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# Relationships among Processing Speed, Attention, and Biochemical Features in Children Identified with Mitochondrial Disease

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# RELATIONSHIPS AMONG PROCESSING SPEED, ATTENTION, AND BIOCHEMICAL FEATURES IN CHILDREN IDENTIFIED WITH MITOCHONDRIAL DISEASE

by

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Under the Direction of Robin D. Morris

## ABSTRACT

Mitochondrial Diseases (MD) are disorders of function in cellular oxidative phosphorylation caused by diverse nuclear DNA and mtDNA mutations and seen in 1/5,000 births. The purpose of this study was to examine relationships across medical indices, biochemical measures, and neurobehavioral functioning in children with MD. Findings from Western Blot, Native Gels, High Resolution Respirometry, and the Nijmegen diagnostic criteria were assessed in relation to children's processing speed and attention, based on the prediction that impaired functioning of proteins, complexes, and cellular respiration, that are critical in ATP production, will impact neurodevelopment and related neuropsychological processes in children with MD. Twenty-five children (ages 4-13) were administered subtests from the DAS-II and NEPSY-II. Results from multiple regression analyses suggest that processing speed and attention deficits may be markers of abnormal protein expression that interferes with the production of ATP in the oxidative phosphorylation process; implications for future research are presented.

INDEX WORDS: Mitochondrial disease, Oxidative phosphorylation, Children, Neuropsychological outcome, Biochemical features

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# CHAPTER 1

## INTRODUCTION

Mitochondrial Disease (MD), also referred to as Oxidative Phosphorylation (oxphos) Disease, is caused by abnormalities in the metabolic process by which an insufficient amount of adenosine triphosphate (ATP) is produced in the mitochondria (Shoffner, 1996). Because ATP is a stored chemical energy that is used for many cellular processes that require energy, such as biosynthesis, locomotion, or transportation of molecules across cell membranes, MD is usually characterized by dysfunction in organ systems, such as the brain and muscles, that have high dependency on the oxidative phosphorylation process for energy (Marcinek, 2004). Since a case of MD was first recognized in 1962, this disease is now reported to be the most common inborn error of metabolism with an estimated frequency of one in 5,000 live births (Thorburn, 2004). However, even this report is most likely an underestimate of the actual prevalence, as many patients with MD are never referred for evaluation and symptoms are often nonspecific and can be disguised as other diseases (Zeviani, Bertagnolio, & Uziel, 1996).

Several hundred variations of MD are classified primarily based on shared clinical presentations. The subtypes of this disease can be classified as 1) myopathic, which includes Chronic Progressive External Ophthalmoplegia (CPEO) and Kearns Sayre syndrome (KSS), or 2) encephalomyopathic, such as Mitochondrial Encephalomyopathy with Lactic Acidosis and Stroke-like Episodes (MELAS), Myoclonic Epilepsy with Ragged-red Fibers (MERRF), and Leigh Syndrome (LS). Symptoms associated with the muscle (myopathy), brain (encephalopathy), or both (encephalomyopathy) are largely attributable to the high dependency of the muscle and brain on the oxidative phosphorylation process for energy (Leonard & Schapira, 2000). For instance, the central nervous system findings include fluctuating

encephalopathy, seizures, stroke-like episodes, mental retardation, dementia, migraine, ataxia, and spasticity (Thorburn, 2004). Myopathic symptoms include hypotonia, exercise intolerance, cardiomyopathy, and muscle pain and weakness. Given that the development and function of all organ systems are dependent on energy, it is not surprising that MD can also cause ocular, vestibular, hepatic, cardiac, renal, gastrointestinal, endocrine, and hematological malfunctions.

A critical problem in doing research in MD is the difficulty quantifying the clinical manifestations in the patient population due to the large spectrum of symptoms presented. The diverse presentation of disease onset, severity, system involvement, and outcome makes diagnosing and predicting the course of the disease challenging (Chinnery et al., 2000). Despite the frequent clinical reports of brain abnormalities and cognitive deficits associated with MD, surprisingly only a few studies have evaluated the psychological and neuropsychological profiles of patients with MD. At this time, there is little known about which characteristics of the disease best predict cognition and general neurobehavioral functioning, or progression of the disease over time (Drummond, 1998; Finsterer, 2006a; Gire et al., 2002; Morava et al., 2006).

Since the discovery of MD about 50 years ago, many researchers have attempted to understand this disease, mostly through biochemical and genetic studies. Several genetic mutations have been identified that are associated with specific clinical profiles (e.g., MELAS, LS, LHON, and MERRF). However, these mutations are not necessarily predictive of their phenotypes and only serve to confirm a diagnosis that has been made through multiple methods of assessments. Furthermore, although abnormalities in certain brain regions have been linked with MD, there is little empirical evidence elucidating how the biochemical abnormalities of MD lead to the brain abnormalities that affect cognition in this population. However, based on maturational theories of brain development, and what is known about brain metabolic activity

from infancy to early childhood, it appears that subcortical and cerebellar areas in the brains, and related white matter connections, are more likely to be interrupted in early-onset MD. Given the neuropsychological sequelae observed in other subcortical and white matter diseases and related brain insults in infancy and early childhood, it is suggested that children with MD should also exhibit deficits in processing speed and attention, which could underlie the delays or deficits in higher order executive functions reported in some cases of MD.

**Diagnostic Criteria, Biochemistry, and Laboratorial Indices of MD**

A challenge in conducting research in MD is the difficulty of finding a relationship pattern among the large spectrum of clinical symptoms presented in this patient population. While some individuals identified with MD display a set of clinical features that fall into a defined clinical syndrome, many individuals do not. MD may only affect a single organ, such as the eye in Leber Hereditary Optic Neuropathy (LHON), or may involve multiple organ systems and present with a variety of neurological and myopathic features. In general, clinicians consider MD for diagnosis when at least two organ systems are affected that cannot be explained by other diseases (Shoffner, 2008).

Given the low diagnostic success rate of mutation analysis and lack of agreement on what are optimal biochemical assays and cut-off lab values, a few different screening scoring systems are used to aid in the diagnosis of patients suspected to have MD. One such diagnostic screening system is the Nijmegen Criteria for Mitochondrial Disease, which was developed by Morava and colleagues (2006) to compare children with established mtDNA or nDNA mutations with other children with non-mitochondrial multisystem disorders. Points are assigned based on 1) neuromuscular presentation (opthalmoplegia, facies myopathica, exercise intolerance, muscle weakness, rhabdomyolysis), 2) CNS presentations (developmental delays, loss of skills, stroke-

like episode, migraine, seizures, myoclonus, cortical blindness, pyramidal signs, extrapyramidal signs, brainstem involvement), 3) multisystem disease (hematology, GI tract, endocrine/growth, heart, kidney, vision, hearing, neuropathy, recurrent/familial), 4) metabolic and imaging studies (elevated lactate, L/P ratio, alanine, CSF lactate, CSF protein, CSF alanine, urinary TA excretion, ethylmalonic aciduria, stroke-like picture, Leigh syndrome), and 5) tissue morphology (ragged red/blue fibers, COX-negative fibers, SDH positive blood vessels, abnormal mitochondrial) (Morava et al., 2006). The specific criteria are provided in Appendix A.

Morava and colleagues (2006) found that all children with genetically established diagnoses of MD had a mitochondrial disease score above 6 (probable mitochondrial disorder) on the Nijmegen, whereas 73% of the children had a score above 8 (definite mitochondrial disorder) when the disease was confirmed through a muscle biopsy. In the non-mitochondrial multisystem disorder group, the score was significantly lower, and no patients reached a score comparable to a definite disorder. Although this scoring system was found to be insufficient for providing a definite diagnosis, the researchers suggested that it can, at least, screen out low-risk groups and prevent unnecessary muscle biopsy from being performed on children (Morava et al., 2006).

Other major clinical classification systems for MD include the Modified Walker Criteria for Mitochondrial Disease Diagnosis (Bernier et al., 2002), Nonaka Criteria for Mitochondrial Encephalomyopathy (Nonaka, 2002), and Wolfson Criteria for Mitochondrial Disease (Nissenkorn et al., 1999). Although the point assignment differs based on the approach, they all focus on identifying mitochondrial clinical phenotypes, Oxphos enzyme deficiency, abnormal mitochondrial ultrastructure, tissue morphology, neuromuscular manifestations, organ system involvement, and metabolic irregularities. Because there is so little consensus on how to

systematically diagnose MD, some medical professionals have opted to use a more traditional, individualized approach for clinically evaluating mitochondrial dysfunction. This process still involves taking family history of MD, physical and neurological examinations, routine and special laboratory tests, and histochemical and biochemical results of muscle biopsy for classic signs and patterns of the disease (Dimauro, Tay, & Mancuso, 2004).

As mentioned, it is usually necessary to do a muscle biopsy to analyze the enzymatic structure and function of the oxidative phosphorylation process, regardless of which clinical classification system or individualized approach is used to diagnose a patient with MD. Oxidative phosphorylation is the terminal process of cellular respiration during which electrons are transferred to molecular oxygen through a series of protein complexes located in the inner mitochondrial membrane (Leonard & Schapira, 2000). Complexes I through IV are responsible for establishing a proton gradient across the mitochondrial membrane. Abnormalities in the oxidative phosphorylation process can interfere with the metabolic pathways, such as glycolysis, pyruvate metabolism, the tricarboxylic acid cycle, protein catabolism, and fatty acid oxidation (Shoffner, 2008). Appendix B provides greater detail about the individual roles the different genes, proteins, and complexes play in the production of ATP in the mitochondria.

One laboratory tool that is commonly used for diagnosis is the Western Blot, which is a method used to assess the integrity of the Oxphos enzymes in a tissue sample (Capaldi, Murray, Byrne, Janes, & Marusich, 2004). Another method is the Natives Gels, which are separation methods with high resolution. These methods can be used to analyze the size, relative abundance, and subunit composition of multiprotein complexes (Swarmy et al., 2006). The Blue Native Gel is used best to visualize the Supercomplex and Complexes III to V, whereas the Clear Native Gel can be used for Complexes I and II. The combination of Blue Native and Clear

Native Gels is an effective method for detecting altered assembly of Oxphos complexes (Bart Devreese, 2002; Munnich & Rustin, 2001; Reif, Voos, & Rassow, 2001; Schagger, Cramer, & Vonjagow, 1994; Schägger & von Jagow, 1991; Shoffner, 2008). Additionally, in recent years, High Resolution Respirometry has been found to be critical for studying the function of oxidative phosphorylation as it provides information about the respiration rate of living cells (Sperl et al., 1997). High Resolution Respirometry is a sensitive electroanalytical method that allows oxygen consumption to be measured within living cells and isolated mitochondria (Barrientos, 2002; Hutter, Unterluggauer, Garedew, Jansen-Durr, & Gnaiger, 2006; Sperl et al., 1997). In this method, specific substrates and inhibitors of respiratory chain enzymes and ATP formation are used to evaluate the functions of individual complexes of oxidative phosphorylation.

No one laboratory technique has yet been shown to adequately diagnose MD. Today, a number of tools, such as the Western Blot, Native Gels, and, more recently, High Resolution Respirometry, are used simultaneously to reach a final diagnosis of this disease. Researchers and medical professionals continue to improve upon these techniques with the hope of creating a more simplified and consistent method of assessing MD. Because research today places greater emphasis on studying the biology and medical presentation of MD, only a handful of clinical studies have been conducted investigating the source of cognitive deficits in this disease. Given there are a number of reported cases in which neuropsychological deficits and psychiatric problems were the primary symptoms of MD, there is a clear need to study this field more aggressively to capture a fuller clinical profile.

**Central Nervous System Dysfunction in MD**

Most symptoms of MD are caused by deficient production of ATP in the mitochondria. The brain, as an organ that is heavily energy-dependent, is especially vulnerable to Oxphos dysfunction (Finsterer, 2006a). Moreover, because the brain has a high oxygen requirement, lower antioxidant enzymes compared to other organs, and high levels of unsaturated fatty acids, it is particularly more vulnerable to the free radicals that are produced by defective mitochondria (Nunomura et al., 2006). A number of neuroimaging studies have been conducted with MD patients investigating the impact of mitochondrial dysfunction on the brain; particular brain regions are implicated in certain subtypes of MD.

The most frequent presentation of mitochondrial dysfunction observed in neuroimaging studies of infants and young children with Leigh Syndrome (LS), a subtype of MD, is degeneration of the brainstem, cerebellum, and basal ganglia (Haas & Dietrich, 2004). One of the earliest case studies reported is that of a 2 year-old girl with brain lesions in the hypothalamus and brainstem and spongiform white matter degeneration (Leigh, 1951), who presented with central apnea, ptosis, and opthalmoplegia, and ataxia. Currently, the diagnosis of Leigh Syndrome requires neurological evidence of bilateral signal hyperintensities in the basal ganglia and brainstem (Dimauro et al., 2004).

Another subtype of MD that has been well-studied through neuroimaging research is MELAS. Stroke-like lesions in the posterior cerebral hemispheres, particularly the occipital lobes, are found to be distinctive in this subtype (Dimauro et al., 2004). For instance, a review of imaging studies of MELAS patients using computed tomography (CT) indicates decreased attenuation of one or both occipital lobes and calcification in the basal ganglia (Haas &Dietrich, 2004). A case study was described by Haas and Dietrich (2004) of a 19 year old man with

MELAS whose Axial CT scan revealed low attenuation in the left occipital lobe, calcification in the thalami, caudate, and lentiform nuclei bilaterally, and high signal intensity in the basal ganglia and thalamus. Furthermore, a case report of a 21 year-old woman with MELAS showed small necrotic foci in the cerebral cortex, amygdala, hippocampus, and cerebellum, and prominent white matter gliosis (Tsuchiya et al., 1999).

Kearns-Sayre syndrome (KSS), a mitochondrial myopathic disorder, results from mutations in mtDNA and is characterized by progressive limitation of eye movement, mild skeletal muscle weakness, short stature, heart block, hearing loss, diabetes, as well as mild cognitive impairments and infrequent seizures. Although KSS is categorized mainly as a myopathic condition, neuroimaging studies have revealed diffuse signal abnormalities of the central white matter and basal ganglia calcification in these patients (Dimauro et al., 2004). For example, in a 9-year-old girl with KSS, Axial Fluid Attenuated Inversion Recovery (FLAIR) images showed high signal intensity in the globus pallidus, dentate nuclei, tectum, and peripheral cerebral white matter (Haas & Dietrich, 2004). Furthermore, Chu and colleagues (1999) presented brain MRI findings on four patients with KSS. Two patients were found to have normal MRI, whereas the other two patients had high-signal lesions bilaterally in the subcortical white matter, thalamus, and brainstem, and atrophy in the cerebellum.

Although central nervous system involvement is a commonly reported feature of MD, only a few studies have attempted to examine neuropsychological features in this population group. Kartsounis and colleagues (1992) were one of the first to study neuropsychological features of MD, administering tests of general intellectual ability, memory, language, and perception to 36 patients with mitochondrial myopathy and encephalomyopathy. Patients were determined to have encaphalopathic features if abnormalities were detected in EEG or CT scans.

Of the 22 patients with encephalomyopathy, 13 patients (59%) displayed mild to severe intellectual disability. Focal deficits were also found in language (50%), memory (64%), and perception (55%). Among the 14 myopathic patients, fewer showed impairment in intellectual ability (14%), language (7%), memory (14%), and perception (14%).

In a similar study, Turconi and colleagues (1999) found developmental delay in 68% of patients (N=16) with mitochondrial encephalomyopathies. Even the 20% of patients who presented with no encephalopathy exhibited mild cognitive delays, including compromised visuospatial abilities and short-term memory deficits. Moreover, performance IQ was found to be significantly lower than verbal IQ in children with mitochondrial disorders. No SPECT evidence of greater impairment of the right hemisphere, however, emerged as had been expected given the observed pattern of non-verbal deficits (Turconi et al., 1999).

In a chart review of 42 children with definite (n=37) and suspected (n=42) MD, Nissenkorn and colleagues (2000) found that 18 children (43%) had normal intelligence, while 24 children (57%) had mental retardation or developmental delay at the time of diagnosis. Over the course of the disease, 17 children (40%) had a static course, while others had acute intermittent encephalopathy (21%), slowly progressive deterioration (29%), or acute deterioration leading to death or vegetative state (10%).

In addition to the neurocognitive deficits observed in MD populations, psychiatric problems have also been commonly reported in MD. These include depression, hallucinations, personality changes, and psychotic episodes (Berio & Piazzi, 2002; Gardner et al., 2003; Kaller et al., 2003). In Chronic Progressive External Ophthalmoplegia (CPEO), bipolar affective disorders were observed to occur (Finsterer, 2006b). Furthermore, a pattern of loss of orientation, paranoid-hallucinatory psychosis, social withdrawal, and odd behavior was evident

in a case study of a male patient with MELAS (Sartor, Loose, Tucha, Klein, & Lange, 2002). Moreover, a growing number of research studies are finding an association between autism spectrum disorders (ASD) and disorders of mitochondrial oxidative phosphorylation (Poling, Frye, Shoffner, & Zimmerman, 2006; Weissman et al., 2008). Other deficiencies include impaired consciousness, decreased alertness, concentration, orientation, and reasoning. In particular, disturbed consciousness was most frequently found in Leigh syndrome and Kearns-Sayre syndrome (Berio & Piazzi, 2002; Crimi et al., 2004).

The wide range of cognitive deficits and psychiatric issues reported in the literature mirrors the heterogeneous presentation of medical problems in the MD population. Although no clear pattern of focal cognitive deficits has yet to emerge for MD patients, the studies to date seem to indicate that at least half of the patients diagnosed with MD are cognitively impaired and that perhaps non-verbal abilities are more heavily impacted than verbal abilities. Although a few studies have investigated neuropsychological aspects of MD, no study to date has attempted to directly quantify relationships between the biochemical and cognitive aspects of mitochondrial dysfunction.

**Early Metabolic Dysfunction and Subsequent Disruption of Neurodevelopment: Implication for deficits in Attention and Processing Speed**

Based on neuroimaging studies, it appears that subcortical and posterior regions of the brain, such as the occipital lobe, cerebellum, brainstem, basal ganglia, and white matter, are more frequently impacted by Oxphos dysfunction in MD. This neuroanatomic profile is consistent with brain regions that are formed in the early stages of neurodevelopment and during periods of high brain metabolic activity.

In the literature of neurodevelopment, it is well supported that subcortical areas mature first, followed by posterior cortical areas, and then anterior regions (Anderson, Northam, Hendy, & Wrennall, 2001). Extensive neuroanatomical studies of cortical development in infants also suggest an inside-out pattern in the neuroanatomical growth of the cortex, specifically in regards to the extent of dendrites, dendritic trees, and myelination. For instance, initial myelination is known to begin at birth in the pons and cerebellar peduncles. The most rapid changes in white matter also occur during the first two years of human life (Johnson, 2004).

Neuroimaging studies have shown that the typical immature brain has considerably higher energy consumption than the adult brain (Skoyles, 2008), particularly in the brainstem, cerebellum, and basal ganglia (Chugani, Phelps, & Mazziotta, 1987). The factors contributing to the increased subcortical metabolic activity in infancy to early childhood include a greater number of synapses in the immature brain and the inefficiency of their axons prior to full myelination. If brain metabolic activity is impaired, it is expected that it will interfere with the proper development of brain regions associated with high energy consumption.

Metabolism, which refers to the biochemical process of energy use, is impaired with dysfunction in the oxidative phosphorylation process. Brain metabolic abnormalities, as measured by decrease in N-acetylaspartate (NAA) and accumulation of lactate, are often detected in MD using proton MR spectroscopy (Detre et al., 1991; Mathews et al., 1993). In a study conducted by Bianchi and colleagues (2003), significant reduction in choline and NAA was observed when MR spectroscopy was positioned in the axial images of deep cerebellar hemisphere, white matter, and the parieto-occipital cortex of either hemispheres of 15 patients with MD. As expected, metabolic dysfunction was detected within the subcortical and posterior brain regions, and in the related white matter.

Clinical studies have shown that disruption in early neurodevelopment, either caused by diffuse disease or brain insults, often lead to cognitive deficits subserved by subcortical brain regions, such as processing speed and attention. For instance, children who have interrupted oxygen intake during sleep due to obstructive sleep apnea syndrome demonstrated increased compensatory cerebral blood flow and slightly impaired processing speed and visual attention (Hill et al., 2006). Furthermore, such neurobehavioral impairments were frequently reported following treatment of childhood cancers with cranial irradiation (CRT). Findings from studies of children treated with CRT prior to age five years showed greatest dysfunction in information-processing speed, a skill associated with subcortical white matter (Anderson, Godber, Anderson, Smibert, & Ekert, 1995). A related study investigated attention and information processing skills in children treated with both CRT and chemotherapy. Anderson and colleagues (2004) found that these children showed deficits in processing speed for complex tasks and selective and shifting attention compared to same age peers (Anderson, Godber, Smibert, Weiskop, & Ekert, 2004) . They suggested that these skills may be impaired due to the vulnerability of cerebral white matter in early childhood.

The development of white matter and gradual myelination and its association with increasing processing speed is well-established in the literature of neurodevelopment. For instance, in a longitudinal study by Kail and Ferrer (2007), a sample of children and adolescents (N= 503) was tested twice over a span of two years using two psychometric measures of processing speed: Visual Matching and Cross-Out. In another sample, children and adolescents (N= 277) were tested four times, every 6 months, on Cross-Out. They examined age-related changes in performance on both tasks and found that processing speed became increasingly faster during childhood, but the rate at which the speed increased slowed in adolescence and

corresponded with changes in brain size and body mass (Kail & Ferrer, 2007). Furthermore, the role of subcortical brain regions in processing speed has also been demonstrated in a number of studies. For example, O'Brien and colleagues (2002) examined the impact of subcortical grey and white matter lesions on cognitive function in a large sample of non-demented elderly adults and found that the extent of subcortical lesions in the caudate and thalamus was significantly correlated with impaired performance on tests of processing speed.

Within the complex set of cortical networks that are involved in attentional processes, particular subcortical brain regions are known to play a critical role. The reticular activating system (RAS) within the brainstem, for instance, is identified as important for arousal and maintaining the alert state in the cerebral cortex. The RAS also plays a key function in filtering out repetitive stimuli and preventing sensory overload (Steriade, 1996). In a PET study, activation of the midbrain reticular formation and thalamic intralaminar nuclei was observed when participants went from a relaxed awake state to an attention-demanding reaction-time task, confirming the importance of RAS in arousal and vigilance (Kinomura, Larson, Gulyas, & Roland, 1996). Moreover, basal ganglia are implicated in attention and often studied in the context of children with ADHD. For instance, Sobel and colleagues (2010) examined the morphologic features of the basal ganglia nuclei in children with ADHD and found significant inward deformations in the caudate, putamen, and globus pallidus. The more prominent inward deformations in the nuclei were associated with the severity of ADHD symptoms.

Neuroimaging studies have shown that the subcortical, posterior, and white matter regions of the brain are often impact by mitochondrial dysfunction. These areas are consistent with the brain regions formed in the early stages of neurodevelopment and during periods of high brain metabolic activity. Given the neuropsychological sequelae observed in other subcortical

and white matter diseases early childhood, it is suggested that children with MD should also exhibit deficits in processing speed and attention.

**Summary**

This study focused on the associations between cognitive functioning and the protein chemistry and structural functioning of the oxidative phosphorylation system in the mitochondria, primarily the complexes that are involved in the production of ATP. The main goal of this study was to investigate processing speed and attentional correlates of biochemical and medical indices in children with MD. Given that MD is caused by the insufficient production of ATP in the Oxphos system, it is possible that data derived using laboratory techniques that directly examine the biochemical structure and function of proteins and complexes of the mitochondrial membrane relate to cognitive and functional abilities.

The laboratory tools and medical data that were used in this study to identify relationships between medical indices of the disease and cognitive measures were the Western Blot, Native Gels, High Resolution Respirometry, and the Nijmegen diagnostic criteria. The findings from these laboratory methods were investigated in relation to measures of processing speed and attention based on the prediction that decreased metabolic activity will interfere with early neurodevelopment and impact related neuropsychological processes.

# CHAPTER 2

# METHODS

## General Overview

All participants for this IRB approved study were recruited from Medical Neurogenetics (MNG). Dr. John Shoffner was the primary diagnostician for this sample, a co-investigator on this project, and a GSU adjunct faculty member in Biology. He is Board Certified in Neurology, Biochemical Genetics, and Molecular Genetics. All medical, laboratory, genetic, and related information about each patient were made available from MNG upon parental consent.

For the study, children (ages 4-13) who received a diagnosis of MD from Dr. Shoffner were recruited. If parents agreed to enroll their children in the study, they were invited to come in for an evaluation which took place either at 1) the Urban Life Building Psychology Clinic at Georgia State University, or at 2) the MNG clinic. A neurobehavioral assessment was completed under Dr. Robin Morris' supervision. The assessment consisted of a clinical interview and clinical and psychological profile questionnaires with the parents, neuropsychological testing with the child, and a feedback session with the parents. The full evaluation took between 3 and 4 hours, with breaks as needed based on the age and needs of the children to reduce fatigue or frustration. After an evaluation report was completed, parents were invited to come back for a feedback session.

## Procedures

A recruitment letter and consent form were either mailed or handed to parents of MD children meeting the study criteria by the staff at MNG. The criteria for participation in the study included: 1) clinical diagnosis of MD, 2) age between 4 to 13 years, 3) English primary

language, and 4) laboratory work-up from MNG. The following exclusionary criteria were used to screen subjects recruited from the initial study based on medical history and parent report: 1) severe, uncorrected hearing or visual impairment and 2) history of significant head injury.

Families interested in participating in the study were asked to mail a signed copy of the consent form to the MNG clinic. Once the consent forms were received, the parents were contacted by phone to answer questions and schedule the parent interview and child testing sessions. The children were asked to sign the assent form upon arrival for the testing session. However, if the child was too cognitively impaired to understand the purpose of the study, only the legal guardian's consent was required. In all situations, an attempt was made to provide a verbal explanation of the research study that was tailored to the participant's level of understanding. For purposes of confidentiality, participant was assigned a unique identifying code number for his or her study records which was separated from his or her name or any other information that could be identifying. All records were stored in a locked office in a locked cabinet.

**Participants**

A total of 25 children with MD, aged 4 through 13 years, participated in this study. Although 38 children were consented into the study, three children were excluded because they failed to meet the criteria for the study and six participants were not able to be scheduled for participation. One participant died prior to the scheduled parent interview and testing. Three participants failed to come back for a testing session after parent interviews were completed due to scheduling difficulties. Although all 25 participants had a basic laboratory work-up done at MNG as required for participation in this study, only 18 western blots, 11 native gels, 3 fibroblast high resolution respirometry, and 9 skeletal muscle high resolution respirometry were

available for this study due to pending laboratory data analysis or lack of sufficient tissue samples obtained. Participants in this sample were evenly distributed between males and females, and had a mean age of 8.8 years at the time of testing. Of the 25 children, 24 were Caucasian and one was Asian. Demographics of the final sample are described in Table 1.

**Medical and Laboratory Measures**

Medical and laboratory results were made available through the patient database at MNG. Only the results from the most recent assessments completed were used for this study.

*Nijmegen Diagnostic Criteria*

The Nijmegen mitochondrial disease criteria form (see Appendix A) was completed for all participants based on their medical records. The diagnostic scores for Clinical, Biochemical, and Genetic Criteria were used in the study as overall medical indices of MD.

*Western Blot (Oxidative Phosphorylation Subunit Proteins)*

Skeletal muscle was obtained from subjects' quadriceps through a muscle biopsy. The testing procedure involved isolating cellular proteins of interest, separating these proteins through gel electrophoresis, transferring them to ploy vinylidene fluoride membrane, and later visualizing them by using antibodies specific to the nuclear DNA and mtDNA that code for specific oxidative phosphorylation subunits. Results of the protein analysis were expressed as the percentage of GAPDH, a constitutively expressed protein. For this study, any of the 5 subunits was deemed abnormal if the subunit/GAPDH ratio was one standard deviation below the mean of the control reference.

*Blue and Clear Natives Gels*

In the Blue Native Gel, intact oxidative phosphorylation supercomplexes and monomeric oxidative phsophorylation enzymes were separated electrophoretically using a 3%-12% gradient

Bis-TRis ployacrylamide gel. The Blue Native Gel was used to qualitatively assess the formation of the Supercomplex (comprised of Complex I, III, and IV) and the monomeric oxphos Complexes III through V. Monomeric oxphos Complexes I and II were assessed with the Clear Native Gel. The presence of abnormalities in these complexes and supercomplex were determined clinically by Dr. Shoffner prior to their use in this study.

*High Resolution Respirometry for Fibroblasts*

Fibroblast respiration was assessed in the presence of physiological substrates. Five ratio measures were developed: the Uncoupling Ratio, defined as $Cr_u$ / Cr, which expresses the respiratory reserve capacity; the Net Routine Flux Control Ratio (Cr/ $Cr_u$), which assesses how closely routine respiration operates to the respiratory capacity of oxidative phosphorylation; the Respiratory Control Ratio (RCR), defined as $Cr_u$ / $Cr_{o,}$ which assesses the uncoupling and oxphos dysfunction; the Leak Flux Control Ratio ($Cr_o$ / $Cr_u$ ), which serves to measure the inverse of RCR and represent proton leak with inhibition of ADP phosphorylation by oligomycin; and Phosphorylation Respiratory Control Ratio (RCRp), defined as (Cr-Cro)/Cru (or 1/UCR-1/RCR, which expresses phosphorylation-related respiration as a function of respiratory capacity. All these ratios were considered abnormal if values were one standard deviation below or above the mean as determined by each ratio's function.

*High Resolution Respirometry for Skeletal Muscles*

For this procedure, live muscle saponin was permeabilized for analysis. This protocol assessed functioning of intact coupled mitochondria as well as the maximum respiratory capacity of the mitochondria in an uncoupled state. Based on MNG's method, the analysis of skeletal muscle respiration was conducted similarly to the fibroblast's described above in that it evaluated the Uncoupling Ratio, Net Routine Flux Control Ratio, Respiratory Control Ratio, and

the Leak Flux Control Ratio. The fifth ratio that was assessed was oxygen consumption when ADP was added to a medium of glutamate and malate as opposed to when it was not. Lastly, the succinate-suplemented media ratio was used to evaluate oxygen consumption with the addition of succinate. Again, abnormal values for these ratios were defined as one standard deviation above or below the mean.

**Processing Speed and Attention Measures**

For this study, it was predicted that processing speed and attention would be primarily impacted by the biochemical abnormalities in the mitochondria of MD patients, which were presumed to have the greatest impact on subcortical and white matter structures in the developing brain. The tests and questionnaires in this study were mainly selected for the cognitive areas they measure, appropriate age ranges they provide, length of testing, and reliability and validity findings. The primary measures were chosen from the following tests.

*Processing Speed*

Two subtests from the *Differential Ability Scales–Second Edition* (DAS-II), Speed of Information Processing and Rapid Naming, were used for this study. The Speed of Information Processing (SIP) subtest measures speed in performing quantitative comparisons; specifically, in each row of items, the child must identify and mark the one circle that has the greatest number of squares. The number of squares within each circle never exceeds four. The basic task of SIP is relatively easy, and almost all children can solve the items correctly. The variation in performance lies in the time the child takes to complete the task (Elliot, 2007). Several studies support the interpretation of the SIP subtest as a measure of general cognitive speed, including a study conducted by Buckhalt and Jensen (1989) using a range of reaction-time measures. In addition, the Rapid Naming (RN) subtest measures speed of lexical access and retrieval. There

are three items within this subtest; in all items, the child names array of colors or pictures as quickly as possible without making mistakes. This test was developed based on the concept of rapid automatized naming (Elliot, 2007). The SIP and RN measures correlate more highly with each other than with any other subtests in the DAS-II.

The DAS-II consists of two age-based versions: the Early Years (ages 2:6-8:11) and School Age (5:0 to 16:11). All participants, excluding one 4- year-9-month old, were given the School Age version of the test for consistency. The results from the 4 year old participant were not included as part of the research data. The normative sample for the DAS-II includes 3,475 children and adolescents who are representative of the US population census for race, gender, community size, and parent education. The reliability coefficient is high ( .95) for the School Age level on the DAS-II. Test-retest reliability scores are very stable for the General Conceptual Ability score and cluster scores, ranging from .79 to .94. Correlations with the McCarthy Scales of Children's Ability (MSCA) are the highest for the Verbal, Perceptual-Performance, or Qualitative scales. For the school age level, all of the DAS composites correlated highly with the WISC-R Full Scale IQ, Verbal IQ for 8 to 10 year olds, and Verbal IQ for 14 to 15 year olds.

*Attention*

The Auditory Attention and Response Set subtest of the NEPSY-II was administered to measure attention. This subtest has two parts; Auditory Attention is designed to assess selective auditory attention and the ability to sustain it (vigilance), whereas Response Set is designed to assess the ability to shift and maintain a new and complex set involving both inhibition of previously learned responses and correctly responding to matching or contrasting stimuli. Only the Auditory Attention component of the test was utilized to measure attention.

The NEPSY-II is a test designed to assess neuropsychological development in children ages 3-16. The 4-year-old participant was also administered the same tests using 5-year-old norms; again, the results were excluded from the research data. The NEPSY-II normative data were collected from 2005 to 2006 using a sample that was stratified on key demographic variables (i.e., age, sex, race/ethnicity, parent education level, and geographic region) according to the October 2003 U.S. census data.

In general, the various reliability measures indicate adequate to good reliability for the different types of scores in NEPSY-II (Korkman & Kemp, 2007). The mean reliability across the age groups for most subtests is between .70 and .90. The test-retest correlations of the different age groups vary between .55 and .81. In addition, correlations between the NEPSY-II and D-KEFS subtests assessing similar processes provide support for the convergent validity of the NEPSY-II. The attention measure of NEPSY-II also correlates with the inattention error score (-.76), visual attention subtest (-.40), and statue subtest (-.59) of the Auditory Continuous Performance Test.

Attention was also assessed using the parent rating of Attention Problems from the *Behavior Assessment System for Children 2nd Edition* (BASC-II). The BASC-II is a diagnostic tool designed to evaluate the behavior of children and young adults, ages two to 25 years (Reynolds & Kamphaus, 2004), based on diagnostic criteria from the *DSM-IV* and *DSM-IV-TR*, as well as other behavioral instruments. The BASC-II is a multi-method tool, since it measures numerous behavioral and personality characteristics.

The BASC-II was normed using two populations: 1) a general population sample of American children and adolescents from various settings, and 2) a clinical norm sample of American children and adolescents (ages 4-18) who were diagnosed with emotional, behavioral,

or physical problems. The general population sample had a total of 4,800 Parent Rating Scales (PRS) reports. In terms of age groups, there were 2,250 pre-schoolers aged 2-5 years, 3,600 children aged 6-11, and 5,500 adolescents (ages 12-18). In terms of gender and ethnic representation, the general sample was very close to U.S. population estimates. The clinical sample had a total of 5,281 reports across the TRS, PRS and SRP scales. The sample comprised 317 pre-school aged (2-5) children, 673 children ages 6-11, and 789 adolescents ages 12-18. The children in this sample had a variety of diagnoses, including specific learning disabilities, speech/language impairments, emotional and behavioral disturbances, hearing impairment and ADD/ADHD.

An analysis of internal consistency yields coefficient alpha reliabilities generally in the .90s for the composite scales, and reliabilities generally in the .80s for individual scales across all forms for both the general sample and the clinical sample (Reynolds & Kamphaus, 2004). When samples of individuals distributed across the three age groups were retested with the BASC-II one to eight weeks after the first administration, the test-retest reliabilities resulted in average correlations in .80s for composite scores and between the .70s and .80s for individual scales across all age groups. For validity measures, the parent rating scale was compared with other behavioral measures such as the ASEBA Child Behavior Checklist for Ages 1-5, the Conners' Parent Rating Scale-Revised, the Behavior Rating Inventory of Executive Functioning (BRIEF), and the BASC-I. Generally, the BASC-II correlated in the .70s and .80s with the first three scales, and in the .90s with the previous BASC-I.

**Supplementary Cognitive Measures**

In addition to the primary processing speed and attention measures that were collected, this study also conducted a more thorough clinical evaluation to ensure that other variables

would be available to contextualize the results. Appendix C provides a list of all the tests that were administered. The tests were administered in the same order for each participant.

*Verbal Functioning*

The Verbal Cluster of the DAS-II was administered; this cluster comprises the Word Definition and Verbal Similarities subtests. The Word Definition subtest measures the ability of the child to define words presented orally by the examiner. As well as requiring access to a previously acquired store of words together with their meanings, Word Definition measures expressive language ability. The Verbal Similarities subtest assesses the ability of the child to identify the common concept linking three words. This subtest is a measure of a verbal development and verbal reasoning (Elliot, 2007).

The Word Generation subtest from the NEPSY-II was also administered. This subtest is designed to assess verbal productivity through the ability to generate words within semantic and initial letter categories (Korkman & Kemp, 2007). The child is given a semantic or initial letter category and asked to produce as many words as possible in 60 seconds.

*Visual-Spatial-Perceptual-Motor Functioning:*

Two subtests from the DAS-II were administered to measure perceptual reasoning: Sequential and Quantitative Reasoning (SQR) and Pattern Construction (PC). The SQR subtest measures the ability to perceive sequential patterns in geometric figures or common rules in numerical relationships. The problems in this subtest are presented visually, with little verbal instruction. Moreover, the PC subtest assesses spatial ability in children by requiring them to copy patterns with wooden blocks, foam squares, and blocks with different patterned and colored sides (Elliot, 2007). The Arrows subtest from the NEPSY-II was also administered to assess

ability to judge line orientation. On this task, the participant was required to look an array of arrows arranged around a target and indicates the arrow that points to the center of the target.

Two sensorimotor tasks were administered from the NEPSY-II: Fingertip Tapping and Imitating Hand Position. Fingertip Tapping has two parts: the first part is designed to assess the child's finger dexterity and motor speed, whereas the second part is used to assess rapid motor programming. The participant was asked to copy a series of finger motions demonstrated by the examiner as quickly as possible. Imitating Hand Position is designed to assess the ability to imitate hand and finger positions. On this task, the participant was required imitate various hand positions demonstrated by the examiner (Korkman & Kemp, 2007).

*Memory Functioning*

Two measures from the DAS-II, Recall of Digits Backward (RDB) and Recall of Sequential Order (RSO), were administered to measure working memory: the ability to process information that is being held in verbal short-term memory. On the RDB subtest, participants were asked to recall in reverse order a list of numbers presented orally by the examiner. In contrast to forward-digit recall tasks (a basic short-term memory process), reverse-digit recall requires deeper processing of the stimulus because the child must be able to both remember and manipulate the information (Elliot, 2007). On the RSO subtest, participants were required to re-sequence an orally-presented list of body parts, arranging the parts from highest to lowest. The early, easier items were done with the aid of a drawing of a human figure, then the picture was removed, requiring the more difficult items to be performed using mental representation alone.

Two memory and learning tasks were also selected from the NEPSY-II: List Memory and Memory for Faces. List Memory was administered to assess verbal learning and memory, rate of learning, and the role of interference in recall for verbal material. On this task, participants were

required to read a list of words several times, recalling them after each presentation. A delayed task assessed long-term memory for words. In addition, the Memory for Faces subtest was administered to assess encoding of facial features, as well as face discrimination and recognition. On this task, participants looked at a series of faces; they were then shown three photographs at a time from which they were required to select a face they had previously seen. Again, a delayed task was given to assess long-term memory for faces.

*Executive Functioning*

Three subtests were administered to assess executive functioning: NEPSY Auditory Attention Response Set, Design Fluency, and Inhibition. The Response Set component of the Auditory Attention subtest is designed to assess the ability to shift and maintain a complex set involving both inhibition of previously learned responses and the ability to correctly respond to matching or contrasting stimuli. Design fluency is a task designed to assess the behavioral productivity in the ability to generate unique designs by connecting up to five dots, presented in two arrays: structured and random. The participants were required to draw as many designs as they could on each array within a specified time limit. Lastly, the Inhibition subtest was administered to assess the ability to inhibit automatic responses in favor of novel response and the ability to switch between response types. On this task, participants looked at a series of black and white shapes or arrows and named either the shape or direction or an alternate response, depending on the color of the shape or arrow.

*Social Perception*

This subtest is designed to assess the ability to recognize affect (happy, sad, anger, fear, disgust, and neutral) from photographs of children's faces in four different tasks. On one task, the participants are simply required to state whether or not two photographs depict faces with the

same affect. On a second task, he or she selects two photographs of faces with the same affect from three to four photographs. On a third task, the child selects one of the four faces that depict the same affect as a face at the top of the page. Finally, the child is briefly shown a face, and from memory, selects two photographs that depict the same affect as the face previously shown (Korkman & Kemp, 2007).

*Behavioral and Adaptive Functioning*

Parents were also asked to rate their children's behavioral and adaptive functioning using the BASC-II and the Vineland Adaptive Behavior Scales-II. In addition to Attention Problems, other areas rated on the BASC-II include Externalizing Problems (Hyperactivity, Aggression, and Conduct Problems), Internalizing Problems (Anxiety, Depression, and Somatization), Atypicality, and Withdrawal. Adaptive Skills are also rated in areas of Adaptability, Social Skills, Leadership, Activities of Daily Living, and Functional Communication.

The Vineland-II Parent/Caregiver version of the structured interview was also conducted with the parents. This measure took between 30 minutes to an hour to complete. This structured interview was designed to measure four domains of adaptive behavior: Communication, Daily Living Skills, Socialization, Motor Skills. Maladaptive behavior (Internalizing and Externalizing Problems) was also assessed. Standardization sampling for the Vineland-II follows the 1980 census data and includes 3,000 subjects from birth through 18 years and 11 months equally divided by sex (Sparrow, Cicchetti, & Balla, 2005).

In terms of reliability, split-half and test-retest reliability coefficients for the Composite scores are good, ranging from median values of .83 for the Motor Skills domain to .94 for the Composite. Inter-rater coefficients, however, are somewhat lower for the same measures (.62 to

.78). Selected standardization subgroups were compared with the original Vineland, K-ABC, and PPVT-R to measure validity. These measures exhibited low to moderate correlations, with generally higher coefficients when the comparisons were made on subjects with handicapping conditions.

*Academic Achievement*

Wechsler Individual Achievement Test-Second Edition (WIAT-II) was used to screen the participants' level of academic achievement (PsychCorp, 2005). Only two subtests from the WIAT-II were administered for the purposes of this study: Word Reading and Numerical Operations.

The WIAT was standardized on 5,586 individuals with two standardization samples drawn for PreK-12 (ages 4-19) and for the college-adult population. Both standardization samples were stratified on the basis of grade, age, sex, race-ethnicity, geographic region, and parent education level, based on the data from the 1998 Bureau of the Census. Internal consistency reliability estimates of the WIAT-II subtests are very high for Word Reading (.97) and Numerical Operations (.91). In the school-aged sample, test-retest correlations for the subtests are consistently above .85 and test-retest correlations for the Composite scores were above .90. In terms of validity, the corresponding subtests of the WIAT and the WIAT-II were strongly correlated (above .80) in the school-aged sample for those subtests with minimal content changes.

**Data Analysis**

Preliminary analyses were conducted to ensure no violation of the assumption of normality, linearity, multicollinearity, homoscedasticity, and independence of residuals. The Kolmogorov-Smirnov test of normality was run for all variables to determine normal

distribution. For normally-distributed continuous variables, two-tailed Pearson's correlations and independent sample t-tests were conducted. Spearman's two-tailed correlations were performed for variables with non-normal distributions. Except for the subdomains of the Vineland that were analyzed using the Vineland-scaled score, all other t-scores, scaled scores, and standard score were converted to z-scores so that most of the data would be on the same quantitative scale to facilitate interpretation of the results.

The primary hypotheses of this study explored associations among processing speed, attention, biochemical abnormalities, and medical indices using multiple regression analysis, independent sample t-tests, and correlations. Separate regression analyses using measures of processing speed and attention were conducted to predict the total number of abnormalities detected through the western blot, native gels, and high resolution respirometry techniques, as well as the Nijmegen clinical and biochemical indices. The DAS-II Processing Speed Cluster, which is a composite score for DAS-II Speed of Information Processing and DAS-II Rapid Naming, was consistently used as a predictor variable in the regression analyses. The BASC-II Attention Problems and NEPSY-II Auditory Attention Total Correct were used in separate regression analyses as a predictor variable in conjunction with the DAS-II Processing Speed Cluster. Collinearity diagnostics (Tolerance and Variance Inflation Factor) between the predictor variables were found to be within acceptable ranges suggesting no multicollinearity.

In addition to the primary measures, correlation analyses and t-tests were performed across select cognitive measures and all standardized biochemical measures to evaluate the potential for other associations outside of the study's primary hypotheses. As in the tests of the primary hypotheses, participants were assigned to an abnormal group (normal vs. abnormal) if

their biochemical z-scores were below or equal to -1. Pearson's correlation was performed on variables that were normally distributed. Otherwise, Spearman's correlation was performed.

On the Nijmegen, the relationships across the main criteria domains (Total Clinical and Total Biochemical), Clinical subdomains (Neuromuscular Manifestations, Central Nervous System and other Organ Involvement, Metabolic and Imaging studies, and Tissue Morphology), and all standardized cognitive measures were investigated through correlation analyses. T-tests were performed to evaluate relationships between the overall clinical, biochemical, and genetic criteria and all cognitive measures. Two groups ("Highly Probable/Probable" and "Possible/Unlikely") were formed for both the Clinical and Biochemical criteria.

Table 1 *Demographic Information (N=25)*

| | *% (n)* |
|---|---|
| Sex | |
| Female | 48 (12) |
| Male | 52 (13) |
| Race | |
| Caucasian | 96 (24) |
| Asian | 4 (1) |
| Handedness | |
| Right | 68 (17) |
| Left | 20 (5) |
| Unknown | 12 (3) |
| Years of Education | |
| None | 4 (1) |
| Pre-kindergarten | 12 (3) |
| Kindergarten | 16 (4) |
| 1st grade | 0 (0) |
| 2nd grade | 20 (5) |
| 3rd grade | 16 (4) |
| 4th grade | 16 (4) |
| 5th grade | 4 (1) |
| 6th grade | 8 (2) |
| 7th grade | 4 (1) |

# CHAPTER 3

# SAMPLE DESCRIPTION

Given the uniqueness of this sample and the complex nature of their clinical profile, a description of the participants' general medical history, laboratory results, and cognitive functioning is provided.

## Medical Review and History

Medical records and parent interviews revealed a history of developmental, behavioral, and psychological problems in a number of participants. Problems during pregnancy and delivery were reported in 40% (n=10) and 44% (n=11) of the participants, respectively. Developmental delays were reported in 72% (n=18) of the participants. Previous and concurrent diagnoses include bipolar disorder (8%, n=2), anxiety (16%, n=4), attentional disorders (12%, n=3), communication disorder (4%, n=1), learning disorder (8%, n=2), autism/aspergers (28%, n=7), obsessive compulsive disorder (12%, n=3), cerebral palsy (8%, n=2), Rett syndrome (4%, n=1), and Gerstmann syndrome (4%, n=1). History of seizures was reported in 32% (n=8) of the participants. Frequently reported medical concerns include fatigue (76%, n=19), motor/muscle problems (76%, n=19), gastrointestinal and eating problems (40%, n=10), and sensitivity to heat and cold (36%, n=9).

### *Complex Abnormalities: Western Blot and Native Gels*

The most commonly diagnosed complex type among the participants was Complex I (68%), followed by Complexes III (36%) and V (20%) as shown in Table 2. This data includes complexes that are part of multicomplex (combination type) diagnoses. Notably, none of the participants were formally diagnosed with Complex II defect, even though abnormal Complex II proteins were detected through the Western Blot in 8 participants as shown in Table 3. This may

be because Complex II abnormalities were not confirmed with the native gels (Table 4). Consistent with diagnoses given to participants, the most commonly detected protein abnormalities on the Western Blot were from Complex I and III, which were present in 60% of the participants for both complex types. The Complex I abnormalities were also the most frequently identified (63.6%) on the Native Gels. In contrast, only 9.1% of the participants were assessed to have abnormal Type III complexes using this method. On the Western Blot, protein abnormalities related to Complexes IV and V were detected in 20% and 4% of the participants, respectively. Moreover, abnormalities in Complexes IV and V were identified in only 9.1% of the participants using the Native Gels.

*Oxygen Consumption: High Resolution Respirometry*

High Resolution Respirometry results were collected from 10 participants to measure oxygen consumption within the living cells. Descriptive statistics are provided in Table 5. When findings from skeletal muscles (n=9) and fibroblasts (n=3) were combined, abnormal Uncoupling and Net Routine Flux Control ratios were found in 60% of the participants, whereas abnormal Respiratory Control and Leak Flux Control ratios were found in only 20%. In addition, 66.7% of participants presented with abnormal ratios of oxygen consumption in the skeletal muscle cells when ADP was added to a glutamate and malate medium and when medium was supplemented with succinate.

*Nijmegen Diagnostic Criteria*

Among the three Nijmegen criteria scores, the Biochemical Criteria was found to be most affected with 60.9% of the participants receiving either a Highly Probable or Probable score. Genetic mutations were identified in less than half of the participants (45%). Notably, only 32%

of the participants met clinical criteria for Highly Probable or Probable mitochondrial disease. Table 6 summarizes the findings.

**Cognitive Measures**

On average, participants performed at least 1 standard deviation below the expected mean across all cognitive subtests, excluding NEPSY-II Word Generation-Semantic, NEPSY-II Memory for Faces Delayed, and WIAT-II Word Reading. The mean scores on the selected cognitive measures ranged from borderline (z-scores from -2.00 to -1.40) to low average (z-scores from -1.39 to -.75). Descriptive summary of performance on the cognitive measures are provided in Table 7.

*Verbal Skills*

The DAS-II Word Definition, DAS-II Verbal Similarities, and NEPSY-II Word Generation subtests were used to measure verbal abilities. The participants' overall performance on the DAS-II Verbal Cluster score was low average with a mean z-score of -1.26. On both of the individual verbal subtests, 45.5% of the participants performed 1 SD below the expected mean, receiving an average z-score of -1.15 and -1.02 on the Word Definition and Verbal Similarities subtests, respectively. The participants' performance on the Word Generation subtest was notable for poorer ability to generate words using initial letter prompts compared to semantic categories in relation to same-aged peers. Specifically, 72.2% of the participants performed 1SD below the expected mean on initial letter prompts compared to 47.8% on semantic categories.

*Visual-Spatial-Perceptual-Motor Functioning:*

On average, the participants demonstrated low average ability to perceive sequential patterns in geometric figures or common rules in numerical relationships as measured by the

Sequential Quantitative Reasoning subtest on the DAS-II. The participants also demonstrated low average ability to judge line orientation as measured by NEPSY-II Arrows. Greater difficulty was observed, however, on the Fingertip Tapping and Imitating Hand Position subtests, with a mean performance in the borderline range on these sensorimotor tasks. Consistently, a lower performance was observed in the borderline range on a perceptual reasoning task with a motor component (DAS-II Pattern Construction), again suggesting greater weakness in fine motor skills.

*Memory and Learning*

Two measures from the DAS-II, Recall of Digits Backward (RDB) and Recall of Sequential Order (RSO), were administered to measure working memory. The participants' performance on the Working Memory cluster was in the borderline range. The average performance on the individual working memory subtests, Recall of Digits Backward and Recall of Sequential Order, were in the borderline and low average ranges, respectively. In terms of rote memory for both immediate and delayed verbal information (NEPSY-II List Memory and List Memory Delayed Total Score), the average participant performed in the low average range with a mean z-score of -1.14. Similarly, on the NEPSY-II Memory for Faces, participants displayed low average ability for both immediate and delayed recall of faces.

*Processing Speed*

Two measures, Speed of Information Processing and Rapid Naming from the DAS-II, were administered to measure Processing Speed. Overall, participants performed in the borderline range on the Processing Speed Cluster, as well as on the individual scores. However, more participants received lower scores on the task measuring the speed of performing

quantitative comparisons (Speed of Information Processing) compared to the task measuring speed of lexical access and retrieval (Rapid Naming).

*Attention*

The NEPSY-II Auditory Attention subtest was administered to assess selective and sustained auditory attention. The mean z-score on this task was -1.13, which is in the low average range. The participants' parents were also asked to rate their children's level of attention problems on the BASC-II. On this measure, participants were slightly elevated, with a mean z-score of -1.13 as shown in Table 8.

*Executive Functioning*

Three subtests were administered to assess executive functioning: NEPSY-II Auditory Attention Response Set, Design Fluency, and Inhibition. On average, the participants performed in the borderline range across these tasks. For instance, the participants received a mean z-score of -1.57 on the NEPSY-II Response Set, a task that measures the ability to shift and maintain a new and complex, inhibit previously learned responses, and correctly respond to matching or contrasting stimuli. The participants also demonstrated difficulty on the NEPSY-II Inhibition subtest, a task assessing the ability to inhibit automatic response in favor of novel responses. Lastly, the average NEPSY-II Design fluency z-score was also in the borderline range, which shows that participants had problems with behavioral productivity.

*Social Perception*

The NEPSY-II Affect Recognition subtest was administered to assess the ability to recognize emotions (happy, sad, anger, fear, disgust, and neutral) in people's faces. The participants performed on the low average range on this task.

*Academic Achievement*

Two subtests from the WIAT-II were administered: Word Reading and Numerical Operations. On average, the participants demonstrated low average skill in basic reading and math.

*Behavior and Adaptive Functioning*

On average, participants were rated by their parents as elevated on the Somatization scale on the BASC-II. The overall high rating on Somatization, however, was not surprising given the many symptoms that these children experienced were due to their medical condition. For example, parents were likely to endorse that their child "complains of pain", "has stomach problems", "gets sick", "vomits", et cetera, all of which are common symptoms of MD. Atypicality ratings were also elevated, on average, indicating a higher level of unusual thoughts and behaviors or disconnection from one's surroundings. Parents endorsed items indicating that participants do "do strange things", "say things that make no sense", "babble", or "act confused". According to Reynolds and Kamphaus (2004), a high Atypicality score may be an indicator of psychotic tendencies, immaturity or developmental delay, mental retardation, or autism spectrum disorders. This is consistent with previous research (Finsterer, 2007) and clinical reports of children, many of whom are developmentally delayed and have cognitive deficits.

Consistently, participants were also rated significantly low across most Adaptive indices on the BASC-II and Vineland-II. For instance, on average, participants were rated low on their overall adaptive skills/behavior index, communication, and daily living skills on both the BASC-II and Vineland-II. Additionally, participants were rated low in Socialization and Motor Skills on the Vineland-II.

Table 2 *Clinical Characteristics*

| | *n* | M | SD | Range |
|---|---|---|---|---|
| Age at Neuropsychological Assessment[a] | 25 | 8.75 | 2.35 | 5-13 |
| Age at Diagnosis[a] | 25 | 5.36 | 3.20 | 0-11 |
| Time since Diagnosis[a] | 25 | 3.00 | 2.43 | 0-10 |

| | % (*n*) |
|---|---|
| Abnormal Enzyme Complexes[b] | |
| I | 68 (17) |
| II | 0 (0) |
| III | 36 (9) |
| IV | 8 (2) |
| V | 20 (5) |

[a]Reported in years . [b]Includes combination types

Table 3 *Descriptive Statistics of Western Blot*

| Variables | z-score | | | | Normal | Abnormal |
|---|---|---|---|---|---|---|
| | *N* | *M* | *SD* | *Range* | *% (n)* | *% (n)* |
| Complex I (ND6) | 15 | -.68 | 1.36 | -2.07-3.18 | 40 (6) | 60 (9) |
| Complex II (30KDa) | 15 | .37 | 1.54 | -1.72-2.98 | 47 (7) | 53 (8) |
| Complex III (Core 2) | 15 | -1.35 | 1.30 | -3.30-1.58 | 40 (6) | 60 (9) |
| Complex IV (COX II) | 15 | -.98 | 0.68 | -2.00- .27 | 80 (12) | 20 (3) |
| Complex V (F1 Alpha) | 15 | -1.15 | 1.41 | -2.95-1.17 | 53 (8) | 4 (7) |
| Total Abnormalities[a] (Max=5) | 15 | 2.40 | 1.96 | .00-5.00 | | |

*Note*. All scores reported in Z-scores except for total abnormalities
[a]Based on -scores $\leq$ -1

Table 4 *Descriptive Statistics of Native Gels*

| Variables | *N* | Normal *% (n)* | Abnormal *% (n)* |
|---|---|---|---|
| Clear Native Complex I | 11 | 36.4 (4) | 63.6 (7) |
| Clear Native Complex II | 11 | 100.0 (11) | 0 (0) |
| Blue Native Complex III | 11 | 90.9 (10) | 9.1 (1) |
| Blue Native Complex IV | 11 | 90.9 (10) | 9.1 (1) |
| Blue Native Complex V | 11 | 90.9 (10) | 9.1 (1) |
| Blue Native Supercomplex (I, III, IV) | 11 | 72.3 (8) | 27.3 (3) |
| | *M* | *SD* | Range |
| Natives Total Abnormalities (Max=6) | 1.18 | 1.47 | 0-5 |

Table 5 *Descriptive Statistics of High Resolution Respirometry*

| Variables | z-scores | | | | Assessment | | |
|---|---|---|---|---|---|---|---|
| | *N* | *M* | *SD* | *Range* | *N* | Normal %(n) | Abnormal %(n) |
| SKELETAL MUSCLES | | | | | | | |
| Uncoupling | 9 | -.78 | 1.43 | -2.29-1.56 | 9 | 44.4 (4) | 55.6 (5) |
| Net Routine Flux Control | 9 | 1.47 | 2.12 | -1.14-4.34 | 9 | 44.4 (4) | 55.6 (5) |
| Respiratory Control | 9 | -.37 | 1.30 | -2.99-1.45 | 9 | 77.8 (7) | 22.2 (2) |
| Leak Flux Control | 9 | .74 | 2.29 | -1.29-6.38 | 9 | 77.8 (7) | 22.2 (2) |
| Glutamate+Malate+ADP | 9 | 1.86 | 1.59 | .00-5.21 | 9 | 33.3 (3) | 66.7 (6) |
| Succinate Supplemented | 9 | 1.68 | 1.13 | .28-3.53 | 9 | 33.3 (3) | 66.7 (6) |
| COMBINED* | | | | | | | |
| Uncoupling | | | | | 10 | 40 (4) | 60 (6) |
| Net Routine Flux Control | | | | | 10 | 40 (4) | 60 (6) |
| Respiratory Control | | | | | 10 | 80 (8) | 20 (2) |
| Leak Flux Control | | | | | 10 | 80 (8) | 20 (2) |

| | *N* | *M* | *SD* | *Range* |
|---|---|---|---|---|
| Total Abnormality[a] (Max=4) | 10 | 1.8 | 1.75 | 0-4 |

*Note.* All scores reported in z-scores except for total abnormalities.
*Combined results from skeletal muscles and fibroblasts
[a]Based on z-scores below or above 1 SD.

Table 6 *Descriptive Statistics of Nijmegen Criteria*

| Variables | *n* | Scoring Evaluation | | | |
|---|---|---|---|---|---|
| | | Highly Probable *% (n)* | Probable *% (n)* | Possible *% (n)* | Unlikely *% (n)* |
| CLINICAL CRITERIA[a] | 25 | 4 (1) | 28 (7) | 56 (14) | 12 (3) |
| BIOCHEMICAL CRITERIA[b] | 23 | 43.48 (10) | 17.39 (4) | 39.13 (9) | 0 (0) |
| | *M* | *SD* | Range | Normal *% (n)* | Abnormal *% (n)* |
| CLINICAL CRITERIA[c] | 2.74 | .75 | 1.00-4.00 | 68 (17) | 32 (8) |
| Neuromuscular Manifestations (max=2) | .96 | .88 | .00-2.00 | | |
| CNS and other Organ Involvement (max=2) | 1.48 | .67 | .00-2.00 | | |
| Metabolic and Imaging Studies (max=4) | 1.35 | 1.37 | .00-4.00 | | |
| Tissue Morphology (max=4) | .13 | .46 | .00-2.00 | | |
| BIOCHEMICAL CRITERIA[c] | 1.96 | .93 | 1.00-3.00 | 39.13 (9) | 60.87 (14) |
| GENETIC CRITERIA (*n*=20) | | | | 55 (11) | 45(9) |

*Note*. [a]Highly Probable (8-12 pts), Probable (5-7 pts), Possible (2-4 pts), Unlikely (1pt)

[b]Highly Probable ($\geq$2 tests abnormal), Probable (2 tests abnormal), Possible (1 test abnormal), Unlikely (all normal)
[c]Normal=Unlikely or Possible; Abnormal=Probable or Highly Probable

Table 7 *Descriptive Statistics of Cognitive Variables*

| Variables | *N* | *M* | *SD* | Range | ≤ -1 *SD* % (*n*) | > -1 *SD* % (*n*) |
|---|---|---|---|---|---|---|
| PROCESSING SPEED (DAS-II) | | | | | | |
| Processing Speed Cluster | 22 | -1.73 | 1.76 | -4.40-1.73 | 68.2 (15) | 31.8 (7) |
| Speed of Information Processing | 22 | -1.51 | 1.52 | -3.70-2.00 | 72.7(16) | 27.3 (6) |
| Rapid Naming | 22 | -1.30 | 1.53 | -4.00-1.00 | 54.5 (12) | 45.5 (10) |
| ATTENTION (NEPSY-II) | | | | | | |
| Auditory Attention Total Correct | 21 | -1.13 | 1.54 | -3.00-1.00 | 57.1(12) | 42.9 (9) |
| VERBAL COMPREHENSION (DAS-II) | | | | | | |
| Verbal Cluster | 23 | -1.26 | 2.12 | -4.60-2.47 | 47.8 (11) | 52.2 (12) |
| Word Definition | 22 | -1.15 | 2.03 | -4.00-2.00 | 45.5(10) | 54.5 (12) |
| Verbal Similarities | 22 | -1.02 | 1.90 | -4.00-2.40 | 45.5(10) | 54.5 (12) |
| WORD RETRIEVAL (NEPSY-II) | | | | | | |
| Word Generation-semantic | 23 | -.91 | 1.62 | -3.00-3.00 | 47.8 (11) | 52.2 (12) |
| Word Generation-Initial Letter | 18 | -1.32 | 1.44 | -2.67-3.00 | 72.2 (13) | 27.8 (5) |
| PERCEPTUAL REASONING (DAS-II) | | | | | | |
| Sequential Quantitative Reasoning | 22 | -1.16 | 1.53 | -3.60-2.20 | 50.0 (11) | 50.0 (11) |
| Pattern Construction | 22 | -1.46 | 1.66 | -4.00-1.20 | 68.2 (15) | 31.8 (7) |
| VISUAL & SENSORIMOTOR (NEPSY-II) | | | | | | |
| Arrows | 22 | -1.23 | 1.55 | -3.00-1.33 | 59.1 (13) | 40.9 (9) |
| FTT-Dominant Hand Combined z score | 20 | -1.63 | 1.41 | -3.00- .67 | 70.0 (14) | 30.0 (6) |
| FTT-Nondominant Hand Combined z score | 20 | -1.75 | 1.52 | -3.00-1.33 | 70.0 (14) | 30.0 (6) |

| | | | | | | |
|---|---|---|---|---|---|---|
| FTT-Repetition Combined z score | 22 | -1.56 | 1.57 | -3.00-1.33 | 63.6 (14) | 36.4 (8) |
| FTT-Sequences Combined z score | 20 | -1.35 | 1.47 | -3.00-1.33 | 60.0 (12) | 40.0 (8) |
| Imitating Hand Position Total z score | 22 | -1.53 | 1.36 | -3.00-1.00 | 68.2 (15) | 31.8 (7) |
| | | | | | | |
| WORKING MEMORY (DAS-II) | | | | | | |
| Working Memory Cluster | 22 | -1.43 | 1.80 | -4.47-1.07 | 59.1 (13) | 40.9 (9) |
| Recall of Sequential Order | 22 | -1.40 | 1.76 | -4.00-1.40 | 50.0 (11) | 50.0 (11) |
| Recall of Digits Backwards | 22 | -1.20 | 1.59 | -4.00- .70 | 40.9 (9) | 59.1 (13) |
| | | | | | | |
| MEMORY (NEPSY-II) | | | | | | |
| List Memory | 17 | -1.14 | 1.59 | -3.67-1.67 | 52.9 (9) | 47.1 (8) |
| Memory for Faces | 22 | -1.09 | 1.49 | -3.00-1.67 | 50.0 (11) | 50.0 (11) |
| Memory for Faces Delayed | 22 | -.80 | 1.56 | -3.00-1.67 | 50.0 (11) | 50.0 (11) |
| | | | | | | |
| EXECUTIVE FUNCTIONS (NEPSY-II) | | | | | | |
| Response Set Total Correct | 17 | -1.57 | 1.63 | -3.00-1.67 | 50.8 (10) | 41.2 (7) |
| Inhibition Naming Combined | 22 | -1.47 | 1.60 | -3.00-1.33 | 63.6 (14) | 36.4 (8) |
| Inhibition Naming Completion Time | 22 | -1.70 | 1.54 | -3.00-1.00 | 68.2 (15) | 31.8 (7) |
| Inhibition Inhibition Combined | 22 | -1.73 | 1.38 | -3.00- .67 | 68.2 (15) | 31.8 (7) |
| Inhibition Inhibition Completion Time | 22 | -1.70 | 1.43 | -3.00-1.33 | 68.2 (15) | 31.8 (7) |
| Inhibition Switching Combined | 19 | -1.56 | 1.56 | -3.00-1.33 | 63.2 (12) | 36.8 (7) |
| Inhibition Switching Completion Time | 19 | -1.65 | 1.76 | -3.00-2.67 | 68.4 (13) | 31.6 (6) |
| Design Fluency | 20 | -1.48 | 1.31 | -3.00- .33 | 60.0 (12) | 40.0 (8) |
| | | | | | | |
| SOCIAL PERCEPTION (NEPSY-II) | | | | | | |
| Affect Recognition | 23 | -1.00 | 1.43 | -3.00-1.33 | 52.2 (12) | 47.8 (11) |
| | | | | | | |
| ACADEMIC ACHIEVEMENT | | | | | | |
| Word Reading | 22 | -.83 | 1.82 | -4.00-1.93 | 40.9 (9) | 59.1 (13) |
| Numerical Operations | 22 | -1.15 | 1.97 | -4.00-2.93 | 54.5 (12) | 45.5 (10) |

Table 8 *Descriptive Statistics of BASC-II*

| Variables | *n* | *M* | *SD* | Range |
|---|---|---|---|---|
| BEHAVIORAL SYMPTOMS INDEX | 20 | .73 | .87 | -.40- 2.60 |
| EXTERNALIZING PROBLEMS | 20 | .13 | .97 | -1.60-2.30 |
| Hyperactivity | 20 | .54 | 1.21 | -1.60-3.40 |
| Aggression | 20 | .16 | .75 | -1.40-1.00 |
| Conduct Problems | 17 | -.16 | .88 | -1.30-2.00 |
| INTERNALIZING PROBLEMS | 20 | .89 | 1.19 | -1.30-2.50 |
| Anxiety | 20 | .53 | 1.09 | -1.60-1.90 |
| Depression | 20 | .48 | 1.15 | -.90-3.00 |
| Somatization | 20 | 1.26 | 1.51 | -1.40-4.00 |
| OTHER | | | | |
| Atypicality | 20 | 1.05 | 1.29 | -.60-5.20 |
| Withdrawal | 20 | .53 | .89 | -1.00-2.60 |
| Attention Problems* | 20 | .93 | 1.10 | -1.10-2.70 |
| ADAPTIVE SKILLS | 20 | -1.17 | 1.32 | -3.10-1.50 |
| Adaptability | 20 | -.63 | 1.36 | -3.00-1.40 |
| Social Skills | 20 | -.48 | 1.19 | 1.90- .48 |
| Leadership | 17 | -.49 | 1.31 | -2.80-1.60 |
| Activities of Daily Living | 20 | -1.32 | 1.22 | -3.50-1.00 |
| Functional Communication | 20 | -1.36 | 1.49 | -3.70-1.60 |

*Note.* All scores reported in z-scores.
*Used in multiple regression analysis

Table 9 *Descriptive Statistics of Vineland-II*

| Variables | *N* | *M* | *SD* | Range |
|---|---|---|---|---|
| ADAPTIVE BEHAVIOR COMPOSITE[a] | 24 | - 1.69 | 1.11 | -4.40- .00 |
| COMMUNICATION[a] | 24 | - 1.37 | 1.29 | -4.27- .33 |
| Receptive[b] | 23 | 11.65 | 3.70 | 3.00-19.00 |
| Expressive[b] | 24 | 11.04 | 3.80 | 4.00-19.00 |
| Written[b] | 24 | 11.46 | 3.41 | 5.00-17.00 |
| DAILY LIVING SKILLS[a] | 24 | -1.92 | 1.13 | -4.40- .33 |
| Personal[b] | 24 | 10.29 | 3.64 | 4.00-18.00 |
| Domestic[b] | 24 | 10.29 | 3.07 | 5.00-16.00 |
| Community[b] | 24 | 9.79 | 3.35 | 3.00-17.00 |
| SOCIALIZATION[a] | 24 | -1.35 | 1.20 | -3.73- .67 |
| Interpersonal Relations[b] | 24 | 11.00 | 3.53 | 4.00-18.00 |
| Play and Leisure Time[b] | 24 | 10.58 | 3.67 | 4.00-17.00 |
| Coping Skills[b] | 24 | 12.33 | 3.43 | 7.00-19.00 |
| MOTOR SKILLS[a] | 24 | -1.86 | 1.36 | -5.00- 1.13 |
| Gross Motor Skills[b] | 24 | 9.58 | 2.96 | 4.00-16.00 |
| Fine Motor Skills[b] | 24 | 11.13 | 4.33 | 1.00-20.00 |
| MALADAPTIVE[c] | 23 | 18.00 | 2.49 | 14.00-16.00 |
| Internalizing Problems[c] | 24 | 18.67 | 2.30 | 15.00-24.00 |
| Externalizing Problems[c] | 24 | 16.62 | 3.29 | 12.00-24.00 |

*Note*. [a]Scores reported in z-scores.
[b]Adaptive Scales reported in Vineland scaled scores: v-ss <13 is well-below to below average, 13-17 is low to high average, >17 is superior to very superior
[c]Maladaptive scales reported in Vineland scaled scores: v-ss <18 is average, 18-20 is elevated, and >20 clinically elevated.

# CHAPTER 4

# RESULTS

## Predicting Biochemical Abnormalities with Processing Speed and Attention Measures

The primary hypotheses of this study explored associations across processing speed, attention measures, and biochemical abnormalities related to MD that were identified in these participants.

### *Western Blot*

Standard multiple regression analyses were conducted with processing speed and attention measure scores as predictor variables and total number of abnormal (at least one standard deviation below the control reference mean) DNA subunit/GADPH ratios associated with Complexes I through V as outcome variables. A maximum of five abnormal ratios from Complex I (ND6), Complex II (30KDa), Complex III (Core 2), Complex IV (COX II), and Complex V (F1 alpha) were possible. When the DAS-II Processing Speed Cluster and the BASC-II Attention Problem z-scores were used as predictor variables, the total variance explained by the model (Table 10) as a whole was 54.3%, $F$ (2, 9) = 5.35, $p$ = .03. Processing speed was a significant predictor of the total number of abnormalities detected through the Western Blot ($\beta$ = -.78, $p$ = .02), as were attention problems ($\beta$ = -.82, $p$ = .02). However, when a separate regression analysis was done using the NEPSY-II Auditory Attention Total Correct z-score as a predictor variable, the model was not significant [$F$ (2, 9) =.51, $p$ =.62] and accounted for only 10.2% of the variance as shown in Table 11.

Correlation analyses were performed in order to clarify the relationships among western blot complex ratios, processing speed, and attention. No results were found to be significant as

shown in Table 12. Independent-sample t-tests were also conducted to further compare performance on processing speed and attention tests for participants with normal and abnormal western blot subunit ratios. The ratios were considered abnormal if the z-scores were equal to or below -1. However, none of the t-test results reached significance. Effect sizes were also small.

*Native Gels*

Multiple regression analysis was again used to determine how much variance in the total number of abnormal supercomplex and monomeric oxphos assemblies was accounted for by the processing speed and attention measures. A maximum of six abnormalities was established through the combined Blue and Clear Native Gels. The DAS-II Processing Speed Cluster and BASC-II Attention Problems measure scores together accounted for only 1% of the variance in the total number of abnormal complex assemblies [$F$ (2,6) = .02, $p$ =.98]. The DAS-II Processing Speed Cluster and NEPSY-II Auditory Attention scores also failed make significant contributions in a separate regression analysis [$F$(2,5) = .09, $p$ = .09]. Lastly, participants with normal complex assemblies were compared to those with abnormal assemblies across all processing speed and attention measures using t-tests; however, none of the results reached significance.

*High Resolution Respirometry*

Multiple regression was used to determine how much variance in the total number of abnormal ratios and oxygen fluxes from high resolution respirometry assessment might be predicted by processing speed and attention measures. A maximum of four abnormal ratios (uncoupling, net routine flux, respiratory control, and leak flux control) could result from the combined high resolution respirometry analysis of fibroblasts and skeletal muscle tissues. The DAS-II Processing Speed Cluster and BASC-II Attention Problems did not account for a

significant proportion of the variance [$F(2,5) = .30$, $p = .76$]. The results from the regression model using the NEPSY-II Auditory Attention measure were also insignificant [$F(2,6) = .15$, $p = .87$] and accounted for only 4.7% of the total variance.

Correlations and t-tests were performed between the High Resolution Respirometry for skeletal muscle ratios, re-calculated as z-scores, and all of the standardized processing speed and attention measures. Table 13 provides a summary of the correlational findings for High Resolution Respirometry of Skeletal Muscles. A strong negative correlation was found between the succinate-supplemented media of skeletal muscles and NEPSY-II Auditory Attention ($r = -.75$, $p = .03$). Moreover, a significant group difference was found on the NEPSY-II Auditory Attention task between participants with normal ($M = 0$, $SD = .89$) and abnormal ($M = -2.33$, $SD = .91$) skeletal muscle succinate-supplemented media ratio; $t(6) = 3.5$, $p = .012$ (two-tailed). The magnitude of the differences in the means (mean difference = 2.33, 95% Cl: .72 to 3.95) was large (eta squared = .67).

*Nijmegen Criteria*

The Nijmegen criteria were used to investigate the relationships between the overall clinical and biochemical features of Mitochondrial Disease and processing speed and attention skills. The Nijmegen criteria that were used to assess the children enrolled in the present study are provided in Appendix C. Multiple regression assessed the ability of DAS-II Processing Speed Cluster and BASC-II Attention Problems to predict the Nijmegen Clinical Criteria Total score and Biochemical Criteria Total score in separate analyses. The total variance of the Clinical Criteria explained by the model as a whole was only 3% [$F(2, 16) = .23$, $p = .80$] and not statistically significant. The two independent variables also failed to reach statistical significance when predicting the Nijmegen Biochemical Criteria Total score [$F(2,15) = .19$, $p =$

.83]. Moreover, DAS-II Processing Speed Cluster and NEPSY-II Auditory Attention measures also failed to significantly account for the variance in the Clinical Criteria regression model [$F(2,18) = .54$, $p = .59$], as well as in the Biochemical Criteria model [$F(2,18) = 1.48$, $p = .26$].

In order to better understand the relationships among all the Nijmegen Clinical criteria subdomains (neuromuscular manifestations, central nervous system and other organ involvement, metabolic and imaging studies, tissue morphology) and the primary cognitive measures of this study, correlation analyses were also conducted. A summary of the results are presented in Table 14. No significant correlations were found between the Nijmegen criteria and the primary cognitive measures.

**Supplementary Analyses**

*Verbal Abilities*

Word Definition, Verbal Similarities, and Word Generation subtests were used to measure verbal abilities. Aside from the group differences that were found for Succinate-supplemented media ratios of skeletal muscle cells on DAS-II Verbal Cluster [$t(6) = 3.23$, $p = .02$, eta squared = .63] using High Resolution Respirometry, no other findings reached significance across all areas.

*Visual-Spatial-Perceptual-Motor Functioning:*

The cognitive measures for this domain include the DAS-II Sequential and Quantitative Reasoning, DAS-II Pattern Construction, NEPSY-II Arrows, NEPSY-II Fingertip Tapping, and Imitating Hand Positions. On Pattern Construction, a significant difference was found between participants with normal ($M = -.52$, $SD = .91$) and abnormal ($M = -2.39$, $SD = 1.24$) Complex II ratios using Western Blot; $t(10) = 2.84$, $p = .02$, eta squared = .45. Correlation analysis revealed a significant association between Pattern Construction and High Resolution Respirometry total

abnormality score of skeletal muscle tissue ($r$ = -.72, $p$ = .04). A significant, positive correlation was also found between Complex II ratio and NEPSY-II Imitating Hand Position ($r$ = .58, $p$ = .04), a motor task.

*Memory Functioning*

The DAS-II Recall of Digits Backward and Recall of Sequential Order Subtests combined to produce an overall Working Memory Cluster score in addition to the individual scores. There were group differences in DAS-II Working Memory Cluster [$t$ (10) =2.70, $p$ =.02, eta squared = .42] between normal ($M$ = -.19, $SD$ =.75) and abnormal ($M$ = -1.99, $SD$= 1.52) Complex II groups using Western Blot. More group differences were found between normal ($M$ = -.10, $SD$ =.58) and abnormal ($M$ = -1.76, $SD$ = 1.36) Complex II groups for Recall of Digit Backwards [$t$ (10) = 2.84, $p$ =.02, eta squared =.45] as well between groups for Succinate-supplemented media ratios of skeletal muscle on Recall of Sequential Order [$t$ (5) = 2.54, $p$ = .05, eta squared = .52]. A significant, positive correlation was found between Complex I ratio and DAS-II Recall of Digits Backwards ($r$ =.59, $p$ =.04), a working memory task using Western Blot.

List Memory and Memory for Faces from the NEPSY-II were also administered to measure memory and learning. No significant relationship was found between these measures and biochemical/medical indices.

*Executive Functioning*

The Auditory Response Set, Design Fluency, and Inhibition subtests from the NEPSY-II were administered as measures of executive functioning. On the Western Blot, a significant difference in scores was also found on the Response Set Total Correct between participants with normal ($M$ = -2.22, $SD$ = 1.20) and abnormal ($M$ = -.83, $SD$ = .24) ratios for Complex IV; $t$ (6) =

-2.67, *p*=.027 (two-tailed). There was a large difference in the means; eta squared = .54, mean z-score difference = -1.39, 95% Cl: -2.67 to -1.09. Moreover, a positive correlation was found between the Complex II ratio and NEPSY-II Design Fluency (*r* =.66, *p* =.01). In support of this finding, there was a significant difference on the NEPSY-II Design Fluency scores between participants with normal (*M* = -.50, *SD* = .64) and abnormal (*M* = -2.29, *SD* = .52) Complex II ratios; *t* (9) = 1.79, *p* = .001. The magnitude of the differences in the means (mean z-score difference = 1.79, 95% Cl: .99 to 2.59) was also large (eta squared = .26). In addition, strong negative correlations were found between leak flux ratio and NEPSY-II Inhibition-Naming Completion Time (rho = -.74, *p* = .04), and respiratory control ratio and NEPSY-II Inhibition-Naming Completion Time (rho = -.74, *p*= .04). No results reached significance with any of the Nijmegen and Native Gel measures.

*Social Perception*

No findings reached significance when correlations and t-tests were conducted between biochemical/medical indices and the NEPSY-II Affect Recognition Subtest.

*Behavioral and Adaptive Functioning*

A significant difference in scores was found on BASC-II Internalizing Problems [*t* (10) = 3.08, *p* = .01, eta squared = .49] between participants with normal (*M* = .89, *SD* = 1.43) and abnormal (*M* = -.50, *SD* = 0) Complex IV ratios on the Western Blot. Likewise, group differences were found on High Resolution Respirometry Succinate-supplemented media ratios of skeletal muscle cells, specifically on BASC-II internalizing problems [*t* (5) = 3.20, *p* = .03, eta squared = .67] and BASC-II Depression [*t* (5) = 3.42, *p* = .02, eta squared = .70]. With t-test analyses, significant group differences were found in the BASC-II Behavioral Symptoms Index scores between participants with normal and abnormal ratios assessing oxygen consumption with

the addition of ADP using High Resolution Respirometry in skeletal muscles; $t(5) = 4.05$, $p = .01$, eta squared = .77. Furthermore, significant associations were found between Nijmegen Neuromuscular Manifestations and BASC-II Withdrawal ($r = -.48$, $p = .03$), and Nijmegen Total Biochemical Criteria and BASC-II Withdrawal ($r = -.66$, $p = .002$).

In regards to Adaptive Functioning, t-test analyses evaluating group differences for Complex I ratios revealed a significant difference in scores on BASC-II Adaptability between participants with normal ($M$=-1.76, $SD$=1.21) and abnormal ($M$ =-.09, $SD$ = 1.08) complexes [t (10) = -2.52, $p = .03$, eta squared = .67] using the Western Blot. Also, On the Nijmegen, significant associations were found between Total Biochemical Criteria and BASC-II Adaptability ($r = -.44$, $p = .05$). Significant difference was found on the Vineland Receptive subdomain for participants with normal ("Possible/Unlikely") and abnormal ("Highly Probable/ Probable) Total Biochemical Criteria [$t$ (19) = 2.69, $p = .01$; eta squared = .28]. Also, group differences were found between participants with and without genetic mutation in the parent ratings of BASC-II Functional Communication [$t$ (14) = 2.46, $p = .027$; eta squared = .29] and Vineland-II Coping Skills [$t$ (17) = 2.16, $p = .05$; eta squared = .22].

*Academic Achievement*

No findings reached statistical significance when correlations and t-tests were conducted among biochemical/medical indices, WIAT-II Word Reading, and Numerical Operation.

Table 10 *Multiple Regression Analyses using BASC-II Attention Problems*

| Biochemical Measures | *N* | *B* | β | *p* | *R* Square |
|---|---|---|---|---|---|
| WESTERN BLOT TOTAL ABNORMALITY[a] | 15 | | | | |
| Model | | | | .03* | .54 |
| DAS Processing Speed | 22 | -.86 | -.78 | .02* | |
| BASC Attention Problems | 20 | -1.45 | -.82 | .02* | |
| NATIVE GEL TOTAL ABNORMALITY[a] | 11 | | | | |
| Model | | | | .98 | .01 |
| DAS Processing Speed | 22 | -.05 | -.06 | .92 | |
| BASC Attention Problems | 20 | .05 | .04 | .95 | |
| HIGH RESOLUTION TOTAL ABNORMALITY[a] | 10 | | | | |
| Model | | | | .76 | .11 |
| DAS Processing Speed | 22 | -.15 | -.15 | .78 | |
| BASC Attention Problems | 20 | -.62 | -.39 | .48 | |
| NIJMEGEN TOTAL CLINICAL SCORE[a] | 25 | | | | |
| Model | | | | .80 | .03 |
| DAS Processing Speed | 22 | -.06 | -.05 | .88 | |
| BASC Attention Problems | 20 | -.38 | -.19 | .53 | |
| NIJMEGEN TOTAL BIOCHEMICAL SCORE[a] | 23 | | | | |
| Model | | | | .83 | .03 |
| DAS Processing Speed | 22 | .09 | .17 | .58 | |
| BASC Attention Problems | 20 | .03 | .03 | .92 | |

*Note*. [a]Dependent variables. ** Significant at the 0.01 level. * Significant at the 0.05 level.

Table 11 *Multiple Regression Analyses using NEPSY-II Auditory Attention*

| Biochemical Measures | *N* | *B* | β | *p* | *R* Square |
|---|---|---|---|---|---|
| WESTERN BLOT TOTAL ABNORMALITY[a] | 15 | | | | .10 |
| Model | | | | .62 | |
| DAS Processing Speed | 22 | -.45 | .58 | -.41 | |
| NEPSY Auditory Attention | 21 | .15 | .66 | .13 | |
| NATIVE GEL TOTAL ABNORMALITY[a] | 11 | | | | |
| Model | | | | .92 | .03 |
| DAS Processing Speed | 22 | -.24 | -.29 | .70 | |
| NEPSY Auditory Attention | 21 | .26 | .28 | .72 | |
| HIGH RESOLUTION TOTAL ABNORMALITY[a] | 10 | | | | |
| Model | | | | .87 | .05 |
| DAS Processing Speed | 22 | .33 | .34 | .63 | |
| NEPSY Auditory Attention | 21 | .38 | -.34 | .62 | |
| NIJMEGEN TOTAL CLINICAL SCORE[a] | 25 | | | | |
| Model | | | | .59 | .06 |
| DAS Processing Speed | 22 | .01 | .02 | .96 | |
| NEPSY Auditory Attention | 21 | -.12 | -.25 | .51 | |
| NIJMEGEN TOTAL BIOCHEMICAL SCORE[a] | 25 | | | | |
| Model | | | | .26 | .14 |
| DAS Processing Speed | 22 | -.22 | -.25 | .49 | |
| NEPSY Auditory Attention | 21 | -.14 | -.14 | .69 | |

*Note*. [a]Dependent variables. ** Significant at the 0.01 level. * Significant at the 0.05 level.

Table 12 *Correlations across Western Blot (N=15), Processing Speed, and Attention*

| | Complex Type | | | | | Total Abnormalities |
|---|---|---|---|---|---|---|
| | I | II | III[a] | IV | V | Based on z-score |
| PROCESSING SPEED | | | | | | |
| Processing Speed Cluster | .51 | .35 | .20 | .41 | -.15 | -.35 |
| Speed of Information Processing | .48 | .51 | .19 | .38 | .02 | -.29 |
| Rapid Naming | .42 | .27 | .21 | .26 | -.22 | -.32 |
| ATTENTION | | | | | | |
| Auditory Attention Correct | .45 | .06 | .10 | .43 | .00 | -.18 |
| BASC-II Attention Problems | .26 | .04 | .46 | .00 | -.44 | -.42 |

*Note.* ** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).
[a]Spearman's correlation was used instead of Pearson's because the distribution was not normal

Table 13 *Correlations across Skeletal Muscles High Resolution Respirometry (N=9), Processing Speed, and Attention*

| | UCR | NRF | RCR | LFC[a] | GMADP | Succinate | Total Abnormality |
|---|---|---|---|---|---|---|---|
| PROCESSING SPEED | | | | | | | |
| Processing Speed Cluster | .15 | -.06 | -.07 | -.19 | -.06 | -.21 | -.13 |
| Speed of Information Processing | .06 | .02 | -.08 | -.05 | .08 | -.07 | .05 |
| Rapid Naming | .19 | -.10 | -.06 | -.24 | -.16 | -.31 | -.26 |
| | | | | | | | |
| ATTENTION | | | | | | | |
| Auditory Attention Total Correct | .36 | -.24 | .39 | -.48 | -.44 | -.75* | -.41 |
| BASC-II attention Problems | .42 | -.40 | -.41 | .05 | -.17 | -.02 | -.17 |

Note. UCR=Uncoupling Ratio; NRF=Net Routine Flux, RCR=Respiratory Control Ratio; LFC= Leak Flux Control, RCRp=Phosphorylation Respiratory Control; GMADP= Glutamate +Malate+ADP.

[a]Spearman's correlation was used instead of Pearson's because the distribution was not normal

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Table 14 *Correlations across Nijmegen Criteria, Processing Speed, and Attention Measures*

| | Clinical Criteria | | | | Total | |
|---|---|---|---|---|---|---|
| | Neuro-muscular | CNS/ Organs | Metabolic/ Imaging | Tissue | Clinical | Biochemical |
| PROCESSING SPEED | | | | | | |
| Processing Speed Cluster | .16 | -.31 | -.03 | -.05 | -.01 | -.13 |
| Speed of Information Processing | .08 | -.29 | .06 | -.11 | -.01 | -.39 |
| Rapid Naming | .17 | -.23 | .17 | .09 | .13 | -.29 |
| ATTENTION | | | | | | |
| Auditory Attention Total Correct | .04 | -.09 | .14 | .04 | .14 | -.33 |
| BASC-II Attention Problems | .01 | .31 | -.32 | -.15 | -.16 | .27 |

*Note.* ** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-ta

# CHAPTER 5

# DISCUSSION

The overarching goal of the current study was to examine relationships among medical indices, biochemical measures, and neurobehavioral functioning of children with mitochondrial disorders. The laboratory biochemical tools and medical data that were used in this study consisted of the Western Blot, Native Gels, High Resolution Respirometry, and the Nijmegen screening criteria. The findings from these laboratory methods were assessed in relation to children's processing speed and attention based on the prediction that impaired functioning of proteins, complexes, and cellular respiration, that are critical in ATP production, will impact neurodevelopment and related neuropsychological processes in children with MD. Supplementary analyses were also conducted to examine relationships among these laboratory measures, diagnostic indices, and other cognitive measures to evaluate whether other explanations were warranted.

Based on maturational theories of brain development and what is known about brain metabolic activity from infancy to early childhood, it was predicted that subcortical and posterior areas in the brain, and related white matter connections, would more likely be interrupted by MD. Moreover, based on the neuropsychological sequalae observed in other subcortical and white matter diseases, and brain insults in infancy and early childhood, it was hypothesized that children with MD would show deficits in processing speed and attention, which could underlie the delays or deficits in higher order cognitive functions reported in some cases of MD.

As predicted, both DAS-II Processing Speed Cluster and BASC-II Attention Problems were significant predictors of the total number of abnormal Complex (I through V) proteins detected through Western Blot analysis. Regression with other cognitive measures, such as

measured by the DAS-II Verbal Cluster, DAS-II Working Memory, DAS-II Pattern Construction, and BASC-II Adaptive Skills, did not show this relationship. These findings suggest that poor processing speed and attention-related problems may be markers of abnormal protein expression of complexes that are central in the oxidative phosphorylation process. The early onset of the disease process, specifically the impaired production of ATP, may disrupt typical development of brain regions that subserve these processing speed and attentional systems.

In contrast to the above relationships, however, processing speed and attention measures were not predictive of other biochemical or medical indices. These predictor variables failed to account for a significant portion of the variance in the Native Gels, High Resolution Respirometry, or Nijmegen Criteria variables. This raises the question of why the relationship exists for the Western Blot but not for other biochemical or medical indices. One possible explanation is that the Western Blot, Native Gels, and High Resolution Respirometry are laboratory tools that measure different aspects of mitochondrial dysfunction. Processing speed and attention may be more sensitive to the differences at the level of protein expression, as measured by the Western Blot, compared to differences at the level of multiprotein (Native Gels) or cellular respiration (High Resolution Respirometry).

It is possible that the significant result with the Western Blot is just a false-positive finding given that most of the relationships measured among processing speed, attention, and other biochemical or medical measures indices were statistically insignificant. However, the sole finding with the Western Blot may partly be explained by the better reliability and higher sensitivity of using this technique compared to others which had a number of measurement-related limitations. For instance, abnormalities on the Native Gels were detected based on a

qualitative assessment (visual detection of abnormalities) as opposed to using normed values like in the Western Blot. While variables from High Resolution Respirometry were based on quantitative values, the sample size was much smaller than the Western Blot. Moreover, the Nijmegen criteria, as medical indices that cover a wide array of disease-related manifestations, may have been too broad and not sensitive enough to capture the subtle differences on these cognitive measures.

Based on findings that suggest that processing speed and attention may be markers for at least certain biochemical dysfunction in MD, additional analyses were performed with other cognitive measures, which were not originally hypothesized to be related, to evaluate whether these findings were representative of more global deficit relationships, or different explanations were warranted. No other regression analysis revealed significant findings. However, explorative analyses showed some deficit relationship between biochemical/medical indices and areas of executive function, working memory, internalizing behavioral problems, and adaptability.

A number of relationships were found between biochemical/medical indices and measures constructed to assess different aspects of executive function. Specifically, there were significant associations between the Western Blot and NEPSY-II Response set Total Correct score, Western Blot and NEPSY-II Design Fluency, and High Resolution Respirometry and NEPSY-II Inhibition Naming Total Completion Time. Whereas the Inhibition Naming Completion Time can be argued to provide a better measure of processing speed, since the task simply measures the ability to rapidly name shapes and direction of arrows, the relationships with the Response Set and Design Fluency tasks were less expected. Furthermore, the associations found among Western Blot measures, DAS-II Recall of Digits Backward, and DAS-

II Recall of Sequential Order were notable when considering that working memory has been suggested to be a component of executive functions (Garon, Bryson, & Smith, 2008). In light of these findings, it is important to explore the interrelationships between executive functions and more basic attentional systems and the possible impact of mitochondrial dysfunction on this network.

A central question, at base, is whether the cognitive framework for understanding mitochondrial dysfunction is one of poor executive control or of compromised lower-order attentional processes. Currently, attention is regarded as a network of interrelated processes that include the properties of selection and control (Burack & Enns, 1997). These attentional properties are thought to be implemented in networks that are hierarchically organized into two distinct levels (Posner & Petersen, 1990). Basic attentional processes, referred to as the posterior attentional systems, are deployed quickly and automatically and usually recruit related system necessary for goal-directed actions. Higher-executive functions, also known as the anterior or supervisory attentional system, provide direction and focus for the operations subserved by the posterior system.

When attention is conceived as a basic building block for the executive function system, it is suggested that attention problems at any point in development will affect emerging executive function abilities (Garon, Bryson, & Smith, 2008). For instance, children with attention problems show deficits in many executive function tasks when compared to typically developing children, as revealed in a number of studies of children with ADHD (Semrud-Clikeman, Walkowiak, Wilkinson, & Butcher, 2010). Furthermore, a hierarchical model of neurodevelopment suggests that early brain damage may have a significant impact on attention development with more global of diffuse implications for later executive function abilities.

Therefore, the understanding that anterior executive functions depend on the efficient execution of the lower-order attentional system, provides a possible explanation for the significant associations found between measures of oxidative phosphorylation dysfunction and measures of executive function in the present study.

Findings from the current study suggest that mitochondrial disease may also detrimentally impact behavior, as implicated by associations found between biochemical and medical indices and measures of internalizing problems, withdrawal, and adaptability. In some regard, it is not surprising that a chronic medical condition, that is known to cause an array of physical and cognitive limitations, will impact how these children perceive themselves and how they choose to interact with others. In fact, the increased risk of major depressive disorder and difficulties in social functioning in children with a chronic medical condition has been well documented in the literature (Curtis & Luby, 2008). Moreover, medical conditions that restrict physical activities or require frequent absences have been associated with social problems, such as being ignored or teased by peers (Lightfoot, Wright, & Sloper, 1999).

Collectively from the study findings, it is unclear whether or not speeded processing and attention are significantly more impacted by biochemical features of MD than other neurocognitive domains. The association found with the Western Blot may reflect the impact that abnormal proteins, that are critical for ATP production, have on processing speed and attention. The significant finding, however, may be also a result of Type I error.

There were a number of considerations when evaluating the study findings. For instance, there were a number of sample-related limitations. First, most of the participants were of average to above average SES and were Caucasian. Therefore, it is unknown how well these findings,

particularly performance on cognitive measures and parent ratings of behavior and adaptive functioning, may generalize to the larger population of MD patients. Second, it is unknown whether parents of children who were higher functioning, compared to parents of children with lower function or greater impairment, may have been more or less invested in participating in this study. Most importantly, the sample size for this study was limited due to the rarity of this condition and children's availability for testing; this was amplified because of missing biochemical data, which limited the extent of regression analyses that could be conducted.

There were also a number of measurement-related limitations to the current study. Most of the attention and processing speed measures had fine motor or oromotor components, which may have slowed the children's performance on these tasks. For instance, 76% of the participants in this study were reported to have motor and muscle-related problems, such as poor balance, motor coordination and strength, and fine motor dexterity. The average participant was rated to have low Motor Skills on the Vineland-II. In addition, some of the children in this study were clearly limited in their language skills, which most likely impacted their abilities to understand some of the more verbal tasks. It is possible that the inclusion of all participants in our analyses, including those who were too impaired to do most of the tasks, may have washed out any subtle distinctions that may have been present for the more functioning children. With a larger sample, statistical analyses could have been controlled for verbal and fine motor skills, which may be potential confounds in this study. Moreover, with a larger sample size, it would have been possible to create a separate group of children who were too severely impaired to be reliably assessed.

Another major issue in this study was mental fatigue. Some of the participants were observed to struggle even when breaks were frequently provided. However, an attempt was

made to minimize the effects of fatigue by testing all participants, with the exclusion of one, in the morning and administering the tests in the same order to make the effects of fatigue more consistent among participants.

**Conclusion**

The current study provides some support for the primary hypotheses that processing speed and attention are markers of abnormal protein expression that interferes with the production of ATP in the oxidative phosphorylation process. To date, no published study has investigated relationships among standardized biochemical features, neuropsychological outcomes, and established diagnostic criteria of Mitochondrial Disease. This study further highlights the level of heterogeneity that exists in the clinical presentation of Mitochondrial Disease. Further studies are needed to better establish relationships between the large spectra of symptoms presented and the underlying neurogenetic and biochemical processes.

Although neural abnormalities, particularly white matter anomalies, have been linked with mitochondrial disease, there is little empirical evidence that elucidates how these abnormalities relate to cognition in this population. Diffusion tensor imaging (DTI) is a new MRI technique that provides non-invasive information about the integrity of white matter by quantitatively analyzing the directionality of axonal fibers (White et al., 2008). DTI has been found to be highly sensitive to subtle white matter abnormalities in neurogenerative disease, even in early stages at which they may be undetectable via conventional MRI. Functional neuroimaging will facilitate further elucidation of the brain-behavior relationships in MD.

Given the wide spectrum of cognitive functioning observed in this study, future studies may also benefit from splitting participants with severe impediments in language functioning or fine motor abilities from those who are higher functioning to capture the subtle relationships

between cognitive and biochemical measures. For instance, participants could be screened using parent ratings on the Vineland-II Functional Communication and Fine Motor Skills. Assessment of children with severe cognitive deficits could be conducted using measures that have lower basal items or relying on parent questionnaires. Lastly, there is a strong need to conduct longitudinal studies to capture the progression of cognitive changes and, at times, loss of acquired skills that is reported in clinical settings but have yet to be well-studied with a large group of patients.

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# Appendix A

## Nijmegen Diagnostic Criteria for Mitochondrial Disease

*Nijmegen Clinical Criteria for Mitochondrial Disease*

| CLINICAL CRITERIA | SCORE |
|---|---|
| Neuromuscular manifestations (Maximum of 2 points) | |
| a. Progressive external ophthalmoplegia (2 points) | |
| b. Ptosis (1 point) | |
| c. Exercise intolerance (1 point) | |
| d. Muscle weakness (1 point) | |
| e. Rhabdomyolysis (1 point) | |
| f. Abnormal electromyogram (1 point) | |
| Central nervous system and other organ involvement (Maximum of 2 points) | |
| g. Isolated central nervous system involvement (1 point) | |
| h. Any other isolated organ system (1 point) | |
| i. Two or more organ systems (2 points) | |
| Metabolic and imaging studies (Maximum of 4 points) | |
| j. Elevated blood lactate on 3 occasions (2 points) | |
| k. Elevated cerebrospinal fluid lactate (2 points) | |
| l. Elevated blood alanine (2 points) | |
| m. Elevated cerebrospinal fluid alanine (2 points) | |
| n. Elevated urine tricarboxylic acid (Kreb) cycle intermediates (2 points) | |
| o. Elevated urine ethylmalonic, 3-methylglutcaonic, or dicarboxylic acids (1 point) | |
| p. Abnormal $^{31}$P-MRS (magnetic resonance spectroscopy) in muscle with reduced Phosphocreatine/$P_i$ ratio (2 points) | |
| q. Abnormal T2 signal in basal ganglia on brain MRI (2 points) | |
| r. Decreased resting metabolic rate or abnormal exercise studies (cycle ergometry protocol) (2 points) | |
| Tissue morphology (Maximum of 4 points) | |
| s. Ragged red fibers on muscle biopsy (2 points if present, 4 points if >2%) | |
| t. Diffuse reduction in cytochrome c oxidase histochemical reaction or scattered COX deficient fibers(4 points) | |
| u. Strongly succinate dehydrogenase positive vessels by histochemistry (1 point) | |
| v. Abnormal mitochondria by electron microscopy (2 points) | |
| **TOTAL** | |

**Scoring for evaluation of Clinical Criteria:**

**Highly probable:** 8-12 points
**Probable:** 5-7 points
**Possible:** 2-4 points
**Unlikely:** 1 point

*Nijmegen Biochemical and Genetic Criteria for Mitochondrial Disease*

| BIOCHEMICAL CRITERIA | SCORE |
|---|---|
| 1. Abnormal high resolution respirometry in muscle or fibroblasts (measurements <5% reference level) (Live (fresh tissue) assessment of Complex V function, coupling and protein leak across the mitochondrial membrane:) | |
| 2. Abnormal OXPHOS subunit immunohistochemistry or immunofluorescece in skeletal muscle tissue sections (Qualitative assessment of OXPHOS enzyme assembly within tissue sections. Defects in OXPHOS enzyme assembly are readily recognized by this testing.) | |
| 3. Abnormal OXPHOS enzymology (single or multiple enzyme defects) (activity measurements <5% reference level) (Testing must be performed on mitochondria isolated from fresh (not frozen) tissue to minimize risk of artifacts caused by freezing skeletal muscle prior to mitochondrial isolation). | |
| 4. Abnormal quantitative Western Blot of Selected OXPHOS subunits from Complexes I-V (levels <5% reference level for subunit) (Western blot can detect defects that are not evident by other techniques. (Oglesbee, Freedenberg, Kramer, Anderson, & Hahn, 2006)) | |
| 5. Abnormal muscle CoQ10 level (<50% of control mean) (Allows assessment for primary defects in Coenzyme Q10 synthesis. (Lopez, et al., 2006)) | |
| 6. Supercomplex evaluation: Optimal OXPHOS function requires aggregation of individual OXPHOS enzymes into supercomplexes which allows efficient and rapid transport of electrons. (Ardehali, Chen, Ko, Mejia-Alvarez, & Marban, 2004; Bianchi, Genova, Parenti Castelli, & Lenaz, 2004; Dudkina, Eubel, Keegstra, Boekema, & Braun, 2005; Genova, Bianchi, & Lenaz, 2003; Schluesener, Rogner, & Poetsch, 2007) Supercomplexes allow efficient formation of an electrochemical (proton) gradient created by Complexes I, III, and IV that is then used by Complex V to synthesize ATP. Supercomplex formation is impaired in a variety of OXPHOS diseases. (Acin-Perez, Fernandez-Silva, Peleato, Perez-Martos, & Enriquez, 2008; Cortes-Hernandez, Vazquez-Memije, & Garcia, 2007; De Meirleir, et al., 2004; Diaz, Fukui, Garcia, & Moraes, 2006; Gerards, et al., 2009; Jesina, et al., 2004; Jonckheere, et al., 2008; Kirby, et al., 2003; Mayr, et al., 2008; Moreno-Loshuertos, et al., 2006; Roels, et al., 2009; Sasarman, Antonicka, & Shoubridge, 2008; Smet, et al., 2009; Ugalde, Janssen, van den Heuvel, Smeitink, & Nijtmans, 2004; Williams, Valnot, Rustin, & Taanman, 2004) | |
| a. Abnormal supercomplex formation (Score 0.5 if present only in Blue Native OR OXPHOS Clear Native Immunoblot. Score 1 if present in BOTH tests.) | |
| b. Abnormal monomeric OXPHOS enzyme complex formation (Score 0.5 if present only in Blue Native OR OXPHOS Clear Native Immunoblot. Score 1 if present in BOTH tests.) | |
| 7. Abnormal in-gel OXPHOS enzyme activity. (Qualitative in-gel assessment of OXPHOS enzyme activity in intact enzymes. This is particularly important in assessment of the ATPase activity of Complex V and Complex V assembly. (Cortes-Hernandez, et al., 2007; De Meirleir, et al., 2004; Jesina, et al., 2004; Jonckheere, et al., 2008; Smet, et al., 2009)) | |
| **TOTAL SCORE** | |

**Scoring for evaluation of Biochemical Criteria**

**Unlikely:** Criteria - A-H are normal
**Possible:** Criteria – a single test is abnormal and the rest are normal or equivocal
**Probable:** Criteria- Two tests are abnormal.
**Highly Probable:** >2 abnormal tests

| GENETIC CRITERIA | SCORE |
|---|---|
| 1. mtDNA depletion (mtDNA copy number <5% reference interval) | |
| 2. Identification of confirmed pathogenic mtDNA or nuclear DNA mutation | |
| 3. Identification of provisional pathogenic mtDNA or nuclear DNA mutation (i.e. mutation requires additional data supporting pathogenicity) | |
| 4. No mutation identified | |

**Scoring for evaluation of Genetic Criteria**
**Definite:** Criteria 1 or 2 are abnormal
**Probable:** Criteria for 3 is abnormal
**Undetermined**: Criteria 4 is present

# Appendix B

## Role of Proteins and Complexes in Oxidative Phosphorylation

Around 90 proteins have been identified that are critical in the oxidative phosphorylation process (Smits, Smeitink, & van den Heuvel, 2010). MD is caused by pathogenic mutations in nuclear or mitochondrial DNA that encode these proteins. In MD, nuclear DNA (nDNA) mutations are passed down by autosomal recessive, autosomal dominant, X-linked, or, more rarely, isodisomy (chromosomes in a pair are inherited from one parent ) mechanism (Shoubridge, 2001). Mitchondrial DNA (mtDNA) is different from nDNA in that it can only be inherited maternally. A mixture of normal and abnormal mtDNAs can be present in MD.

Understanding the variable nature of the genotype-phenotype relationship in MD is a challenging process (Smits, et al., 2010). For instance, the same mutation in a gene can present with very different clinical manifestations, whereas the same clinical phenotype can be caused by different mutations (Bernier, et al., 2002; Lamont, et al., 1998). The variability in symptoms may be due to several factors, including the ratio of wild-type to mutant mtDNA, varying thresholds of biochemical expression for both the mutation and the tissue involved, and the controlling effect of nuclear and other mitochondrial genes (Leonard & Schapira, 2000). Given that studies are still underway to locate the genes that are responsible for mitochondrial dysfunction, the identification of a pathogenic mutation is not required in the diagnosis of MD as long as there is sufficient evidence of clinical and biochemical anomalies related to oxphos defects (Morava, et al., 2006).

The Table below provides a summary of the individual roles the different complexes play in ATP production.

| | |
|---|---|
| Complex I | Removes electrons from NADH and transfers them to ubiquionone, a lipid-soluble carrier. It is also responsible for moving protons across the membrane to produce a proton gradient. |
| Complex II | Serves to channel additional electrons into the quinone pool by removing electrons from electron donors, such as succinate, and transferring them to ubiquinone. |
| Complex III | Removes electrons from ubiquinol, which is the reduced product from Complex I, and transfers them to cytochrome c, a water-soluble electron carrier located within the inter-membrane space When electron transfer is delayed by a high membrane potential, point mutations, or respiratory inhibitors, Complex III leaks electrons to oxygen resulting in the formation of highly toxic superoxide, which is thought to contribute to the pathology of a number of diseases. |
| Complex IV | Involved in removing electrons from cytochrome c and transferring them to molecular oxygen, producing molecules of water. Furthermore, it contributes to the proton gradient by moving protons across the membrane. |
| Complex V | As the protons pass through Complex V, the osmotic energy of the gradient is converted into chemical energy in the form of ATP. This transmembrane proton gradient is used to drive the endergonic reaction of ATP synthesis. |

# Appendix C

## List of Tests Administered Based on Age

*Tests Administered*

| DOMAIN/Subtests | Administered Ages |
|---|---|
| PROCESSING SPEED | |
| Speed of Information Processing (DAS-II) | 5-13 |
| Rapid Naming (DAS-II) | 5-13 |
| ATTENTION | |
| Auditory Attention (NEPSY-II) | 5-12 |
| EXECUTIVE FUNCTIONING | |
| Response Set (NEPSY-II) | 5-12 |
| Inhibition (NEPSY-II) | |
| Naming | 5-13 |
| Inhibition | 5-13 |
| Switching | 7-13 |
| Design Fluency (NEPSY-II) | 5-13 |
| VERBAL COMPREHENSION | |
| Word Definition (DAS-II) | 5-13 |
| Verbal Similarities (DAS-II) | 5-13 |
| PERCEPTUAL REASONING | |
| Sequential Quantitative Reasoning (DAS-II) | 5-13 |
| Pattern Construction (DAS-II) | 4-13 |
| WORKING MEMORY | |
| Recall of Sequential Order (DAS-II) | 5-13 |
| Recall of Digits Backwards (DAS-II) | 5-13 |
| VISUAL AND SENSORIMOTOR | |
| Arrows (NEPSY-II) | 5-13 |
| Fingertip Tapping (NEPSY-II) | 5-13 |
| Imitating Hand Positions (NEPSY-II) | 4-12 |

| | |
|---|---|
| WORD RETRIEVAL | |
| Word Generation (NEPSY-II) | |
| Semantic | 4-13 |
| Initial Letter | 7-13 |
| MEMORY | |
| List Memory (NEPSY-II) | 7-12 |
| Memory for Faces (NEPSY-II) | 7-12 |
| SOCIAL PERCEPTION | |
| Affect Recognition (NEPSY-II) | 4-13 |
| READING | |
| Word Reading (WIAT-II) | 4-13 |
| MATH | |
| Numerical Operations (WIAT-II) | 5-13 |