbal skills were on track. By 3 years of age extensive testing led to no definitive diagnosis, and many dietary interventions were not successful. Visual issues became more troublesome and she was declared legally blind.

Initially hypotonic as an infant and toddler, Case 8 developed hypertonia that was treated via dorsal rhizotomy. While described as having spastic diplegia at the time, she was not given a diagnosis of CP because it did not fit her myriad of impairments.

Case 10 continued to be a sickly child and minor illnesses caused major complications during dehydration. While a muscle biopsy was attempted at the age of 3 during an orthopedic procedure, the results were inconclusive. It is important to note that this subject is older than most subjects in this work thus her muscle biopsy was over 15 years ago when the science was not as precise. She became more stable from ages 9-15 years of age, but by 16 her spasticity worsened and migraines began. Peripheral neuropathies followed and with this came depression and anxiety. Implantation of a Baclofen pump at 18 years of age assisted with pain relief and spasticity but further impaired her independence. Dysarthria and dysphagia became new concerns, and her migraines accelerated. 'Stroke-like' episodes were also a new and troubling manifestation.

Case 10's parents sought new perspective on her declining functioning and mitochondrial disease as considered. A metabolic geneticist recommended a trial of a mitochondrial cocktail based on her medical history. Her 'stroke-like' episodes diminished significantly.

Case 10 needs assistance with all activities of daily living (ADLs) and requires attendant care to go to college. She is now wheelchair dependent. She

has abnormal posturing of bilateral index fingers. She is on a diabetic diet to manage her weight. She was diagnosed with neurogenic bladder and decreased pulmonary function. Her physicians believe that the interplay of medications contribute to her level of function and weight gain.

Her family traveled to the National Institutes of Health so Case 10 could be assessed by the Undiagnosed Disease team, and it was suggested there that she may have a variant of hereditary spastic paraplegia or a new disease altogether. No conclusive diagnosis was found.

Case 10's clinical indicators: 3 or more organ systems involved, nystagmus and other visual issues, respiratory issues, hypertonia, dysarthria, dysphagia, neurogenic bladder, peripheral neuropathy, and migraines.

APPENDIX E

TABLES 2a & 2b

Table 2a

Contingency Table for Clinical Indicators of CP Demonstrates Clinical Indicators

	Yes	No	Total
CP	6	0	6
Not CP	0	27	27
Total	6	27	33

Table 2b

Contingency Table for Clinical Indicators of RTT Demonstrates Clinical Indicators

	Yes	No	Total
RTT	4	1	5
Not RTT	0	28	28
Total	4	29	33

APPENDIX F

FIGURE 3

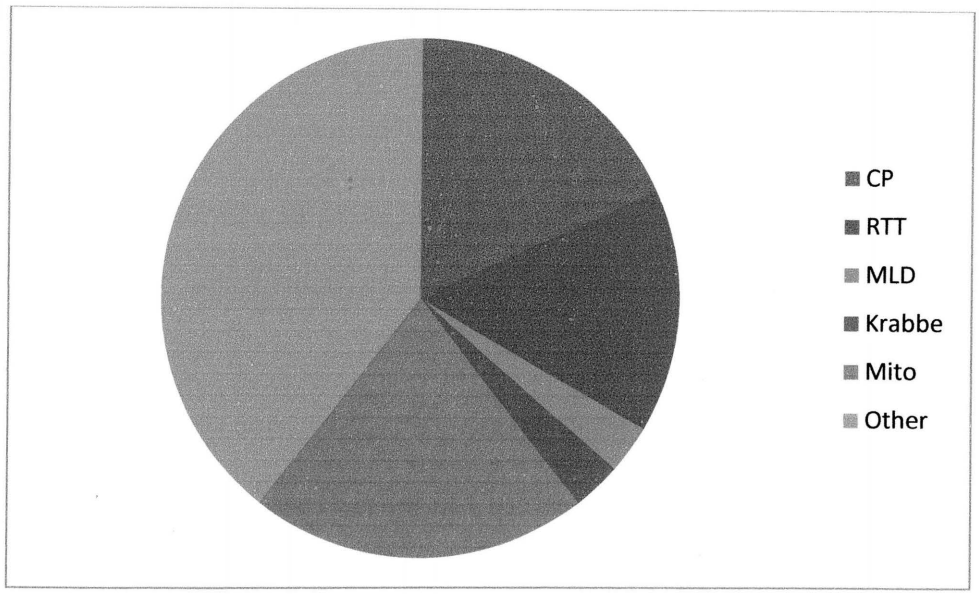

Figure 3: Frequency of Know Diagnoses

APPENDIX G

Case Studies of Children with Known Diagnoses

Case 1

Case 1 was a male born via induction following an uncomplicated pregnancy 10 days after his estimated due date. He weighed 7 pounds 1 ounce with Apgars of 9, 9. Perinatal history significant for lack of urination for the first 24 hours followed by orange colored urine containing a powder like substance.

Case 1 appeared to develop typically during his first year of life with appropriate weight gain and attainment of developmental milestones. However, it was noted that he did not eat as much as his peers, and had 4 or more bowel movements per day while urinating less than a typical infant. He reportedly was a light sleeper (needed to be held to nap).

At 18 months Case 1 was demonstrating good language development and attention to books and puzzles, but he was not walking independently. He cruised with his feet turned inwards, and had weak trunk strength. He had recurrent orange urine that was not related to an acute illness.

At 20 months Case 1's physical therapist (PT) reported bilateral foot pronation, decreased trunk control, and weakness. In the same month a pediatric orthopedist ruled out any skeletal problem, and suspected an underlying pathology of muscle weakness. By 22 months first blood work ordered by the neurologist led to no definitive diagnosis. Magnetic resonance imaging (MRI) of his brain at 23 months was unremarkable. By 2 years of age this subject was visibly weak and thin, and had an episode of violent vomiting that was ruled as a

stomach virus. He started having difficulty crawling (with hands fisted) because his knees would "lock up," and by the next month he could no longer pull to stand or cruise. He was in an active regression.

At 26 months the neurologist was concerned about elevated tone in Case 1's bilateral lower extremities and ordered a muscle biopsy and lumbar puncture (LP); the latter indicated decreased folate and probable cerebral folate deficiency. Leucovorin was prescribed to supplement folate. Case 1 continued to regress and by the end of 27 months could no longer sit independently or control his hands. He stopped speaking soon after.

At 29 months Case 1 underwent a further evaluation. After reviewing the medical records including the MRI, and evaluating this subject, a skin biopsy was performed which confirmed the diagnosis of metachromatic leukodystrophy (MLD). This male continued to demonstrate regression. He lost head control and had increased tone in his bilateral lower extremities, but demonstrated truncal hypotonia. He had trouble eating, managing only pureed foods, and lost the ability to track visually. He also had random vomiting episodes. At the end of 32 months he had white powder in his urine.

Physical therapy was re-initiated at home when Case 1 was 3 years old. While no quantitative gains had been made since his rapid regression in his second year of life he was gaining weight and appearing more comfortable. He had frequent seizure activity as noted by eye and extremity movements. He

demonstrated decorticate and decerebrate postures. His vision and cognition continued to be impaired, but he had no significant illnesses or setbacks until he was 5 years old. Two bouts of respiratory distress necessitated hospitalization; the second stay indicated the increasing risk for aspiration and a gastrostomy tube (G tube) was placed. He was able to recover both times with minimal intervention. He was enrolled in hospice and passed away at 6 years 7 months of age. His sister was born 2 weeks later, and while a carrier for MLD, she is not affected by the disorder.

Case 1's clinical indicators: developmental delay in attainment of gross motor milestones (walking) indicating an initial gait disturbance, regression in all aspects of development, feeding difficulties, discolored urine, hypertonicity, hypotonicity, seizures, decorticate/decerebrate posturing, and nystagmus.

Case 2

Case 2 is a female was born at 37 weeks gestation after an uneventful pregnancy and natural delivery. She weighed 5 pounds 13 ounces and her Apgars were 9, 10. Her mother reports that she was a great breastfeeder and hit her early developmental milestones as follows: laughed at 5 months; rolled over at 6 months; sat independently at 7 months; clapped hands at 8 months; waved "bye-bye" at 10 months; said her first word, "duck," at 12 months; transitioned into sitting independently at 13 months; and was able to crawl in a "bunny hop" style at 14 months.

At 13 months this subject showed the first stages of regression, losing speech, the ability to wave, etc. Her mother noted that her cognitive development slowed, she stopped saying "duck," waving "bye-bye," and playing patty-cake. She also did not turn to look when her name was called, and started staring at pictures on the wall and did not interact with her toys. Gross motor skills were always delayed and she never developed fine motor skills beyond simple grasping, which she lost by 12 months. Her regression was marked at 13 months by strange changes per her mother. She started crossing her eyes, tilting her pelvis repetitively, playing with her saliva, biting herself and others, and not able to make eye contact. Her mother described her appearance as "very autistic like." By 19 months she stopped crawling (bunny hop), babbling, and did not smile or interact, becoming totally withdrawn. At 22 months she was diagnosed with a seizure disorder and began a regimen of steroids which temporarily caused an improvement in crawling, smiling, babbling, and interaction. However, she started clapping her hands non-stop, which concerned her parents as they were suspicious she had Rett syndrome (RTT).

At 2 years of age Case 2 was diagnosed with atypical RTT. During the next year her mother sent blood samples to several labs that were looking for the gene. After the announcement that MECP2 was the causative gene in 1999 when she was 2 years old, her parents received a call that her sample was positive for the

most common mutation on that gene, T158M. As she developed seizures and bruxism developed.

Case 2's clinical indicators: developmental delay in attainment of gross motor milestones (walking), regression in all areas of development, seizures, bruxism, hand wringing, and an ataxic and apraxic gait.

Case 3

Case 3 is a male that was the product of a full term pregnancy and delivered via Caesarian section (C-section). He weighed 7 pounds 11 ounces. Prenatal complications included a history of a 'vanishing twin' with early bleeding and two amniotic sacs, but only one embryo. There were no complications during delivery. At 4 months of age his mother noted that he did not use his left hand and routinely "curled" his left foot, and his synergistic pattern was that of spastic hemiplegia. At 8 months of age he was given the diagnosis of cerebral palsy (CP) without diagnostic studies. At 9 months imaging showed evidence of a right cerebral peduncle stroke; this area is supplied by the right posterior cerebral artery. Timing and cause is still questionable, but he does not appear to have a blood disorder that would have caused a thrombosis. A diagnosis of CP still correlates given the diagnostic definitions and clinical indicators.

At 11.5 months Case 3's physical therapy evaluation found the following clinical indicators: no cognitive impairments; abnormal postures of left upper and lower extremities; left upper extremity held in classic Erb palsy positioning;

left lower extremity with bias towards internal rotation of hip, knee hyperextension, plantar grasp, and "W" sit in play; developmental delay; and non-classical hemiplegia. Additional neuromuscular assessments added the descriptor of dystonia.

Following cord blood transplant with his own cord blood as well as constraint-induced therapy in his preschool years Case 3's parents report improved function in speech and motor skills.

Case 3's clinical indicators: no cognitive impairments for age, abnormal postures of left upper and lower extremities, hypertonia, hemiplegia, dystonia, developmental delay, and hemiplegia.

Case 4

Case 4 is a male who was born at 35 weeks gestation and weighed 5 pounds 13 ounces with Apgars of 8, 9. The only unusual finding is his prenatal history was a double placenta. Parents were "in denial" per mother's report that anything was wrong. When they approached the pediatrician with their concerns about his development at the 6 month check-up they were told to give him some more time since he was born prematurely.

By 7 months Case 4 was not making any gains in sitting, crawling, or pulling up. His rolling was inconsistent and he did not "look right" to his parents. By 8 months his parents' concerns were validated by a family member who is a nurse. At the 9 month check-up the pediatrician noted elevated tone and

developmental delay and ordered an MRI. The MRI showed periventricular leukomalacia (PVL) which was then confirmed by a neurologist. Physical therapy was consulted and the following was noted at his evaluation at 9 months: extensor bias, fisting with cortical thumb, tongue thrust, resistance to passive range of motion in all extremities/hypertonia, gross motor development at 3 months 20 days with scatter skills in other developmental domains between 4-7 months further indicating a developmental delay. His mother also reported that he had the "shivers" 2-3 times per day. The physical therapist recommended additional consults as she suspected CP. At 1 year of age the diagnosis of CP was confirmed by another physician. He was started on an aggressive therapy program, underwent an electroencephalogram (EEG) that ruled out seizures (secondary to report of "shivers"), and went through hyperbaric treatment. He has a brother who is 3 years younger and is developing typically.

Case 4's clinical indicators: hypertonia (extensor bias, cortical thumb), developmental delay, and spastic quadriplegia.

Case 5

Case 5 is a male who was born a week early secondary to maternal infection. He was born via C-section and a knot was noted in his umbilical cord. He weighed 8 pounds 7 ounces. He had difficulty breastfeeding and formula was supplemented; he developed jaundice for which he was treated.

Case 5 was a calm and quiet infant per his mother. By 4 months his parents noticed he was not developing like his older brother had at the same age; he was not making any attempts to roll. At 5 months he had his first noted seizure that lasted 1.5 hours. After emergency transport and administration of Ativan to stop the seizure, diagnostics began. MRI indicated a leukodystrophy, and genetic testing confirmed Alexander disease.

Physicians and family noted significant regression of this subject at 20 months of age, and the regression hit a critical point at 23 months after G tube placement. Case 5 is noted to have macrocephaly, significant gross motor regression, seizure disorder, and episodic gagging and vomiting despite gastrojejunostomy (G-J) tube placement at almost 6 years of age. Botox injections to his salivary glands and medications have not been able to alleviate the gagging and vomiting which causes significant seizure breakthrough secondary to loss of medications. His peak gross motor skills were at 18 months as he could scoot and crawl, but by 5 years of age he was totally dependent on others for care. He continues to demonstrate fluctuating tone with periods of decorticate positioning.

Case 5's clinical indicators: macrocephaly, regression, seizures, hypotonia, hypertonia, initial gait disturbance, decorticate posturing, and episodic gagging and vomiting.

Case 6

Case 6 is a male who was born weighing 7 pounds 8 ounces. Within an hour of delivery he experienced cardiac arrest, the first of 3 in his initial 24 hours of life. At 10 months of age he was diagnosed with CP; etiology was a stroke in utero at 28 weeks gestation. While the family saw this as a cumulative diagnosis, his condition changed over the next few years. He was evaluated by many specialists throughout the country and finally a definitive diagnosis, Complex III dysfunction with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes disorder (MELAS), confirming mitochondrial disease.

Case 6 also was diagnosed with Ehlers-Danlos secondary to ligamentous laxity; cardiac anomalies including a patent ductus arteriosus (PFO) which contributed to a transient ischemic attack (TIA) when he was 9 years old; autonomic dysfunction including poor temperature regulation; café au lait spots; vision issues; peripheral neuropathy; sleep apnea; hypotonia; and chronic fatigue necessitating use of a power wheelchair. He also has a documented developmental delay as he did not walk until he was 3 years old. He has left hemiparesis and wears ankle foot orthotics (AFOs).

Case 6's clinical indicators: developmental delay, hypotonia, left hemiparesis, café au lait spots, chronic fatigue, vision disturbances, peripheral neuropathy, and with 3 or more organ systems affected.

Case 7

Case 7 is a female born at 38 weeks gestation, weighing 7 pounds 2 ounces. She was noted to have microcephaly after birth but discharged as a typically developing infant. Her gestation was unremarkable and she has two older siblings who are typically developing. At 2 weeks of age she was readmitted to the hospital secondary to feeding issues. Over the next 2 months she was fed via nasogastric (NG) tube and then a G tube was placed with Nissen fundoplication. She was diagnosed with bilateral sensorineural hearing loss, cortical visual impairment, mild hypoplasia of the corpus callosum, failure to thrive (FTT), and developmental delay. Physical therapy evaluation at 2 months of age noted dysmorphic facial features with microcephaly, hypotonia, frequent extensor posturing with head rotation to the right, decorticate posturing, immature and abnormal movement patterns in all positions, weak palmar grasp, unable to elicit plantar grasp, and poor state regulation/irritability.

Parents sought multiple consults to discern etiology of Case 7's impairments. They were concerned about her apparent pain/distress that was initially attributed to gastroesophageal reflux disease (GERD). At approximately 1 year of age a diagnosis of Complex I mitochondrial disorder was confirmed. Consequent to this diagnosis she was re-admitted to her local hospital secondary to continued concerns about pain management. EEG ruled out seizures and an upper gastrointestinal (GI) scope ruled out GERD. Medications added included

Neurontin, Scopolamine, and Loritab, and significant strides were made in patient comfort. Physicians postulated pain was GI and neurologic in nature. Family and therapists noted increased state regulation, head control, and attention to the environment with hopes of improved function. Further imaging refuted the earlier finding of the underdeveloped corpus callosum, but added the diagnosis of Dandy-Walker malformation.

Case 7's clinical indicators: microcephaly, developmental delay, decorticate/extensor posturing, irritability, primary feeding issues, hypotonia, blindness, deafness, dysmorphism, with 3 or more organ systems affected.

Case 8

Case 8 is a female who was born at 39 weeks gestation, weighing 7 pounds 2.5 ounces, to healthy parents and has a typically developing sister who is 2 years younger. Her mother reports that the pregnancy was normal except for decreased fetal movements in comparison to her other pregnancy. Apgars were 9, 10 and this subject had mild jaundice that resolved without intervention. Her parents first became concerned when she was 6 months old and pinched her fingers in a drawer, but did not show a pain response. Until this time she had met all of her developmental milestones, in fact she had been advanced in some domains: sitting independently, holding a book right side up, imitating her mother when she read to her, and pointing at 5 months old.

By 10 months Case 8 could identify some shapes in a book and her parents stated that these advanced skills were as "equally concerning" as the holes in her development, e.g., not being able to transition into sitting. By 16 months she was not pulling to stand or walking, and her parents documented a regression from 10-16 months. She had lost the 10 words she had been able to say at her first birthday. By 22 months differential diagnosis included RTT and leukodystrophies.

By 3 years of age Case 8 had a provisional RTT diagnosis; she was able to walk with assistance, sit in cross leg position, swat at a switch, and use eye gaze to communicate. Her distinctive aberrant hand movements manifested as hand to mouth. Between 3-4 years of age these movements morphed into patting of her clavicles; at baseline this is a soft tapping but when she is agitated/ill/off baseline in any way this movement becomes scratching. At 5 years of age genetic testing confirmed RTT. She requires assistance for gait due to apraxia/ataxia.

Case 8's clinical indicators: developmental delay, regression, hand stereotypies, and apraxia/ataxia.

Case 9

Case 9, a male, was born at 36 weeks 4 days via C-section after a gestation significant for preterm labor. He weighed 7 pounds 8 ounces and his Apgars were 5, 7, and 8. At birth it was noted that he was having consistent seizure activity that could not be stopped with anti-convulsants. Initial diagnoses included aortic

stenosis, GERD, and laryngomalacia. Imaging studies indicated polymicrogyria. He demonstrated hypotonia, developmental delay, feeding difficulties, and frequent seizures. He died at 7 months of age.

Case 9's clinical indicators: hypotonia, developmental delay, feeding difficulties, and seizures.

Case 10

Case 10 is a female whose initial gestation was unremarkable. An ultrasound at 30 weeks indicated hydrocephalus, so she was delivered via C-section at 38 weeks. She weighed 6 pounds 11 ounces with Apgars 5, 8. An MRI was performed and it was discovered that the hydrocephalus was being caused by alobar holoprosencephaly (HPE), a failure of forebrain division. Genetic studies indicated this was a de novo mutation of the ZIC2 gene. A shunt was placed to treat the hydrocephalus. She has healthy parents and two younger brothers who are typically developing.

Case 10 is dependent in all aspects of care. She fed orally until about 18 months when a G tube was placed secondary to aspiration concerns. This subject demonstrates developmental delay, elevated startle response, hypertonia, dysmorphic features, and seizures. Complications from her HPE include diabetes insipidus.

Case 10's clinical indicators: developmental delay, elevated startle reflex, hypertonia, dysmorphic features, and seizures.

Case 11

Case 11 is a male delivered via C section at 38 weeks. Gestation was significant for polyhydraminos and preterm labor at 30 weeks. He weighed 9 pounds 3 ounces and demonstrated transient tachypnea. Other issues include atypical torticollis, irritability, short stature, developmental delay, hypertonia, diaphoresis of unknown origin, unexplained fevers, and hydrocele. He also has dysmorphic features including posteriorly rotated ears and enlarged tongue as well as some hand deviations. Respiratory and feeding issues include FTT, dysphagia, GERD, vomiting episodes, pylorus spasms, delayed gastric emptying, difficulty with respirations following anesthesia, and stridor. He is also sensitive to sunlight. Due to difficulty feeding he first received a nasogastric (NG) tube but ultimately required a G tube. Initial diagnostic suspicion was Krabbe disease which was disputed by lysosomal storage analysis, but chromosomal analysis found a variation in the HRAS G12S gene which indicates Costello syndrome.

Case 11's clinical indicators: atypical torticollis, irritability, short stature, developmental delay, hypertonia, diaphoresis, unexplained fevers, hydrocele, dysmorphic features, respiratory issues including stridor, feeding issues, FTT, dysphagia, GERD, vomiting episodes, and 3 or more organ systems affected.

Case 12

Case 12 is a female whose gestation was complicated by maternal hypertension and pre-eclampsia. Labor was induced at 37 weeks; her Apgars

were 7, 9. At birth it was noted that there was a 'knot' in the umbilical cord and her bilateral upper extremities were hypotonic. She was referred to physical therapy at 7 months of age secondary to hypotonia and developmental delay. PT reported developmental delay and hypotonia. Secondary to her sister having a complicated perinatal period with hypotonia and seizures, an MRI and metabolic testing were ordered. A neurologist ruled out metabolic disorders via this testing and diagnosed her with benign congenital hypotonia.

Case 12's clinical indicators: hypotonia and developmental delay.

Case 13

Case 13 is a male born full term weighing 7 pounds 14 ounces. While his mother has epilepsy and had some seizure activity around the time of his birth, this was not reported to affect the infant. Case 13 had multiple hospitalizations in the first year of life including stays for respiratory syncytial virus (RSV) and rotavirus. At 2 years of age he was hospitalized for a "pseudo-stroke" following a high fever which resulted in significant right hemiparesis. Diagnoses also include Arnold Chiari malformation requiring decompression, hydrocephalus requiring shunt placement, hypotonia, and developmental delay. Developmental delay was noted with the following late attainments of motor milestones: rolling prone to supine at 10 months; rolling supine to prone at 12 months; independent sitting at 12 months; crawling at 17 months; pull to stand at 20 months; cruising at 21 months; and independent ambulation at 28 months. His therapists also noted

pervasive joint hypermobility, bilateral genu recurvatum, right sided weakness, and balance deficits.

Genetic reports also mention ventricular septal defect (VSD), anisocoria, poor growth, and cryptorchidism. At 2 years 11 months Case 13 was given the diagnosis of Noonan syndrome despite negative gene test as he fits the clinical phenotype thus demonstrates genetic variant.

Case 13's clinical indicators: hypotonia, developmental delay, right hemiparesis, and balance deficits.

Case 14

Case 14 is a female born at 26 weeks gestation after a difficult pregnancy. At birth she was noted to have hydrocephalus requiring a ventriculoperitoneal (VP) shunt and Pierre-Robin sequence. During her neonatal intensive care unit (NICU) course she required a tracheostomy secondary to respiratory compromise including bronchopulmonary dysplasia (BPD), and a G tube for feeding secondary to both craniofacial anomalies and respiratory compromise. Following a bout of RSV at 3 years of age she was referred for physical therapy.

Physical therapy documented hypertonicity in all extremities, but this subject's lower extremities were more involved. She also demonstrated significant developmental delay and abnormal postures, e.g., left upper extremity synergies. Her PT recommended a neuromuscular consult and Case 14 was given

a diagnosis of CP secondary to prematurity, hydrocephalus, and static encephalopathy.

Case 14's clinical indicators: hypertonicity, spastic quadriplegia, developmental delay, respiratory issues, feeding issues, dysmorphism, and abnormal postures.

Case 15

Case 15 is a male born at 39 weeks 5 days gestation, weighing 7 pounds 15 ounces with Apgars of 9, 9. Pregnancy was significant for maternal hypertension. At 8 weeks it was noted that there was blood in his stool and it was determined he had a milk allergy. Case 15 also developed congenital muscular torticollis, plagiocephaly, severe constipation, ptosis, and strabismus in the first year. There were also language development concerns; while he could say "mama" and "dada" by 6 months, he never waved or used "yes" or "no." By 2 years of age his speech was echolalic and he did not demonstrate the ability to social reference. By 27 months the diagnosis of autism was confirmed. Gross motor development was always delayed secondary to hypotonia while fine motor was on target or advanced. Additional testing via EEG indicated abnormalities but no seizure activity; immunology results indicated antibody deficiencies. With multiple systems involved, Case 16 was evaluated for mitochondrial disease and was found to have a nuclear derived Complex I mitochondrial disorder.

Case 15's clinical indicators: torticollis with plagiocephaly (resolved), autism, food allergy, immune deficiency, developmental delay of gross motor milestones (walking), hypotonia, and 3 or more organ systems involved.

Case 16

Case 16 is the younger brother of Case 15. He was born at 37.5 weeks weighing 6 pounds 10 ounces. The pregnancy was unremarkable and his Apgars were good. His mother reported that he was always more affected than his older brother. He had weak muscles that his mother states were "like jello," which indicates an underlying hypotonia. He demonstrated right facial dropping and severe drooling. He walked at 15 months. Case 16 had severe feeding problems including dysphagia with laryngeal penetration that required a G tube. He also was found to have motility issues. Like his brother he has a compromised immune system. He was given a diagnosis of failure to thrive (FTT). He also suffers with chronic diarrhea and fatigue. His gross and fine motor skills are delayed. Case 16 was also found to have a nuclear-derived mutation of Complex I and IV.

Case 16's clinical indicators: hypotonia, primary feeding issues, FTT, immune compromise, developmental delay, and 3 or more organ systems involved.

Case 17

Case 17 is a female whose delivery was induced at 38 weeks gestation secondary to maternal hypertension. She weighed 5 pounds 14 ounces with Apgars of 8, 9. Early concerns noted by her mother included screaming episodes that were attributed to colic at 2 weeks of age, thus breastfeeding was stopped and formula was started. Her mother then noted she had a poor seal around the bottle nipple and lost a lot of formula. Case 17 "could not fight gravity" per her mother's report and also had a strange tendency to do "sit ups" when in supine and shake when in supported standing around 9-10 months. Therapy was initiated at 10 months and the diagnostic journey began.

Descriptors of Case 17 include trunk rocking in sitting, hand to mouth behaviors, developmental delay, regression, and abnormal movement patterns including a strange mode of commando crawling. Over time therapists noted a tightening in the left upper extremity indicating some muscle/tendon shortening. Gait was described as ataxic and apraxic. Additional findings included delayed myelination and thin corpus callosum per MRI, abnormal EEG indicating epilepsy, G tube placement, and negative tests for RTT (MECP2 and CDKL5), Angelman, mitochondrial disorders, and muscular dystrophy.

Case 17 demonstrated a decrease in seizure activity and an increase in attention/alertness per mother following a change in diet and supplemental nutrients/compounds. She is no longer on G tube formula and eats table food.

Despite no genetic marker Case 17 has a clinical diagnosis of atypical RTT confirmed by multiple specialists. It is surmised that she has a variant of one of the genes causing RTT.

Case 17's clinical indicators: developmental delay, regression, and hand to mouth behaviors, and gait apraxia/ataxia.

Case 18

Case 18 is a male born weighing 8 pounds 14 ounces via scheduled C section at 39 weeks 5 days secondary to prenatal diagnosis of cardiac issues at 19 weeks gestation (by ultrasound). He is the 9th child born to his parents and the only one to have any significant medical problems. His Apgars were 6, 9 following vigorous stimulation and positive pressure ventilation for 90 seconds. Following birth he was definitively diagnosed with hypoplastic right heart syndrome, transposition of the great arteries, tricuspid atresia, and Goldenhar syndrome. He demonstrated facial asymmetry due to Goldenhar and was born without an external right ear, hypoplastic right mandible, and possible right facial palsy. He had vocal cord paralysis, feeding difficulty requiring G tube, right torticollis, and a subsequent developmental delay due to spending the first few months of his life in the hospital. Two surgeries were performed during that time to correct critical cardiac anomalies.

Physical therapy evaluation at 3 months indicated developmental delay, right torticollis, normal tone, decreased strength for age, but no abnormal

reflexes. Secondary to low oxygen saturation (approximately 82% at rest) cardiovascular endurance was also impaired as evidenced by quick cyanosis when upset or over exertion. Further assessments indicated some abnormal movement patterns, e.g., crawling, which were attributed to asymmetries. Gross motor developmental issues mostly resolved by 15 months of age; however feeding issues persist.

Case 18's clinical indicators: developmental delay, right torticollis, abnormal movement patterns, dysmorphic features including asymmetry, poor endurance, and primary feeding issues.

Case 19

Case 19 is a female born at 23 weeks gestation weighing 1 pound 2 ounces and had an initial Apgar of 2. She required mechanical ventilation and was in the NICU for 4.5 months. She was diagnosed with hydrocephalus requiring taps until she was large enough for a shunt placement at 4 months of age. She suffered a right intraventricular hemorrhage (IVH) Grade IV and a left IVH Grade III.

Case 19 demonstrated developmental delay and had inconsistent therapies as her parents moved frequently due to military obligations. Her mother reports that at 2 years of age she noted that this child was making progress; she developed head control, was able to feed herself, and use a sippy cup. She commando crawled around 5 years of age and sat independently when placed at 7

years old. At this time she had a dorsal rhizotomy secondary to lower extremity tone.

Physical therapy reports indicated pervasive hypertonicity in all extremities with the left more involved than the right; bilateral hip and knee flexion contractures secondary to tone; and impaired righting reactions and protective responses. By 13 years of age Case 19 used rolling and commando crawling as means of independent mobility, and was non-ambulatory. Her presentation/diagnosis is spastic quadriplegic CP.

Case 19's clinical indicators: hypertonicity, developmental delay, and spastic quadriplegia.

Case 20

Case 20 is a female born full term following an unremarkable gestation. She weighed 7 pounds 14 ounces and her Apgars were 7, 9. She remained in the hospital for 2 days to resolve a mild jaundice. Developmental milestones are as follows: rolled at 14 weeks, sat at 6 months, commando crawled at 7 months, crawled at 9 months, and walked at 15 months. At 10 months she developed a high fever and following this her mother noted a decline in acquisition of age appropriate gross motor milestones. At 14 months old she stopped saying the few words she had spoken to date. By 15 months her mother had significant concerns and changed pediatricians to get a second opinion.

Physical therapy evaluation at 17 months indicated delayed and atypical development and an apraxic/ataxic gait. Subsequent evaluations confirmed these issues, and a neurologist and geneticist were consulted. Differential diagnoses included autism, food allergies, RTT, and Angelman syndrome. A diagnosis of RTT was confirmed via MECP2 testing when Case 20 was 23 months old. Hand to mouth movements began at 26 months and feeding difficulties worsened. She received a G tube when she was 3.5 years old. At this age she was noted to develop bruxism. Her gait pattern was described as apraxic and ataxic.

Case 20's clinical indicators: regression, developmental delay, apraxic/ataxic gait, hand to mouth movements, and bruxism.

Case 21

Case 21 is a male born one week after his due date. While the pregnancy was unremarkable, his delivery was significant for slowing of his heart rate necessitating a forceful extraction. He weighed 7 pounds 11 ounces and reportedly had good Apgars. At 6 months his lack of use of his right upper extremity raised concern and he was tentatively given a diagnosis of a brachial plexus injury. Over the next few months, however, it was noted that his development was delayed. An MRI indicated that Case 22 had suffered a cerebrovascular accident (CVA) in utero which resulted in a right hemiplegia.

Case 21 per mother's report was late in receiving referrals to therapy, and continued to show developmental delay; he did not walk until 17 months. Private

physical and occupational therapy were initiated at 3 years of age when he aged out of early intervention. This corresponded with the initiation of botulinum toxin (Botox) injections for primarily the right upper extremity.

Physical therapy evaluation at 3 years of age indicated hypertonicity in the right upper extremity with synergy posturing; persistent palmar grasp of right hand; delayed protective response to right; gait disturbance with internal rotation of right hip and decreased active dorsiflexion of right ankle indicating spastic hemiplegia.

Case 21's clinical indicators: developmental delay, hypertonicity, and spastic hemiplegia.

Case 22

Case 22 is a female born following pre-implantation genetic diagnostics via in vitro fertilization. Her gestation was significant for preterm labor at 28 weeks followed by maternal chorioamnionitis resulting in her mother having a high fever which induced maternal seizures and birth. Case 22 was delivered at 32 weeks weighing 4 pounds 3 ounces with Apgars of 6, 9. She was diagnosed with meconium staining, sepsis, and respiratory compromise, and she was febrile secondary to pneumonia. She required ventilator assistance and NG feeding during the early phase of her NICU stay.

Early development was significant for poor state regulation, sensory defensiveness, poor emotional development, motor hyperactivity, and feeding

difficulties. Throughout infancy and into her toddler stage Case 22 continued to demonstrate a unique constellation of signs and symptoms, including not being able to sleep in supine or tolerate rides in the car. She was described as clumsy. Early differential diagnoses included CP which was eliminated; autism which was ruled out; and developmental coordination disorder (DCD). At 2 years 10 months of age a physical therapy evaluation noted sensory dysfunction most notable for excessive movement, mild hypotonia, and behavior that was not typical for her age. The PT suggested that Case 22 may have significant vestibular involvement as she overly relied on visual and somatosensory input and craved intense vestibular input. The parents of Case 22 had already traveled all over the United States for diagnostics, but were willing to pursue this new theory. A year later, Case 22 was confirmed with a diagnosis of bilateral vestibular loss which is suspected to be a result of Gentamicin poisoning. Indications included disturbance in the vestibular ocular reflex (VOR) and a lack of post-rotatory nystagmus. This child and her mother (while in labor) were given high doses of Gentamicin which is a known vestibular toxin.

Treatment for Case 22 included decreasing anxiety by allowing her to sleep with the lights on to have visual stabilization; limiting car travel; allowing for frequent rest breaks during play and pre-school due to her overreliance on vision and somatosensory input which causes extreme fatigue and thus behavioral

outbursts. Her parents continue to travel to get answers and advice on how to manage this unique pediatric disorder.

Case 22's clinical indicators: mild hypotonia, poor state regulation, non-age appropriate fatigue, vestibular dysfunction, and fine motor delay (developmental) secondary to the above.

Case 23

Case 23 is a female born at 38 weeks gestation weighing 6 pounds 7 ounces after an unremarkable gestation. Delivery was significant for fetal heart rate deceleration and cord compression. She had jaundice and was treated over the first week of life; it resolved. Her parents said she was a "hairy" and "fussy" baby. She was small for her age during the first two years of life. She never crawled. At her 18 month appointment a speech delay was noted, however hearing tests indicated she had no issues with her auditory system. Early childhood intervention evaluated her and discerned that she had global developmental delays. A differential diagnosis of pervasive developmental disorder, not otherwise specified (PDD-NOS) was proposed in her second year of life.

The parents of Case 23 were not satisfied with that diagnosis and sought additional consults and testing including another hearing test and MRI. The MRI confirmed congenital bilateral perisylvian polymicrogyria. Seizures began as Case 23 entered adolescence and this was related to the onset of hormone changes.

Case 23's clinical indicators: developmental delay with the greatest delay in speech, and seizures.

Case 24

Case 24 is a female born at 35 weeks gestation after preterm labor complicated the pregnancy at 29 weeks. She weighed 4 pounds 8 ounces with an Apgar of 10. Her mother describes her as a sleepy infant who was easy and somewhat detached. First signs of concern were the fact that Case 24 could be placed in sitting but did not make any attempts to transition. Her mother describes that it was as if she had a motor arrest around 15 months. After a series of evaluations she was diagnosed with RTT at 2 years of age via the MECP2 gene. Around that time her hand stereotypies developed with a midline clapping, then a clapping of the wrists, and then hand to mouth. Arm splints were used from 2-5 years and then the hand gestures ceased. Her gait is described as apraxic and ataxic.

Case 24's clinical indicators: developmental delay, hand stereotypies, and apraxic/ataxic gait.

Case 25

Case 25 is a female twin born at 24 weeks gestation following IVF. Delivery was due to a placental abruption. She weighed 1 pound 4 ounces with Apgars of 2, 2, and 4. During her 4 month NICU stay she was diagnosed with bilateral grade III IVH resulting in PVL. She had a PDA which resolved. Other diagnoses

included chronic lung disease, apnea, and retinopathy. Initial physical therapy evaluation following discharge from the hospital noted developmental delay, hypotonia, flexion bias of lower extremities in both prone and supine, extensor posturing, impaired state regulation, decreased eye contact and tracking, and impaired sensory processing.

By 2 years of age additional concerns arose about neurological and developmental status. Case 25 was having 'spells' that were thought to be seizures but EEG results were normal. Additional testing indicated a continued developmental delay. She did not say "mama" until 11 months and did not walk until 22 months. At 18 months she began some sensory stimulation behaviors and continued to mouth toys into her second year. However, no regression in any area of development was detected. Hypotonia was also noted.

Differential diagnoses were mild cerebral palsy; progressive developmental disorder; pervasive developmental disorder not otherwise specified; and sensory processing disorder (SPD). SPD is the working diagnosis at the time of writing is the latter.

Case 25's clinical indicators: developmental delay, hypotonia, abnormal movement patterns as an infant (resolved), visual disturbances, and sensory processing issues.

Case 26

Case 26 is a female twin born at 32 weeks gestation due to maternal elevated blood pressure causing severe headaches that could not be managed with medication. She weighed 3.5 pounds and had Apgars of 6, 9. This pregnancy was a result of fertility enhancement and was complicated by twin to twin transfer (determined at 20 weeks gestation) resulting in Case 26 having an in utero CVA. This prenatal CVA led to lissencephaly (diagnosed at 2 weeks of age), cortical visual impairment, and intractable atonic seizures which began at 11 months of age. She demonstrated left hemiparesis and developmental delay.

Physical therapy evaluation at 16 months documented decreased active range of motion, strength, and protective reactions on the left side. Her palmar grasp was persistent on the left and she had an extensor bias. Gross motor skills were in the 7 month range demonstrating a developmental delay. At 18 months Case 27 underwent a hemispherotomy to disconnect the right part of her brain causing the seizures from the rest of her brain. This was performed after numerous consultations and magnetoencephalography (MEG). While the surgery stopped the seizures it did cause increased weakness in Case 26's left hemibody.

Case 26's clinical indicators: developmental delay, hemiparesis, seizures (resolved), and visual disturbances.

Case 27

Case 27 is a male born at 35 weeks gestation via C-section secondary to breech position and oligohydramnios. Apgars were 9, 9 and he weighed 5 pounds. Following birth he has some respiratory distress thought to be related to pneumonia. He was intubated and extubated a few times and treated for jaundice, but he stabilized and was discharged at 3 weeks of age. Due to a congenital hemangioma on his scalp additional imaging was ordered. At 2 months of age a cranial ultrasound indicated PVL with right involvement greater than the left. Subsequent evaluations indicated the infant had hypertonicity in bilateral upper and lower extremities, developmental delay, and bilateral fisting. A diagnosis of spastic quadriplegia CP was confirmed. Surgeries performed later in life include G tube placement and implantation of a Baclofen pump.

Case 27's clinical indicators: developmental delay, hypertonicity in bilateral upper and lower extremities and fisting, and spastic quadriplegia.

Case 28

Case 28 is a male born full term weighing 6 pounds 6 ounces after an uneventful gestation. At 2 weeks of age he was admitted to the hospital for upper extremity tremors, but all testing was negative for pathology. At 4 months he was again admitted with a diagnosis of FTT. His mother had noted a slowing of development at 2 months, as exemplified with his loss of ability to smile. Initial MRI imaging did not indicate any abnormalities.

Therapeutic evaluations during Case 28's early months state that he demonstrated decorticate posturing, developmental delay, regression, irritability, poor state regulation, abnormal persistence of primitive reflexes, extensor thrust, and hypertonicity in all extremities with hypotonicity in his trunk. He also demonstrated frequent upper extremity tremors, difficulty tracking, nystagmus, and feeding difficulty/vomiting. Differential diagnoses included Smith-Lemli-Opitz syndrome (SLOS), Leigh syndrome, mitochondrial disease, or a lysosomal storage disorder. At 7 months he received a G tube with Nissen fundoplication. His seizure disorder was also confirmed at this time.

At 9 months Case 28 went into status epilepticus and was intubated and placed on a ventilator. CT of his brain indicated generalized atrophy and enlargement of the sulci and ventricles. An MRI further elucidated abnormalities in the brainstem, cerebellum, basal ganglia, with perfuse cerebral white matter involvement (leukodystrophy) indicative of an inborn metabolic disorder. Mitochondrial disease interventions were initiated until definitive etiology was determined. Also documented as this time were clonus in the right lower extremity, horizontal nystagmus, and tongue fasciculations.

Case 28 expired in his sleep at 10.5 months. Two weeks following his death results from lysosomal storage disorder testing indicated that his diagnosis was Krabbe disease (leukodystrophy); both his parents were tested and carried the autosomal recessive gene for Krabbe.

Case 28's clinical indicators: decorticate posturing, developmental delay, regression, irritability, poor state regulation, abnormal persistence of primitive reflexes, extensor thrust, hypertonicity, hypotonicity, upper extremity tremors, difficulty tracking, nystagmus, primary feeding issue including vomiting, and seizures.

Case 29

Case 29 is a male who was born at 40 weeks gestation after an uncomplicated gestation and birth. He weighed 6 pounds 3 ounces. Shortly after delivery he was transferred to the NICU secondary to respiratory distress. He developed feeding issues and was diagnosed with FTT, thrush, bilateral conjunctivitis, hypercalciuria, and hypercalcemia. Family history is positive for two (out of six) siblings with small congenital cataracts that had not at that time of his birth required intervention.

By 14 months he only weighed 13 pounds and was noted to have oral aversion, oral leukoplakia, photophobia, corneal clouding, continuous red, inflamed eyes, and hearing loss. Differential diagnoses included biotinidase deficiency, chronic granulomatous disease, and hereditary benign intraepithelial dyskeratosis. At 5 months of age a G tube was placed, but by 26 months of age his oral intake of pureed foods had improved and thus routine tube feedings were not necessary. Signs and symptoms include FTT, bilateral hearing loss requiring hearing aids, oral aversion, progressive ocular abnormalities, and

oral/espophageal abnormalities. He could not see beyond four feet in dim light. He was also found to have decreased amounts of insulin growth factor 1 (IGF-1) supporting a possible hypothesis of lack of growth due to autoimmunity. He was started on growth hormone therapy. By the age of 4 years Case 29 continued on growth hormone therapy and weighed 25 pounds. He no longer relied on the G tube for feeds but had difficulty with solids and relies on liquid nutrition. His visual sensitivity continues, but cognitively he seems unimpaired.

Two years after Case 29's assessment by the Undiagnosed Disease Program (UDP) at the National Institute of Health (NIH) a diagnosis of hereditary benign intraepithelial dyskeratosis (HBID) was confirmed secondary to a de novo duplication of chromosomal material on the long arm of chromosome 4. His case is unique in that his symptoms of FTT, hearing loss, and lack of growth hormone are not explained by this diagnosis.

Case 29's clinical indicators: feeding difficulties with FTT and delayed growth, hearing loss, visual disturbances, and three or more organ systems involved.

Case 30

Case 30 is a male born at 41 weeks gestation weighing 8 pounds 4 ounces with Apgars of 8, 9. The pregnancy was complicated by a fall by his mother at 33 weeks gestation. Early concerns arose about the difference in functioning in the right and left sides of his body. At 1 year of age an MRI revealed an area of

concern in the deep parietal white matter, left more involved than right, which could be indicative of PVL. Further EEG, mitochondrial, and metabolic work ups showed slight aberrations but no significant pattern of pathology. Working diagnoses include gross and fine motor delays, mild myopathy, and motor apraxia. However, additional diagnoses of Asperger disorder, attention deficit disorder (ADD), seizure disorder, mild right hemiparesis, and verbal apraxia have been reported as well.

Case 30's clinical indicators: developmental delay, apraxia, autism, ADD, seizures, and right hemiparesis.

Case 31

Case 31 is the sister of Case 30, and is 2 years 8 months younger than her brother. She was born full term weighing 8 pounds 8 ounces with Apgars of 9, 9. Pregnancy was complicated by maternal malaise and significant maternal weight gain. Case 31 was notably hypotonic at birth and was a poor feeder. Since birth her parents have been concerned about her respiratory status, specifically shallow breathing. Nerve conduction velocity/electromyography (NCV/EMG) testing at 18 months did not show significant pathology. However, upon a repeat of these tests with muscle biopsy a year later there was a mild distal myopathy. She tested negative for RTT. Over the next 2 years concerns about GI issues, specifically constipation, and arose. By 4 years 8 months she was given the diagnoses of hypotonia with hypermobility, with some suspicion of a connective

tissue disorder such as Ehlers-Danlos syndrome. She wears bilateral AFOs for stability.

Case 31's clinical indicators: hypotonia and hypermobile joints.

During the writing of this work a new test for mitochondrial disorders became available and both Case 30 and 31 demonstrated decreased Complex I activity via buccal swab analysis. While this science is in its infancy this information provides at least a working diagnosis for these siblings.

Case 32

Case 32 is a female born vaginally weighing 6 pounds 11 ounces with Apgars of 9, 9. Mother had gestational diabetes and premature labor; however, induction was required for delivery. Case 32 had mild jaundice, small bumps on skin, and decreased movements and passed newborn hearing screen on the fourth attempt. She could not breastfeed due to a poor latch, and at 5 weeks projectile vomiting began. At 4 months solid foods were introduced but were not tolerated well, and she was only taking 4 ounces in a bottle per feed.

At 8 months of age Case 32 was admitted to the hospital with FTT. A modified barium swallow (MBS) showed GERD and dysphagia and an NG tube was placed. By 10 months she could not roll and did not tolerate prone. She was beginning to sit independently. Feeding and gastrointestinal issues continued. At 11 months a repeat MBS showed worsening of issues but she did begin to roll.

A week after her first birthday Case 32 was readmitted and continued to have respiratory and gastrointestinal illnesses. Gagging on food became a major issue and pica developed. She began to crawl in an uncoordinated manner. Neurological concerns began to solidify. Since the age of 4 months "staring" episodes had developed. No official seizure diagnosis was made at that time; however, movement patterns also pointed to neurological issues. By 13 months plantar grasp was pervasive and she would not bear weight through her lower extremities.

Another hospitalization followed due to continued bouts of vomiting; thus a G tube was placed and a muscle biopsy performed due to concerns about metabolic disease. Muscle biopsy results indicated some abnormalities suggesting a fatty acid or mitochondrial disorder.

It was noted that with every increase in mobility came a concomitant GI exacerbation in Case 32. By 15 months she was noted to have abnormal postures, e.g., extension of all extremities with fist clenching when excited or angry. Balance in sitting and crawling was impaired. Behavior was also an issue with physical refusals and behavioral outbursts. In addition, self-stimulation behaviors developed such as spinning in sitting, arm flapping, and hand movements. Due to these movements and perseverative play, autism is still being considered as a clinical indicator despite her social nature. At 16 months a metabolic geneticist did not see mitochondrial or metabolic features in Case 32,

but did suspect a syndrome; all testing was negative. Pulling to stand emerged with a wide base of support. She was noted to have hypotonia, frontal bossing, and a depressed nasal bridge.

The family history is rich with a myriad of issues that contribute to Case 32's clinical picture. Maternal grandmother had a cousin who died at 8 years of age after a lack of motor and speech development secondary to suspected prenatal syphilis exposure. Mother's sister has a learning disability. Mother's cousin on her father's side has mental delay with childlike behaviors as an adult, but no official diagnosis. Maternal aunt on father's side had multiple sclerosis. Paternal uncle has either bipolar or ADD. All of the uncle's children have developmental delay of motor or speech. Paternal grandfather's family has many members demonstrating complex, yet undiagnosed issues. One male cannot ambulate, has G-J tube, and pica. One female was originally given the diagnosis of CP but that was withdrawn. Another female had a probable diagnosis of a shrinking cerebellum.

Case 32 continued to be evaluated by physicians and researchers across the United States. Genetic studies first revealed a gain on the short arm of chromosome 12 (12p13.31) that her mother also carries. This A625G variant is related to a short-chain acyl-CoA dehydrogenase (SCAD) deficiency. Testing at the UDP at NIH when she was 3 years of age found a decrease in normal levels of Complex II and III in the electron transport chain (mitochondrial disorder)

which in combination with the SCAD variant causes a phenomenon called synergistic heterozygosity.

Case 32's clinical indicators: hypotonia, developmental delay, FTT, dysmorphism, autistic like behaviors, primary feeding issues, and three or more organ systems involved.

Case 33

Case 33 is a female born vaginally in the breech position at 40 weeks gestation. She weighed 8 pounds 12 ounces with an Apgar of 8. She gained head control around 3-4 months and rolled supine to prone at 6 months. At 11 months it was noted that she had a hypotonic trunk with hypertonic extremities. She would demonstrate periodic regression of certain skills with noted developmental delay.

At 22 months Case 33 began having detectable seizures. She also developed eczema related to gluten exposure. Testing was done throughout the country with the only common finding being MRI evidence of hypomyelination indicating a leukodystropy. Her lysomal storage panel negative for MLD, Krabbe, as well as other known disorders causing leukodystrophy. Cortical visual impairment also noted.

Case 33's clinical indicators: hypotonia, hypertonia, developmental delay, regression, initial gait disturbance, cortical visual impairment, and seizures.

APPENDIX H

TABLES 3a & 3b

Table 3a

Contingency Table for Clinical Indicators of Leukodystrophy Demonstrates Clinical Indicators

	Yes	No	Total
Leudystrophy	3	0	3
Not Leukodystrophy	0	30	30
Total	3	30	33

Table 3b

Contingency Table for Clinical Indicators of Mitochondrial Disorders Demonstrates Clinical Indicators

	Yes	No	Total
Mitochondrial disorder	4	3	7
Not Mitochondrial disorder	0	26	26
Total	4	29	33

APPENDIX I

TABLE 4

Table 4

Primary Clinical Indicator Tool, Revised

Clinical Indicators	Cerebral Palsy	Rett Syndrome	Leukodystrophy	Krabbe Disease	Mitochondrial Disorders	Complex
Disorders of Movement						✓✓
Hypertonia (↑DTRs)						
One of the following -plegias						
-Quadriplegia						
-Hemiplegia						
-Diplegia						
One of the following dyskinesias						
-Spastic						
-Dystonia						
-Choreo-athetosis						
-Hypotonia with ataxia*						
Hypotonia (↓DTRs)						✓✓✓✓✓
Ataxia						
Apraxia						
Initial gait disturbance						
Hand stereotypies						
Development						
Regression						
Irritability						✓
Body Systems						
3+ organ systems 1° affected						✓✓
Feeding issues 1°						✓
Dysmorphism**						✓✓✓

*Hypotonia in combination with ataxia in CP

**Including arthrogyposis, macrocephaly, and microcephaly

APPENDIX J

Cases Studies of Children with Known Diagnoses, Blinded

Case 1

Case 1 is a female who was initially diagnosed with developmental delay and left torticollis. She demonstrates seizures, hypotonia at rest, extremity shaking, decorticate posturing, hypertonicity in her extremities, initial gait disturbance, regression, visual disturbances, and respiratory compromise. She can communicate through crying and move extremities away from painful stimuli. She is neurologically impaired and her medications cause lethargy. Adaptive equipment is needed for positioning and she is maximally dependent in all aspects of care. She was diagnosed at 9 months of age.

Case 2

Case 2, a female, was the product of a pregnancy significant for maternal illness and bedrest. She was born at home with a midwife, and was diagnosed a few days after delivery. Clinical indicators include hypotonia, open mouth posturing with tongue protrusion, cervical stacking, externally rotation of bilateral tibias with feet in supination, poor postural control, ligamentous laxity, and developmental delay. Medical concerns included small patent ductus arteriosus (PDA) at 6 months of age, idiopathic rib fracture at 8 months, and recommendation of growth hormone at 16 months.

Case 3

Case 3 is a male who developed typically until 3 ½ year of age when he sustained an injury. He demonstrates a flexed posture, open mouth posturing

with decreased saliva control, hypertonicity in bilateral lower extremities (BLE), left elbow flexion contracture, muscle weakness, compensatory strategies for movement, decreased balance and coordination for age, and difficulty acquiring age appropriate gross motor skills. He is able to ambulate independently, play ball, and ride a bike, but atypically.

Case 4

Case 4 is a male who developed typically until he went into congestive heart failure (CHF) at 10 months of age, he was stabilized, but then relapsed at 13 months. He was intubated on the second admission but self-extubated causing a near-death experience. This resulted in a blood clot in the abdominal aorta with decreased blood flow to BLE and cortical blindness. He demonstrates hypotonia, GI issues requiring gastrostomy tube (G tube), primary feeding issues, sensory aversion, periodic extensor posturing to gain postural control, hypertonia in distal extremities, developmental delay, and muscle weakness. He did require heart transplant in the first year of life.

Case 5

Case 5 is a female who was born following a pregnancy complicated by twin-to-twin transfusion syndrome resulting in the death of the other twin. She is G-tube fed and has respiratory and sleeping issues. She demonstrates seizures, abnormal postures, muscle weakness, developmental delay, cardiac issues, GI

issues, visual disturbances, hypertonia, and three or more organ systems involved.

Case 6

Case 6 is a male born without left eye and underdeveloped right eye leading to poor vision. He demonstrates hypotonia, muscle weakness, abnormal postures, developmental delay, feeding issues, and sensory/behavior issues.

Case 7

Case 7 is a male twin born at 26 weeks gestation weighing 1 pound 15 ounces. He had surgery to correct strabismus at age 3 and now wears glasses. He demonstrates hypertonicity in his BLE and muscle weakness. His gait is crouched indicating spastic diplegia. He has sensory and attention issues.

Case 8

Case 8 is a male demonstrating right sided weakness as well as decreased weight-bearing on that side. He has a crouched gait with elevated tone in his BLE indicative of spastic diplegia, developmental delay, cognitive/language/social challenges, visual motor issues, and tongue thrust.

Case 9

Case 9 is a male who was noted at 2 months to have an adhesion on his left hamstring tendon, mild hypoplasia of his mandible and a high palate. He demonstrates hearing loss, hypertonicity greater on the right side of his body, upper extremity tremor with movement (right greater than left), flexion postures,

right hip dysplasia, muscle weakness, poor tolerance to riding in the car, visual disturbances, hearing loss, developmental delay, preference to right rotation of head, right parietal plagiocephaly, and abnormal postures.

Case 10

Case 10 is a male born 5 weeks premature. He demonstrates apraxia, decreased balance, coordination, and strength for age. He fatigues easily. He struggles with motor planning, attention to task, and sensory integration issues including oral/texture aversions. He has developmental delays in all areas.

Case 11

Case 11 is a male who requires use of bracing and assistive devices for mobility secondary to joint impairments. He has a narrow shoulder girdle, protracted scapulae, abnormal posturing including bilateral elbow extension, and left hip dislocation. He uses compensatory patterns for movement. He requires assistance for transitions and uses bilateral hip-knee-ankle-foot orthoses (HKAFOs) and a platform walker for upright movement. Independent mobility is achieved on the floor where he is able to scoot on his bottom and roll.

Case 12

Case 12 is a female born without a left lung. She presents with an atypical gait pattern noted for a wide base of support. Her posture is significant for stacked neck and shoulders and left torticollis. She demonstrates developmental delay, weak upper extremities including hand intrinsics, sensory issues including

oral aversions, visual disturbances, primary feeding issues requiring G tube, impaired motor planning/apraxia, and three or more organ systems involved.

Case 13

Case 13 is a female with hypertonia in her right hemibody in the presentation of spastic hemiplegia. She demonstrates developmental delay, visual disturbances, stuttering, and distractibility.

Case 14

Case 14 is a male born following an unremarkable pregnancy. He demonstrates significant reflux, left lateral flexion of head with right rotation indicative of a left torticollis, and age-appropriate development. The combination of torticollis and reflux is indicative of Sandifer syndrome.

Case 15

Case 15 is a male with flexion contracture patterning throughout his body. He demonstrates scoliosis, right hip dislocation, and limited independent movement. His right side is more involved than his left. He has poor head control and no trunk control. He demonstrates spastic quadriplegia, developmental delay, visual disturbance including cortical visual impairment, seizures, and inability to speak. He has a percutaneous endoscopic gastrostomy (PEG) tube and urinary catheter. At 14 years of age underwent release of right hip adductors and fusion of right ankle and foot. He is dependent in all aspects of care.

Case 16

Case 16 is a male born at 36 weeks 2 days weighing 5 pounds 3 ounces. Pregnancy complicated by incompetent cervix and a non-reactive fetal non-stress test (NST). Delivery was via Caesarian section (C-section) secondary to nonreassuring fetal heart rate tracing (NRFHT), and infant had meconium staining. Apgars were 7, 8 and infant required intubation. He was admitted to the neonatal intensive care unit (NICU) secondary to hypothermia, hyperbilirubinemia requiring phototherapy, and poor feeding. At 1 week of age vomiting became a significant issue and his oral intake was stopped (NPO) and he was fed via intravenous feedings (IVF). Gastroesophageal reflux (GERD) was noted but no obstruction was found. Seizures were diagnosed and treated.

In the first year of life Case 16 developed preference for right head rotation secondary to tight left sternocleidomastoid (indicative of left torticollis), plagiocephaly, a mild developmental delay in his motor skills, hypertonicity in left upper and lower extremity, and left hemiplegia.

Case 17

Case 17 is a female who presented at 9 years 10 months of age with bilateral edema including face, visual impairment, fatigue with movement, and pain. She was diagnosed at 7 years of age, and underwent hospitalization for 2 months for exacerbation of symptoms prior to initial assessments. She has a history of vision and kidney issues, muscle weakness, and pain. She is being

treated for depression and receives weekly steroid treatments. Muscle tightness due to self-limited movements secondary to pain is noted.

Over the next year Case 17 lost over 20 pounds with cessation of steroid treatments and depressive symptoms improved; however, pain and movement restrictions continued. The following year was not as positive with decreased mobility leading to a more sedentary lifestyle. Patient reports increased pain, nausea, and edema with steroid injections. Bone density study indicated multiple old fractures. Cataracts diagnosed and attributed to steroids; abdominal distention and pitting edema in bilateral lower extremities also of concern.

Case 18

Case 18 is a male born naturally and full term but complicated by twin-to-twin transfusion syndrome. His early months were significant for GERD for which he was medicated. At 6 months his parents were concerned about his development and initiated therapy services. He received a diagnosis at 1 year of age.

At 5 years 5 months he uses a manual wheelchair and bilateral ankle foot orthoses (AFOs). He demonstrates hypertonia, movement patterns consistent with spastic quadriplegia, muscle weakness, visual disturbances, developmental delay, and requires assistance in all aspects of care.

Case 19

Case 19 is a male born full term after an uncomplicated pregnancy. He was admitted to the NICU 4 days after birth secondary to pulmonary hypertension. Mother became concerned at 2-3 months when patient did not make eye contact. Diagnosis received at 10 months. Gross motor skills delayed: rolled at 8 months, sat independently at 16 months, and crawled at 24 months. He demonstrates seizures for which he is medicated, hypertonicity, movement patterns consistent with spastic quadriplegia, developmental delay, modified crawling movements, difficulty with transitions, mouthing of toys, and muscle weakness.

Case 20

Case 20 is a female twin born via C-section. She was in breech position prior to delivery. She weighed 5 pounds 8 ounces and demonstrated multiple ventricular septal defects (VSDs), left radial dysplasia with a club hand, horseshoe kidney, and anal stenosis. She remained in the NICU for a month secondary to failure to thrive (FTT) requiring a feeding tube and oxygen support.

At 23 months of age Case 20 had pollicization of the right index finger to create a thumb. She has undergone release of a tethered cord. She was not able to crawl secondary to the right extremity deformities, but walked around 2 years of age. Her gait is significant for a wide base of support with excessive hip abduction and external rotation. She uses abnormal and compensatory movement patterns.

She demonstrates primary feeding issues, three or more organ systems involved, and dysmorphism.

Case 21

Case 21 is a male born at 23 weeks gestation; his twin delivered 3 weeks prior and did not survive. He remained in the NICU for 3 months requiring a tracheostomy and G tube, and was transferred to another NICU for one more month where he received his diagnosis. He was on a ventilator until 2 years of age and continues to have recurrent respiratory issues. He demonstrates hypertonicity and muscle weakness indicative of spastic quadriplegia, developmental delay, and seizures.

APPENDIX K

TABLE 5

Table 5

Primary Clinical Indicator Tool, Revised

Clinical Indicators	Cerebral Palsy	Rett Syndrome	Leukodystrophy	Krabbe Disease	Mitochondrial Disorders	Complex
Disorders of Movement						
Hypertonia (↑DTRs)						✓
One of the following -plegias						
-Quadriplegia						
-Hemiplegia						
-Diplegia						
One of the following dyskinesias						
-Spastic						
-Dystonia						
-Choreo-athetosis						
-Hypotonia with ataxia*						
Hypotonia (↓DTRs)						✓
Ataxia						
Apraxia						
Initial gait disturbance						
Hand stereotypies						
Development						
Regression						
Irritability						
Body Systems						
3+ organ systems 1° affected						✓✓✓
Feeding issues 1°						✓✓
Dysmorphism**						✓✓

*Hypotonia in combination with ataxia in CP

**Including arthrogyposis, macrocephaly, and microcephaly

APPENDIX L

IRB Approval Letter

TWU
TEXAS WOMAN'S UNIVERSITY
DENTON DALLAS HOUSTON

Office of Research
6700 Fannin Street
Houston, TX 77030-2343
713-794-2480 Fax 713-794-2488

September 24, 2008

Ms. Mary Parker
School of Physical Therapy - Sharon Olson Faculty Advis
6700 Fannin Street
Houston, TX 77030

Dear Ms. Parker:

Re: *"Differential Diagnosis in Pediatric Physical Therapy: A Primer of Mitochondrial Disorders and Other Complex Metabolic Pathologies"*

The above referenced study has been reviewed by the TWU Institutional Review Board (IRB) and was determined to be exempt from further review.

Any changes in the study must receive review and approval prior to implementation unless the change is necessary for the safety of subjects. In addition, you must inform the IRB of adverse events encountered during the study or of any new and significant information that may impact a research participant's safety or willingness to continue in your study.

Sincerely,

John D. Radcliffe

Dr. John Radcliffe, Chair
Institutional Review Board - Houston

TEXAS WOMAN'S UNIVERSITY
DENTON DALLAS HOUSTON
6700 Fannin, Houston, Texas 77030 713/794-2480

MEMORANDUM

TO: Dr. Sharon Olson
Mary E. Parker TWU #0011885

FROM: IRB

DATE: October 6, 2009

SUBJECT: Modification to currently approved proposal

Proposal Title: **"Differential Diagnosis in Pediatric Physical Therapy: A Primer of Mitochondrial Disorders and other Complex Metabolic Pathologies"**

New Proposal Title: "Differential Diagnosis in Children with Multisystem Involvement: Mitochondrial and Other Complex Metabolic Pathologies"

Your modifications, as per your attached memo of September 20, 2009 to the currently IRB approved protocols have been approved.

John D. Radcliffe

John Radcliffe, PhD.
Chairperson