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Distinct Neuropsychological Profile and Associated Neurochemical Changes in Individuals with Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-Like Episodes (MELAS)

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DISTINCT NEUROPSYCHOLOGICAL PROFILE AND ASSOCIATED NEUROCHEMICAL CHANGES IN INDIVIDUALS WITH MITOCHONDRIAL ENCEPHALOMYOPATHY, LACTIC ACIDOSIS, AND STROKE-LIKE EPISODES (MELAS)

By

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ABSTRACT

Distinct Neuropsychological Profile and Associated Neurochemical Changes in Individuals with Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-Like Episodes (MELAS)

by

Emily B. Leaffer, MA, MPH

Advisor: Nancy S. Foldi, PhD
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Background: Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like Episodes (MELAS) is a maternally inherited progressive multisystemic disorder. Occurrence of seizure and/or stroke-like episode, as well as classic biomarkers (i.e., cerebral lactic acidosis, depleted N-acetylaspartate (NAA)), have been linked to neuropsychological deficit and trigger the developmental cascade of neurodegeneration affecting posterior prior to anterior brain regions. While a pattern of global deterioration has been reported, systematic examination in a large MELAS cohort has not been conducted.

Objective: First, we examined verbal and visual memory function and its relationship to brain metabolites (lactate, NAA) in individuals with MELAS. Second, we hypothesized that MELAS would perform worse overall and show a selective profile of decline based on localization of seizure/stroke-like episode and increased lactate/decreased NAA, such that posterior-localized visually-mediated functions will show faster decline compared with anterior-localized verbally-mediated functions.

Methods: Cognition was examined over time in 35 MELAS, 78 mt3243A>G carriers, and 28 controls (16-80 years). Composite z-scores were used for global mental status, attention, speed,
mental flexibility, reasoning, language, verbal and visual memory, and visual-spatial skills, as well as for visually-mediated and verbally-mediated tasks. MR Spectroscopy (NAA, Lactate), Chi-square, t-tests, Pearson correlations, ANOVA, and Generalized Estimating Equations were run ($\alpha=0.05$).

**Results:** When compared to carriers and controls, MELAS patients had: 1) most impaired memory functions (Visual: $p=0.0003$; Verbal: $p=0.02$), 2) greater visual than verbal memory impairment, 3) highest brain lactate levels ($p<0.0001$), and 4) lowest brain NAA levels ($p=0.0003$). At baseline, MELAS performed significantly worse across all cognitive domains ($p=0.00$) and performance correlated with neurochemical biomarkers ($p=0.00$). MELAS exhibited faster decline in visual-spatial skills ($p=0.00$), speed ($p=0.045$), and global mental status ($p=0.02$); moreover, visually-mediated tasks declined faster in MELAS ($p=0.02$), while verbally-mediated tasks did not ($p=0.77$).

**Conclusions:** Individuals with MELAS are at increased risk for poor memory, and although both verbal and visual memory are impaired, visual memory is both preferentially affected and more clearly associated with brain metabolite levels. In MELAS, there is an early vulnerability of the posterior brain structures, which results in a distinctive progressive cognitive phenotype with a posterior to anterior hemispheric gradient, such that visual memory is targeted before verbal memory. MELAS show worse cognitive function overall and faster decline in global mental status, speed, and visual-spatial skills. Faster decline in visual, not verbal, tasks supports the notion that a distinct neurodegenerative profile exists affecting posterior brain regions and associated cognitive functions in particular.
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DEDICATION

To my husband, Matthew –

I could not have done this without you. Thank you for everything. Love you more.
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BACKGROUND AND SIGNIFICANCE

Introduction.

Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) is a progressive multisystemic disorder of the central nervous system (Hirano, 1992; Sproule, 2008; Kaufmann, 2009). MELAS is defined by six cardinal characteristics: 1) exercise intolerance, 2) onset prior to age 40 years old, 3) stroke, 4) seizures, 5) ragged-red fibers, and 6) lactic acidosis (Hirano, 1994). Secondary manifestations affecting cardiac, renal, endocrine, and gastrointestinal systems, as well as cognitive and psychiatric involvement, also occur in most individuals diagnosed with the disorder (Hirano, 1992; Sproule, 2008; Kaufmann, 2009).

MELAS results from mitochondrial DNA (mtDNA) mutations of genes encoding for transfer RNA (tRNA) proteins. The mtDNA is passed down through matrilineal relatives. In about 80% of the cases, MELAS is due to an Adenosine (A) to Guanine (G) transition at nucleotide mt3243A>G in the tRNA. Individuals with the mt3243A>G gene mutation develop with affected mitochondria that essentially work much less efficiently than those without the mutation. Over time, the developmental consequences can be devastating, due to a complex series of underlying neurochemical changes. In the brain, this effectively results in decreased energy supplies to affected neurons, glia and blood vessels, leading to decreased cellular oxygen consumption, increasing lactic acid levels, neuronal hyper-excitability, increased capillary permeability, and eventually resulting in seizures and/or stroke-like episodes, which in turn may set off additional metabolic cascades with lethal neurodegenerative consequences.

However, not all individuals with the mt3243A>G mutation develop the full MELAS phenotype with seizures and stroke-like episodes, and even among carriers there is wide variation in presentation. Carriers experience some of the early underlying neurochemical
changes, and they are at increased risk for symptoms of migraine, deafness, muscle weakness, and diabetes; but it is unknown whether select cognitive sequela occurs. Outcomes of individuals with MELAS involve a wide spectrum of disability, significant morbidity, and potential early mortality (Kaufmann, 2009). Outcomes of carriers who do not convert to the full MELAS phenotype are also associated with variable morbidity. For both groups, little is known about the neuropsychological consequences.

First, this paper reviews established knowledge about MELAS with regards to underlying genetics and pathophysiology, clinical phenotype, disease prognosis, and cognition. The purpose is to demonstrate the neuropsychological significance of the disorder and lay the groundwork for neuropsychological study. Individuals with MELAS provide a model for study of brain localization of function and progressive cognitive decline. Carriers of the mt3243A>G mutation allow for investigation of possible prodromal cognitive characteristics that may impact the cognitive phenotype. Second, given the paucity of neuropsychological data on individuals affected with this condition, the study presented will provide invaluable information both in understanding brain-cognition and brain-behavior relationships and aiding characterization of the functional consequences of the mutation.

History. MELAS was originally identified in 1984 with the identification of cardinal characteristics including ragged red fibers (abnormal muscle cells identified on staining with thickened edge, reflecting accumulation of abnormal mitochondria below the plasma membrane of the muscle fiber) found in muscle, short stature, seizures, hemiparesis, hemianopia, cortical blindness, and frequently a build-up of lactate (Hirano, 1992; Pavlakis, 1984). Early case report descriptions were used to first classify primary clinical features of the disease (Askanas, 1978;
Skoglund, 1979; Hart, 1977; Shapira, 1975; Pavlakis, 1984) and additional cases displayed some, but not all, of the identified frequently occurring symptoms (Hackett, 1973; Markesbery, 1979; Monnens, 1975). Subsequent seminal accounts identified the necessary criteria for a MELAS diagnosis to include 1) a stroke-like episode prior to age 40, 2) encephalopathy expressed as seizures or dementia or both, and 3) lactic acidosis (a neurochemical reaction of insufficient oxygen to power mitochondrial ATP conversion, and subsequent increased glycolysis, resulting in lactic acid blood and/or cerebrospinal fluid (CSF) build-up and lowering of overall pH) or ragged-red fibers (Hirano, 1992). A study of the clinical features in 110 cases of MELAS further refined the top six cardinal manifestations of the disease to include: 1) exercise intolerance, 2) onset prior to age 40 years old, 3) stroke, 4) seizures, 5) ragged-red fibers, and 6) lactic acidosis (Hirano, 1994). Since its original inception over 30 years ago, the clinical spectrum of MELAS has been well-defined (Hirano, 1992; Hirano, 1994; Damian, 1995; Morovvati, 2002; Huang, 2002; Chinnery, 1997; Chinnery, 1998; Kaufmann, 2009).

**Epidemiology.** The exact prevalence of individuals affected with MELAS is unknown, in part due to the variation in the clinical presentation of the disease contributing to difficulty in clinical diagnosis (Lightowlers, 2015), sensitivity of tests for MELAS-specific mutation, patient and family desire to know whether mutation is present (Majamaa, 1998), as well as the differences in research methodology, participant groups, and geographic location (Sproule, 2008).

The original epidemiological study of adults with the MELAS mutation was conducted in Finland and revealed an estimated prevalence of more than 10.2 per 100,000 individuals; if it is correctly assumed that all first-degree maternal relatives also carry the mutation, estimated
prevalence increases to 16.3 per 100,000 individuals (Majamaa, 1998). Prevalence of the MELAS mutation in the North East of England ranges from 0.95 per 100,000 (Chinnery, 2000a) to 3.65 per 100,000 individuals (Shaefer, 2008), to the most recent estimate of 3.5 per 100,000 individuals (Gorman, 2015). These findings indicate the overall stability in mitochondrial disease rates over time and within geographic location, and the MELAS mutation remains the highest in Swedish (8.9 in 100,000; Darin, 2001) and Finnish (18.4 per 100,000; Uusimaa, 2007) children. A recent epidemiological population-based study in Australia revealed the prevalence of MELAS to be 236 per 100,000 individuals (Manwaring, 2007), a pattern which is much higher than previously reported, suggesting that (a) the contribution of varying prevalence rates based upon differing methodologies and geographic locations or (b) most likely, that the mt3243A>G mutation is frequently overlooked and subsequently under-diagnosed. Nonetheless, MELAS remains one of the most prevalent mitochondrial disorders. Of those who carry the MELAS mutation, the prevalence of those clinically affected was found to be 5.71 per 100,000 individuals (Majamaa, 1998), suggesting a disease burden that needs to be further examined and addressed.

**Mitochondrial Function.**

**Typical Mitochondria Function, Genetics, and Pathophysiology.** Mitochondria are energy-generating structures located inside cells within the cytoplasmic fluid surrounding the cell nucleus. Mitochondria are necessary for oxygen usage, protein production, cellular transport, and power provision to cells and the rest of the body. Each mitochondrion is made up of its own genetic material called mitochondrial deoxyribonucleic acid (mtDNA). This type of deoxyribonucleic acid (DNA) is separate from the majority of DNA that is typically found in the
cell’s nucleus. The primary function of mitochondria is metabolic by which energy is produced from food, facilitating a process called oxidative phosphorylation (OXPHOS). OXPHOS utilizes oxygen and simple sugars to transform adenosine diphosphate (ADP) to adenosine triphosphate (ATP). ATP is the molecule that is used to transfer and expend the energy produced by mitochondria (DiMauro, 2008; DiMauro, 2013a). The impaired OXPHOS process is believed to have given rise to many mitochondrial diseases.

Human mtDNA consists of 37 genes, 13 structural proteins, two ribosomal ribonucleic acids (rRNA), and 22 transfer ribonucleic acids (tRNA). Each of the structural proteins is composed of subunits within five different complexes (Complex I, II, III, IV, and V) (Figure 1), which work together to achieve this necessary energy production (Davis, 2011). Complexes I through IV are connected by subcomponents, coenzyme Q and cytochrome c, and together they compose the mitochondrial respiratory chain. The mitochondrial respiratory chain, located in the inside of the mitochondria membrane, is responsible for facilitating the process of ATP production (Davis, 2011). While OXPHOS is the process responsible for the oxidation of NADH and FADH₂, Complex I (NADH: ubiquinone oxidoreductase) is the first enzyme within the respiratory chain and contributes electrons for NADH and Complex II (Succinate: ubiquinone oxidoreductase) provides electrons for FADH₂. Complexes I, III (Ubiquinol: ferricytochrome c oxidoreductase), and IV (Ferricytochrome c: oxygen oxidoreductase) transfer electrons and translocate protons across the mitochondria membrane to the organelle. Complex III is the middle segment of the respiratory chain, also known as the cytochrome bc₁ complex. Complex IV is also the end of the respiratory chain by which electrons are transformed to oxygen and reduced to water in order to purge expended low-energy electrons; however, protons continue to be translocated by this enzyme complex. During the movement of protons, Complex
V (ATP synthase) independently creates heat and transforms ADP to ATP. Thus, Complex V may be primarily responsible for ATP synthesis; however, OXPHOS is the result of all enzymes arranged within all five multiprotein lipid complexes necessary for energy production. Notably, Complex I, III, IV, and V are encoded by both nuclear DNA (nDNA) and mtDNA, whereas Complex II is only encoded by nDNA (Taanman, 2002; DiMauro, 2008; DiMauro, 2013a).

![Mitochondrial respiratory chain](image)

**Figure 1.** Mitochondrial respiratory chain  
Source: DiMauro, 2008

Three characteristics are specific to mtDNA: maternal inheritance, heteroplasmy, and mitotic segregation. *Maternal inheritance* refers to the fact that all mtDNA mutations are transmitted from the mother to her offspring. This is due to the mitochondrial DNA being localized to mitochondria in the cell’s cytoplasm, which is entirely passed on by the egg gamete and not the sperm. As such, only females will then pass the mutation onto future offspring. *Heteroplasmy* occurs when mutant mtDNA are present with normal mtDNA. Normally, all copies of functioning mtDNA are identical, a state termed homoplasmy. However, with mutation, the mutated copies are mixed with the normal mtDNA throughout the egg cell’s cytoplasm, thus being mixed in cells throughout the developing organism’s body. The higher the percentage of heteroplasmy (0 – 100%) that is present, the greater the mitochondrial dysfunction
that will result. A threshold effect has been postulated that reflects the point where the number of mutated mtDNA exceeds the point where mitochondrial function is unchanged and results in clinical manifestations. Mitotic segregation explains that the ratio of normal to mutant mtDNA in the mother’s cell may not be the same as the ratio present in the offspring, such that the mother could have a higher percentage of mutant mtDNA (and thus expression of greater mitochondrial dysfunction with presentation of a more severe phenotype), while the offspring has a lower percentage, or vice-a-versa (Davis, 2011). Each of these three principles applies to the mitochondrial dysfunction that underlies MELAS transmission, acquisition, and expression. The combined effects of heteroplasm, threshold and mitotic segregation contribute to the intra-individual and inter-individual variation of phenotypes.

**Mitochondrial dysfunction in MELAS.** MELAS is a polygenetic disorder, typically characterized by a mutation in the MT-TL1 gene that causes an adenine to guanine transition at the nucleotide position 3243, commonly referred to as mt3243A>G (Figure 2). This particular mutation gives rise to approximately 80% of MELAS cases; however, MELAS has also been associated with the involvement of several other genes and at least 29 different point mutations have been identified on mitochondrial tRNA and protein coding genes (Wong, 2007; DiMauro, 2008; DiMauro, 2013a; DiMauro, 2013b). A point mutation is a single change in the DNA sequence that could involve a base pair change, insertion, or deletion (gene review glossary). tRNA is required for protein synthesis (Scaglia, 2012), and these identified genes associated with MELAS give instructions for making the tRNA molecules necessary to produce proteins in the mitochondria. Given that the type of mutation associated with the MELAS syndrome is a tRNA point mutation, mitochondrial protein synthesis is reduced (Davis, 2011). These genes are also
associated with providing instructions to create proteins in Complex I, a process essential for OXPHOS to occur. The phenotype associated with mtDNA point mutations has not only been identified in the MELAS population, but also in other mitochondrial disorders such as myoclonic epilepsy with ragged red fibers (MERRF), Leber’s hereditary optic neuropathy (LHON), and Leigh syndrome (LS). Although these mitochondrial syndromes share common genetic mutations, variations in clinical features make them distinct from one another (Wong, 2007; Sproule, 2008).

Mitochondrial DNA point mutations found in MELAS prevent proper protein creation, resulting in decreased oxygen usage and energy production. The effects of these genetic mutations associated with MELAS are neurochemical defects within the respiratory chain of complexes I, II, III, and IV, as well as oxidative phosphorylation of complex V. From a neurochemical perspective, deficient Complex I activity appears to be most commonly responsible for many of the phenotypic characteristics of MELAS. However, altered Complex III activity, and cytochrome b in particular, has been associated with the development of myopathy (or weakness) in the MELAS population. Reduced Complex IV activity has been implicated as well (Zeviani, 2007; Leonard, 2000; Schapira, 2002; Taanman, 2002; Schon, 2002; DiMauro, 2008; DiMauro, 2013a; DiMauro, 2013b).
There is a detrimental cascading effect that results from mitochondrial dysfunction, such that essentially all cells housing mutated mitochondria may not have enough energy to function optimally. When this happens, mitochondrial disorders such as MELAS may result.

**Mechanism underlying of brain function.**

The percentage of mutated mitochondria within cells varies from person to person (heteroplasmy) and gives rise to the spectrum of the mt3243A>G-associated phenotype, such that the more mutated mitochondria present within each cell and the more cells that house
mutated mitochondria, the worse the phenotype. Additionally, there are differences in severity among individuals (even within the same family) and differences in severity across organ systems (even within the same individual). These differences are likely due to mitotic segregation, such that the cytoplasmic location where the mutated mitochondria are in the fertilized egg at the start of cell division may influence where they end up at the end of development and cellular differentiation. One study identified higher overall proportion of gene mutation in MELAS patients compared with asymptomatic gene carriers (Vydt, 2007). Therefore, some carriers and affected individuals may have more pronounced brain involvement while others have minimal involvement, despite the same genetic basis for disease.

Differential heteroplasmy and disease severity may well account for the variation in brain involvement across mt3243A>G carriers. Underlying neurochemical changes due to decreased energy production of the mutated mitochondria are presumed to interfere with optimal neuronal and brain function, and the severity is likely related to percentage of mutated mitochondria within cells within brain tissue. There may be a threshold effect where the cells function adequately with some mutated mitochondria, but should the level exceed threshold, detrimental neurochemical changes result. Should these changes result in seizures and stroke-like episodes, then the individual becomes diagnosed with MELAS. And, with seizures and stroke-like episodes, further neurodegeneration and dementia may result as a consequence.

Mitochondrial disorders are frequently diagnosed when the presence of high lactate levels is identified in blood and ventricle CSF, a pattern present in approximately 94% of MELAS patients (Sproule, 2008). This classic chemical feature of cerebral lactic acidosis causes further cell damage due to the low amount of energy and the cells are unable to maintain normal functions necessary for cellular health, such as excreting waste products efficiently or bringing in
necessary nutrients through active transport. As a result of these detrimental effects, cerebral lactate has been linked to neuropsychological deficits in the MELAS population, such that the higher the level of ventricular lactate, the higher the rate of global neuropsychological dysfunction and the lower the level of neurological functioning (Kaufmann, 2004).

Brain morphological findings in individuals with MELAS include atrophy (degeneration or wasting away of body tissue or organ), calcifications (accumulation of calcium salts in body tissue), hyperintensities (heightened focal or diffuse white and/or grey matter regions) (Finsterer, 2006). The stroke-like episodes and associated decreased utilization of oxygen likely results from the deficient OXPHOS process that contributes to: 1) poor vasodilation (widening of blood vessels) secondary to mitochondrial angiopathy (disease of the blood vessels), and 2) cytotoxic damage secondary to mitochondrial cytopathy (disease state of organs of continuing energy failure without sufficient ATP production) (Koenig, 2016; Ohama, 1987; Kishi, 1988; Sproule, 2008; Xie, 2014). Episodes are likely due to neuronal hyper-excitability resulting from extracellular ion concentration imbalance or membrane instability due to mitochondrial dysfunction in either neuronal (neurons or astrocytes) or capillary endothelial cells (Iizuka, 2005).

Functionally, there are many CNS abnormalities that have been identified in the population of mitochondrial disorders with dementia, including MELAS. More specifically, hypoperfusion (decreased blood and oxygen flow to organs and tissues, also known as shock) observed on single-photon emission computed tomography (SPECT), focal hypometabolism (impaired cerebral glucose uptake) observed on fluorodeoxyglucose-positron emission tomography (FDG-PET), increased lactate, decreased choline, decreased creatine, decreased N-acetylaspartate (NAA) observed on MR spectroscopy (MRS), focal or diffuse slowing and
paroxysms in the occipital and parietal regions brain observed on electroencephalogram (EEG), and prolonged latencies and decreased amplitudes on visual evoked potential tests (VEP) (Finsterer, 2009).

As noted, once a mt3243A>G carrier experiences a stroke-like episode or a seizure, their clinical diagnosis changes and the individual becomes classified as a fully symptomatic MELAS patient. It is postulated that the underlying neurochemical changes stimulated by mitochondrial dysfunction resulting in increased build-up of lactic acid precede these distinct phenotypic outcomes. Deficient OXPHOS process function paired with increased brain lactate production has been evident in both symptomatic carriers and MELAS patients (Dubeau, 2000); however, the rate of accumulation is higher in MELAS patients compared to carriers (Kaufmann, 2011). One large prospective proton MRS study indicated that MELAS patients had higher lactate and lower NAA, while mutation carriers had higher lactate, NAA and total creatine when each group was compared to unaffected healthy controls. More specifically, the amount of ventricular lactate increased with presentation, such that unaffected healthy controls had the least amount of lactate, followed by mt3243A>G mutation carriers who later who did not convert to MELAS when followed over time, followed by mt3243A>G carriers who subsequently converted to the full MELAS phenotype, and lastly the most lactate was found in the MELAS patients. Gray matter creatine and choline were also higher in converters compared to controls. In connection with cognitive and behavioral outcome, lower NAA correlated with worse neuropsychological functioning, whereas higher ventricular lactate was associated with poorer neurological functioning (Weiduschat, 2014). These results are suggestive of an active disease progression reflecting underlying neurochemical changes among carriers, preceding stroke-like episodes and seizures necessary for a clinical MELAS diagnosis.
Alternatively, Iizuka and Sakai (2005) have proposed that stroke-like episodes are due to an increased neuronal excitability resulting from changes in intracellular ion homeostasis and increased membrane permeability. By examining serial brain MRIs in patients with MELAS who suffered stroke-like episodes, they observed changes that extended beyond the initial localized brain region where the stroke-like episode occurred, such that over time adjacent neurons depolarized resulting in epileptic activities in the surrounding cortex (Iizuka, 2005).

Among individuals with MELAS, it has been noted that seizures frequently primarily occur in occipital and parietal brain regions (Veggiotti, 1995; Canafoglia, 2001; Iizuka, 2003; Iizuka, 2005) and stroke-like episodes typically occur first in the occipital lobe and then eventually progress to temporal and parietal regions of the brain (Sue, 1998; Iizuka, 2003; Iizuka, 2005). Thus, the underlying neurochemical changes that lead up to the presenting phenotypic features of stroke and seizure appear to result in a preferentially localized target – one that first affects the rear portions of the brain before more frontal structural involvement. This may be related to the normal distribution of the mitochondria throughout the brain; mitochondria have been shown to more densely populate occipital regions, perhaps leaving them more susceptible to damage when the mitochondria are impaired (Turnbull, 2010; Berti, 2014). Moreover, resultant consequences of seizures and/or strokes also lead to detrimental biochemical cascades including excess calcium and glutamate release from the damaged cells, which, in turn, increases calcium influx into surrounding cells, causing excitotoxicity, failure of the mitochondria, apoptosis, and damage to the blood-brain barrier (causing brain swelling and tissue damage) (Iizuka, 2005). The experience of seizures and stroke-like episodes, combined with the subsequent cascade of metabolic changes, results in progressive brain damage and
neurodegenerative process likely leading to disability, morbidity, and in some cases, premature death (Figure 3).

**Figure 3.** mt3243A>G mutation inheritance and progression

While mitochondrial dysfunction may affect most, if not all, energy processes and bodily systems, those specific to MELAS are described below. Particular attention is given to the discussion of the cognitive impairment and psychiatric disturbances experienced by the MELAS
population, followed by the limited available literature on the cognitive phenotype of the mt3243A>G carriers who have not converted to the full MELAS phenotype.

Clinical Presentation: MELAS and mt3243A>G carriers.

Affected MELAS.

Those affected with MELAS express primary clinical characteristics that are accompanied by varying degrees of secondary neurological and non-neurological symptomatology, with 90% of cases presenting with symptoms between 20 and 40 years of age (Hirano, 1994). More specifically, headache/migraines, balance difficulty, clumsiness, progressive muscle impairment (e.g., ataxia, limb weakness, myopathy), myoclonus (sudden involuntary jerking of a muscle or group of muscles), peripheral neuropathy (or nerve damage), loss of sensation, developmental symptoms (e.g., perinatal difficulties, motor developmental delay, speech developmental delay, reading/learning difficulties, special education classes), cardiac symptoms (e.g., cardiomyopathy resulting from high-energy demands that may not be met, conduction defects), and systematic symptoms [e.g., exercise intolerance, loss of hearing and hearing aids (Vandana, 2016), gastrointestinal disturbance (e.g., constipation, discomfort, hepatopathy), growth failure manifesting as short stature, hirsutism (unwanted, male-pattern hair growth in women), ptosis (droopy eyelids), diabetes mellitus, renal disease, optic atrophy and night blindness] are not uncommon. Rare instances of dermatologic (e.g., vitiligo, or skin loses pigment, and other pigment changes) and pulmonary (hypertension) symptoms have also been reported. Additionally, earlier onset of medical concerns (e.g., migraines, clumsiness, myoclonus), cognitive difficulties (e.g., memory problems) and psychiatric symptoms (e.g.,
depression, hallucinations, delusions) are frequently reported when compared with carrier relatives and the general population (Sproule, 2008; Kaufmann, 2009).

Cognition & Behavior in Mitochondrial Encephalopathy. Mitochondria encephalopathy has been associated with a specific type of cognitive disorder entitled Mitochondrial Dementia (MD), evident in 40-90% of individuals with MELAS, which likely results from both cortical injury due to seizures and stroke and pre-existing neuronal dysfunction and deterioration caused by impaired mitochondrial function (Hirano, 1994; Yatsuga, 2012; DiMauro, 2013a). The clinical presentation of MD is typically characterized by a diagnosis of cognitive impairment based upon neuropsychological testing and neuroimaging techniques, as well as various behavioral manifestations (Finsterer, 2008). However, surprisingly little work has thoroughly investigated the neuropsychological profile of the dementia associated with MELAS, and more careful delineation of the profile is necessary to better understand the course of the illness. The work that has been published will be reviewed below.

Cognition in MELAS. Individuals with MD present with impaired cognitive functioning across domains of attention, executive functioning, language, visual-spatial ability, and memory (Finsterer, 2008). The presence of cognitive impairment in individuals diagnosed with MELAS has been reported based upon clinical report (Kartsounis, 1992), single case studies, longitudinal examination of single cases, and a few cross-sectional studies, many of which are limited by methodological weakness (e.g., small sample size, assessed by clinical means only, described in
general terms). Nonetheless, these accounts describe initial cognitive impairment with profound deterioration of cognitive function over time among individuals diagnosed with MELAS.

More specifically, primary case studies have reported observed difficulties in domains of executive functioning (e.g., mental flexibility, set-shifting) (Coomans, 2011; Neargarder, 2007; Sartor, 2002; Hirano, 1994; Lang, 1995), visuo-spatial ability (Sartor, 2002; Neargarder, 2007; Kiejna, 2002; Pachalska, 2002), language (Neargarder, 2007; Kiejna, 2002; Pachalska, 2002), memory (Neargarder, 2007; Kiejna, 2002; Pachalska, 2002), attention (Neargarder, 2007), processing speed (Sproule, 2008) and motor skills (Neargarder, 2007). Although one study identified a relative strength in verbal memory ability in two patients with MELAS, performance in this area was still impaired compared to normative data (Neargarder, 2007). Additional cognitive manifestations observed in cases of MELAS include prosopagnosia, topographical disorientation (Funakawa, 1994) and auditory agnosia (Miceli, 2008; Johns, 1993; Kamada, 2001; Tawankanjanachot, 2005; Chinnery, 2000b). Further, findings also indicate that individuals with MELAS exhibit global cognitive impairment with deterioration over time in both intellectual and cognitive abilities (Neargarder, 2007; Majamaa-Voltti, 2006; Chu, 2012; Lacey, 2008; Kiejna, 2002; Pachalska, 2002; Kaufman, 2010; Kaufmann, 2011; Lang, 1995; Nesbitt, 2013) as well as demonstrate a progressive decline in mental status (Kiejna, 2002).

Two longitudinal case studies of MELAS patients, one tested four times over the course of two years (Pachalska, 2002) and another tested twice with 5 months in between (Kiejna, 2002) described a profound decline in both verbal and non-verbal intelligence, memory (i.e., immediate/delayed logical memory and visual recall), visuospatial functioning (i.e., line orientation, facial recognition), and language ability (i.e., verbal expression, reading, writing) (Pachalska, 2002; Kiejna, 2002). A four-year follow-up study of a single patient with MELAS
revealed reduced basic attention, working memory, executive functioning (e.g., figural fluency, planning, problem solving), and visuo-con constructive abilities; in contrast, verbal learning and memory remained preserved and previously impaired language (e.g., comprehension, repetition, writing) appeared to have improved between baseline and follow-up evaluations (Sartor, 2002). A two-year follow-up study of a patient with MELAS (associated mutation different from mt3243A>G) found reduced cognitive function characterized by language (e.g., initiative, fluency, comprehension, communication) and executive functioning (e.g., set-shifting) impairment, while verbal/visual learning and memory and aspects of attention and praxis remained reduced compared to normative data though no decline was observed over time (Salsano, 2011).

Compared with other mitochondrial encephalopathies, individuals with MELAS appear to exhibit more significant cognitive deficits. Specifically, in a study that compared MELAS patients with four other ME (i.e., progressive external ophthalmoplegia (PEO), Kearns-Sayre syndrome (KSS), myoclonus epilepsy with ragged red fibers (MERRF)), individuals with MELAS were found to have greater cognitive impairment in areas of non-verbal intelligence, orientation, attention, executive ability, memory, generative language, arithmetic ability, and drawing skills (Lang, 1995). Another comparison study of cognition in MELAS versus MERRF revealed that individuals with MELAS displayed greater deficit in areas of abstract reasoning, language (e.g., naming, fluency), attention, visual-spatial ability, and verbal and visual memory (Kaufmann, 2004).

As part of a natural history study of 35 patients with MELAS associated with the mt3243A>G mutation, Kaufmann et al. reported a global neuropsychological score, ranging from 0 to 3, such that the categorical score ranged from normal cognitive performance (= 0) to
severely impaired cognitive performance (= 3), which prevented formal testing from being performed. When examined at baseline and follow-up at years 1 and 4, findings indicated that progressive cognitive decline was present in all individuals affected by MELAS. Notably, decline was not observed in their carrier relatives. The neuropsychological global score was an aggregate of performance on standardized tasks in areas of memory, language, attention, reasoning skills, and visual-spatial functioning (Kaufmann, 2011). These data are the best representation of what occurs over time in a group with confirmed MELAS currently available, yet the process of assigning categorical scores and making a global composite allows for generalized findings, but does not allow for detailed exploration of the neuropsychological profile. More in depth analyses of these data will allow for exploration of whether selected domains may be preferentially compromised.

**Behavior in MELAS.** The effects of MELAS as a multi-systemic chronic illness on behavior are evident in the literature. Patients commonly experience depression (Magner, 2014; Angelin, 2012; Scaglia, 2010; Ryu, 2009), anxiety (Miyaoka, 1997; Angelin, 2012), apathy (Salsano, 2011; Koller, 2003), and personality changes (Amemiya, 2000; Angelin, 2012). A longitudinal case report revealed increasing symptoms of depression, anxiety, and irritability over time (Pachalska, 2002; Kiejna, 2002). In addition to increased aggression (Kaufman, 2010; Koller, 2003), loss of impulse control (Koller, 2003), and an increased prevalence of frontal lobe syndrome (personality change, loss of initiative, psychomotor retardation, social withdrawal, odd behavior, decreased activity, reduced volition, apathy, blunted affect, and diminished self-care), was identified this population (Angelin, 2012; Inagaki, 1997; Koller, 2003; Sartor, 2002).
Symptoms of obsessive-compulsive disorder (Lacey, 2008) and mutism (Coomans, 2011) were also reported in individuals who have the mt3243A>G mutation.

Common psychiatric disturbances reported by patients with MELAS include psychotic symptoms (Magner, 2014; Angelin, 2012), including hallucinations (e.g., visual) (Scaglia, 2010; Kiejna, 2002; Sartor, 2002; Kaufman, 2010), delusions (Scaglia, 2010; Kaufman, 2010), and confusion or delirium (Magner, 2014; Coomans, 2011; Kaufman, 2010; Feddersen, 2003). Encephalopathic psychosis has also been specifically identified in this population (Suzuki, 1997; Sharfstein, 1999; Amemiya, 2000; Patel, 2007), as well as episodes of aggression and confusion (Feddersen, 2003). The prevalence of psychosis in patients with MELAS has been found to range from 7% (Oexle, 1997) to about 17% (Iizuka, 2007). It is important to note that many instances of psychotic symptoms are present during stroke-like episodes (Sano, 1995; Sartor, 2002); however, evidence supports the presence of psychiatric symptoms that were frequently reported prior to a diagnosis of MELAS (Fattal, 2006). Angelin et al. have proposed that identified white matter abnormalities present in a majority of MELAS patients possibly interrupt the networks involved in the regulation of emotion and behavior (Angelin, 2012).

Taken together, individuals affected with MELAS experience behavioral and psychiatric disturbances to a greater extent than their carrier relatives and the general population. Additionally, onset of psychiatric symptoms (e.g., hallucinations, delusions) occurs earlier in MELAS patients than in other groups (Kaufmann, 2009).

Prognosis in MELAS. The prognosis of individuals affected with MELAS typically entails progression of clinical symptoms, with medical providers treating the individual systemic
manifestations. It is widely recognized that the MELAS population is highly susceptible to
cognitive impairment across domains during the course of illness, and these deficits presumably
impact ability to function in daily life. The age of onset of cognitive problems, in particular
memory difficulties, is significantly earlier among the fully affected MELAS group when
compared to carrier relatives and the general population (Kaufmann, 2009). With progression of
disease and CNS involvement, these individuals tend to exhibit greater cognitive and intellectual
dysfunction with more global deficits developing over time, suggestive of MD (Kaufmann,
2004). As such, the more appropriate diagnosis, to better characterize cognitive dysfunction
following the progression, would be *Major Neurocognitive Disorder*, defined by Diagnostic and
Statistical Manual - Fifth Edition (DSM-V) as: 1) “evidence of significant cognitive decline from
a previous level of performance in one or more cognitive domains based on concern for the
individual of significant decline and a substantial impairment in cognitive performance,” 2) “the
cognitive deficits interfere with independence in everyday activities,” 3) “the cognitive deficits
do not occur exclusively in the context of a delirium,” and 4) “the cognitive deficits are not
better explained by another mental disorder” (American Psychiatric Association, 2013). As
demonstrated by existing case studies, there likely exists this progressive decline in
neuropsychological function. While some MELAS patients may not initially meet criteria for a
diagnosis of dementia or Major Neurocognitive Disorder (Sartor, 2002), they possibly will later
on, as they exhibit further cognitive deterioration as the disease progresses. As reviewed here,
the systematic research on the change in cognition over time in the MELAS population is
compelling, yet limited.

In a series of studies reported by Kaufmann and colleagues, neurological,
neuropsychological, and functional ability in a large sample of patients with MELAS has been
examined. The group reported on the common, and devastating, multisystemic phenotypic characteristics of 45 fully symptomatic MELAS patients (Kaufmann, 2009). They demonstrated progressive decline in global neurological and neuropsychological functioning in 30 affected individuals (Kaufmann, 2004). Moreover, they conducted the largest prospective natural history study of the mt3243A>G mutation, following 31 fully symptomatic MELAS patients as well as 54 individuals with asymptomatic (having no symptoms) or oligosymptomatic (having few/minor symptoms) presentations paired with the genetically confirmed mutation over a 20+ year period, confirming progression of neurological, cognitive, and functional deterioration, worsening structural abnormalities on MRI, and increased lactic acidosis (Kaufmann, 2011). These findings agree with clinical report of progression of medical concerns, neurological impairment, and cognitive decline expressed by the MELAS population over time.

Notably, the fully symptomatic MELAS syndrome represents only a small portion of the population who have the mt3243A>G mutation. Many relatives of identified MELAS patients who carry the mt3243A>G mutation may exhibit similar clinical and diagnostic features with varying degrees of severity (McFarland, 2002), and may covert to the full MELAS phenotype over time (Kaufmann, 2011; Weiduschat, 2014).

*Carriers of mt3243A>G mutation.*

Carrier relatives of those affected may manifest some symptoms described in patients with the full phenotype of the MELAS disorder, and they are sometimes referred to oligosymptomatic carriers. Other carriers of the mt3243A>G mutation are asymptomatic. According to Kaufmann et al. (2009), oligosymptomatic carriers display similar features as do
patients with MELAS. Examining symptom characteristics in 78 genetically identified carriers, researchers found neurological symptoms (e.g., headaches or migraines, limb weakness, loss of sensation, balance difficulty, clumsiness, and myoclonic jerks), non-neurologic symptoms (e.g., perinatal difficulties, motor and speech delays, difficulty chewing or swallowing), physical attributes (e.g., being underweight, short stature, and having microcephaly), neuroimaging results (e.g., MRS ventricular lactate), and laboratory results (e.g., levels of hemoglobin, urine creatine, and triiodothyronine). Within this study sample, more than 60% of carrier relatives experienced symptoms (most commonly, exercise intolerance, hearing loss, gastrointestinal problems, and diabetes) in at least two organ systems consistent with those manifestations of the fully symptomatic MELAS patient (Kaufmann, 2009). In addition to these symptoms, oligosymptomatic individuals have also been observed to have cerebellar atrophy and ataxia, myopathy, cardiomyopathy, and nephropathy (Damian, 1995). Another study attempted to characterize the clinical spectrum of symptoms associated with the mt3243A>G mutation among 50 genetically identified individuals. While many of the symptoms noted above were identified in the sample, additional identified symptoms include pseudobulbar syndrome, ocular involvement, and thyroid gland disease (Dvorakova, 2016). As such, there is a wide phenotypic presentation among carriers who have experienced neither seizures nor strokes.

**Cognition in mt3243A>G mutation carriers.** As a group, carrier relatives appear to experience similar difficulties in cognition compared with the fully symptomatic MELAS patients; however, these deficits likely have a later onset and are less severe in nature (Kaufmann, 2009). Similar progression of cognitive difficulties in carriers (asymptomatic and oligosymptomatic, separately) and fully symptomatic patients has been demonstrated
(Kaufmann, 2004), as well as stable neurological, neuropsychological, and functional status over a four-year follow-up period (Kaufmann, 2011). When performance on different tests of neuropsychological function was compared between fully symptomatic MELAS patients (n=31), carrier relatives (n=76) and unaffected familial controls (n=28), carriers performed significantly better than the MELAS group across all neuropsychological domains, but they did not differ from the controls (Hinton, 2012). Although there were no identified neuropsychological problems within the carrier group, the authors did not segregate the sample of carriers further into those with physical symptoms versus those without, so the group may have reflected a mixed population and any subtle effects were washed out. More thorough and careful analyses looking within the carrier group is necessary to tease out whether any cognitive or behavioral problems may exist in this group.

In a multigenerational pedigree study of three MELAS cases and 26 genetic carriers of the mt3243A>G mutation, two oligosymptomatic individuals were identified to have some level of dementia or retardation (Damian, 1995). Intellectual disability has also been identified in a small subset of carrier relatives (Dvorakova, 2016). A large prospective study of 135 individuals with the mt3242A>G mutation examined global neurological and neuropsychological scores in those who later converted to MELAS and those who did not. Although there was a range of presentations among the individuals, no between group differences were observed (Weiduschat, 2014).

The longitudinal progression of cognitive symptoms in the carrier population has not yet been elucidated. A 3-year follow-up study of 33 carriers reported increased disease severity, increased thickening of the left ventricular wall, worsening of hearing loss, and changes on EEG in occipital and parietal regions. Notably, over this three-year period, only four had seizure
activity and one had a stroke-like episode. While overall worsening of neuropsychological functioning was rarely observed during the three-year study period, cognitive decline was reported in areas of verbal and non-verbal intelligence, verbal memory, attention, language, visual-spatial skills, and motor performance (Majamaa-Voltti, 2006). Perhaps initial symptoms of focal and/or global cognitive dysfunction emerge in the oligosymptomatic individuals; however, these deficits do not appear to interfere significantly with daily functioning early on such that mt3243A>G mutation carriers may initially meet criteria for *Mild Neurocognitive Disorder*, defined by the DSM-V (American Psychiatric Association, 2013) as 1) “evidence of modest cognitive decline from a previous level of performance in one or more cognitive domains based on concern for the individual of mild decline and a moderate impairment in cognitive performance,” 2) “the cognitive deficits do not interfere with capacity for independence in everyday activities,” 3) “the cognitive deficits are not better explained in the context of a delirium,” and 4) “the cognitive deficits are not better explained by another mental disorder” (American Psychiatric Association, 2013). While Kauffman et. al. (2011) did not find significant global cognitive decline or neurological deterioration over a one and four year period of time, careful examination of this neuropsychological data in such a large group of genetically identified carriers is necessary to better characterize the nature of the cognitive problems these individuals are at risk for.

**Behavior in mt3243A>G mutation carriers.** Little is known about the behavioral manifestations of the asymptomatic and oligosymptomatic mt3242A>G carriers. While various psychiatric symptoms were present in carrier relatives, those features most frequently reported were low frustration threshold, distractibility, and depression (Kaufmann, 2009). Another study
identified depression in a small number of carrier relatives (Dvorakova, 2016). Impaired quality of life has also been reported (Verhaak, 2016).

**Prognosis in mt3243A>G mutation carriers.** With regard to prognosis, some carriers will go on to convert to the full MELAS presentation, while others will not. Individuals with the mt3243A>G mutation can vary in cognitive presentation, ranging from no identified cognitive impairments to evidence of some cognitive and behavioral difficulties, but whether there are progressive declines in selective cognitive domains is unknown. Should the carrier convert to the full MELAS phenotype, then a neurodegenerative process will ensue, resulting in global impairments. To determine whether a more subtle degenerative process started before conversion, sequential neuropsychological testing is necessary. Further exploration of neuropsychological and behavioral characteristics of the carriers will allow for improved understanding of their clinical phenotype.

Given the greater proportion of the population who have the mt3243A>G mutation compared with the MELAS syndrome, exploration of the neuropsychological and behavioral manifestations in the carriers is crucial to understanding the full spectrum of disease. Determining whether there may be a characteristic neuropsychological profiles associated with the mt3243A>G mutation carriers, determining whether it gets progressively worse with time, and examining whether the profile may predict who will and will not convert to the fully symptomatic MELAS are all unanswered questions that need to be addressed in future research.
Comorbidities that may impact cognition and behavior. Cognitive and psychiatric burden in the MELAS population arises from a variety of potential etiologies. Prominent manifestations of MELAS, such as stroke-like episodes occurring in 99% and seizures occurring in 96% of MELAS patients (Sproule, 2008), are clinical events that classify the transition from carrier of the mt3243A>G mutation to the fully symptomatic affected phenotype. Additional manifestations of MELAS, including diabetes mellitus, cardiac manifestations, fatigue, and sleep may also individually contribute to the expression of cognitive deficits. Whether cognition and behavior outcomes observed in MELAS patients follow a characteristic course over time or are associated with particular physical and/or psychosocial co-morbid symptomology is unknown.

Physical comorbidities.

Diabetes Mellitus (DM). The role the mutation associated with MELAS may have in the development of DM ranges from 0.5 to 60% depending on geographic regions including Finland (Majamaa, 1998), England (Newkirk, 1997), and Japan (Otabe, 1994; Odawara, 1995; Kadowaki, 1994). Individuals with MELAS are frequently diagnosed with DM as a comorbid condition.

Individuals with DM without mitochondrial disease have been found to be at increased risk for cognitive dysfunction (e.g., Type 1 DM: lower general intelligence, psychomotor speed, and mental flexibility; Type 2 DM: lower general cognitive ability, memory, processing speed, and executive functioning) compared with individuals without DM, and the progression of decreased cognition slowly progresses (Koekkoek, 2015). Behavior is also affected in these
individuals, such that higher rates of depression (Holt, 2014) and serious psychological distress (Egede, 2012) have been reported.

With regard to neuropsychological function among MELAS patients, one study found that individuals with MELAS who had a prior DM diagnosis were more likely to experience cognitive decline than MELAS patients without DM, specifically in young patients (Murakami, 2016). In terms of psychiatric disturbance, one MELAS case with DM presented with transient auditory hallucinations and frontal lobe syndrome (Inagaki, 1997) while four other patients with the same comorbid diagnoses exhibited mental illness (i.e., recurrent major depression, panic disorder with agoraphobia, social phobia, and simple phobia) (Miyaoka, 1997).

Cardiac Manifestations. Manifestations of cardiac dysfunction are commonly observed in MELAS patients, including hypertrophic cardiomyopathy progressing to dilated cardiomyopathy (present in one third of patients) with potential progression of disease to heart failure, pre-excitation syndromes, and conduction block. Notably, variation in cardiac features in MELAS may be related to the heteroplasmy observed in mitochondrial disorders (Hsu, 2016). A study of eight MELAS patients and four asymptomatic mt3243A>G mutation carriers demonstrated that cardiac abnormalities, particularly left ventricular involvement, were only present in the fully symptomatic MELAS patients while the carriers remained free of cardiac symptoms (Vydt, 2007). Patients with cardiac disease and/or cardiovascular risk factors without mitochondrial disease have been found to be at increased risk for cognitive decline, mild cognitive impairment, and dementia, providing evidence for the relationship between heart and brain function (Qiu, 2015). Interestingly, the cascade of events of decreased brain function over time noted in dementia may be parallel to that of decreased heart function over time identified in individuals with cardiomyopathy. Additionally, similar patterns of decline may also be observed in the
developmental trajectory of cognitive and vascular reserve (Picano, 2014). Among patients with both MELAS and cardiac dysfunction, further investigation of the relationship between cardiac involvement and neurocognitive sequela is warranted, as there is the potential for cardiac involvement to contribute to the neuropsychological phenotype.

Fatigue. Fatigue is commonly reported in individuals with mitochondrial disorders (Mancuso, 2012). Single case studies (Coelho-Miranda, 2000; Petrovic, 2012), small sample case reports (Hammans, 1995) and direct clinical report have indicated that individuals with the mt3243A>G mutation are easily fatigued. One study reported perceived fatigue in 78% of the 72 individuals with the mt3243A>G mutation who completed self-report surveys (Verhaak, 2016). Fatigue is a symptom associated with the development of myopathy, or the progressive muscle weakness that results from the deficient oxidative phosphorylation process that occurs in mitochondrial disorders. Exercise intolerance, also frequently reported in mitochondrial disorders (Taivassalo, 2003), is a likely manifestation of the myopathy and subsequent to the tendency to become easily fatigued, thus it is frequently used as a measure of fatigue. It was one of the most common systemic symptoms reported in a natural history study of the MELAS population (Kaufmann, 2011). In one study, both fully symptomatic MELAS patients (93%) and carrier relatives (38%) report exercise intolerance significantly more so than controls (Kaufmann, 2009). Another study described exercise intolerance present in 34.7% of individuals with the mt3243A>G mutation (Mancuso, 2012). Fatigue may well impact on task performance and mood in patients with MELAS and carriers, but to date, no study has examined this association directly.

Sleep. Given that sleep disturbances in patients without mitochondrial disease are associated with a wide range of cognitive and behavioral risk in children and adolescents (Beebe,
2011), adults (Pace-Schott, 2011; Yaffe, 2014), and individuals with dementia (Porter, 2015), these difficulties may well contribute to some of the observed cognitive and behavioral problems found in MELAS patients. Sleep pathology may be underreported in the mitochondrial population (Ramezani, 2014). Presumed effects of the neurological, neuromuscular, and psychiatric manifestations of mitochondrial disorders may involve poor sleep-wake cycles, sleep apnea, sleep disordered breathing, and generally disrupted sleep. The integrity of the nervous system depends on normal sleep patterns and mutations present in mitochondrial disorders may have an effect on the individual’s sleep behavior; as such, there may be a strong relationship between mitochondrial dysfunction and sleep pathology. Ramezani et al. identified 54 individuals in the literature who have proven or suspected mitochondrial disease, of which 87% had abnormal findings on polysomnography, 22% exhibited signs of central sleep apnea, and 44% displayed depressed ventilatory drive as a result of hypoxia and/or hypercapnia. Of those participants who underwent extensive study, 75% endorsed nocturnal sleep dysfunction and 30% reported excessive daytime sleepiness (Ramezani, 2014). Given that the responsibility of mitochondria involves respiratory function, patients with obstructive sleep apnea exhibit alterations in mitochondria DNA that appears to be associated with increased levels of oxidative stress (Lacedonia, 2015). Perhaps the increased oxidative stress can damage the patient’s sleep patterns but also damage the patient’s mitochondrial function. One report of 14 of 20 patients with mitochondrial disorders endorsed symptoms of depression or anxiety and some of the patients reported cognitive impairment; many of these patients also endorsed sleep-related problems (Ramezani, 2014). Few studies have identified sleep pathology specific to the MELAS population. In single cases of MELAS, delayed sleep phase syndrome (Suzuki, 2007) and hypoxic ventilator depression (Osanai, 2001) were identified.
Although the above list of DM, cardiac problems, fatigue and sleep disturbances reflects only a few of the potential comorbid physiological problems that may negatively affect cognition in the population with MELAS, they do represent serious complications that need to be considered when investigating the neuropsychological profile of the disease. MELAS is a complex disorder, affecting multiple organ systems that may each independently contribute to impaired cognitive function.

*Psychosocial comorbidities.*

Similar to neurodegenerative disorders (e.g., Huntington’s disease, Parkinson’s disease, Alzheimer’s disease), a considerable disease burden exists in the mitochondrial disorder population. Not only do these individuals experience chronic physical and cognitive difficulties, but they are also clearly affected from a psychosocial perspective. As MELAS may be one of the more debilitating mitochondrial disorders, consideration of psychosocial contributions and effects is quite important. MELAS affects not only the individual, but also many family members – both those who inherited the mt3243A>G mutation and those who care for the fully symptomatic family members. Within the multigenerational affected families, the offspring who are carriers of the mutation may also be providing care for family members who are fully symptomatic and may become concerned about their unknown future disease progression. The burden of caregiving is enormous, especially when an oligosymptomatic carrier has some level of impairment and remains caring for the fully symptomatic family member. As observed in inherited neurodegenerative disorders such as Huntington’s disease (Domaradzki, 2015), there is a negative impact on the family as a whole (e.g., loss of meaningful relationship or companionship) between the caregiver or other family members and the affected family member.
Given that the mutation is maternally inherited, affected mothers may become impaired which may also affect the family dynamic and roles of the household.

Mitochondrial disorders, including MELAS, are chronic neurodegenerative diseases with similar psychosocial concerns as movement disorders and dementias. Individuals who carry the mt3243A>G mutation are at risk for great psychosocial burden including disease or diagnosis acceptance, identity and role changes and transitions, psychological effect of physical/cognitive/functional limitations, increased dependency on others, isolation from healthy individuals, effect on various relationships, perceived stigma, and many other aspects of living life with a chronic illness (Groves, 2005). Additionally, predictive genetic testing for inherited disorders may give rise to psychological distress and worsening of quality of life, as have been observed in the Huntington’s disease population (Hayden, 2005). Such psychosocial challenges affect all aspects of life, including behavior, financial status, access to healthcare, reproductive decisions, and education and occupational decisions.

The Study of the Neuropsychological Phenotype and Neurochemical Abnormalities in the MELAS population.

Although the presentation of MD is generally consistent in those with MELAS compared with symptomatic dementias and more commonly neurodegenerative disorders, there is a better understanding of the mechanism in the MELAS disorder because of its known genetic etiology. With utilization of the neuroscience reductionist model, following from the point mutation in the mtA3243 gene to cognition, the effects at each level of development can be considered to impact outcome. For example, there exists the possibility that cognitive deficits, specifically executive
dysfunction and memory difficulties, are not solely due to the stroke-like episodes and/or seizures. It is well known that in MELAS patients, the posterior portions of brain are typically affected by the stroke-like episodes while the frontal lobes remain particularly spared (Barkovich, 1993; Sproule, 2008). Due to the locations of the stroke-like episodes and seizures, prediction of a different course of cognitive decline may apply well to the MELAS population. Presumably, the earlier affected functions are localized posteriorly while the later affected functions are localized more so anteriorly. If this were the case, decline would first be observed in visually-based domains (e.g., tasks requiring visual organization) and spatial orientation followed by language (e.g., comprehension), learning and memory. Later affected domains of higher-order cognitive functions including attention, executive processes, language processing, speech production, and motor control would be observed.

The literature on the neuropsychological profile has clearly identified cognitive impairment across domains in patients with MELAS, and limited clinical assessment and case reports have demonstrated a decline in functioning over time. Kaufmann et. al. has certainly demonstrated a global decline over time in both neurological and neuropsychological functioning (Kaufmann, 2004; Kaufmann, 2011); however, no systematic longitudinal study has examined this progressive decline in a comprehensive fashion with detailed exploration of each cognitive domain, nor has the relationship between neurochemical metabolic function and cognition been explored to further delineate the nature of cognitive deficits in the MELAS population. Study of individuals with the mt3243A>G mutation, with focus on symptomatic MELAS patients, will allow for testing of key neuropsychological concepts, and both brain-cognition and brain-behavior relationships in this population, that the current literature is lacking.
This study investigated a sample of individuals identified with the mt2343A>G mutation (both symptomatic MELAS and carrier relatives) and healthy control participants. Among those who are fully symptomatic with MELAS, the change in cognition over time was studied, by examining progression across cognitive domains compared with carrier relatives and healthy control participants to determine whether a characteristic neuropsychological profile exists and whether neurochemical anomalies are associated with cognition in the MELAS population.

Specific Aims.

Paper 1.

Aim 1. To examine memory performance in a cross-sectional sample of individuals with MELAS, carrier relatives, and unaffected control participants. Given the predominance of stroke-like episode in the posterior regions (e.g., occipital lobe) of the brain in individuals with MELAS, we hypothesize that visual memory will be more impaired than verbal memory.

Aim 2. To examine the association of neurochemical metabolites with neuropsychological performance in a cross-sectional sample of individuals with MELAS, carrier relatives, and unaffected control participants. We hypothesize that increased brain lactate and decreased NAA will have detrimental effects on memory.

Paper 2.

Aim 1. To examine neuropsychological functioning in a large sample of MELAS, carrier relatives, and control participants based on cross-sectional data. We hypothesize that MELAS
patients will demonstrate worse performance across cognitive domains compared to carrier relatives and controls at baseline.

**Aim 2.** To examine the relationship between neurochemical metabolites and cognition (i.e., domains of attention, processing speed, executive functioning/mental flexibility, language, memory, reasoning, visuospatial skills) in the mt3243A>G mutation. We hypothesize that ventricular and occipital lactate will be highest and NAA will be lowest in MELAS and that these levels will correlate with performance, such that higher levels of lactate and lower levels of NAA are associated with worse cognitive performance.

**Aim 3.** To examine the cognitive trajectory over time and further delineate the cognitive phenotype associated with the MELAS population. We hypothesize that a selective profile of decline will be observed, such that functions associated with posterior brain regions (i.e., visually-mediated tasks) will show a faster decline compared with anterior brain regions (i.e., verbally-mediated tasks).

**METHODS**

For the current project, data were utilized from two separate studies approved by the institutional review board at Columbia University Medical Center (IRB #AAAB1425) and supported by grants from the NIH (grant number P01-HD080642; P01-HD32062).

Recruitment of potential participants involved telephone calls to patients previously known to the principal investigator and other researchers affiliated with the research study,
private patients of the physicians involved in the study, flyers, and advertisements on relevant websites and at meetings of patient organizations.

There were three groups of participants: fully symptomatic MELAS (probands), carrier relatives carrying the mt3243A>G mutation, and unaffected control participants. Specifically, participants were selected based on the presence of the mtDNA mutation 3243A>G in the probands, individuals presenting with focal brain involvement (i.e., stroke and/or seizure) in addition to cerebral lactic acidosis. Carriers are designated as individuals related to the proband through the maternal lineage who carry the mt3243A>G mutations confirmed by pedigree analysis and have not had any stroke or seizure. Familial controls include fathers of the probands, other paternal family members who do not carry the mt3243A>G mutation, and spouses with the rationale that these controls would be a proxy control for environment, genetic make-up, and psychosocial issues. Controls were recruited when available; thus, matched comparison groups were not used.

Participants.

The initial prospective cohort study was a natural history observational study during which longitudinal data were collected for a sample of 123 matrilineal relatives from 45 families, from which 35 fully symptomatic MELAS patients, 78 carrier relatives, and 28 control participants were included. Participants were enrolled in the study from December 1995 through January 31, 2008. From this larger sample, 32 (91.4%) MELAS patients, 73 (93.6%) carrier relatives, and 28 (100.0%) controls underwent cognitive testing as part of a comprehensive medical evaluation. Excluded participants included individuals who were under 16 years of age. For those
participants who attempted to complete the cognitive assessment but were not able to complete all components due to limited cognitive ability, they were determined to have a very low score on the Mini Mental Status Exam (MMSE) and as such, the lowest score possible was assigned for all other tasks that were attempted and not completed. While global neuropsychological scores on this population have been examined (Kaufmann, 2011), we investigated specific cognitive domains with the hypothesis that posteriorly-located functions (e.g., visual-based domains) would be differentially affected by case status. These data were used to examine differences at baseline and the rate of change over time in cognition in the fully symptomatic MELAS patients compared with carrier relatives and control participants.

**Procedures.**

Once identified, potential participants were offered participation in the study. Participants, legally authorized representatives of individuals not able to consent for themselves, and parents of enrolled children (<18 years) gave written informed consent for study participation. For all carriers and probands, the presence mutated mitochondria and percentage of mtDNA point mutations was determined through blood samples and cultured skin fibroblasts. In addition to completing the neuropsychological evaluation, all participants underwent a neurological exam, structured medical history interview (i.e., developmental, medical, familial, social, educational, and behavioral history), Karnofsky functional living scale to assess daily living skills, six-minute walk test, laboratory tests, as well as and magnetic resonance spectroscopy (MRS) to obtain neurochemical metabolite levels. For the purposes of the current study, the primary outcomes included neuropsychological evaluation data and neurochemical metabolite values.
Evaluators who were trained and overseen by a neuropsychologist, administered the neuropsychological evaluation to all participants at baseline and follow-up visits. The battery of neuropsychological tests to be administered examined a range of cognitive domains, including general cognitive function and specific domains of attention, executive function (e.g., mental flexibility, set-shifting), language, visual-spatial skills, and memory. Each evaluation’s duration was between one and a half to two hours long. In general, all measures were administered during each evaluation; however, a shortened evaluation was administered for very impaired patients. Data were recorded on paper forms during evaluation and files were stored in locked filing cabinets. Data were entered into a password-protected database.

The majority of evaluations took place at Columbia University Medical Center (CUMC) in New York, New York. Participants traveled to CUMC from locations across the United States. When necessary, neuropsychological evaluations were conducted in the families’ homes for those who live in the New York metropolitan area and had difficulty making it to the medical center.

Outcomes.

Cognitive performance was the primary outcome for both papers and associated specific aims. For Paper 1, the primary outcomes were 1) visual memory as measured by the Benton Visuospatial Retention Test (BVRT), 2) verbal memory as measured by the Selective Reminding Test (SRT), and 3) neurochemical metabolites (e.g., ventricular lactate, occipital lactate, occipital NAA).
For Paper 2, in addition to the measured neurochemical metabolite levels (e.g., ventricular lactate, occipital lactate, occipital NAA), the following cognitive domain structures were composed of the following measures (Table 1):

**Table 1. Cognitive Domain Composition**

<table>
<thead>
<tr>
<th>Cognitive Domain Composite Scores</th>
<th>Measure</th>
<th>Quantitative Value Used</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Attention</strong></td>
<td>MMSE Attention/Calculation*</td>
<td>Total score (max: 7)</td>
</tr>
<tr>
<td></td>
<td>Cancellation: TMX Omissions</td>
<td>Total omissions</td>
</tr>
<tr>
<td></td>
<td>Cancellation: Shape Omissions</td>
<td>Total omissions</td>
</tr>
<tr>
<td><strong>Processing Speed</strong></td>
<td>Trailmaking Test A Time</td>
<td>Total time (seconds)</td>
</tr>
<tr>
<td></td>
<td>Cancellation: TMX Time</td>
<td>Total time (seconds)</td>
</tr>
<tr>
<td></td>
<td>Cancellation: Shape Time</td>
<td>Total time (seconds)</td>
</tr>
<tr>
<td><strong>Executive Functioning / Mental Flexibility</strong></td>
<td>Trailmaking Test B Time</td>
<td>Trails B total time (seconds) - Trails A total time (seconds)</td>
</tr>
<tr>
<td><strong>Language</strong></td>
<td>MMSE Language*</td>
<td>Total score (max: 7)</td>
</tr>
<tr>
<td></td>
<td>Letter Fluency (CFL)</td>
<td>Total correct</td>
</tr>
<tr>
<td></td>
<td>Category Fluency (Animals)</td>
<td>Total correct</td>
</tr>
<tr>
<td><strong>Memory</strong></td>
<td>SRT Total Recall*</td>
<td>Total correct (max: 12)</td>
</tr>
<tr>
<td></td>
<td>SRT Delayed Recall*</td>
<td>Total correct (max: 12)</td>
</tr>
<tr>
<td></td>
<td>SRT Delayed Recognition*</td>
<td>Total correct (max: 12)</td>
</tr>
<tr>
<td></td>
<td>BVRT Recall*</td>
<td>Total correct (max: 10)</td>
</tr>
<tr>
<td></td>
<td>BVRT Recognition*</td>
<td>Total correct (max: 10)</td>
</tr>
<tr>
<td><strong>Reasoning</strong></td>
<td>Wechsler Similarities</td>
<td>Total correct (max: 28)</td>
</tr>
<tr>
<td></td>
<td>Odd Man Out Total</td>
<td>Total correct (max: 40)</td>
</tr>
<tr>
<td><strong>Visual-Spatial</strong></td>
<td>BVRT Copy*</td>
<td>Total correct (max: 10)</td>
</tr>
<tr>
<td></td>
<td>Hooper Visual Organization Test</td>
<td>Total correct (max: 30)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Brain Region Composite Scores</th>
<th>Measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visually-Mediated Tasks</td>
<td>BVRT Recall*</td>
</tr>
<tr>
<td>(Posterior Localization)</td>
<td>BVRT Recognition*</td>
</tr>
<tr>
<td>Odd Man Out Total</td>
<td>BVRT Copy*</td>
</tr>
<tr>
<td>-------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Hooper Visual Organization Test</td>
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</tr>
<tr>
<td>MMSE Attention/Calculation*</td>
<td></td>
</tr>
<tr>
<td>MMSE Language*</td>
<td></td>
</tr>
<tr>
<td>Letter Fluency (CFL)</td>
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<tr>
<td>Category Fluency (Animals)</td>
<td></td>
</tr>
<tr>
<td>SRT Total Recall*</td>
<td></td>
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<td>SRT Delayed Recall*</td>
<td></td>
</tr>
<tr>
<td>SRT Delayed Recognition*</td>
<td></td>
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<tr>
<td>Wechsler Similarities</td>
<td></td>
</tr>
</tbody>
</table>

**Verbally-Mediated Tasks (Anterior Localization)**

*MMSE: Mini Mental Status Exam  
SRT: Selective Reminding Test  
BVRT: Benton Visual-Spatial Retention Test
Paper 1
The relationship between neurochemical metabolites and memory performance in individuals with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS)

Introduction
Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) is a maternally-inherited progressive multisystemic polygenic disorder, originally identified in 1984 (Pavlakis, 1984), with estimated prevalence ranging from 0.95-236 per 100,000 (Majamaa, 1998; Chinnery, 2000a; Darin, 2001; Uusimaa, 2007; Manwaring, 2007; Shaefer, 2008; Gorman, 2015). MELAS is characterized by six cardinal symptoms, including onset prior to 40 years old, stroke-like episodes, seizures, exercise intolerance, ragged-red fibers, and lactic acidosis (Hirano, 1994).

Mitochondrial disorders arise when the oxidative phosphorylation process (OXPHOS), facilitating metabolic function by transforming adenosine diphosphate (ADP) to adenosine triphosphate (ATP), is impaired yielding diminished energy production (Taanman, 2002; DiMauro, 2008; Davis, 2011; DiMauro, 2013a). In MELAS, 80% of cases are caused by an MT-TL1 gene mutation, such that adenine transitions to guanine at nucleotide position 3243 (mt3243A>G) (Wong, 2007; Scaglia, 2012; DiMauro, 2013b). While carriers of the mutation experience none to few symptoms, the conversion to MELAS is clinically defined by the occurrence of seizure and/or stroke-like episode triggering, the developmental cascade of MELAS. Of note, is that most seizures and stroke-like episodes are localized to posterior brain regions (Veggiotti, 1995; Canafoglia, 2001; Iizuka, 2003; Ito, 2008).

Lactic acidosis, defined as increased cerebral lactate production, is a classic biomarker of the mt3243A>G mutation resulting from the deficient OXPHOS process (Castillo, 1995;
Mathews, 1993; Yatsuga, 2012; Kaufmann, 2004; Wilichowski, 1999; Moller, 2005; Dubeau, 2000). Likewise, N-acetylaspartate (NAA) is also associated with the mt3243A>G mutation. Specifically, symptomatic MELAS patients have higher lactate and lower NAA levels in brain when compared with carrier relatives and control participants (Weiduschat, 2014). Increased lactate and decreased NAA in the brain have also been linked to overall neuropsychological deficits (Kaufmann, 2004; Weiduschat, 2014).

Memory impairment, in particular, is consistently observed in symptomatic MELAS patients (Kaufmann, 2004; Kaufmann, 2009; Kaufmann, 2011; Hinton, 2012; Finsterer, 2008; Kartsounis, 1992; Pachalska, 2002; Kiejna, 2002; Sartor, 2002; Salsano, 2011; Neargarder, 2007), yet not in carriers (Kaufmann, 2004; Weiduschat, 2014; Kaufmann, 2009; Kaufmann, 2011; Hinton, 2012). However, the relationships between verbal and visual memory, and between brain neurochemicals and memory, have not been explored. We hypothesized that: 1) given the predominance of stroke-like episode in the posterior regions (e.g., occipital lobe) of the brain, visual memory will be more impaired than verbal memory, and 2) given the association of brain neurochemicals with neuropsychological performance, increased brain lactate and decreased NAA will have detrimental effects on memory.

Method

A natural history study of individuals with MELAS associated with the mt3243A>G mutation was conducted from December 1995 through January 2008 at Columbia University Medical Center (Kaufmann, 2009; Kaufmann, 2011). The study was approved by the institutional review board at Columbia University Medical Center (IRB# AAAB1425) and supported by grants from
the National Institutes of Health (grant numbers P01-HD080642 and P01-HD32062) to Drs. Darryl C. De Vivo and Salvatore Di Mauro.

Participants.

As part of the natural history observational study (Kaufmann, 2009; Kaufmann, 2011), 123 matrilineal relatives from 45 families were enrolled. 96 participants who completed measures of verbal and visual memory were included in the current analysis separated into three groups: 1) 18 fully symptomatic individuals with MELAS (termed MELAS), 2) 59 carriers of the mt3243A>G mutation (termed carriers), and 3) 20 controls without the mt3243A>G mutation who were patrilineal relatives or relatives by marriage (termed controls). MELAS were defined as individuals with the mt3243A>G mutation, lactic acidosis, and features of the clinical phenotype such as stroke-like episodes and/or focal seizures. Carriers were defined as asymptomatic or oligosymptomatic individuals with the mt3243A>G mutation who have not had stroke-like episodes or seizures. Controls do not carry the mt3243A>G mutation.

Procedures.

The full prospective cohort study procedures have been described elsewhere (Kaufmann, 2009; Kaufmann, 2011). The study was conducted at Columbia University Medical Center in New York, New York. All participants gave written informed consent prior to participation in the study.
Each participant underwent a comprehensive neuropsychological evaluation to assess cognitive functioning across domains, including attention, processing speed, executive function, language, visual-spatial ability, and memory. For the purposes of this analysis, verbal and visual memory performance was examined as the primary outcomes.

Measures.

*Demographics.* Each participant was classified by age (in years), sex (male, female), and education (total years).

*Verbal Memory.* Verbal learning and memory was assessed using the Selective Reminding Test (SRT; Bushke, 1974). This is a rote list-learning task of 12 words with six learning trials, a delayed recall trial administered 15 minutes following last learning trial, and a multiple-choice recognition trial administered immediately following the delayed recall trial.

*Visual Memory.* Visual learning and memory was assessed using the Benton Visuospatial Retention Test (BVRT; Benton, 1955). This task involves an initial copy trial of 10 designs, a 10-second immediate recall trial of 10 different designs, and a multiple-choice recognition trial of all 10 designs from the immediate recall trial.

*Neurochemical Variables.* Neurochemical metabolites were measured using multi-slice proton magnetic resonance spectroscopic imaging (MRS). For the purposes of this study, biochemical values estimated within the lateral ventricles and occipital lobe gray matter were measured based upon two 15-mm axial oblique brain segments; full method is described elsewhere (Kaufmann, 2004; Kaufmann, 2009; Kaufmann, 2011; Duyn, 1993). Occipital lactate and N-acetylaspartate
(NAA) were measured in the tissue of the gray matter of the occipital lobe. Ventricular lactate was measured within the fluid of the lateral ventricle. All values were measured in institutional units (i.u.). Values were characterized based upon comparison with the amount (mean and standard deviation) of each neurochemical measured in the healthy control sample.

Statistical Analysis.

Descriptive statistics were examined using frequencies and percentages for demographic characteristics, lactic acidosis levels, and verbal/visual memory scores among the three clinical groups – 1) symptomatic, 2) carrier, and 3) controls. To compare these variables among groups, ANOVA were used for continuous variables and chi-squared tests were used for categorical variables. Cut-offs were identified for the two primary cognitive measures and the three biochemical variables. Lactate levels were separately represented by three categories: “within normal limits” (ventricular: ≤4.8 i.u.; occipital: ≤4.5 i.u.), “high” (ventricular: 4.8-5.7 i.u.; occipital: 4.5-5.4 i.u.), and “very high” (ventricular: ≥5.7 i.u.; occipital: ≥5.4 i.u.). Occipital NAA was categorized as “low” (≤14.0 i.u.), “within normal limits” (14.0 i.u.-17.6 i.u.), and “high” (≥17.6 i.u.). Two ratios were created, 1) occipital lactate to occipital NAA and 2) ventricular lactate to occipital NAA, to examine the relationships between biochemical values and memory performance. For both verbal and visual memory, test scores were converted to percentiles and categorized as “within normal limits” for scores above the 16th percentile, “mildly impaired”: for scores between 16th and 2nd percentile (or between one and two standard deviations from the population mean), and “severely impaired” for scores below the 2nd percentile (more than two standard deviations below the population mean). Chi-squared tests were used to determine the difference in amount of occipital and ventricular lactate, occipital
NAA, verbal memory performance, and visual memory performance among the three clinical groups and separately between MELAS and carriers, MELAS and controls, and carriers and controls. Verbal and visual memory performances were compared within each group using Pearson correlations and paired t-tests.

The relationships between verbal and visual memory and biochemical variables (occipital lactate, ventricular lactate, occipital NAA) were examined in two ways. First, six separate Pearson correlations were run to examine the relationship between 1) verbal memory and each biochemical variable separately, and 2) visual memory and each biochemical variable separately. Second, using the pre-identified cut-offs indicated above, Chi-squared tests were used to categorically examine the relationship between the biochemical values and memory, separately for verbal and visual memory. Third, four separate Pearson correlations were run to examine the relationship between each memory variable (verbal, visual) and each ratio variable (occipital lactate / occipital NAA, ventricular lactate / occipital NAA).

Alpha was set at 0.05. All analyses were conducted via SAS 9.4 (SAS Institute, Cary, NC, U.S.A.).

**Results**

Demographic data are presented in Table 2. The mean age of the overall sample was 42.5 (SD = 14.7) years, ranging from 16 to 76 years old. Education ranged from 9 to 20 total years with an average of 14.7 (SD = 2.7) years. The sample was 61.5% female and 88.5% Caucasian. Although groups differed by age (p = 0.002) and sex (p<0.0001), no differences were identified with respect to ethnicity or total years of education. Stroke-like episodes were reported in 83.3%
of MELAS compared with none in carriers or controls (p<0.0001). Seizures were reported in 83.3% of MELAS compared with 3.6% of carriers and no controls (p<0.0001).

**Ventricular Lactate.**
Overall, the ventricular lactate levels ranged from 2.0–14.4 i.u., with a mean of 5.3 i.u. (SD = 2.4) and significantly differed across the groups (p<0.0001), ranging from 2.4–14.4 i.u. in MELAS, 2.0–8.6 i.u. in carriers, and 2.4–5.6 i.u. in controls (Figure 4). Ventricular lactate significantly differed between MELAS and carriers (p<0.0001), MELAS and controls (p<0.0001), and carriers and controls (p = 0.02). Categorization of ventricular lactate levels by group is summarized in Table 3.

**Occipital Lactate.**
Overall, the occipital lactate levels ranged from 1.2–10.3 i.u., with a mean of 4.2 i.u. (SD = 1.8). Occipital lactate significantly differed across the three groups (p<0.0001), ranging from 2.5–10.3 i.u. in MELAS, 1.2–9.8 i.u. in carriers, and 2.2–5.3 i.u. in controls (Figure 4). Occipital lactate levels significantly differed between MELAS and carriers (p<0.0001) and MELAS and controls (p = 0.0002), but not between carriers and controls (p = 0.49). Categorization of occipital lactate levels by group is summarized in Table 3.

**Occipital NAA.**
Overall, the occipital NAA levels ranged from 8.4–26.4 i.u., with a mean of 16.4 i.u. (SD = 3.7). Occipital NAA significantly differed across the three groups (p = 0.0003), ranging from 8.4–20.6 i.u. in MELAS, 9.5–26.4 i.u. in carriers, and 12.5–18.5 i.u. in controls (Figure 4). Occipital
lactate levels significantly differed between MELAS and carriers (p = 0.0003), MELAS and controls (p = 0.03), and carriers and controls (p = 0.05). Categorization of occipital NAA levels by group is summarized in Table 3.

Verbal Memory versus Visual Memory.

A strong positive correlation was found between verbal and visual memory in the MELAS group (r = 0.68; p = 0.002), but not in carriers (r = 0.22; p = 0.09) or controls (r = 0.16; p = 0.50). Worse visual memory performance compared with verbal memory was identified in MELAS (p = 0.01) and carriers (p=0.03); however, this difference was not observed in controls (p = 0.09).

Visual Memory.

Overall, visual memory performance ranged from 0.05%ile to 98.0%ile, with a mean of 31.3%ile (SD = 32.1%ile). Visual memory performance significantly differed across the groups (p = 0.0003) (Table 4); specifically, differences were identified between MELAS and carriers (p = 0.01), MELAS and controls (p<0.0001), and carriers and controls (p = 0.01). Categorization of visual memory by group is summarized in Table 3.

Visual Memory & Biochemical Values.

Occipital Lactate: A weak negative correlation between occipital lactate and visual memory was found (r = -0.27; p = 0.007). Visual memory differed by the amount of occipital lactate (p =
0.01), such that the higher the lactate amount present, the worse the observed visual memory performance (Table 5).

Ventricular Lactate: A moderate negative correlation between ventricular lactate and visual memory was found ($r = -0.37; p = 0.0002$). Visual memory differed by the amount of ventricular lactate ($p<0.0001$), such that the higher the lactate amount present, the worse the observed visual memory performance (Table 5).

Occipital NAA: A weak positive correlation between occipital NAA and visual memory ($r = 0.20; p = 0.05$). Visual memory performance differed by the amount of occipital NAA ($p = 0.0009$), such that the higher the lactate amount present, the worse the observed visual memory performance (Table 5).

A moderate negative correlation was found between visual memory and 1) the ratio of occipital lactate to occipital NAA ($r = -0.33; p = 0.001$) and 2) the ratio of ventricular lactate to occipital NAA ($r = -0.37; p = 0.0002$) (Figure 5).

**Verbal Memory.**

Overall, verbal memory performance ranged from 0.9%ile to 99.0%ile, with a mean of 38.4%ile (SD = 31.5%ile). Verbal memory performance significantly differed across the three clinical groups ($p = 0.15$) (Table 4), and no differences reached significance between pairs: MELAS and carriers ($p = 0.06$), MELAS and controls ($p = 0.29$), or carriers and controls ($p = 0.46$). Categorization of verbal memory by group is summarized in Table 3.
Verbal Memory & Biochemical Values.

Occipital Lactate: Occipital lactate did not correlate with verbal memory performance (r = -0.15; p = 0.16), nor did verbal memory performance differ by the amount of occipital lactate (p = 0.40) (Table 5).

Ventricular Lactate: A weak negative correlation between ventricular lactate and verbal memory was found (r = -0.24; p = 0.02); however, verbal memory performance did not differ by the amount of ventricular lactate (p = 0.34) (Table 5).

Occipital NAA: Occipital NAA level did not correlate with verbal memory performance (r = -0.05; p = 0.62), nor did verbal memory performance differ by the amount of occipital NAA (p = 0.44) (Table 5).

Examination of the ratio of occipital lactate to occipital NAA was not correlated with verbal memory performance (r = -0.16; p = 0.12); however, a weak negative correlation between verbal memory performance and the ratio of ventricular lactate to occipital NAA was found (r = -0.24; p = 0.02) (Figure 6).

Discussion

This study examined the relationship between verbal and visual memory function and neurochemical variables (i.e., ventricular lactate, occipital lactate, occipital NAA) in a cohort of 18 MELAS, 58 carrier relatives of the mt3243A>G mutation, and 20 control participants. The data supported our hypotheses that visual memory would be preferentially impaired and that lactic acidosis was associated with detrimental effects on memory functioning. We proposed that higher lactate levels in both the ventricles and occipital lobe would be associated with worse
memory performance overall, whereas normal lactate levels would be associated with memory performance within expectations. Similarly, NAA has also been associated with the mt3243A>G mutation and cognitive deficits (Weiduschat, 2014); as such, we proposed that the ratios of occipital lactate to occipital NAA and ventricular lactate to occipital NAA would be associated with worse memory performance. The findings supported this.

Prior work noted that the MELAS related stroke-like episodes and seizures are predominantly localized to posterior brain regions while anterior regions remain relatively spared (Castillo, 1995). As mitochondria are more heavily localized to the occipital cortex in the brain, the mitochondrial dysfunction in MELAS may thus place increased metabolic demand in posterior brain regions resulting in more detrimental impact in these regions (Turnbull, 2010; Berti, 2014). Because of this, we reasoned that memory that relied on visual material subserved in part by the posterior occipital brain regions, would be preferentially affected. Consistent with our hypothesis, visual memory was worse than verbal memory among MELAS. This was also seen among carriers, but not controls. Memory impairment was present in a greater proportion of individuals with the mt3243A>G mutation (MELAS and carriers) compared to controls, with a higher percentage of individuals with the mt3243A>G mutation having impaired visual memory (83.4% MELAS, 47.4% carriers) compared with verbal memory (55.6% MELAS, 25.4% carriers). Verbal memory differed across the three clinical groups, with more than a quarter of MELAS performing in the severely impaired range compared with less than 10 percent of carriers and controls combined. Most striking, was that nearly three-quarters of MELAS performed in the severely impaired range on the visual memory task compared with less than 20 percent of carriers and controls combined.
In addition, and as expected (Weiduschat, 2014), MELAS were found to have the highest lactate levels, lowest NAA levels, and most impaired memory. Specifically, 83.3% fell in the high and very high ventricular lactate ranges compared with 51.8% of carriers and 20.0% of controls. Occipital lactate was similarly distributed with 77.8% of MELAS in high or very high categories compared with 22.4% of carriers and 15.0% of controls. Occipital NAA was lowest in 61.1% of MELAS compared with 15.5% of carriers and 25.0% of controls. This distinct pattern of neurochemical abnormalities contributed to the cognitive presentation in the MELAS population.

Results showed that occipital and ventricular lactate to NAA ratios were both moderately correlated with visual memory performance. Furthermore, individuals with higher lactate levels and lower NAA levels demonstrated greater visual memory dysfunction; the majority of these individuals were in the MELAS clinical group. In contrast, only a weak correlation was identified between the occipital lactate to NAA ratio and verbal memory performance and no association between ventricular lactate to NAA ratio and verbal memory was identified. Prior work suggests that NAA decreases while cerebral lactate accumulates (Weiduschat, 2014), based on the location of stroke-like episodes it may be that the neurochemical biomarkers follow the same pattern of posterior to anterior progression. The current findings of more severely affected visual than verbal memory tasks in MELAS thus likely reflect this underlying brain localization pattern; visually-mediated tasks are dependent on visual cortex in the occipital lobe (posterior), while verbally-mediated tasks are primarily associated with more anterior brain regions.

Taken together, our results indicated that visual memory performance is sensitive to cerebral lactate (occipital and ventricular) and NAA levels, and discriminates the groups across these neurochemical metabolites. In contrast, verbal memory performance correlated with
ventricular lactate only. These findings suggest that the majority of individuals with high lactate and low NAA perform poorly on the visual memory test, yet some individuals in this range are still able to perform within normal limits on the verbal memory test. Of particular interest was that visual memory performance was poor even within the carrier group, suggesting that even before there are clear clinical manifestations of seizures and stroke-like episodes, underlying brain neurochemical changes may be affecting subtle cognitive changes. Almost half of the carrier group (48%) scored within the impaired range on the visual memory test, and 19% scored within the severely impaired range. As well, for the carriers there was a wide range of neurochemical levels. These findings suggest that using underlying neurochemical changes as a marker for disease state may well be a more sensitive biomarker for predicting clinical involvement than the current clinical characterization based on presentation of seizure or stroke-like episode.

While our MELAS sample size is limited due to the nature of studying a rare disease and the consideration that various psychosocial, medical, and physical comorbidities may also impact cognition, this study is the first to examine the relationship between neurochemical metabolites and specific neuropsychological domains (i.e., memory) in this population. Our findings suggest a distinct cognitive phenotype likely attributed to the posteriorly localized nature of biochemical features and clinical characteristics associated with MELAS.

Specifically, our findings support that the increased lactate and decreased NAA localized to posterior brain regions not only contribute to characteristic seizures and stroke-like episodes, but also play a role in the presence of such posteriorly localized cognitive deficits (i.e., poor performance on visually-mediated tasks). This is definitively seen in the MELAS group and there is a suggestion that this may also be the case among the mt3243A>G carrier population
who have not experienced stroke-like episodes. As such, further research is needed to examine neurochemical biomarker specificity within the mt3243A>G population.
### Table 2. Participant characteristics.

<table>
<thead>
<tr>
<th></th>
<th>MELAS (n = 18)</th>
<th>Carriers (n = 58)</th>
<th>Controls (n = 20)</th>
<th>Between Group Comparison</th>
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</thead>
<tbody>
<tr>
<td><strong>Age (in years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>16.0 - 61.0</td>
<td>17.0 - 76.0</td>
<td>32.0 - 73.0</td>
<td><strong>F = 6.84</strong> p = 0.002</td>
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<tr>
<td>Mean (SD)</td>
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<td>41.6 (14.7)</td>
<td>51.6 (11.5)</td>
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<tr>
<td><strong>Sex: N (%)</strong></td>
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<tr>
<td>Male</td>
<td>7 (38.9)</td>
<td>14 (24.1)</td>
<td>16 (80.0)</td>
<td><strong>p &lt; 0.0001</strong></td>
</tr>
<tr>
<td>Female</td>
<td>11 (61.1)</td>
<td>44 (75.9)</td>
<td>4 (20.0)</td>
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<tr>
<td><strong>Education (in years)</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>9.0 - 18.0</td>
<td>10.0 - 20.0</td>
<td>10.0 - 18.0</td>
<td><strong>F = 0.43</strong> p = 0.65</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>14.3 (2.9)</td>
<td>14.9 (2.8)</td>
<td>14.5 (2.4)</td>
<td></td>
</tr>
<tr>
<td><strong>Ethnicity: N (%)</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>15 (83.3)</td>
<td>54 (91.4)</td>
<td>17 (85.0)</td>
<td><strong>p = 0.44</strong></td>
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<td>Black</td>
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<td>0 (0.0)</td>
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<td>Hispanic</td>
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<td>Asian</td>
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<td>1 (5.0)</td>
<td></td>
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<tr>
<td>American Indian</td>
<td>2 (11.1)</td>
<td>2 (3.4)</td>
<td>1 (5.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Stroke-like Episode: N (%)</strong>*</td>
<td></td>
<td></td>
<td></td>
<td><strong>p &lt; 0.0001</strong></td>
</tr>
<tr>
<td>Yes</td>
<td>15 (83.3)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>3 (16.7)</td>
<td>56 (100.0)</td>
<td>19 (100.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Seizure: N (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>p &lt; 0.0001</strong></td>
</tr>
<tr>
<td>Yes</td>
<td>15 (83.3)</td>
<td>2 (3.6)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>3 (16.7)</td>
<td>54 (96.4)</td>
<td>19 (100.0)</td>
<td></td>
</tr>
</tbody>
</table>

*information not available for all participants (Stroke-like Episode n = 93; Seizure n = 93)
Table 3. Neurochemical metabolites and memory performance across clinical groups.

<table>
<thead>
<tr>
<th></th>
<th>MELAS (n = 18)</th>
<th>Carriers (n = 58)</th>
<th>Controls (n = 20)</th>
<th>Between Group Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Verbal Memory: N (%)</strong>&lt;sup&gt;*&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>within normal limits (&lt;16th %ile)</td>
<td>8 (44.4)</td>
<td>44 (75.8)</td>
<td>13 (65.0)</td>
<td></td>
</tr>
<tr>
<td>mildly impaired (16th - 2nd %ile)</td>
<td>5 (27.8)</td>
<td>12 (20.7)</td>
<td>6 (30.0)</td>
<td><strong>p = 0.02</strong></td>
</tr>
<tr>
<td>severely impaired (&lt;2nd %ile)</td>
<td>5 (27.8)</td>
<td>2 (3.5)</td>
<td>1 (5.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Visual Memory: N (%)</strong>&lt;sup&gt;§&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>within normal limits (&lt;16th %ile)</td>
<td>3 (16.7)</td>
<td>31 (53.5)</td>
<td>16 (80.0)</td>
<td><strong>p &lt; 0.0001</strong></td>
</tr>
<tr>
<td>mildly impaired (16th - 2nd %ile)</td>
<td>1 (5.6)</td>
<td>16 (27.6)</td>
<td>2 (10.0)</td>
<td></td>
</tr>
<tr>
<td>severely impaired (&lt;2nd %ile)</td>
<td>14 (77.8)</td>
<td>11 (18.9)</td>
<td>2 (10.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Occipital NAA Levels: N (%)</strong>&lt;sup&gt;°&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low (≤14.0 i.u.)</td>
<td>11 (61.1)</td>
<td>9 (15.5)</td>
<td>5 (25.0)</td>
<td></td>
</tr>
<tr>
<td>within normal limits (14.0 - 17.6 i.u.)</td>
<td>4 (22.2)</td>
<td>22 (37.9)</td>
<td>12 (60.0)</td>
<td><strong>p = 0.0008</strong></td>
</tr>
<tr>
<td>high (≥17.6 i.u.)</td>
<td>3 (16.7)</td>
<td>27 (46.6)</td>
<td>3 (15.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Occipital Lactate Levels: N (%)</strong>&lt;sup&gt;§&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>within normal limits (≤4.5 i.u.)</td>
<td>4 (22.2)</td>
<td>45 (77.6)</td>
<td>17 (85.0)</td>
<td><strong>p = 0.006</strong></td>
</tr>
<tr>
<td>high (4.5 - 5.4 i.u.)</td>
<td>5 (27.8)</td>
<td>8 (13.8)</td>
<td>3 (15.0)</td>
<td></td>
</tr>
<tr>
<td>very high (≥5.4 i.u.)</td>
<td>9 (50.0)</td>
<td>5 (8.6)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Ventricular Lactate Levels: N (%)</strong>&lt;sup&gt;°&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>within normal limits (≤4.8 i.u.)</td>
<td>3 (16.7)</td>
<td>28 (48.2)</td>
<td>16 (80.0)</td>
<td><strong>p = 0.0005</strong></td>
</tr>
<tr>
<td>high (4.8 - 5.7 i.u.)</td>
<td>1 (5.5)</td>
<td>15 (25.9)</td>
<td>4 (20.0)</td>
<td></td>
</tr>
<tr>
<td>very high (≥5.7 i.u.)</td>
<td>14 (77.8)</td>
<td>15 (25.9)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
</tbody>
</table>

*Significantly differed between MELAS and carriers only (p = 0.0008)
§Significantly differed between MELAS and carriers (p < 0.0001) and MELAS and controls (p = 0.000) only
°Significantly differed between MELAS and carriers, MELAS and controls, and carriers and controls (all p < 0.05)
Table 4. Comparison of visual and verbal memory performance within and between clinical groups.

<table>
<thead>
<tr>
<th></th>
<th>MELAS</th>
<th>Carriers</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 18</td>
<td>n = 58</td>
<td>n = 20</td>
</tr>
<tr>
<td>Visual Memory: Mean (SD)*</td>
<td>11.33 (23.48)</td>
<td>31.27 (31.20)</td>
<td>52.25 (32.44)</td>
</tr>
<tr>
<td>Verbal Memory: Mean (SD)§</td>
<td>26.31 (31.52)</td>
<td>42.69 (32.06)</td>
<td>36.75 (28.07)</td>
</tr>
<tr>
<td>Visual - Verbal: Mean (SD)</td>
<td>-14.97 (23.19)</td>
<td>-11.42 (39.48)</td>
<td>15.50 (39.36)</td>
</tr>
<tr>
<td>Within Group Comparison</td>
<td>t = -2.74 (p = 0.01)</td>
<td>t = -2.20 (p = 0.03)</td>
<td>t = 1.76 (p = 0.09)</td>
</tr>
</tbody>
</table>

*Significantly different among groups (p = 0.0003)
§No difference among groups (p = 0.15)
Table 5. Relationship between neurochemical metabolites and memory performance

<table>
<thead>
<tr>
<th></th>
<th>within normal limits (&gt;16th %ile)</th>
<th>mildly impaired (16th - 2nd %ile)</th>
<th>severely impaired (&lt;2nd %ile)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ventricular Lactate and Verbal Memory Performance:</strong> ( p = 0.40 )</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>within normal limits ((\leq 4.8 \text{ i.u.}))</td>
<td>34</td>
<td>11</td>
<td>2</td>
<td>47 (49.0)</td>
</tr>
<tr>
<td>high ((4.8 - 5.7 \text{ i.u.}))</td>
<td>14</td>
<td>5</td>
<td>1</td>
<td>20 (20.8)</td>
</tr>
<tr>
<td>very high ((\geq 5.7 \text{ i.u.}))</td>
<td>17</td>
<td>7</td>
<td>5</td>
<td>29 (30.2)</td>
</tr>
<tr>
<td>Total (%) ( 65 (67.7) )</td>
<td>23 (24.0)</td>
<td>8 (8.3)</td>
<td>n = 96</td>
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</tr>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>within normal limits (&gt;16th %ile)</th>
<th>mildly impaired (16th - 2nd %ile)</th>
<th>severely impaired (&lt;2nd %ile)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ventricular Lactate and Visual Memory Performance:</strong> ( p &lt; 0.0001 )</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>within normal limits ((\leq 4.8 \text{ i.u.}))</td>
<td>29</td>
<td>10</td>
<td>8</td>
<td>47 (49.0)</td>
</tr>
<tr>
<td>high ((4.8 - 5.7 \text{ i.u.}))</td>
<td>16</td>
<td>2</td>
<td>2</td>
<td>20 (20.8)</td>
</tr>
<tr>
<td>very high ((\geq 5.7 \text{ i.u.}))</td>
<td>5</td>
<td>7</td>
<td>17</td>
<td>29 (30.2)</td>
</tr>
<tr>
<td>Total (%) ( 50 (52.1) )</td>
<td>19 (19.8)</td>
<td>27 (28.1)</td>
<td>n = 96</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>within normal limits (&gt;16th %ile)</th>
<th>mildly impaired (16th - 2nd %ile)</th>
<th>severely impaired (&lt;2nd %ile)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Occipital Lactate and Verbal Memory Performance:</strong> ( p = 0.45 )</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>within normal limits ((\leq 4.5 \text{ i.u.}))</td>
<td>45</td>
<td>17</td>
<td>4</td>
<td>66 (68.7)</td>
</tr>
<tr>
<td>high ((4.5 - 5.4 \text{ i.u.}))</td>
<td>11</td>
<td>4</td>
<td>1</td>
<td>16 (16.7)</td>
</tr>
<tr>
<td>very high ((\geq 5.4 \text{ i.u.}))</td>
<td>9</td>
<td>2</td>
<td>3</td>
<td>14 (14.6)</td>
</tr>
<tr>
<td>Total (%) ( 65 (67.7) )</td>
<td>23 (24.0)</td>
<td>8 (8.3)</td>
<td>n = 96</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>within normal limits (&gt;16th %ile)</th>
<th>mildly impaired (16th - 2nd %ile)</th>
<th>severely impaired (&lt;2nd %ile)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Occipital Lactate and Visual Memory Performance:</strong> ( p = 0.01 )</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>within normal limits ((\leq 4.5 \text{ i.u.}))</td>
<td>40</td>
<td>14</td>
<td>12</td>
<td>66 (68.7)</td>
</tr>
<tr>
<td>high ((4.5 - 5.4 \text{ i.u.}))</td>
<td>7</td>
<td>3</td>
<td>6</td>
<td>16 (16.7)</td>
</tr>
<tr>
<td>very high ((\geq 5.4 \text{ i.u.}))</td>
<td>3</td>
<td>2</td>
<td>9</td>
<td>14 (14.6)</td>
</tr>
<tr>
<td>Total (%) ( 50 (52.1) )</td>
<td>19 (19.8)</td>
<td>27 (28.1)</td>
<td>n = 96</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>within normal limits (&gt;16th %ile)</th>
<th>mildly impaired (16th - 2nd %ile)</th>
<th>severely impaired (&lt;2nd %ile)</th>
<th>Total (%)</th>
</tr>
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<tbody>
<tr>
<td><strong>Occipital NAA and Verbal Memory Performance:</strong> ( p = 0.44 )</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low ((\leq 14.0 \text{ i.u.}))</td>
<td>15</td>
<td>6</td>
<td>4</td>
<td>25 (26.0)</td>
</tr>
<tr>
<td>within normal limits ((14.0 - 17.6 \text{ i.u.}))</td>
<td>27</td>
<td>10</td>
<td>1</td>
<td>38 (39.6)</td>
</tr>
<tr>
<td>high (≥ 17.6 i.u.)</td>
<td>23</td>
<td>7</td>
<td>3</td>
<td>33 (34.4)</td>
</tr>
<tr>
<td>-------------------</td>
<td>----</td>
<td>---</td>
<td>---</td>
<td>-----------</td>
</tr>
<tr>
<td>Total (%)</td>
<td>65 (67.7)</td>
<td>23 (24.0)</td>
<td>8 (8.3)</td>
<td>n = 96</td>
</tr>
</tbody>
</table>

**Occipital NAA and Visual Memory Performance:** \( p = 0.002 \)

<table>
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<tr>
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<th>mildly impaired (16th - 2nd %ile)</th>
<th>severely impaired (&lt;2nd %ile)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>low (≤14.0 i.u.)</td>
<td>10</td>
<td>1</td>
<td>14</td>
<td>25 (26.0)</td>
</tr>
<tr>
<td>within normal limits</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(14.0 - 17.6 i.u.)</td>
<td>19</td>
<td>13</td>
<td>6</td>
<td>38 (39.6)</td>
</tr>
<tr>
<td>high (≥ 17.6 i.u.)</td>
<td>21</td>
<td>5</td>
<td>7</td>
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<td>Total (%)</td>
<td>50 (52.1)</td>
<td>19 (19.8)</td>
<td>27 (28.1)</td>
<td>n = 96</td>
</tr>
</tbody>
</table>
Paper 1 Figures.

**Figure 4.** Neurochemical metabolites by group
**Figure 5.** Relationship between visual memory and neurochemical ratios
Figure 6. Relationship between verbal memory and neurochemical ratios
Paper 2

Progression of cognitive decline and neurochemical changes in individuals with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS)

Introduction

Originally identified in 1984 (Pavlakis, 1984), mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) is a maternally-inherited disease that results from a mitochondrial gene mutation involving an adenine to guanine transition on nucleotide position 3243 (mt3243A>G) (Hirano, 1992; Hirano, 1994; Sproule, 2008; Kaufmann, 2009). First based upon case reports and seminal accounts (Pavlakis, 1984; Hirano, 1992; Hart, 1977; Askanas, 1978; Skoglund, 1979; Shapira, 1975; Damian, 1995; Morovvati, 2002; Huang, 2002; Chinnery, 1997; Chinnery, 1998), classification of MELAS has long been characterized by six cardinal symptoms, including onset prior to 40 years of age, stroke, seizures, exercise intolerance, ragged-red fibers, and lactic acidosis (Hirano, 1994). Current prevalence estimates range from 0.95 to 236 per 100,000 (Majamaa, 1998; Chinnery, 2000a, Darin, 2001; Uusimaa, 2007; Manwaring, 2007; Shaefer, 2008; Gorman, 2015). Although MELAS is considered a progressive disorder of the central nervous system, multiple secondary manifestations affect cardiac, renal, endocrine, and gastrointestinal systems in addition to cognitive and psychiatric comorbidity (Hirano, 1992; Sproule, 2008; Kaufmann, 2009; Vandana, 2016). While an individual can carry the gene mutation and experience none to few symptoms (referred to as asymptomatic or oligosymptomatic carriers, respectively), the conversion to MELAS is clinically defined, such that a seizure and/or stroke-like episode has occurred (referred to as symptomatic MELAS) (Kaufmann, 2009; Damian, 1995; Vandana, 2016; Dvorakova, 2016).
Typically functioning mitochondria provide energy to the cells of the human body and are vital for oxygen usage, protein production, and cellular transport. These metabolic processes are facilitated by oxidative phosphorylation (OXPHOS) resulting in energy production following the transformation of adenosine diphosphate (ADP) into adenosine triphosphate (ATP). However, in the case of MELAS, the OXPHOS process is impaired resulting from a biochemical reaction of insufficient oxygen to power mitochondrial ATP conversion yielding increased glycolysis and cerebral lactate as well as lowered pH and N-acetylaspartate (NAA). Consequently, decreased energy production results and normal functioning of the mitochondria is not achieved. This trajectory is associated with the mitochondrial tRNA gene mutation mt3243A>G in 80% of individuals diagnosed with MELAS (Taanman, 2002; DiMauro, 2008; Wong, 2007; Davis, 2011; Scaglia, 2012; DiMauro, 2013a; DiMauro, 2013b; Dubeau, 2000).

Mitochondrial Dementia, occurring in 40-90% of the MELAS population, is due to progressively impaired mitochondrial function impacting on neuronal function and eventual cortical injury due to seizures and stroke-like episodes (Hirano, 1994; DiMauro, 2013a; Yatsuga, 2012; Finsterer, 2008). Although much of the literature on cognition in the MELAS population is limited to clinical accounts, case reports, and cross-sectional studies, neuropsychological functioning has been characterized by global impairment and a progressive decline in mental status, intelligence, and cognitive abilities (Hirano, 1994; Sproule, 2008; Kartsounis, 1992; Lang, 1995; Sartor, 2002; Kiejna, 2002; Pachalska, 2002; Majamaa-Voltti, 2006; Neargarder, 2007; Lacey, 2008; Coomans, 2011; Chu, 2012; Nesbitt, 2013). The largest prospective natural history study found that MELAS patients exhibited decline in a global neuropsychological score and the onset of cognitive difficulties was earliest the affected MELAS (Kaufmann, 2004; Kaufmann, 2009; Kaufmann, 2011; Weiduschat, 2014). Although it appears that MELAS patients
demonstrate cognitive performance consistent with mitochondrial dementia (Kaufmann, 2004), no study has looked at individual cognitive domains to determine whether a selective cognitive phenotype exists within the MELAS population and whether progression of cognitive decline is distinct from other neurodegenerative disorders.

In order to better understand the course of the illness from a cognitive perspective, we aimed to further delineate the neuropsychological profile associated with MELAS compared with carrier relatives of the mt3243A>G mutation and unaffected control participants. Location of cerebral lactic acidosis, depletion of NAA, and occurrence of seizures and stroke-like episodes are known to occur first in the back regions of the brain and later affect more frontal regions as the MELAS disease symptoms progress over time (Veggiotti, 1995; Sue, 1998; Iizuka, 2003; Iizuke, 2005). Our previous work demonstrated that visual and verbal memory are sensitive to lactate and NAA; however, only visual memory performance discriminates across groups, such that visual memory was worse in affected MELAS yet no difference was observed in verbal memory compared with carrier relatives and control participants (Leaffer, in preparation). These findings suggest that perhaps individuals with MELAS demonstrate a distinct neurodegenerative profile preferentially affecting posterior brain regions and associated cognitive functions.

This study has three aims and testable hypotheses. (1) To examine neuropsychological functioning in a large sample of MELAS, carrier relatives, and control participants based on cross-sectional data. We hypothesize that MELAS patients will demonstrate worse performance across cognitive domains compared to carrier relatives and controls at baseline. (2) To examine the relationship between neurochemical metabolites and cognition in the mt3243A>G mutation. We hypothesize that ventricular and occipital lactate will be highest and NAA will be lowest in
MELAS and that these levels will correlate with performance, such that higher levels of lactate and lower levels of NAA are associated with worse cognitive performance. (3) To examine the cognitive trajectory over time and further delineate the cognitive phenotype associated with the MELAS population. We hypothesize that a selective profile of decline will be observed, such that functions associated with posterior brain regions (i.e., visually-mediated tasks) will show a faster decline compared with anterior brain regions (i.e., verbally-mediated tasks).

Method

Participants. The study cohort is described elsewhere (Kaufmann, 2011). Briefly, 123 matrilineal relatives from 45 families were enrolled in the natural history prospective cohort study of the mt3243A>G genotype between December 1995 and January 2008. From this larger study, our sample is limited to individuals who underwent longitudinal neuropsychological assessment as part of a comprehensive medical evaluation. Fully symptomatic MELAS patients were clinically defined by the presence of the mt3243A>G mutation, focal brain involvement (i.e., stroke-like episode and/or seizure), and cerebral lactic acidosis. Carrier relatives were related to the individual with MELAS through maternal lineage, carry the mt3243A>G mutation confirmed by pedigree analysis, and were either asymptomatic or oligosymptomatic at baseline. Fathers of MELAS, other paternal family members, and spouses who do not carry the mt3243A>G mutation served as the control group. Only data from individuals age 16 and above are included in the current analyses.

The institutional review board at Columbia University Medical Center approved this study (IRB#AAAB1425); written informed consent was obtained. The study was supported by
grants from the National Institutes of Health (P01-HD080642; P01-HD32062) to Drs. Darryl C. De Vivo and Salvatore Di Mauro. This study was exempt from the institutional review board at Queens College of the City University of New York.

**Procedures.** All participants underwent comprehensive neuropsychological evaluations (1.5-2 hours in length) to measure attention, processing speed, executive functioning, language, visual-spatial ability, and memory. In general, all measures were administered; however, a shortened evaluation was administered for very impaired patients. For individual cognitive tests that were attempted, but the participant was unable to complete due to cognitive impairment, the lowest possible value for that task was assigned.

**Measures.**

**Demographics.** We classified participants by age, sex, total education, and ethnicity. Clinical characteristics were also recorded for each participant, including whether a stroke-like episode and/or seizure had occurred.

**Cognitive measures.** Composition of cognitive domain composites is outlined in Table 6.

Folstein Mini Mental Status Exam (MMSE; Folstein, 1975): Brief assessment of mental status; maximum score is 30 points. From this measure, two different scores were used: 1) language score comprised of naming, repetition, comprehension, and writing (maximum 7
points), and 2) attention/calculation score based upon serial 7s, spelling world backwards, and adding change (maximum 7 points).

Similarities Subtest from the Wechsler Adult Intelligence Scale – Revised (WAIS-R; Wechsler, 1981): Measure of abstract reasoning and semantic knowledge, during which a participant was given two words and asked to describe how they are alike.

Selective Reminding Test (SRT; Bushke, 1974): Measure of verbal learning and memory through rote list-learning task of 12 words. Three trials were completed: 1) six learning trials, 2) delayed recall trial after 15 minutes, and 3) multiple-choice recognition trial following delayed recall.

Benton Visual Retention Test (BVRT; Benton, 1955): Measure of visual perception, visual-spatial drawing construction, and visual memory; Three trials were completed: 1) copy of 10 designs, and 2) 10-second recall trial of 10 designs, and 3) multiple-choice recognition trial of all 10 designs from the recall trial.

Verbal Fluency (Letters: CFL and Semantic: Animals; Benton, 1994): Measure of retrieval of information that requires executive control and behavioral initiation, consisting of three one minute trials to name as many words the subject can think of that begin with the letters C, F, and L. A single semantic/category trial to name as many animals as the participant can think of beginning with any letter.

Trail Making Test A & B (TMT A, TMT B; Army Individual Test Battery, 1944; Reitan, 1985): Measure of attention and executive functioning. TMT A assessed visual attention and processing speed, involving drawing a line connecting the numbers in order from lowest to
highest. TMT B assessed executive functioning, specifically set-shifting, involving drawing a line connecting numbers and letters in order, switching from number to letter to number, etc.

Hooper Visual Organization Test (HVOT; Hooper, 1958): Measure of visual integration by which participants were asked to name pictures of objects that have been cut up and rearranged. This task consists of 30 items.

Cancellation Test (Sano, 1984): Measure of processing speed and vigilance, during which participants were instructed to cross out all the target stimuli presented in an array of either shapes (target: diamonds) or letter triads (target: TMX) as quickly as possible. The shape condition denotes ‘shape time’ for task completion and ‘shape omissions’ for the number of target shapes not crossed out. The letter triad condition denotes ‘TMX time’ for task completion and ‘TMX omissions’ for the number of target letter triads not crossed out.

Odd Man Out Test (Flowers, 1985): Measure of reasoning that required the participant to determine which set of letters or numbers differs from the other sets. A total score is calculated based on the number of correct responses across four trials.

**Neurochemical Variables.** Participants underwent multi-slice proton magnetic resonance spectroscopic imaging (MRS) to estimate lactate and NAA quantities in the fluid of the lateral ventricles and occipital lobe gray matter; full method is described elsewhere (Kaufmann, 2004; Kaufmann, 2009; Kaufmann, 2011; Weiduschat, 2014; Duyn, 1993). Values were measured in institutional units (i.u.) and categorized in comparison to the average amount of metabolite present in the unaffected control participants (Table 7).
Statistical Analysis.

Demographic characteristics were summarized with frequencies/percentages for categorical variables and mean/standard deviations for continuous variables. Model assumptions (i.e., normality, linearity, homoscedasticity, and homogeneity) were examined for all variables. Chi-squared tests were used to determine statistical significance of categorical variables and t-tests/ANOVAs were used to determine statistical significance of continuous variables. Baseline predictor variables (i.e., age, sex, total education, ethnicity) were examined across participant groups. Significance level was set at 0.00625 (0.05/8) for all analyses after adjusting for multiple comparisons (8 individual cognitive scores for our primary hypotheses).

Cut-offs were identified for neurochemical variables. Lactate was separately represented by three categories: “within normal limits” (ventricular: ≤4.8 i.u.; occipital: ≤4.5 i.u.), “high” (ventricular: 4.8-5.7 i.u.; occipital: 4.5-5.4 i.u.), and “very high” (ventricular: ≥5.7 i.u.; occipital: ≥5.4 i.u.). Occipital NAA was categorized as “low” (≤14.0 i.u.), “within normal limits” (14.0 i.u.-17.6 i.u.), and “high” (≥17.6 i.u.). Two ratios were created, 1) occipital lactate to occipital NAA and 2) ventricular lactate to occipital NAA, to examine the relationships between neurochemical values and cognitive performance across domains.

Cognitive domain composites were based upon Siedlecki et. al.’s (2008) five-factor model, with adjustments made depending on the available data in the current study. To obtain one score for each domain composite: 1) individual test scores were converted into a z-score; 2) mean z-scores for each measure were determined based on group performance; and 3) mean z-scores were computed for each pre-determined cognitive domain using all measures that are classified within that domain. Baseline performance was examined across groups for each cognitive domain.
Generalized estimating equations (GEE) were utilized to examine the rate of cognitive change over time across groups. As such, the linear relationship over multiple evaluations from baseline through year four on eight cognitive domain scores was examined. The main goal was to look at disease status (i.e., case status) as the primary predictor variable. In the GEE model, predictor variables including age, sex, and total education, a two-level ethnicity variable (i.e., white vs. non-white), and family number to approximate environment were controlled for to determine whether they were associated with the rate of change in cognition across groups. The primary outcomes were the repeated individual cognitive domain scores. In each of these separate cognitive domain models, predictor variables of case status, time since baseline evaluation, and case status X time interaction were included in the model. A significant interaction indicated that participants with different case status have differential rates of cognitive change over time. An exploratory post-hoc sensitivity analysis was completed to determine whether the interaction was significant or not.

To explore visual and verbal functioning separately, two additional composite scores were derived and rate of change over time was examined utilizing the same GEE model specified above. A verbal composite comprised all verbally-mediated measures, including MMSE attention/calculation, MMSE language, letter/category fluency, SRT total recall, SRT delayed recall, SRT delayed recognition, and the Wechsler similarities subtest. A visual composite comprised visually-mediated measures, including BVRT recall, BVRT recognition, Odd Man Out total, BVRT copy, and the Hooper Visual Organization Test.
**Results**

Demographic and clinical characteristics are summarized in Table 7. Of the 141 participants included in this analysis, there were 35 MELAS, 78 carriers, and 28 controls. At baseline, the total sample ranged from 16 to 80 years, with mean age of 41.4 (SD = 14.8) years. The sample was 40.4% male and 90.8% Caucasian. Total education in the sample ranged from seven to 20 years, with mean education of 14.4 (SD = 2.9) years. Participant groups differed by age and sex only. Stroke-like episodes and seizures occurred in 85.3% and 85.7% of MELAS, respectively. Seizures were noted in 2.7% of carriers.

*Baseline cognitive performance across domains.*

Baseline cognitive performance differed across the three groups in all domains of functioning (p = 0.000) (Table 8; Figure 7). Post-hoc analyses revealed that the MELAS and carrier groups differed across all domains (p = 0.00) as did the MELAS and control participants (p = 0.00). However, none of the cognitive domains differed between carrier and control groups.

Verbal and visual composites differed across groups at baseline (p = 0.000) (Table 8; Figure 8). Similar to domain-specific findings, significant differences were observed for verbal and visual composite scores between MELAS and carriers (p = 0.000) and MELAS and controls (p = 0.000), but not between carriers and controls.

*Neurochemical metabolites at baseline across groups.*

Across the total sample, ventricular lactate ranged from 1.97 – 15.35 i.u. with a mean of 5.75 i.u. (SD = 2.87). Occipital lactate ranged from 1.23 – 14.56 i.u. with a mean of 4.61 i.u. (SD = 2.37).
Occipital NAA ranged from 2.83 – 26.40 i.u. with a mean of 15.53 i.u. (SD = 4.38). Groups significantly differed by ventricular lactate (p = 0.000), occipital lactate (p = 0.000), and occipital NAA (p = 0.000). The MELAS group had the highest percentage of participants falling in the ‘very high’ category for ventricular lactate (83.3%) and occipital lactate (60.0%), and the ‘low’ category for occipital NAA (73.3) (Table 7).

*Relationship between cognitive domains and neurochemical metabolites at baseline.*

Measured neurochemical metabolites were significantly associated with each cognitive domain at baseline (Table 9). Both lactate variables were negatively correlated with cognition, such that performance on cognitive tasks decreases as lactate increases. Ventricular lactate was highly correlated with language, moderately correlated with processing speed, reasoning, and visual-spatial skills, and weakly correlated with mental flexibility, and memory (p = 0.00); no association was observed with attention (p = 0.17). Occipital lactate was moderately correlated with processing speed, language, memory, reasoning, and visual-spatial skills (p = 0.00), and weakly correlated with attention (p = 0.02) and mental flexibility (p = 0.00). Occipital NAA was positively correlated with cognition, such that performance on cognitive tasks increases as NAA increases. NAA was moderately correlated with processing speed, language, memory, reasoning, and visual-spatial skills, and weakly correlated with attention, mental flexibility (p = 0.00).

For visually-mediated tasks, moderate negative correlations with ventricular lactate and occipital lactate, and a moderate positive correlation with occipital NAA were observed (p = 0.00). For verbally-mediated tasks, moderate negative correlations with ventricular lactate
and occipital lactate, and a moderate positive correlation with occipital NAA were observed 
(p = 0.00) (Table 9).

Both neurochemical ratios were significantly associated with each cognitive domain at baseline (p = 0.00). Moreover, the associations remained and were stronger, suggesting that this ratio is the most sensitive way to think about the neurochemical changes underlying the disease (Table 9).

Rate of change over time across cognitive domains.

Compared to control participants, individuals with MELAS showed a significantly faster decline in processing speed (p = 0.045) and visual-spatial skills (p = 0.003), while no difference in the rate of change over time was observed for other domains (Figure 9). The rate at which carrier cognitive performance changed over time did not differ from controls (Figure 9).

Individuals with MELAS exhibited a significantly faster decline in performance on visually-mediated tasks over time compared to control participants (p = 0.016), whereas no difference in rate of change over time on verbally-mediated task performance was observed (Figure 10). The rate at which carrier performance changed over time did not differ from controls with respect to visually-mediated tasks or verbally-mediated tasks (Figure 10).

Discussion

This study examined the progression of cognitive function and neurochemical changes in a cohort of 35 individuals with MELAS, 78 carrier relatives, and 28 control participants. We
hypothesized that MELAS patients would demonstrate worse cognition across domains, and
given that the disease selectively targets posterior brain regions, a selective profile of decline will
be observed, such that functions associated with posterior brain regions (i.e., visually-mediated
tasks) would show a faster decline compared with anterior brain regions (i.e., verbally-mediated
tasks).

Compared with carrier relatives and control participants, individuals with MELAS
demonstrated worse performance across all cognitive domains at baseline, including attention,
processing speed, mental flexibility, language, memory, reasoning, and visual-spatial ability; no
difference was observed between carriers and controls. Likewise, performance on visually-
mediated tasks and verbally-mediated tasks were also worse in individuals with MELAS.
Neurochemical metabolites (i.e., ventricular lactate, occipital lactate, and occipital NAA)
differentiated MELAS from carriers and controls, such that higher proportions of the MELAS
group had “high” and specifically “very high” lactate levels and “low” NAA levels. Lactate and
NAA also correlated with each cognitive domain and visual/verbal composites at baseline,
indicating that as ventricular/occipital lactate increased and NAA decreased, cognition declined.
Moreover, the associations with the two ratios of ventricular/occipital lactate to occipital NAA
remained and were stronger, suggesting that this ratio is the most sensitive way to think about the
neurochemical changes underlying the disease.

Over an average of almost three years, MELAS exhibited a faster decline in processing
speed, and visual-spatial ability compared with control participants, whereas carrier relatives did
not. Not only was there a trend of worse performance on visually-mediated tasks at baseline,
performance declined at a faster rate in the MELAS group only. There was no difference in the
rate of change on verbally-mediated task performance. Our prior work demonstrated that
individuals with MELAS have the highest lactate and lowest NAA localized within posterior brain regions, and these levels differentiate visual memory performance but not verbal performance. Taken together, these findings provide evidence for a selective cognitive profile associated with MELAS, such that both the severity of impairment and the rate of decline observed on visually mediated tasks are worse than that seen on verbally mediated tasks.

The findings of a pathognomonic, disease specific, pattern of cognitive involvement is likely due to the anatomical underpinnings of the disease. In the brain, the distribution of mitochondrial is normally densest in the occipital lobe region. In MELAS, decreased mitochondrial function presents with characteristic neurochemical changes that are also primarily associated with posterior brain regions, likely reflecting the consequence of mitochondrial distribution. For patients with MELAS, seizures, and stroke-like episodes have also been shown to occur primarily in posterior brain regions. Thus, the disease mechanism preferentially targets the posterior regions of the brain resulting in detrimental effects on cognitive functions (i.e., visually-mediated tasks) localized to these regions. With time, the neurodegeneration becomes more global, and affecting more anteriorly localized cognitive functions (i.e., verbally-mediated tasks).

These findings are of great importance to understanding the neuropsychological consequences of MELAS; however, other contributions to cognitive outcome should be considered. Specifically, detrimental multisystemic effects, psychosocial, and physical comorbidities may have also impacted cognitive performance. While we attempted to best categorize each task into cognitive domains, individual tasks may have multiple components and realistically could have been placed in more than one domain; however, we were guided by a pre-determined factor structure (Siedlecki, 2008), and also further discriminated between visual
and verbal-based tasks to better address our hypotheses. Specific to our study sample, the differing time between evaluations among participants was addressed by GEE modeling and discrepant sample sizes within each cognitive domain is a weakness inherent to the limited abilities of the MELAS sample to complete the full evaluation. Despite these limitations, our findings contribute to improving scientific knowledge by determining a specific cognitive phenotype associated with the disease.

This study was the first to examine cognitive decline and neurochemical changes in a large cohort of the MELAS population. We identified worse performance in all cognitive domains, highest lactate and lowest NAA levels, and a faster rate of decline in processing speed, visual-spatial skills, and on visually-mediated tasks across domains in the individuals with MELAS. These findings support the notion that a distinct trajectory of cognitive decline is associated with this specific neurodegenerative disease. While the neuropsychological profile of Alzheimer’s disease is characterized by language and memory impairments (Weintraub, 2012), and executive function deficits are consistently observed in Parkinson’s disease (Petrova, 2012), the cognitive phenotype associated with MELAS presents in a posterior to anterior progressive fashion with underlying visually-mediated performance deficits appearing prior to verbal impairments.
Paper 2 Tables.

Table 6. Cognitive domain composition

<table>
<thead>
<tr>
<th>Domain</th>
<th>Measure</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Attention</strong></td>
<td>MMSE Attention/Calculation*</td>
</tr>
<tr>
<td></td>
<td>Cancellation: TMX Omissions</td>
</tr>
<tr>
<td></td>
<td>Cancellation: Shape Omissions</td>
</tr>
<tr>
<td><strong>Processing Speed</strong></td>
<td>Trailmaking Test A Time</td>
</tr>
<tr>
<td></td>
<td>Cancellation: TMX Time</td>
</tr>
<tr>
<td></td>
<td>Cancellation: Shape Time</td>
</tr>
<tr>
<td><strong>Executive Functioning / Mental Flexibility</strong></td>
<td>Trailmaking Test B Time</td>
</tr>
<tr>
<td><strong>Language</strong></td>
<td>MMSE Language*</td>
</tr>
<tr>
<td></td>
<td>Letter Fluency (CFL)</td>
</tr>
<tr>
<td></td>
<td>Category Fluency (Animals)</td>
</tr>
<tr>
<td><strong>Memory</strong></td>
<td>SRT Total Recall*</td>
</tr>
<tr>
<td></td>
<td>SRT Delayed Recall*</td>
</tr>
<tr>
<td></td>
<td>SRT Delayed Recognition*</td>
</tr>
<tr>
<td></td>
<td>BVRT Recall*</td>
</tr>
<tr>
<td></td>
<td>BVRT Recognition*</td>
</tr>
<tr>
<td><strong>Reasoning</strong></td>
<td>Wechsler Similarities</td>
</tr>
<tr>
<td><strong>Visual-Spatial</strong></td>
<td>Wechsler Similarities</td>
</tr>
<tr>
<td></td>
<td>BVRT Copy*</td>
</tr>
<tr>
<td></td>
<td>Hooper Visual Organization Test</td>
</tr>
<tr>
<td><strong>Visually-Mediated Tasks</strong></td>
<td>BVRT Recall*</td>
</tr>
<tr>
<td></td>
<td>BVRT Recognition*</td>
</tr>
<tr>
<td></td>
<td>Odd Man Out Total</td>
</tr>
<tr>
<td></td>
<td>BVRT Copy*</td>
</tr>
<tr>
<td></td>
<td>Hooper Visual Organization Test</td>
</tr>
<tr>
<td><strong>Verbally-Mediated Tasks</strong></td>
<td>MMSE Attention/Calculation*</td>
</tr>
<tr>
<td></td>
<td>MMSE Language*</td>
</tr>
<tr>
<td></td>
<td>Letter Fluency (CFL)</td>
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<td></td>
<td>Category Fluency (Animals)</td>
</tr>
<tr>
<td></td>
<td>SRT Total Recall*</td>
</tr>
<tr>
<td></td>
<td>SRT Delayed Recall*</td>
</tr>
<tr>
<td></td>
<td>SRT Delayed Recognition*</td>
</tr>
<tr>
<td></td>
<td>Wechsler Similarities</td>
</tr>
</tbody>
</table>

*MMSE: Mini Mental Status Exam  
SRT: Selective Reminding Test  
BVRT: Benton Visual-Spatial Retention Test
Table 7. Participant demographic and clinical characteristics at baseline.

<table>
<thead>
<tr>
<th></th>
<th>Total Sample</th>
<th>MELAS</th>
<th>Carriers</th>
<th>Controls</th>
<th>Between Group Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (in years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>16.0 - 80.0</td>
<td>16.0 - 61.0</td>
<td>16.0 - 80.0</td>
<td>23.0 - 73.0</td>
<td>F = 10.6 p = 0.000</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>41.4 (14.8)</td>
<td>34.3 (11.9)</td>
<td>41.4 (15.2)</td>
<td>50.5 (12.4)</td>
<td></td>
</tr>
<tr>
<td><strong>Sex: N (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>57 (40.4)</td>
<td>16 (45.7)</td>
<td>19 (24.4)</td>
<td>22 (78.6)</td>
<td>p = 0.000</td>
</tr>
<tr>
<td>Female</td>
<td>84 (59.6)</td>
<td>19 (54.3)</td>
<td>59 (75.6)</td>
<td>6 (21.4)</td>
<td></td>
</tr>
<tr>
<td><strong>Education (in years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>7.0 - 20.0</td>
<td>8.0 - 18.0</td>
<td>9.0 - 20.0</td>
<td>7.0 - 20.0</td>
<td>F = 3.1 p = 0.05</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>14.4 (2.9)</td>
<td>13.4 (2.6)</td>
<td>14.9 (2.9)</td>
<td>14.4 (3.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Ethnicity: N (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>128 (90.8)</td>
<td>31 (88.6)</td>
<td>72 (92.3)</td>
<td>25 (89.3)</td>
<td>p = 0.53</td>
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<tr>
<td>Black</td>
<td>1 (0.7)</td>
<td>1 (2.9)</td>
<td>0 (0.0)</td>
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<tr>
<td>Hispanic</td>
<td>6 (4.3)</td>
<td>1 (2.9)</td>
<td>4 (5.1)</td>
<td>1 (3.6)</td>
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<tr>
<td>Asian</td>
<td>1 (0.7)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
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<tr>
<td>American Indian</td>
<td>5 (3.5)</td>
<td>2 (5.7)</td>
<td>2 (2.6)</td>
<td>1 (3.6)</td>
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<tr>
<td><strong>Stroke: N (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>29 (21.3)</td>
<td>29 (85.3)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>p = 0.000</td>
</tr>
<tr>
<td>No</td>
<td>107 (78.7)</td>
<td>5 (14.7)</td>
<td>75 (100.0)</td>
<td>27 (100.0)</td>
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<tr>
<td><strong>Seizure: N (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>32 (23.4)</td>
<td>30 (85.7)</td>
<td>2 (2.7)</td>
<td>0 (0.0)</td>
<td>p = 0.000</td>
</tr>
<tr>
<td>No</td>
<td>105 (76.6)</td>
<td>5 (14.3)</td>
<td>73 (97.3)</td>
<td>27 (100.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Occipital NAA Levels: N (%)</strong></td>
<td>112</td>
<td>30</td>
<td>62</td>
<td>20</td>
<td>χ² = 40.2 p = 0.000</td>
</tr>
<tr>
<td>low (≤14.0 i.u.)</td>
<td>35 (31.3)</td>
<td>22 (73.3)</td>
<td>8 (12.9)</td>
<td>5 (25.0)</td>
<td></td>
</tr>
<tr>
<td>within normal limits (14.0 - 17.6 i.u.)</td>
<td>42 (37.5)</td>
<td>4 (13.3)</td>
<td>26 (41.9)</td>
<td>12 (60.0)</td>
<td></td>
</tr>
<tr>
<td>high (≥ 17.6 i.u.)</td>
<td>35 (31.3)</td>
<td>4 (13.3)</td>
<td>28 (45.2)</td>
<td>3 (15.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Occipital Lactate Levels: N (%)</strong></td>
<td>111</td>
<td>30</td>
<td>61</td>
<td>20</td>
<td>χ² = 45.9 p = 0.000</td>
</tr>
<tr>
<td>within normal limits (≤ 4.5 i.u.)</td>
<td>69 (62.2)</td>
<td>5 (16.7)</td>
<td>47 (77.0)</td>
<td>17 (85.0)</td>
<td></td>
</tr>
<tr>
<td>high (4.5 - 5.4 i.u.)</td>
<td>19 (17.1)</td>
<td>7 (23.3)</td>
<td>9 (14.8)</td>
<td>3 (15.0)</td>
<td></td>
</tr>
<tr>
<td>very high (≥ 5.4 i.u.)</td>
<td>23 (20.7)</td>
<td>18 (60.0)</td>
<td>5 (8.2)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Ventricular Lactate Levels: N (%)</strong></td>
<td>111</td>
<td>30</td>
<td>61</td>
<td>20</td>
<td>χ² = 43.1 p = 0.000</td>
</tr>
<tr>
<td>within normal limits (≤ 4.8 i.u.)</td>
<td>49 (44.1)</td>
<td>4 (13.3)</td>
<td>29 (47.5)</td>
<td>16 (80.0)</td>
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<tr>
<td>high (4.8 - 5.7 i.u.)</td>
<td>20 (18.0)</td>
<td>1 (3.3)</td>
<td>15 (24.6)</td>
<td>4 (20.0)</td>
<td></td>
</tr>
<tr>
<td>very high (≥ 5.7 i.u.)</td>
<td>42 (37.8)</td>
<td>25 (83.3)</td>
<td>17 (27.9)</td>
<td>0 (0.0)</td>
<td></td>
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</tbody>
</table>
Table 8. Cognition by domain at baseline.

<table>
<thead>
<tr>
<th>Cognitive Domain</th>
<th>MELAS*</th>
<th>Carriers§</th>
<th>Controls</th>
<th>Between Group Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean (SD)</td>
<td>Range</td>
<td>n</td>
</tr>
<tr>
<td>Attention</td>
<td>28</td>
<td>-0.32 (0.58)</td>
<td>-0.91-0.96</td>
<td>70</td>
</tr>
<tr>
<td>Processing Speed</td>
<td>30</td>
<td>-0.71 (1.11)</td>
<td>-2.09-0.68</td>
<td>71</td>
</tr>
<tr>
<td>Mental Flexibility</td>
<td>32</td>
<td>-0.59 (1.06)</td>
<td>-3.49-0.87</td>
<td>72</td>
</tr>
<tr>
<td>Language</td>
<td>23</td>
<td>-0.70 (0.80)</td>
<td>-1.83-0.88</td>
<td>69</td>
</tr>
<tr>
<td>Memory</td>
<td>29</td>
<td>-0.70 (0.93)</td>
<td>-1.98-0.84</td>
<td>70</td>
</tr>
<tr>
<td>Reasoning</td>
<td>24</td>
<td>-0.63 (0.90)</td>
<td>-2.08-0.72</td>
<td>71</td>
</tr>
<tr>
<td>Visual-Spatial</td>
<td>30</td>
<td>-0.77 (1.09)</td>
<td>-2.00-0.77</td>
<td>73</td>
</tr>
<tr>
<td>Visually-Mediated Tasks</td>
<td>28</td>
<td>-0.77 (0.96)</td>
<td>-1.84-0.79</td>
<td>69</td>
</tr>
<tr>
<td>Verbally-Mediated Tasks</td>
<td>21</td>
<td>-0.54 (0.74)</td>
<td>-1.99-0.69</td>
<td>67</td>
</tr>
</tbody>
</table>

*MELAS and carrier relatives differed by all domains (p = 0.00); MELAS and control participants differed by all domains (p = 0.00)

§Carrier relatives and control participants did not differ by any domain
Table 9. Relationship between cognitive domains and neurochemical metabolites at baseline visit.

<table>
<thead>
<tr>
<th>Cognitive Domain</th>
<th>Ventricular Lactate</th>
<th>Occipital Lactate</th>
<th>Occipital NAA</th>
<th>Ventricular Lactate / Occipital NAA</th>
<th>Occipital Lactate / Occipital NAA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>r</td>
<td>p-value</td>
<td>n</td>
<td>r</td>
</tr>
<tr>
<td>Attention</td>
<td>100</td>
<td>-0.1</td>
<td>0.174</td>
<td>100</td>
<td>-0.4</td>
</tr>
<tr>
<td>Processing Speed</td>
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<td>-0.5</td>
<td>0.000</td>
<td>101</td>
<td>-0.6</td>
</tr>
<tr>
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<tr>
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<tr>
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<td>0.000</td>
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<tr>
<td>Visually-Mediated Tasks</td>
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<tr>
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Table 10. Rate of change in cognition over time in MELAS vs. controls and carriers versus controls.

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<th>Cognitive Domain GEE Models*</th>
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<th>β§</th>
<th>p-value</th>
<th>β</th>
<th>p-value</th>
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<td>0.555</td>
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<td><strong>Carriers vs. Controls</strong></td>
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<td>0.770</td>
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<td>0.085</td>
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</table>

*Adjusted for age in years, gender, years of education, ethnicity, family

§ Beta value represents the interaction of clinical group and time
Paper 2 Figures.

**Figure 7.** Cognitive performance at baseline across domains.
**Figure 8.** Visually-mediated and verbally-mediated task performance at baseline.
Figure 9. Rate of change over time across groups in each cognitive domain.
Figure 10. Rate of change over time on visually-mediated and verbally-mediated task performance.
Discussion & Conclusions.

This dissertation examined cognitive function and neurochemical biomarkers as part of a large longitudinal natural history study of individuals with MELAS, carrier relatives of the mt3243A>G mutation, and unaffected control participants. Our goal was to determine whether a selective neuropsychological profile associated with MELAS exists and whether neurochemical anomalies are associated with cognition in the MELAS population. Our work and hypotheses were driven by the notion of a posterior to anterior progression of distinct clinical features, including seizures, stroke-like episodes, and neurochemical variations (i.e., cerebral lactate and NAA) that have predominantly been localized to posterior brain regions in association with the MELAS population, and the mt3243A>G mutation in particular.

First, we examined memory performance and the relationship between neurochemical metabolites and memory function. We hypothesized that posteriorly located seizures and stroke-like episodes (e.g., in the occipital lobe) would yield greater visual memory deficit compared with verbal memory, and that increased cerebral lactate and decreased NAA will be associated with worse memory performance. We found that compared with carrier relatives and controls, individuals with MELAS had the highest ventricular and occipital lactate, lowest NAA, and most impaired verbal and visual memory performance; however, neurochemical levels only substantially differentiated visual memory performance. Verbal memory performance, though appearing weakest in the MELAS group likely related to overall cognitive decline associated with the disease, was no more than weakly associated with neurochemical variations. Noteworthy is the finding that worse visual memory compared with verbal memory performance was found not only in the MELAS group but also in the carrier relatives, and carrier relatives also demonstrated worse visual memory compared with control participants. These findings, in
combination with neurochemical variations observed between carrier relatives and control participants (e.g., significantly different occipital NAA and ventricular lactate levels) suggest the presence of an underlying neurochemical profile contributing to a more subtle phenotype within this group.

Second, we examined cognition across domains (e.g., attention, processing speed, executive functioning/mental flexibility, language, reasoning, visual-spatial skills), the relationship between neurochemical metabolites and cognitive performance across these domains, and the rate of change over time for each cognitive domain. We hypothesized that individuals with MELAS will have: a) worse cognitive performance in all domains at baseline, b) the lowest NAA and highest lactate levels, which will correlate with poorer cognitive performance, and c) a longitudinal cognitive progression characterized by a faster rate of decline in visually-based functions associated with posterior brain regions compared with carrier relatives and control participants. To further elucidate the effect of the posterior localization of seizures, stroke-like episodes, and variations in neurochemicals, we created visually-mediated (posteriorly located functions) and verbally-mediated (anteriorly located functions) composite scores to examine at baseline, in association with neurochemicals, and their rate of change over time. We found that individuals with MELAS demonstrate worse cognitive performance across domains of attention, processing speed, executive functioning/mental flexibility, language, reasoning, and visual-spatial skills. Again, neurochemical metabolites differentiated MELAS (i.e., highest lactate, lowest NAA) from carrier relatives and control participants, supporting the assumption that as ventricular/occipital lactate increased and occipital NAA decreased, cognition declined. Moreover, when ratios of lactate to NAA were utilized to examine the relationship between neurochemicals and cognitive performance, the associations became more evident and
stronger, thus indicating that this is the most sensitive and best way to examine the effect of these biomarkers in relation to disease outcome.

Consistent with our hypotheses, MELAS demonstrate a wider range and worse average performance on visually-mediated tasks compared with verbally-mediated tasks within group and compared with carrier relatives and control participants. Furthermore, the rate of change over time was significantly different between MELAS and controls in areas of processing speed, and visual-spatial skills such that a faster decline was observed in these areas only; all other domains of functioning appear to change in a similar fashion to controls. Likewise, and in support of the posterior to anterior progression of cognitive deterioration, visually-mediated tasks declined significantly faster in the MELAS group compared with control participants whereas no difference was observed for verbally-mediated tasks. Although carrier relatives and control participants did not differ with respect to cognitive performance across domains, a pattern of greater deficit on visually-mediated tasks (e.g., wide range of performance) in particular combined with evidently higher NAA (which will drop to lowest value range should these individuals convert) and lactate levels compared with controls, is further suggestive of a more mild phenotype associated with the mt3243A>G mutation that may be helpful in predicting conversion to the MELAS syndrome.

MELAS as a neuroscience reductionist model.

In addition to the identified strengths, and in light of the known study limitations, the known genetic etiology of MELAS provides an advantage to studying the cognitive and behavioral consequences of such a devastating developmental disorder. MELAS can be
described in terms of a neuroscience reductionist model, allowing for the consideration of the effect of the genetic mutation on cognition and behavior across systems and throughout development. In MELAS, the typical mt3243A>G mutation yields a reduction in mitochondrial tRNA, which limits protein synthesis, and which, in turn, impacts on mitochondrial function. Such mitochondrial dysfunction results in decreased energy production and deficient OXPHOS process. While the brain must continue to develop with the context of mitochondrial dysfunction, it is brain function that suffers. With the build-up of lactic acidosis, neuronal hyperexcitability, and occurrence of stroke-like episodes and seizures, the brain structure and function becomes less resilient. This cascade of events, in combination with the effects of the individual’s environment and multisystemic involvement, triggers the deterioration of cognition and behavior in the fully symptomatic MELAS (Figure 11). The localization of mitochondria known to be more densely located within the brain’s occipital cortex may contribute to the overall disease manifestation. Mitochondrial dysfunction within the posterior brain regions may affect cognitive and functional outcome as areas that normally may have higher energy demands will likely suffer the greatest impact when the energy supply is limited. As such, cognitive functions localized to these particular posterior brain regions will be adversely affected (Trumbull, 2010; Berti, 2014).
Figure 11. Cascade of events from gene mutation through cognitive and behavioral effects

There are phenotypic differences across individuals who are genetically identified as having mt3243A>G mutations, which has resulted in characterizing them as two separate groups – carriers and those who have the fully symptomatic MELAS phenotype. Among the carrier group, there are some individuals who appear to asymptomatic and many others who present with a wide range of symptoms. This difference in presentation, despite the same underlying genetic etiology, makes studying the disorder particularly complex. There is an evolution in

Source: Adapted from V.J.H.
symptomatology over time, such that the disease course can be thought to occur as a developmental progression. Prior to the conversion from carrier to affected, more benign symptoms are frequently experienced (e.g., headaches, DM). The clinical diagnosis of MELAS is only assigned when certain clinical features are experienced (e.g., stroke-like episodes and seizures). The progression of the clinical syndrome and medically symptomatic decline is well-documented. However, from a neuropsychological perspective, minimal work has systematically examined the progression of cognitive deterioration. Such change has been documented by clinician report, few longitudinal case studies, and the examination of global neurological and neuropsychological function over time as part of the natural history study by Kauffman et. al. (2004). The observed progressive decline in cognitive and behavioral functioning, both type and severity is likely impacted by the multisystemic deterioration, seizures, stroke-like episodes, and vascular pathology.

**MELAS as a model disease for lifespan neuropsychology.**

From a lifespan developmental neuropsychology perspective, MELAS can be examined in terms of the evolution of brain-behavior relationships through which physical, cognitive, behavioral, and psychosocial changes manifest over time. MELAS is a complex multisystemic disorder affecting the CNS (as well as other systems) with symptoms varying case by case. The influence of external factors within the environment on the cognitive and behavioral outcome of an individual with MELAS is vast. There exists a dynamic bidirectional relationship between one’s genetic makeup and one’s environment, and early experiences shape later experiences along one’s developmental trajectory. These notions contribute to the outcome of the individual with MELAS. In particular, MELAS is maternally inherited and the extent of heteroplasmy and
mitotic segregation contributes to the clinical phenotypic spectrum of disease. The development of the CNS is likely compromised in the setting of deficient genetic product, which leads to developmental and functional differences as well as cognitive and behavioral consequences in this population. More specifically, the disease mechanism underlying cognitive outcome associated with the MELAS population is related to the presence of seizures, stroke-like episodes, and neurochemical variations (e.g., high lactate, low NAA) that primarily occur in posterior brain regions (e.g., occipital cortex). The considerable density of mitochondria in the occipital cortex and observed mitochondrial dysfunction in posterior brain regions places the brain at great risk for diminished energy production in the context of high energy demand. This results in detrimental cognitive outcome with specific impact on posteriorly-localized cognitive functions (i.e., visually-mediated tasks). Assuming a posterior to anterior progression of cognitive decline, more anteriorly-localized cognitive functions (i.e., verbally-mediated tasks) and subsequent more global deficits will subsequently occur. The effects of the mt3243A>G mutation can be followed throughout life.

*MELAS as a model for a neurodegenerative disorder.*

Mitochondrial oxidative phosphorylation generates energy as ATP, is the major source of energy in many tissues, has complex genetics, and declines with age (Wallace, 1992; Hirano, 1994). Given that the hypothesis of mitochondrial dysfunction and oxidative phosphorylation defects may contribute to the rapid manifestation of neurodegenerative disorders, including Parkinson’s, Alzheimer’s, and Huntington’s diseases (Wallace, 1992), a similar mechanism may underlie the early presence of cognitive decline in individuals with MELAS. These specific neurodegenerative disorders as well as others (e.g., Lewy-body dementia, vascular dementia,
fronto-temporal dementia, multi-system atrophy, corticobasal degeneration, supranuclear palsy, Cerebral Autosomal-Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy known as CADASIL in addition to symptomatic dementias (e.g., Creutzfeld-Jacob disease, acute demyelinating encephalomyopathy, normal pressure hydrocephalus, HIV-related dementia, pontine- or extrapontine myelinolysis) are differential diagnoses used when considering a diagnosis of MD (Finsterer, 2008).

The current findings support the notion that MELAS is a neurodegenerative disorder with a distinctive cognitive phenotype primarily affecting posterior brain regions and associated cognitive functions. Although the mechanism of mitochondrial dysfunction and oxidative phosphorylation defects underlying the rapid manifestation of neurodegenerative disorders (e.g., Alzheimer’s Disease, Parkinson’s Disease, Huntington’s Disease) (Wallace 1992) may be similar to that of MELAS, the neurochemical abnormalities differ among these diseases. Specifically, our findings of highest lactate and lowest NAA levels in MELAS and the association between these levels and cognitive performance is selective to MELAS. These neurochemical variations are different from those found in other neurodegenerative disorders; for example, individuals with Alzheimer’s disease are estimated to have high lactate levels in more generalized areas (i.e., cerebrospinal fluid) and decreased whole brain NAA has been found as well as more anteriorly in the cingulate cortex and frontal lobes (Redjems-Bennani, 1998; Moffett, 2007; Glodzik, 2015). Moreover, we observed a faster rate of decline in visually-mediated tasks, in addition to processing speed, and visual-spatial skills in absence of differential decline in areas dependent on language and verbally-mediated measures. These findings support the notion that a distinct trajectory of cognitive decline is associated with this specific neurodegenerative disease. It is well established that the cognitive phenotype associated with Alzheimer’s disease is
characterized by deficits in language and memory (Weintraub, 2012), and dysexecutive syndrome in Parkinson’s disease (Petrova, 2012), the cognitive phenotype associated with MELAS presents in a posterior to anterior progressive fashion with underlying visually-mediated performance deficits appearing prior to verbal impairments.

Presumably, carriers meet criteria for Mild Neurocognitive Disorder according to DSM-5 (American Psychiatric Association, 2013), and should conversion occur (noting that it does not occur in every carrier), the typical decline in functioning eventually yields a diagnosis of Major Neurocognitive disorder. This developmental progression of symptoms, both the observed cognitive and functional deterioration, is also commonly seen in individuals with other pathologies who exhibit the change from Mild Neurocognitive Disorder (formerly mild cognitive impairment) to Major Neurocognitive Disorder, or MD. Although our findings showed between group differences between carrier relatives and control participants on a visual memory task, but not other cognitive tasks across domains, the range of performance was greater and some of the carriers were impaired. Furthermore, in terms of neurochemical differences, the majority of carriers have the highest NAA levels (which will likely drop to the lowest should conversion to MELAS occur) and more than 50% have higher than expected lactate levels. As such, this pattern of findings subtly suggests that there is neurochemical involvement that may be more helpful than the clinical manifestation of seizures and/or stroke-like episodes in determining who will and who will not convert to the full MELAS syndrome. These measurable neurochemical biomarkers may be able to predict those at greatest risk for the transition to MELAS, which is currently being studied.

As such, individuals with MELAS meet criteria of MD and represent a model for a distinct neurodegenerative disorder. Individuals initially diagnosed with Mild Neurocognitive
Disorder, which may eventually progress to the development of Alzheimer’s disease (Major Neurocognitive Disorder), demonstrate the same deficient OXPHOS process in both the less severe and more severe versions of the disorder (Wang, 2006). This is analogous to the mechanism of mitochondrial dysfunction observed between mt3243A>G carriers and fully symptomatic patients. Not only do individuals with MD experience progressive cognitive difficulty, it is also not uncommon for these individuals to present with accompanying psychiatric manifestations (e.g., hallucinations) that have also been observed in other types of dementia (e.g., Alzheimer’s disease, Lewy Body dementia, Vascular dementia, Parkinson dementia).

**Strengths and Limitations.**

This is the first longitudinal study to examine the progression of cognition and relationship to neurochemical metabolites in a large cohort of individuals with MELAS compared with carrier relatives of the mt3243A>G mutation, and unaffected control participants. The possible alternative contributions of the devastating impact of the accompanying multisystemic disease manifestations, as well as environmental, psychosocial, and physical comorbidities, should be considered and further elucidated in future research as these are likely contributing to the worse cognitive outcome and faster cognitive decline observed in individuals with MELAS overall. Methodological limitations including small proband sample size due to the nature of rare disease, differing number of neuropsychological evaluations per participant, and differing time lapsing between these evaluations. These limitations were addressed and best overcome with the guidance of a prior factor analysis (Siedlecki, 2008) for the composition of cognitive domains and the use of generalized estimating equations to account for evaluation.
differences. Additionally, discrepant sample sizes across the cognitive domain analyses were due to the very limited abilities of some of the participants; as such the lowest possible score was assigned. Despite these weaknesses, this study is the largest cohort of the mt3243A>G mutation for which cognitive performance has been examined. Our findings have illuminated a distinct neuropsychological profile associated with MELAS, which has not been done prior. Moreover, the cognitive phenotype of carrier relatives remains to be refined; however, as indicated above, the findings in carriers of worse visual memory, as well as underlying neurochemical variations when compared with control participants suggest a selective “milder” profile may exist in determining who will convert to the full MELAS syndrome.

Conclusions.

MELAS is a quintessential model for developmental neuropsychology that can be mapped from gene mutation to cognitive phenotype, highlighting the development and degeneration of cognitive skills across domains. The observed visually-based performance deficits are in line with localized clinical symptoms (e.g., seizures and/or stroke-like episodes) and clear associations between cognition and underlying neurochemical changes (e.g., high occipital/ventricular lactate, low occipital NAA) primarily affecting posterior brain regions were noted. Taken together, MELAS is a MD syndrome with a clear distinctive and pathognomonic cognitive phenotype that does not implicate other well-known dementia syndromes as the cause of the cognitive deficits. The fact that carrier relatives appear to have subtle cognitive-related distinctions and neurochemical variations allows for the identified high lactate and NAA levels to be used as biomarkers that can predict disease severity and potential conversion to MELAS syndrome. Perhaps the distinction between MELAS, carrier relatives, and controls that we have
found allows for potential treatments, including gene therapy to correct the underlying mutations or treatments aimed at controlling lactic acidosis and NAA build up and depletion as a means to possibly prevent neurodegeneration.
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